Early Histogenesis of Transplanted Mouse Mammary Glands
I. Within 21 Days following Isografting*

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Summary. Mammary duct-segments, each 0.6 mm long, from virgin female mice were isografted into the mammary gland-free fat pads of young female mice by Hoshino’s quantitative transplantation technique. The hosts were sacrificed at intervals from 24 hours to 21 days. No recognizable mammary structures were recovered earlier than 72 hours after transplantation. In hosts treated with estrogen and progesterone before and after transplantation, regenerating mammary gland tissues were recovered from just over 50% of the grafts, whether or not the donor was pretreated with 3-methylcholanthrene. Mitoses were more often observed in regenerating mammary glandular tissues, which had been transplanted from donors pretreated intraperitoneally with 20 mg of 3-methylcholanthrene nine hours before donation to hormone-treated hosts, and some of the mitoses exhibited chromosomal bridges and polyploid mitoses. Estrogen-progesterone enriched environment apparently enhanced regeneration of mammary transplants. The present experiments suggest that the transplanted mammary tissue seemed to dissociate to individual cells before 72 hours following transplantation and regenerate from them into single mammary glands, and that one dose of 3-methylcholanthrene may cause histologically-visible changes in epithelial cells of regenerating mammary grafts.

Key Words: Mammary glands—Histogenesis—Regeneration—Transplantation—Mouse.

Introduction

Transplantation of mammary tissues, whether transplantable tumors, hyperplastic alveolar nodules or normal glands, has been studied mainly in respect to mammary carcinogenesis (Faulkin et al., 1958; Fischer, 1937; Hoshino et al., 1965; Mühlbock, 1956; Prehn, 1953; Shimkin et al., 1946). Fewer reports have been concerned with transplantability and growth potentiality of grafted normal mammary tissues (Daniel et al., 1968; Hoshino, 1962, 1963b, 1964b, 1967; Hoshino et al., 1967). The earliest recovery of transplanted normal mammary tissue recorded in the literature is apparently 11 days after transplantation (Hoshino, 1962). As far as we know, there are no reports concerning earlier histogenesis during regeneration of normal mammary isografts.

The effects of 3-methylcholanthrene on mitosis and chromosomes have been reported with normal cells in tissue culture (Biesele et al., 1956; Kato, 1968), but not with normal mammary tissues in an in vivo system.

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The accelerating effects of estrogen and progesterone upon growth of mammary glands have been reported in \textit{in situ} glands (Hoshino, 1963a, 1964a) and in mammary grafts (Hoshino, 1962, 1964b).

The fate of mammary isografts in mice was studied histologically at varying intervals, 24 hours to 21 days, after transplantation with or without estrogen-progesterone treatment of the host mice. The influence of the pretreatment of mammary tissue with 3-methylcholanthrene while in the donors upon regeneration of such grafts was also investigated. A preliminary report has previously been presented (Hoshino \textit{et al.}, 1966).

\section*{Materials and Methods}

The mice used were female mice of two F-1 hybrids: (C57BL $\times$ CBA)$_{F-1}$ and (CBA $\times$ BC$_{B}$)$_{F-1}$ hybrids. The incidence of spontaneous mammary cancer in these hybrids as well as their parental strains in our laboratory were previously reported (Hoshino, 1964c). The rates of successful isografting of mammary glands in these hybrid mice were also reported (Hoshino, 1962, 1964b; Hoshino \textit{et al.}, 1967). All animals were maintained under uniformly controlled environmental conditions and provided with Purina Lab Chow and water \textit{ad libitum}.

The main site of mammary transplantation was the fourth mammary gland-free fat pad prepared when proposed host mice were three weeks of age, by the method developed by \textit{DeOme et al.} in 1959 with moderate modification (Hoshino, 1962). At intervals of from 3 to 15 days after the preparation of the transplantation sites, mammary duct-segments, each 0.6 mm long, were isografted by Hoshino's quantitative transplantation technique (Hoshino, 1963b).

This paper describes the results of two experiments undertaken at different times.

\textit{Experiment I.} From two 4-month-old virgin female CBA mice, 114 mammary duct-segments were excised and isografted into 38 female (CBA $\times$ BC$_{B}$)$_{F-1}$ mice at three sites of transplantation; one in the right fourth mammary gland-free fat pad and the other two grafts in the dorsal subcutaneous areas of both sides. In this group, the host mice received no hormonal treatments. They were sacrificed in groups of three at 24 and 48 hours, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, and 21 days following transplantation. The fat pads and dorsal subcutaneous tissues at the sites of transplantation were removed and prepared as paraffin sections. