

Effects of Estrogen or Progesterone on Prepuberal Ovaries Implanted in Castrated Adult Male Rats

Ovarian graft in the systemic circulation of an adult male rat develops as a polycystic ovary. Fragments of vagina grafted in these animals show permanent oestrus¹. Similar results are obtained by administration of testosterone to the female new-born rat². These and other observations led to the schema suggested by HARRIS³ of a cyclic pattern of the hypothalamus in both sexes altered to become continuous in the male by its own androgens or by administration of exogenous androgens in the female.

KEMPF⁴ finds that estrogens and progesterone are able to induce ovulation in ovarian grafts of adult female to adult male castrated rat. The same treatment did not evoke ovulating response in the testosterone sterilized rat⁵.

Our interest was to know the response of prepuberal ovarian grafts (which are void of corpora lutea) in the adult castrated male rats, to estrogen or progesterone.

Material and methods. 41 adult male rats, from the Rattus Norvegicus AxC strain weighing between 150 and 200 g, maintained under controlled conditions, were castrated under ether anesthesia. At the time of operation, 1 ovary of a prepuberal female (15–25 days old) was grafted into the anterior chamber of the eye.

After 15 days of evolution, the animals, taken at random, were separated into 3 groups and treated with oestradiol dipropionate, progesterone and an untreated group (see Table).

The 3 groups were killed by bleeding under ether anesthesia, on the 21st day after operation; grafts were removed, fixed in Bouin fixative, embedded in paraffin, serially sectioned at 10 μ and stained with HE. The remaining endocrine organs were dissected and weighed. The pituitary glands were fixed in ELFTMAN's fluid⁶, serially sectioned and stained with PAS-orange G-Methyl Blue. Statistical results were analyzed by the Student's *t*-test.

Results. Most of the grafts succeeded. The histological observations of ovary grafts in all groups are shown in Figure 1. The pituitary glands showed scarce castration cells in the control group. No castration cells were found in groups treated with estrogen or progesterone.

Administration of estrogen to male castrated rats caused hypertrophy of the pituitary, adrenal and thyroid

glands (Table). In the group of animals treated with progesterone, hypertrophy is traced only in adrenals. No mammary hypertrophy was evidenced in any of the experimental groups.

Discussion. Our observations confirm former results obtained by KEMPF⁴, and prove clearly that the injection of a single dose of estrogens, or 2 of progesterone, cause the appearance of ovulation in prepuberal (us) of adult (KEMPF⁴) ovaries grafted in adult male castrated rats. Ovulatory action of progesterone under these conditions could be explained by the lowering effect of progesterone on the nervous excitability threshold, regulating the cyclic mechanism of gonadotrophic secretion in the male, described by INOUE⁷ to a secretory system similar to

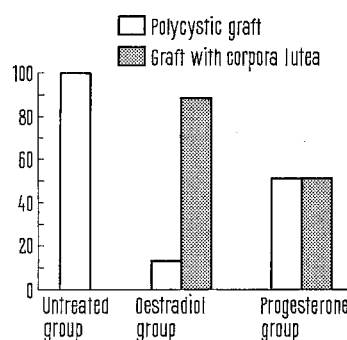


Fig. 1. Percent of graft with corpora lutea in male castrated adult rat with oestradiol dipropionate (500 μ g) or progesterone (1 mg twice, days 15 and 19).

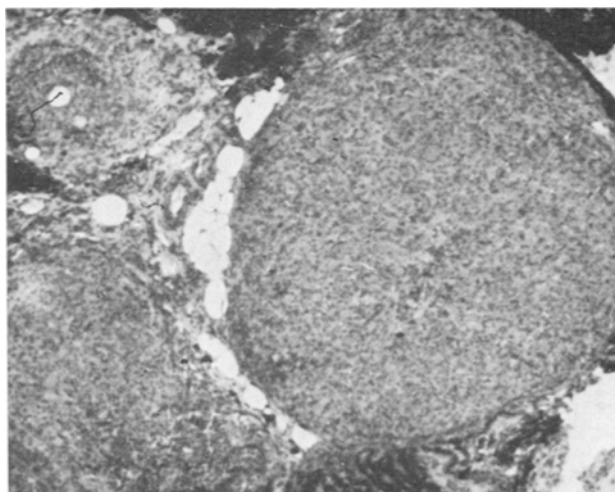


Fig. 2. Corpus luteum, in ovary grafted in male castrated rat, treated with 500 γ of estradiol dipropionate.

