The Direct Effect of Pituitary Isografts on Mammary Gland Development in the Mouse.* (27628)

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Multiple(1,2) or single(3,4) pituitaries transplanted into intact mice at sites distant from the diencephalon stimulate a lobulo-alveolar development of host mammary glands and an alteration of the host's estrous cycles. The latter response is manifested by the occurrence of vaginal cornification every 10-14 days in grafted mice rather than every 4-6 days in non-grafted controls(2). In addition, pituitary transplants decrease the latent period and increase the incidence of mammary tumors in strains of mice with or without assayable mammary tumor agent(1,2,3). These findings have been explained by the release of prolactin from the pituitary grafts which exerts both a direct trophic action on the mammary glands and a luteotrophic effect on the ovaries(2). The demonstration of local lobulo-alveolar development of mammary parenchyma adjacent to subcutaneous implants of pituitary glands(5) has supported the postulated mammotrophic effect of the pituitary graft, but did not show the extent to which the ovaries participated in the observed mammary gland growth.

This experiment demonstrates the importance of the ovary in the development of the mammary gland produced by the direct action of a pituitary isograft.

Materials and methods. Thirty-two Strong A virgin female mice were divided equally into 4 experimental groups: ovariectomized controls, ovariectomized-pituitary grafted, intact controls, intact-pituitary grafted. Ovariectomies were performed at 38 days of age. Single whole isologous pituitaries were transplanted into 60-day-old host mice from donors of the same age and sex. Pituitary transplants were placed subcutaneously in the right inguinal region in immediate proximity to the mammary gland. All animals were killed at 120 days of age with ether. The skins bearing the mammary glands were removed, pinned on paraffin blocks, and fixed in Bouin's fluid. Whole mounts were prepared of the mammary glands removed from the right and left inguinal regions of each mouse. After examination of the whole mounts, areas containing pituitary grafts were removed, embedded in paraffin, sectioned at 5 μ, and stained with hematoxylin and eosin.

Results. Pituitary transplants were identified grossly in the right inguinal region of 6 of 8 ovariectomized and 6 of 8 intact-grafted mice. Transplants were from ¼ to ¾ the size of an intact mouse pituitary. Examination of the hematoxylin and eosin stained sections revealed that all of the surviving grafts contained viable cells from the pars distalis and pars intermedia.

In all the intact animals bearing histologically viable subcutaneous transplants the glandular tissue of right inguinal mammary glands in close proximity to the pituitary grafts showed a marked lobulo-alveolar and duct development (Fig. 1) whereas the mammary glands from the left inguinal region of the same intact-grafted hosts (Fig. 2) showed little if any end-bud development greater than the non-grafted intact controls. The area covered by the ducts of the mammary glands from the inguinal region of ovariectomized animals was not as great as in the intact groups. However, the inguinal mammary glands from the graft-bearing side of the ovariectomized hosts (Fig. 3) covered a greater area (1½ to 1¾ times) than the glands from the opposite side of the same hosts (Fig. 4). A slight but constant end-bud proliferation was observed adjacent to the transplants in the ovariectomized mice (Fig. 3); on the side opposite the grafts the mammary glands were similar to those from non-grafted ovariectomized animals.

Discussion. Vaginal cycle alteration(2)

* Supported by U.S.P.H.S. Research Grant.
† Trainee, U.S.P.H.S. Cancer Training Grant.

