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Mammary Lobulo-Alveolar Growth in Adreno-Ovariectomized Rats Following Transplantation of "Mammotropic" Pituitary Tumor.* (29512)

P.K. TALWALKER[†] AND J. MEITES[‡]

Department of Physiology, Michigan State University, East Lansing

The ovarian hormones, estrogen and progesterone, have been shown to induce mammary lobulo-alveolar (L-A) growth in intact or ovariectomized rats and to synergize with prolactin and growth hormone (STH) in adreno-ovariectomized-hypophysectomized rats(1). Ovarian hormones themselves have little or no effect on rat mammary L-A development in absence of the anterior pituitary (AP), whereas our laboratory has recently reported that AP hormones (STH and prolactin) alone induced mammary L-A growth in the absence of the ovaries, adrenals and pituitary(2). This suggested that STH and prolactin are of primary importance for mammary L-A growth in the rat.

Furth and his co-workers(3,4) have developed many types of transplantable pituitary tumors. One of these is a monomorphous pituitary "mammotropic" tumor, strain F₄ (MtT.F₄), originally induced by estrogen(5). This tumor secretes prolactin, STH and corticotropin (ACTH) but no FSH-LH or TSH in the host animal(6-10). The tumor provides a continuous source of prolactin, STH and ACTH, which are secreted in increasing amounts as the tumor grows in the host. It was of interest, therefore, to determine the effect of a MtT.F₄ transplant on mammary growth in the rat in which the sources of estrogen and progesterone have been elim-

inated by removal of the ovaries and adrenals.

Methods. Highly inbred, virgin female Fischer rats of the CDF strain (Charles River Breeding Laboratories, Brookline, Mass.), 3 months old, were fed *ad libitum* on Wayne Lab Blox pellets, supplemented with canned dog food (Dash) and slices of oranges. Rats in Groups 1 and 2 (Table I) were sham-operated, while those in Groups 3 and 4 were adreno-ovariectomized. Following each operation, the rats were injected once subcutaneously with 30,000 units of penicillin G. The completeness of adrenalectomy was assessed in 2 ways: (a) after killing the rats at the end of experiment, they were carefully examined with a magnifying lens for adrenal remnants, and (b) 9 rats of the same strain, age, and weight were adreno-ovariectomized and given saline (1% NaCl) in their drinking water for 7 days. From the 8th day onward, the rats were given distilled water instead of saline. The day on which each rat died, after saline withdrawal, was recorded.

Seven days after the operation, rats in Groups 2 and 4 were transplanted subcutaneously with a Furth pituitary "mammotropic" tumor (MtT.F₄, passage 42) in the dorsal neck region. Each MtT.F₄ was removed aseptically, cut into small pieces with iris scissors, and the tumor mince was suspended in an equal volume of medium 199 (pH 7.4, Difco Laboratories, Detroit, Mich.). This was injected in a 0.1 ml volume. The tumors attained palpable size within 4-5 weeks. Vaginal smears were taken from the adreno-ovariectomized rats to test for estrogenic activity.

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[†] Present address: Research Division, Pharmaceutical Dept., CIBA Ltd., Basel, Switzerland.

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TABLE I. Mammary Lobulo-Alveolar Growth in Adreno-Ovariectomized Rats Following Transplantation of Pituitary "Mammotropic" Tumor, Strain F₄ (MtT.F₄).

G Group	Treatment*	No. of rats	Avg MtT.F ₄ wt (g)	Mammary growth rating†					Mammary secretion rating†				
				I	II	III	IV	V	0	1	2	3	4
1	Intact controls	9	—	2	7	0	0	0	0	0	0	0	0
2	Intact, MtT.F ₄ -transplant	14	6.1 ± 1.0‡	0	0	0	0	14	0	0	0	0	14§
3	Adrex-ovax, no MtT.F ₄ transplant	13	—	12	1	0	0	0	0	0	0	0	0
4	Adrex-ovax with MtT.F ₄ transplant	12	9.9 ± 1.3	0	0	2	4	6	2	4	3	3	0

* Adrenalectomized = Adrex, Ovariectomized = Ovax.

† See text for rating system.

‡ Mean ± standard error of mean, Group 2 vs 4: P < 0.01.

§ Mammary secretion in these rats was far greater than indicated by rating of 4.

