extracts (fig. 2a). These manipulations in the case of the 25 °C animals extended the period of responsiveness to the hormone to 12 h posteclosion. Furthermore, the animals stored at 10 °C showed an even longer period of responsiveness to the DH and were capable of a strong diuretic response to the hormone at 48 h posteclosion. These experiments indicate that in the apparent absence of intrinsic DH, there is a temperature dependent loss of sensitivity to DH injections. The delayed loss of DH sensitivity noted in neck-ligatured animals stored at 10 °C could prove useful as an assay system for future studies.

Since removal of intrinsic DH coupled with storage at 10 °C enhanced the period of responsiveness in neck-ligatured animals, the effect of multiple injections of DH on animals neck-ligatured and held at 10 °C was examined. These animals were divided into 2 groups. Each animal in the 1st group was injected with DH extract at 0, 12, and 24 h posteclosion. Animals in the 2nd group were given a single injection at either 0, 12, or 24 h posteclosion. As indicated in figure 2b animals given multiple injections began to show a decline in sensitivity to the DH extract after the 2nd injection, furthermore, by 24 h posteclosion the sensitivity of these animals to the DH had been eliminated. By contrast, animals given single injections over this time period still respond to the DH injection.

The above experiments demonstrated that, even though DH is present in all adult monarchs[1], the sensitivity of the butterfly to DH is restricted to a short period of adult life. Furthermore it appears that this loss of sensitivity to the DH is both temperature-dependent and DH-sensitive. It therefore seems conceivable that this system may represent an invertebrate example of the desensitization phenomena which have been commonly reported in vertebrate systems[4]. If so, it would be of interest to obtain additional data concerning the molecular details of this process in the monarch.

1 R.M. Dores, S.H. Dallmann and W.S. Herman, J. Insect Physiol. *25*, 897 (1979).
2 S.W. Nicolson, J. Insect Physiol. *22*, 1347 (1976).
3 W.S. Herman and S.H. Dallmann, J. Insect Physiol., in press (1981).
4 K.J. Catt, J.P. Harwood, G. Aguilera and M.L. Dufau, Nature *280*, 109 (1979).

Effects of prolactin (PRL) on gonadotropin release in mice with congenital PRL deficiency[1]

A. Bartke, T.M. Siler-Khodr and F. Bex

Department of Obstetrics & Gynecology, The University of Texas Health Science Center, San Antonio (Texas 78284, USA), and Endocrine Section, Wyeth Laboratories Inc., P.O. Box 8299, Philadelphia (Pennsylvania 19101, USA), 30 July 1980

Summary. In prolactin (PRL)-deficient male dwarf mice, treatment with PRL stimulates the release of FSH without affecting plasma LH levels. We now report that this effect of PRL is not mediated by the testes and that PRL does not modify FSH or LH release in female dwarf mice.

In addition to regulating its own release[2], prolactin (PRL) has been shown to suppress secretion of pituitary gonadotropins in a number of mammalian species[3]. However, in male dwarf *(dw/dw)* mice with hereditary PRL deficiency[4], treatment with ovine PRL or with PRL-secreting ectopic pituitary grafts produced significant increase in plasma FSH levels[5]. We now report that this unexpected effect of PRL in dwarf mice is not mediated through the testes, and that PRL does not modify gonadotropin release in female dwarf mice.

Dwarf mice *(dw/dw)* were raised in our own colony and maintained in a room with 14 h of light: 10 h of darkness with continuous access to food and water. 16 males were castrated and 9 of them were given a transplant of 1 pituitary gland each from a normal (non-dwarf) adult female under the renal capsule. 36 female dwarf mice were divided into 4 treatment groups as follows: 12 were given pituitary grafts, 9 were sham-operated, 9 were injected s.c. daily with 125 µg ovine PRL (NIH-P-S12) in 0.05 ml saline, and 6 were injected with saline alone. 2 weeks after the onset of these treatments, blood samples were collected by cardiac puncture under ether anesthesia and the animals were sacrificed. Pituitary glands were homogenized in cold 0.01 M phosphate buffered saline, pH = 7.6. The concentration of FSH and LH in plasma and pituitaries was determined by NIAMDD rat FSH radioimmunoassay and ovine: ovine LH radioimmunoassay[6] and expressed in terms of NIAMDD rat standards FSH-RPI and LH-RPI. These procedures have been validated for use in the mouse[7]. Results obtained in saline-injected and sham-operated controls were combined for statistical analysis.

