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Differential Responsiveness of A and C3H Mouse Mammary Tissues to Somatotropin-Containing Hormone Combinations.* (26826)

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Somatotropin has previously been reported to act as a lobuloalveolar mammogen and as a lactogen in the C3H/Crgl strain of mice, when administered along with appropriate ovarian hormones and adrenocortical hormones, respectively(1). In the C3H/Crgl strain, bovine somatotropin and ovine mammotropin were found to be interchangeable in the experimental production of mammogenesis as well as of lactogenesis. The lactogenic effect of somatotropin had not been reported in any other species; accordingly, we determined the mammary responsiveness of various other inbred strains of mice to somatotropin-containing and mammotropin-containing hormone combinations. Treatment with estradiol + progesterone + mammotropin followed by cortisol + mammotropin was effective in inducing milk secretion in the mammary glands of hypophysectomized-ovariectomized female mice of the 10 inbred strains and sublines tested(2). Estradiol + progesterone + somatotropin followed by cortisol + somatotropin initiated milk secretion in all except the A strains of mice.

The A/Crgl and C3H/Crgl strains both carry the mammary tumor agent and both have a high incidence of spontaneous mammary tumors(2). The high mammary tumor incidence in A/Crgl mice is restricted, however, to breeding females, whereas the incidence is high in virgin as well as in breeding females of the C3H/Crgl strain. The difference in mammary gland response to the somatotropin-containing hormone combination may be due to a difference in the genetic constitution of the 2 strains resulting in (a) a difference in rate of metabolism and/or excretion of the administered hormone or (b) a difference in responsiveness of the mammary tissue itself. The present investigation was undertaken to distinguish between these two possibilities.

Materials and methods. A/Crgl and C3H/Crgl mice and their reciprocal hybrids (A ×

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C3H, C3H × A) were used. The growing mammary gland parenchyma, occupying only a small portion of the inguinal (#4) mammary fat pads of 3-week-old hybrids, was surgically removed. Approximately 1 mm^3 pieces of primary ducts from donor A and C3H female mice were immediately transplanted into these mammary gland-free fat pads by a technic described previously(3). Each hybrid host received one C3H transplant in one fat pad and one A transplant in the contralateral fat pad. Three-week-old females, 9-month-old virgin females, and 4-month-old nulliparous pregnant females at the 12th to 14th day of pregnancy were used as donors; tissues transplanted into a given host animal were taken from donors of the same age. In this manner, genetically different mammary tissues of similar age and physiological state could be followed under identical hormonal conditions in the same host. The host mice were hypophysectomized-ovariectomized at the age of 5 weeks, and treated beginning on the first post-operative day with estradiol-17β (E) + progesterone (P) + somatotropin (STH) for 21 days to induce lobuloalveolar development. This regimen was followed by injection of cortisol acetate (F) + STH for 5 days to initiate milk secretion. All hormones were administered as daily subcutaneous injections of aqueous suspension (steroids) or solution (STH). The steroids E and P were combined in a single injection. Daily doses of E, P, and F were 1 μg, 1 mg, and 125 μg, respectively. The STH was prepared from bovine pituitaries according to the method of Li(4) and was reported to contain approximately 0.5% mammotropin and less than 1% of other pituitary hormones. Animals were treated with increasing doses of STH as follows: 100 μg (days 1-7), 500 μg (days 8-14), and 1000 μg (days 15-26). Half of the 8-10 animals in each experimental group were killed on the 22nd day to study the extent of lobuloalveolar growth; the other half were terminated on the 27th day to determine lactogenic response. At autopsy, the mammary glands were prepared as whole-mounts and stained with hematoxylin. The state of alveolar development was determined from study of stained whole-mount preparation. Presence or absence of milk secretion was ascertained by examination of exposed mammary glands prior to fixation.

Results and discussion. Significant differences in lobuloalveolar and lactogenic response to the hormone treatment were observed between the 2 types of transplanted tissue, and between transplants and host (hybrid) mammary tissue. Among the C3H transplants used, response of mammary tissues from 4-month-old nulliparous pregnant females was slightly greater than that from virgin females. However, no appreciable differences were observed in the response of transplants from A donors in spite of variations in age or physiological state.

Treatment with E + P + STH resulted in development of some alveoli and a few lobules in the hybrid mammary glands. The transplants from A donors grew to occupy 30 to 100% of the fat pad, and consisted generally of ducts, many end buds, a few isolated alveoli, and occasional small lobules (Fig. 1). The amount of growth seen in C3H transplants was similar to that seen in A transplants, but there were many more lobules in the mammary parenchyma of the C3H transplants than in either A transplants or hybrid mammary glands.