Eight weeks after tumor transplantation, all rats in Groups 1-4 were killed. Both inguinal mammary pads from each rat were dissected free, spread flat on cork and fixed in Bouin's fluid. One mammary pad from each animal was stained by a standard procedure(2) for gross examination, and rated for development according to the following scale:

- I. = Few ducts; few or no end buds
- II. = Moderate duct growth; moderate number of end buds
- III. = Numerous ducts and branches; many end buds.
- IV. = Numerous ducts and branches; moderate lobulo-alveolar growth
- V. = Numerous ducts with many branches; dense lobulo-alveolar growth, as in mid or late pregnancy

The other mammary gland pad from the same animal was prepared for histological examination, as described earlier(11). Lactation ratings of 0 to 4 were assigned to each gland, depending on the amount of secretion observed microscopically(11). The MtT.F₄ from each of the tumor bearing rats was also removed and weighed.

Results. The data are summarized in Table I. The mammary glands from intact controls (Group 1) consisted of moderately developed ducts with few end buds (Fig. 1A) and no secretion (Fig. 1B). MtT.F₄ transplantation in intact rats (Group 2) induced extensive mammary lobulo-alveolar growth and intense mammary secretion (Rating 4),

far greater than that observed during normal lactation.

Adreno-ovariectomy alone, without an MtT.F₄-transplant, (Group 3) resulted in marked regression of the mammary duct and end bud system (Fig. 1C). Transplantation of an MtT.F₄ in adreno-ovariectomized rats (Group 4) induced extensive mammary duct growth and branching, and moderate to extensive L-A development (Fig. 1D). These rats showed a variable but small degree of mammary secretion (Fig. 1E and F).

The average weight of the tumors from the adreno-ovariectomized rats (9.9 g) was significantly greater (P < 0.01) than the tumors removed from the intact rats (6.1 g). Vaginal smears of all adreno-ovariectomized rats failed to show any evidence of estrogenic activity. The 9 adreno-ovariectomized control rats (separate from those used in Groups 1-4) died after withdrawal of saline and substitution with distilled water, as follows: one rat on day 5, 2 on day 7, 2 on day 8, and one each on days 11, 12, 14, and 15.

Discussion. In previous *in vivo* studies, using adreno-ovarectomized-hypophysectomized rats and mice(1), or *in vitro* cultures of mammary glands of mice(12), estrogen and progesterone were needed in addition to prolactin and STH to induce mammary L-A growth. In the present study, mammary L-A growth was induced by the hormones secreted by MtT.F₄. This is in agreement with the similar findings of Clifton and Furth(13).

We have reported that MtT.F₄ secretes

prolactin *in vivo* as well as *in vitro* cultures (6). Other *in vivo* studies(7-10) have demonstrated that it also secretes STH and ACTH but no FSH-LH or TSH. This indicates that mammary L-A growth in adreno-ovariectomized rats bearing a MtT.F₄ is primarily if not entirely due to secretion of prolactin and

STH by the tumor. ACTH has not been shown to directly stimulate mammary growth. We previously demonstrated that only prolactin and STH were needed to induce full mammary L-A development in adreno-ovariectomized rats or adreno-ovariectomized-hypophysectomized rats(2), suggesting that