In castrated pituitary-engrafted male dwarf mice, plasma FSH levels were twice as high as in castrated controls ($p < 0.025$) (table 1). In contrast, plasma LH levels were nearly identical in the 2 groups. Treatment of female dwarf mice with PRL or grafts had no effects on plasma FSH and

Table 1. Effects of pituitary grafts on FSH and LH levels in castrated male dwarf *(dw/dw)* mice

	Control (7)	Grafts (9)
Plasma FSH (ng/ml)	824 ± 240	1708 ± 198*
Plasma LH (ng/ml)	119 ± 61	104 ± 35
Pituitary FSH (ng/mg)	3786; 2836	4930; 4304
Pituitary LH (ng/mg)	2786; 1391	1448; 1530

Values are means ± SE for individual plasma samples or values obtained in pools of 3-4 pituitaries. Number of animals in parenthesis. *Significantly different from controls; $p < 0.025$.

Table 2. Effects of prolactin (PRL) and pituitary grafts on FSH and LH levels in female dwarf *(dw/dw)* mice

	Control (15)	PRL (9)	Grafts (12)
Plasma FSH (ng/ml)	120 ± 16	144 ± 3	108 ± 8
Plama LH (ng/ml)	24 ± 10	43 ± 16	< 8
Pituitary FSH (ng/mg)	3058 ± 485	4203 ± 1234	2401 ± 296
Pituitary LH (ng/mg)	9069 ± 1125	6605 ± 712	4540 ± 631

Samples from 3 mice were pooled for each determination and the results are reported as means ± SE. Number of animals in parenthesis. Significance of the differences in the text.

LH levels or on the concentration of FSH in the pituitary (table 2). Pituitary LH levels were reduced by grafts ($p < 0.025$) but not by PRL. This latter discrepancy between the results obtained with pituitary transplants and with injected PRL could be due to different patterns of peripheral PRL levels after these treatments (sustained vs intermittent elevations) or to species specificity of PRL (murine vs ovine hormone).

Increase in plasma FSH levels in castrated dwarf mice treated with PRL-producing pituitary grafts indicates that the previously demonstrated ability of PRL to stimulate testicular growth and function in these animals[4,5] does not account for its effect on FSH release. Sexual dimorphism in the response of FSH release to PRL is evident from comparison of results obtained in males[5] and in females given identical treatments in the present study. This dimorphism probably cannot be explained by higher androgen levels in male animals, because castration did not abolish the ability of PRL to stimulate FSH release in male dwarfs, or by the effects of ovarian steroids since *dw/dw* females do not undergo sexual maturation and have atrophic uteri and no corpora lutea in the ovaries[4,8]. Perinatal masculinization of the CNS by testicular androgens is believed responsible for sexual dimorphism in numerous neuro-endocrine characteristics and therefore it may account also for the difference between males and females in the response of FSH regulatory mechanisms to PRL.

Significant increase in pituitary FSH levels in intact engrafted male dwarfs[5] and a similar, although not significant, trend observed in castrated males in the present study (table 1) suggest that PRL was exceedingly unlikely to modify plasma FSH levels by reducing its clearance. The mechanism by which PRL may stimulate FSH synthesis and release in male dwarf mice is unknown. However, we suspect that dopaminergic neurons may mediate this action of PRL, because PRL can increase dopamine turnover in the hypothalamus[9] and pharmacological blockade of dopaminergic receptors appears to prevent increase in FSH levels in pituitary-engrafted hamsters (Bartke and Ojeda, unpublished).

Results obtained in golden hamsters[10,11] suggest that the ability of PRL to stimulate the release of FSH but not LH in male mammals is not limited to laboratory mice or to animals with PRL deficiency. In addition to suggesting a role for PRL in regulating FSH release, the present findings indicate that stimulation of testicular function by PRL[5,12] may involve both direct effects of PRL on the Leydig cells[12,13] and consequences of PRL-induced increase in plasma FSH levels.