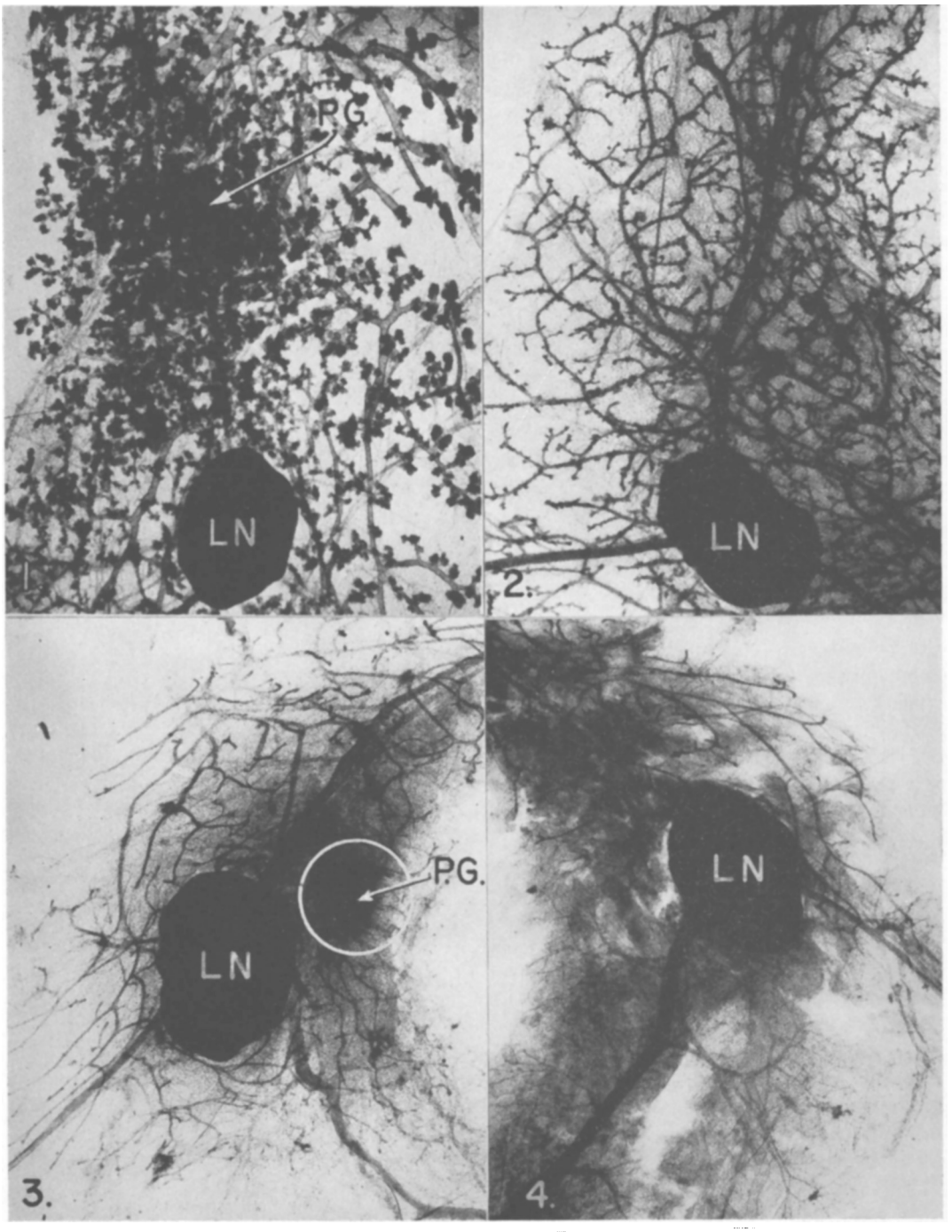

FIG. 1-4: Photographs of whole mounts of mammary glands removed from pituitary-grafted mice. (P. G.—pituitary graft; LN—lymph node) ×10.4.

FIG. 1. From right inguinal region of intact mouse. Extensive alveolar and duct growth next to pituitary graft.

FIG. 2. From left inguinal region of same mouse as in Fig. 1. Minimal end-bud development.

FIG. 3. From right inguinal region of ovariectomized mouse. Minimal end-bud development next to pituitary graft.