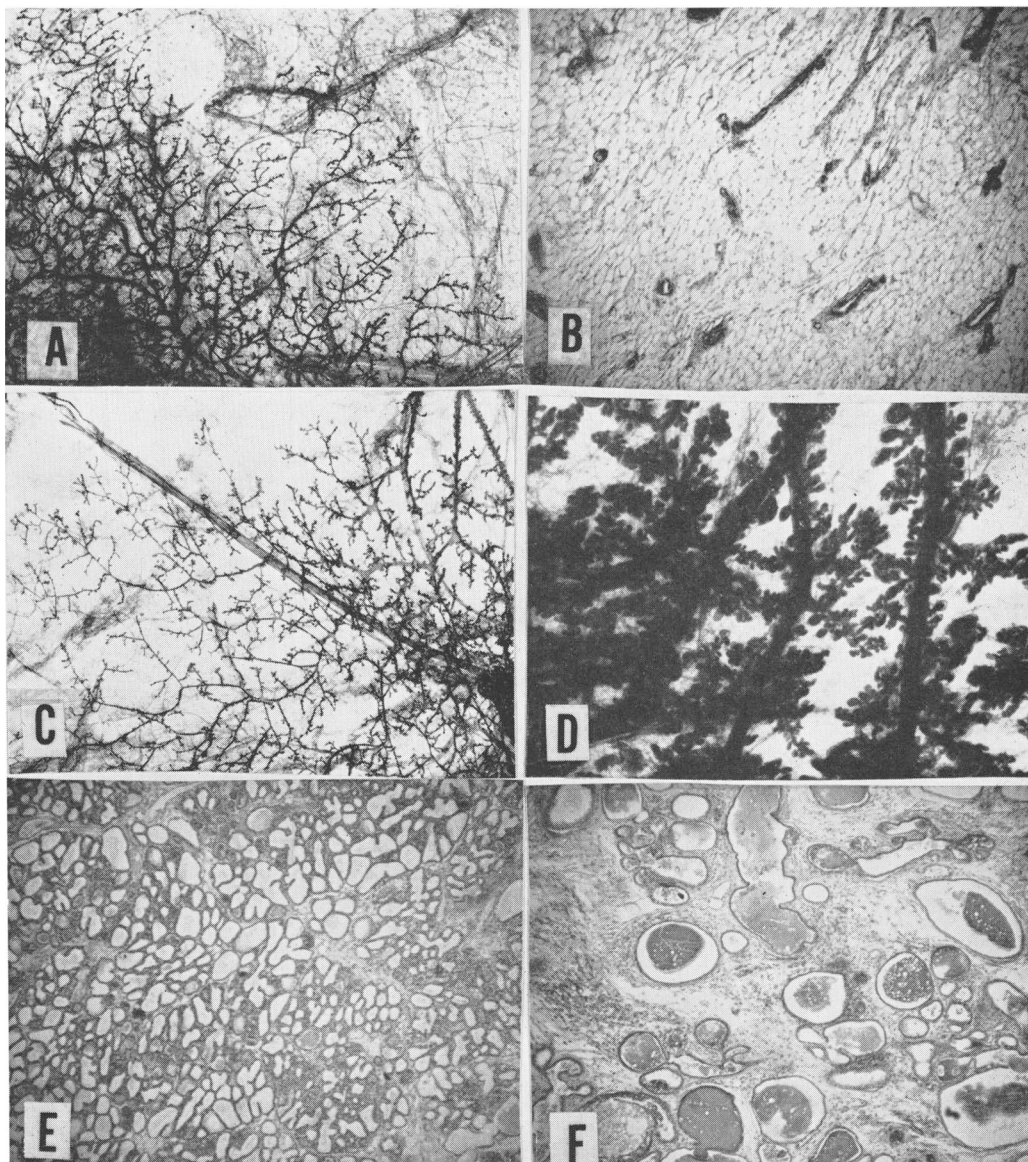


FIG. 1. A. Intact control. Moderate duct development and few end buds ($\times 9$). B. Mammary gland section from the same animal as in A. No mammary secretion present ($\times 100$). C. Adreno-ovariectomized rat without MtT.F₄ transplant. Ducts with little branching ($\times 9$). D. Adreno-ovariectomized rat with MtT.F₄ transplant. Extensive lobulo-alveolar growth ($\times 9$). E. Same type of rat as in D, showing slight mammary secretion ($\times 100$). F. Same type of rat as in D showing moderate mammary secretion ($\times 100$).

prolactin and STH were primarily responsible for mammary L-A growth in the rat. Lasfargues(14) also reported that L-A growth could be induced in mouse mammary gland cultures by addition of STH or prolactin to the culture medium, and that addition of estrogen was not essential.

Ovariectomy and adrenalectomy appeared to be complete in these rats. Vaginal smears of these rats did not reveal any evidence of estrogenic activity, indicating that estrogen was not present to synergize with prolactin and STH to stimulate mammary L-A growth. At necropsy, gross examination did not reveal any presence of adrenal remnants.

The 9 adreno-ovariectomized control rats died within 15 days after saline withdrawal. Most of the adreno-overiectomized rats with MtT.F₄ showed a slight degree of mammary secretion. Since prolactin and adrenal cortical hormones are believed to represent the minimal requirements for initiation of mammary secretion in the rat(1), it is possible that slight amounts of adrenal cortical hormones were present, insufficient to maintain life without saline but enough to synergize with the large amounts of prolactin secreted by the MtT.F₄.

Summary. 1. The influence of an autonomous, transplanted "mammatropic" pituitary tumor (Furth, MtT.F₄) which secretes large amounts of prolactin, STH and ACTH, was tested on mammary lobulo-alveolar (L-A) growth in adreno-ovariectomized (doubly operated) Fischer rats. The MtT.F₄-transplant stimulated extensive mammary L-A growth in intact or doubly-operated rats. Intact tumor bearing rats showed intense mammary secretion, while doubly operated tumor-bearing rats revealed only slight amounts of mammary secretion. The average weight of MtT.F₄ transplants in doubly operated rats was higher than in intact controls. 2. Completeness of adrenalectomy and ovariectomy was verified, as follows: (a) 9 control doubly-

operated rats failed to survive more than 15 days after withdrawal of saline; also examination of the experimental rats after sacrifice failed to reveal any presence of adrenal remnants (b) vaginal smears did not indicate the presence of estrogen. It is concluded that full mammary L-A growth can be induced following adreno-ovariectomy in Fischer rats by prolactin and STH released from a MtT.F₄ tumor.

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