1 This work was supported by the National Institute of Child Health and Human Development through a grand HD12642 and RIA Core of grant HD10202. We thank NIAMDD and Drs G.D. Niswender and L.E. Reichert, Jr, for reagents used in radioimmunoassays.

2 R.M. MacLeod and J.S. Lehmeyer, Cancer Res. *34*, 345 (1974).
3 G. Kann, J. Martinet and A. Schirar, in: Prolactin and Human Reproduction, p.47. Ed. P.G. Grosignani and C. Robyn. Academic Press, London 1977.
4 A. Bartke, Mem. Soc. Endocr. *15*, 193 (1966).
5 A. Bartke, B.D. Goldman, F. Bex and S. Dalterio, Endocrinology *101*, 1760 (1977).
6 G.D. Niswender, A.R. Midgley, Jr, S.E. Monroe and L.E. Reichert, Jr, Proc. Soc. exp. Biol. Med. *128*, 807 (1968).
7 W.G. Beamer, S.M. Murr and I.I. Geschwind, Endocrinology *90*, 823 (1972).
8 E. Howe, S. Lintern-Moore, G.P.M. Moore and J. Hawkins, Biol. Reprod. *19*, 959 (1978).
9 T. Hokfelt and K. Fuxe, Neuroendocrinology *9*, 100 (1972).
10 A. Bartke, B.D. Goldman, F.J. Bex and S. Dalterio, Int. J. Androl. suppl. *2*, 345 (1978).
11 A. Bartke, T.M. Siler-Khodr, M.P. Hogan and P. Roychoudhury, Endrocrinology *108*, 133 (1981).
12 A. Bartke, Fedn Proc. *39*, 2577 (1980).
13 E.H. Charreau, A. Attramadal, P.A. Torjesen, K. Purvis, R. Calandra and V. Hansson, Molec. cell. Endocr. *6*, 303 (1977).

The role of calcitonin in hypocalcemia in acute experimental pancreatitis

P.D. Broulík, J. Škrha and V. Pacovský

IIIrd Medical Clinic, Faculty of General Medicine, Charles University, U nemocnice 1, Prague 2 (Czechoslovakia), 9 June 1980

Summary. Experimental pancreatitis in rats was accompanied by hypocalcemia. Thyroidectomy did not abolish the fall in serum calcium observed in intact animals during pancreatitis. Data presented in our study suggest that the thyroid gland and calcitonin are not important factors in causing the hypocalcemia observed in rats.

A number of theories have been advanced to explain the hypocalcemia which is frequently observed in acute pancreatitis[1-3]. Edmondson proposed that the hypocalcemia might be due to calcium deposition in and around the necrotic pancreatic tissue[3,4]. This is not a likely explanation for the prolonged hypocalcemia observed since induced hypocalcemia is normally followed by an increase in parathormone concentration and in a return of serum calcium to normal levels within 12 h[5,6]. A hormonal basis has been postulated for the hypocalcemia commonly seen in pancreatitis. It has been suggested that the hypocalcemia of acute pancreatitis may be an effect of glucagon which is increased in pancreatitis[7,8]. Other possible causes of hypocalcemia include increased secretion of calcitonin, which has a hypocalcemic effect[9-11]. The present studies were undertaken to test whether acute pancreatitis can stimulate calcitonin secretion which may then be involved in the pathogenesis of the hypocalcemia that complicates acute pancreatitis.

Materials and methods. 45 male rats (Wistar, 300-400 g) were kept on a standard diet and divided into several groups: controls, controls with pancreatitis, parathyroidectomized, parathyroidectomized with pancreatitis, thyroparathyroidectomized, and thyroparathyroidectomized with pancreatitis. Acute experimental pancreatitis was produced as follows: under mild ether narcosis, 0.6 ml of 5% sodium cholate (lot 0369071/73 Spofa, Prague) was administered interstitially into the pancreas[12]. Rats were thyro-