Relative weight (mg/100 g body weight) of pituitary, thyroid and adrenals of castrated male rats grafted with ovary and treated with estradiol dipropionate or progesterone

Group	No. of animals	Pituitary	Thyroid	Adrenals
Castrated male + ovary graft	10	4.3 $\pm$ 0.3 ^a	5.8 $\pm$ 0.4	15 $\pm$ 1.0
Castrated male + ovary graft + estradiol dipropionate (500 μ g on day 15)	15	7.7 $\pm$ 0.6 ^b	10.3 $\pm$ 0.5 ^b	22 $\pm$ 0.9 ^b
Castrated male + ovary graft + progesterone (1 mg twice, on day 15 and 19)	16	4.4 $\pm$ 0.6	6.5 $\pm$ 0.2	19 $\pm$ 0.3 ^b

^a S.E.; ^b *P* < 0.01.

¹ TH. MARTINS, C. r. Soc. Biol. 109, 134 (1932).

² C. A. BARRACLOUGH, Endocrinology 68, 62 (1961).

³ G. W. HARRIS, Endocrinology 75, 627 (1961).

⁴ R. KEMPF, Archs Biol. 61, 59 (1950).

⁵ C. A. BARRACLOUGH, S. YRRARAZAVAL and R. HATTON, Endocrinology 75, 838 (1964).

⁶ H. T. ELFTMAN, Stain Tech. 32, 25 (1957).

⁷ S. INOUE, Gunma Symp. Endocr. 2, 79 (1965).

that of the female, probably due to an antagonism to the estrogens secreted by the graft. Progesterone had the ability to facilitate ovulation in the adult rat⁸ and in the androgeinized rat when it was submitted to electrical stimulation of the hypothalamus^{9,10}. However, in the androgeinized rat, the sole administration of progesterone did not evoke an ovulating response¹¹. This difference in response may derive from the possibility that the effect of its own androgens on the hypothalamus in the newborn male rat, does not perform a 'whole or none' result, which apparently occurs in the androgeinized rat. This is supported by the fact that ovulation of ovarian graft in male castrated rat, may be elicited by direct stimulation of the preoptic area without progesterone pretreatment¹².

The ovulatory action of estrogens could thus provide an explanation either by a 'rebound' mechanism of the sudden fall and further increase of LH, or by a facilitating effect on the output of folliculotrophins by estrogens performed at certain levels of the central nervous system¹³ or directly at the level of the pituitaries^{14, 15, 16}.

Résumé. Des greffons d'ovaire de rat impubère placés dans la chambre antérieure de l'œil du mâle castré, après traitement avec 0,5 mg de dipropionate d'œstradiol ou avec deux doses de 1 mg de progestérone, ont montré une évidente ovulation. On interprète ce fait comme dû

à l'action des hormones injectées dans le système nerveux central et qui favorisent le mécanisme de l'ovulation.

LAURA RIBONI, HORTENSIA D'ALBORA,
EMILIA CARLEVARO¹⁷ and R. DOMINGUEZ¹⁸

*Facultad de Medicina,
Departamento de Histología y Embriología,
Montevideo (Uruguay), 7 January 1969.*

⁸ J. W. EVERETT, *Physiol. Rev.* 44, 373 (1964).

⁹ C. A. BARRACLOUGH and R. A. GORSKI, *Endocrinology* 68, 68 (1961).

¹⁰ R. A. GORSKI and C. A. BARRACLOUGH, *Endocrinology* 73, 210 (1963).

¹¹ C. A. BARRACLOUGH, in *Neuroendocrinology* (Eds. L. MARTINI and J. GANONG; Academic Press, New York 1966), chap. 19, p. 61.