FIG. 4. From left inguinal region of same mouse as in Fig. 3. Less duct development.

and mammary gland growth(1,2,6) were observed in intact mice following implantation of pituitary isografts. Strong A mice with intraocular and subcutaneous pituitary transplants exhibited characteristic 10-14-day alteration of vaginal cycles. The mammary glands of Strong A mice, however, were shown to be relatively unresponsive to the systemic effects of pituitary implantation since only slight end-bud growth occurred in graft-bearing hosts(6). This was further demonstrated in the present experiment by the minimal growth of the mammary glands on the side opposite the graft in the intact host. Since the amount of hormones released into the systemic circulation of Strong A mice by single pituitary grafts has little effect on the mammary glands, this strain is particularly well suited for the study of the direct local effect of pituitary grafts upon the mammary tissue.

Loeb *et al.*(1) believed that the mechanism underlying the development of mammary glands in pituitary-grafted hosts was dependent upon an intact and functioning ovary since little or no mammary development occurred in males or ovariectomized females of the A strain. In this experiment the local growth of the mammary gland around a single subcutaneous pituitary transplant in ovariectomized as well as intact mice suggested that mammary gland development in pituitary-graft-bearing mice can be explained in part by a direct mammotrophic effect as proposed by Muhlbock and Boot(2). The ovary however is important in promoting this direct action of a single pituitary transplant since mammary tissue adjacent to a pituitary isograft did not show significant lobulo-alveolar growth in ovariectomized hosts.

Local mammary gland growth around a pituitary graft in intact mice is similar to that which occurs locally at the site of prolactin injections(7,8). Growth hormone induces duct branching when injected into adrenogonadectomized mice(8). Recently fractions of bovine anterior pituitary glands have been isolated which have little or no lactogenic activity but definite mammogenic activity when injected with estradiol benzoate in ovariectomized mice(9). Mammotrophic hormones secreted by pituitary tumor transplants stimulate lobulo-alveolar development of the mammary glands of adreno-gonadectomized male rats(10). Recently it has been demonstrated that duct growth and lobulo-alveolar growth can be induced in rats by combined treatment with bovine somatotrophic hormone and bovine prolactin in the absence of ovaries and adrenals(11). The relationship between these findings and the results of the present study warrant further investigation.

Summary. A single pituitary gland was transplanted adjacent to the mammary gland of the right inguinal region in intact and ovariectomized Strong A mice. Lobulo-alveolar and duct development occurred locally around the pituitary graft in intact hosts while duct growth and minimal end-bud development were observed in the glandular tissue adjacent to the graft in ovariectomized hosts. These data indicate that the ovary is necessary for the maximal response of mammary tissue to the direct, local effect of a pituitary isograft in Strong A mice.

1. Loeb, L., Kirtz, M. M., *Am. J. Cancer,* 1939, v36, 56.

2. Muhlbock, O., Boot, L. M., *Cancer Research,* 1959, v19, 402.

3. Liebelt, A. G., Liebelt, R. A., *ibid.,* 1961, v21, 86.

4. Boot, L. M., Muhlbock, O., Popcke, G., *Arch. Int. Physiol.,* 1960, v68, 851.

5. Gardner, W. U., *Proc. Am. Assn. Cancer Research,* 1960, v3, 113.

6. Bardin, C. W., Liebelt, A. G., *Anat. Rec.,* 1961, v139, 205.

7. Lyons, W. R., PROC. SOC. EXP. BIOL. & MED., 1942, v51, 308.

8. Nandi, S., *Univ. Calif. Publications in Zoology,* 1959, v65, 1.

9. Damm, H. C., Turner, C. W., PROC. SOC. EXP. BIOL. & MED., 1958, v99, 471.

10. Clifton, K. H., Furth, J., *Endocrinology,* 1960, v66, 893.

11. Talwalker, P. K., Meites, J., PROC. SOC. EXP. BIOL. & MED., 1961, v107, 880.

Received March 23, 1962. P.S.E.B.M., 1962, v110.