Treatment with E + P + STH followed by F + STH induced occasional milk secretion in parts of the mammary glands of a few hybrid hosts (Fig. 2). This same treatment initiated milk secretion in most of the C3H transplants but in none of the A transplants. The majority of the C3H transplants had dilated lobules filled with milk (Fig. 3). The mammary parenchyma of the A transplants consisted primarily of ducts and regressed alveoli (Fig. 4), although occasional lobules were maintained.

These results clearly demonstrate that the difference in mammary responsiveness of A/Crgl and C3H/Crgl mice to the somatotropin-containing hormone combination is due to the difference in genetic make-up of the mammary tissues, and cannot be explained on the basis of differences in rate of metabolism and/or excretion of hormones in the 2 strains. Gardner and Argyris(5) came to a

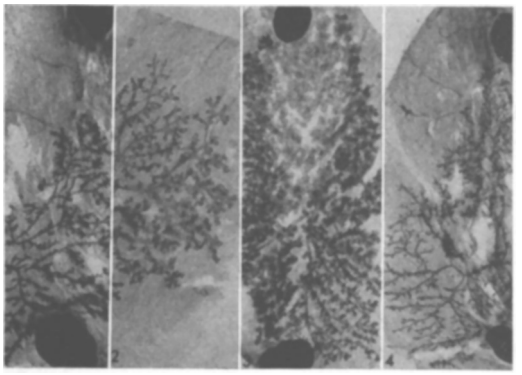

FIG. 1. Outgrowth from a piece of mammary duct from a 4-mo-old nulliparous pregnant A mouse transplanted into a female A × C3H hybrid. Host hypophysectomized-ovariectomized at 5 wk of age and treated with estradiol + progesterone + somatotropin for 21 days. Note presence of a few alveoli. Hematoxylin. × 3.5.

FIG. 2. Host gland from A × C3H female, hypophysectomized-ovariectomized at 5 wk of age and treated with estradiol + progesterone + somatotropin for 21 days followed by cortisol + somatotropin for 5 days. Note presence of some milk-filled alveoli. Hematoxylin. × 5.

FIG. 3. Outgrowth from a piece of mammary duct from a 4-mo-old nulliparous pregnant C3H female, transplanted into the same hybrid host described in Fig. 2. Note presence of large number of milk-filled lobules. Hematoxylin. × 3.5.

FIG. 4. Outgrowth from a piece of mammary duct from a 4-mo-old nulliparous pregnant A mouse transplanted into the same hybrid host described in Fig. 2. Note absence of milk-filled alveoli. Hematoxylin. × 3.5.

similar conclusion in their studies of the strain differences in estrogen sensitivity of the vaginal mucosa of mice.

Summary and conclusions. Mammary tissues from A/Crgl and C3H/Crgl strains of mice were transplanted into mammary gland-free fat pads of female hybrids (C3H × A, A × C3H). The hosts were hypophysectomized-ovariectomized, and treated with estradiol + progesterone + somatotropin for 21 days. Half of them received an additional 5 days treatment with cortisol + somatotropin. On the basis of the lobuloalveolar mammogenic and lactogenic responsiveness of the A, C3H, and hybrid mammary tissues to the hormone combinations used, the following general conclusions were drawn: 1. There is a quantitative difference in lobuloalveolar mammogenic response of A/Crgl and C3H/Crgl mammary tissues to the somatotropin-containing hormone combination, degree of response being greater in the C3H/Crgl mammary gland. 2. There appears to be a qualitative difference in lactogenic response of A and C3H mammary tissue to the somatotropin-containing combination. 3. The occurrence of milk secretion in mammary glands of hybrids (A × C3H, C3H × A) and in C3H mammary transplants, and its absence from A transplants, indicate that the genetic determinant for mammary gland sensitivity to the somatotropin-containing hormone combination must lie in the mammary tissue itself, and that this can be transmitted by either male or female C3H parent to the hybrid offspring.

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Effect of Enol Etherification on Antiestrual and Contraceptive Activity of Some Progestins in Rats. (26827)

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A series of several enol ethers of Δ^4-3-ketosteroid hormones recently synthesized in our laboratories(1) has shown more potent hormonal activity when administered orally than their respective parent compounds(2-4). Enhancement of the activity was dependent upon the nature of the alcohol chain. The present investigation was designed to study