EFFECTS OF THYROPARATHYROIDECTOMY ON IMPLANTATION AND EARLY GESTATION IN RATS

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It has been demonstrated that thyroxine is essential for normal fertility in rats and rabbits (Chu, 1944–46; Krohn & White, 1950); a study by Parrott, Johnston & Durbin (1960) included parathyroid hormone (PTH) as well as thyroxine ($L-T_4$). The present preliminary study was undertaken to investigate whether thyrocalcitonin (TCT) and PTH have any influence on implantation and early gestation in rats.

Thirty, 2-month-old, female Charles River rats (Charles River Breeding Laboratory, Waltham, Mass.) with an average body weight of 208 g were randomly allocated to six equal groups. They were housed in an air-conditioned room, exposed to 14 hr of light and had free access to Purina lab pellets and tap water. On the 1st day of pregnancy (the day when spermatozoa were detected in vaginal smears after the females were placed overnight with males of proven fertility), five groups were thyroparathyroidectomized according to the method described by Hirsch, Gauthier & Munson (1963). The rats in the remaining group were subjected to sham operation and served as the controls. Replacement studies were initiated after the operations.

Thyroxine (synthetic sodium L -thyroxine, Flint Laboratories) and parathyroid extract (Eli Lilly & Co.) were dissolved and diluted, respectively, with normal saline. Thyrocalcitonin (calcitonin, Armour Pharmaceuticals) was dissolved in acidic saline adjusted to pH 4.5 with dilute hydrochloric acid. The respective doses of $L-T_4$, PTH and TCT were 2.5 μ g, 15 USP. U and 50 MRC mU/100 g body weight. Injections of PTH and TCT were given twice daily until the 13th day of gestation. All drugs were administered subcutaneously in a volume of 0.1 ml. An equal volume of acidic saline was used as the control injection.

On Day 7 of gestation, the number of implants (beads) in both horns of the uterus was recorded for each animal at laparotomy under ether anaesthesia. The animals were returned to individual cages after the abdominal muscles were sutured with fine silk thread and the skin closed by a wound clip. On Day 14, the animals were similarly anaesthetized and the numbers of embryos in the uteri were recorded. Blood samples were taken from the abdominal aorta in each rat and stored in centrifuge tubes under ice. The animals were then killed by opening the diaphragms. Embryos were dissected out individually, with

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Table 1. Effects of thyroparathyroidectomy and replacement therapy upon implantation, mean weights of embryos, pituitaries, ovaries, adrenals and serum calcium levels in rats

Groups and treatments	Day 7		Day 14							
	Initial body wt (g)	Body wt (g)	No. of implants	Body wt (g)	No. of embryos	Wt of embryo† (mg)	Wt of pituitaries (mg/100 g)	Wt of ovaries (mg/100 g)	Wt of adrenals (mg/100 g)	Serum calcium (mg/100 ml)
1. TPTX L-T ₄ , 2.5 µg PTH, 15 USP.U	209	219	11.6±0.50	240	10.8±0.4	770 ± 18	4.3±0.5	44.5±3.7	38.8±4.5*	10.9±0.1
2. TPTX L-T ₄ , 2.5 µg	201	213	11.5±0.51	237	11.2±0.4	664 ± 84	4.7±0.4	42.1±4.8	38.7±4.9*	9.1±0.1*
3. TPTX PTH, 15 USP.U	207	211	6.2±0.60*	231*	6.0±0.2	906*±58	5.2±0.7	46.0±4.0	27.3±3.6	11.2±0.1
4. TPTX Acid saline	210	211	5.8±0.60*	221*	5.2±0.4	931*±37	4.9±0.9	38.9±4.1	26.1±5.6	8.2±0.9*
5. Sham operation Acidic saline control	217	222	13.4±0.91	255	12.0±0.3	690 ± 43	4.1±0.9	42.4±7.5	28.7±3.3	10.4±0.1
6. TPTX L-T ₄ , 2.5 µg PTH, 15 USP.U TCT, 50 mU	206	216	12.2±0.70	241	11.0±0.4	685 ± 42	4.1±0.1	40.2±4.0	35.0±4.9*	10.5±0.1

L-T₄ = L-thyroxin; PTH = parathyroid hormone; TCT = thyrocalcitonin; TPTX = thyroparathyroidectomy. Doses used: L-T₄/100 g body wt, daily; PTH and TCT/100 g body wt, twice daily. Each group contained five rats. All data, except those relating to body weight, are expressed as means ± S.E.

* Significantly different when compared to control ($P < 0.05$); multiple range test (Duncan, 1955).

† Mean of the averages for each rat in the group.

the intact amniotic sac and a portion of the uterine wall, and weighed. Adrenals, ovaries and pituitary glands were removed, freed from fat and weighed.

Calcium was estimated by diluting 0.2 ml serum with 9.8 ml diluting fluid (0.1% Lanthanum in 0.06 N-HCl) and analysed in a Perkin-Elmer Atomic Absorption Spectrophotometer Model 290B (Perkin-Elmer Corp., 1971).

The results presented in Table 1 demonstrate that TCT as well as PTH had no apparent effect on implantation and early gestation up to Day 14. The numbers of implants in TCT- and/or PTH-deficient rats (Groups 1 and 2) were not different from the controls or other treated groups, except for Groups 3 and 4 in which $L-T_4$ was not administered. Serum calcium levels in the PTH-deficient groups (2 and 4) were lower ($P < 0.05$) than control or PTH-replacement groups. The dose of PTH used in this experiment was sufficient to keep the calcium level of blood within a normal physiological range. The apparent hypocalcaemia observed in the PTH-deficient groups had no visible effect on implantation processes and the early development of embryos. The severity of PTH deficiency was more apparent in Group 4 in which $L-T_4$ was also lacking. In those groups (3 and 4) lacking $L-T_4$, the number of implants was significantly lower ($P < 0.05$) when compared to the controls and all other groups. Though the number of implantation sites was reduced in the $L-T_4$ -deficient groups, the mean weight of embryos on Day 14 was higher ($P < 0.05$) when compared to the controls and other groups. This might have been due to the fact that as the number of developing embryos in the uterus decreased, their weights increased as a result of better nourishment (Wilson, Runner & Zarrow, 1963).

Pituitary and ovarian weights in the control and treated groups did not change, but adrenal weights in groups given $L-T_4$ (1, 2 and 6) increased significantly ($P < 0.05$) when compared to the control, thyroparathyroidectomized and PTH groups (3, 4 and 5). Exogenous $L-T_4$ probably triggered ACTH release in these animals (Contopoulos & Koneff, 1963). The body weights of Groups 3 and 4 ($L-T_4$ -deficient) decreased significantly ($P < 0.05$) on Day 14 when compared to the control or $L-T_4$ -treated groups, indicating one of the effects of hypothyroidism in rats.

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