¹² J. MOLL and G. ZEILMAKER, *Acta endocr. (Copenh.) suppl.* 100, 146 (1965).

¹³ S. KANEMATSU and C. A. SAWYER, *Endocrinology* 72, 243 (1963).

¹⁴ E. M. BOGDANOV, *Endocrinology* 73, 696 (1963).

¹⁵ Acknowledgment. Estradiol dipropionate was kindly supplied by the CIBA laboratories Montevideo and Progesterone by Schering, Co. A.G., Berlin (Germany).

¹⁶ With the technical assistance of Mr. D. ZIPITRÍA.

¹⁷ Fellow of the University of R.O.U. (Comisión de dedicación total).

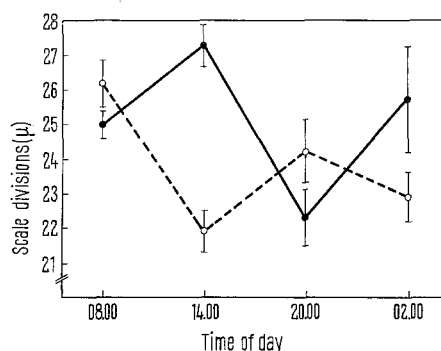
¹⁸ School of Medicine, Montevideo (Uruguay).

Circadian Fluctuations in Tibia Cartilage — Assayable Pituitary Homogenates of Fed and Starved Weanling Female Rats¹

Circadian fluctuations of several metabolites in weanling rats have been reported previously². Each one of these has been at some time reported to be related to the metabolic activity of growth hormone (GH). The present communication reports on fluctuations observed in the activity of pituitary homogenates as measured by the tibia test³ and presumably reflecting GH activity.

Female weanling Holtzman rats were accommodated in single cages in a temperature-controlled room (22.5°C) with 06.00–18.00 h light and 18.00–06.00 h dark cycles. Lab chow and tap water were given and libitum. On the 4th day the rats were divided into 8 groups of 8 rats each. Groups 1, 3, 5 and 7 were starved 24 h prior to sacrifice (08.00, 14.00, 20.00 and 02.00 h, respectively). Groups 2, 4, 6 and 8 were allowed to eat ad libitum. This was done because of the well-known relation between food intake, fasting and GH levels⁴. At the indicated sacrifice time the rats were quickly killed by decapitation and the pituitaries of a given group were weighed and pooled in a tissue homogenizer and stored at –5°C. After removal from storage the tissue was disrupted by alternate thawing (45°C) and freezing and after addition of saline the glands were homogenized. Before final dilution (0.7 mg of fresh pituitary in 0.2 ml of extract) the pH was adjusted to 10. The dosage of 0.2 ml/rat was injected s.c. into young hypophysectomized rats once daily for 4 consecutive days. The assay rats were killed on the 5th day and the tibia cartilage-widening activity of the extracts was determined⁵. Standards of GH were not used since the objective was the comparison of the groups sacrificed at different intervals. Significance of difference in epiphyseal cartilage width (one scale division = 1 μ) was done using Student's *t*-test.

Fed rats: The pituitary content of tibia-active material rose from the late morning levels to a peak at 14.00 h ($p < 0.01$, Figure). There was a significant depletion by



Circadian fluctuations in the pituitary content of epiphyseal cartilage width assayable substance. Solid black line, fed rats; broken line, animals fasted 24 h prior to sacrifice. Ordinate shows epiphyseal cartilage width in micra, abscissa indicates time of sacrifice. Vertical bars depict standard error of the mean.

¹ This investigation was supported by U.S.P.H.S. Grant No. HD 03331, National Institute of Child Health and Human Development.

² M. S. BAHORSKY and L. L. BERNARDIS, *Experientia* 23, 634 (1967).

³ F. S. GREENSPAN, C. H. LI, M. E. SIMPSON and H. M. EVANS, *Endocrinology* 45, 455 (1949).

⁴ J. ROTH, S. M. GLICK, R. S. YALOW and S. A. BERSON, *Science* 140, 987 (1963).