GROWTH OF OVARIAN FOLLICLES IN RATS IN THE ABSENCE OF GONADOTROPHINS

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Ovarian follicles grow and develop to the antral stage in rats and guinea-pigs, even after hypophysectomy (Dempsey, 1937; Paesi, 1949), indicating that gonadotrophic hormones are not essential for the early growth of follicles. Since these findings were based only on the histological appearance of the ovary after hypophysectomy, the present investigation was to determine the effect of hypophysectomy on the granulosa cells of growing follicles by using autoradiography after pulse-labelling with tritiated thymidine (Peters & Levy, 1966; Ryle, 1969a, b; How *et al.*, 1970; Pedersen, 1970).

Female Sprague–Dawley rats were hypophysectomized at 22 days of age and kept in an air-conditioned room (22°C) under controlled lighting (12 hr light/24 hr) and with free access to food and water. Tritiated thymidine was injected intraperitoneally (2 $\mu\text{Ci/g}$ body wt) at 32 days of age, and a constant 1-hr labelling time was used. At autopsy the ovaries were removed and fixed in Bouin's fluid. Autoradiographs were prepared from 5 μm paraffin-wax sections coated with Sakura NR-M2 emulsion by the dipping method. The exposure time was 2 weeks at 4°C in dry air. The image was developed with Sakura Konidol X and sections were stained with haematoxylin and eosin.

The number of labelled granulosa cells as a proportion of the total number of granulosa cells in a growing follicle was determined in the cross-section in which the nucleolus of the oocyte was seen, and the regression lines and correlation coefficients were computed statistically. Cells were considered labelled if they had four or more grains over the nucleus. Background labelling never exceeded 0.5 grains/100 μm^2 .

Serum concentrations of FSH and LH were measured by the double antibody radioimmunoassay of Daane & Parlow (1971), with some modifications.

After hypophysectomy, degenerative changes were seen in the ovary. The interstitial cells had shrunken nuclei and reduced cytoplasm. Although many follicles were atretic, a few follicles with more than one layer of granulosa cells persisted.

Autoradiography showed that the granulosa cells had taken up [^3H]thymidine, and there was a statistical correlation between the total number of granulosa cells and the number of labelled granulosa cells in growing follicles in hypophysectomized and intact rats (Text-fig. 1).

Serum FSH and LH levels were undetectable at 32 days of age.

The nature of the factor responsible for the initial development of an im-

TEXT-FIG. 1. Relationship between the total count of granulosa cells and the count of labelled granulosa cells in growing follicles of the ovary of the (a) intact rat, and (b) hypophysectomized rat.

mature ovarian follicle is still unknown, but it is clear that gonadotrophins are not essential.

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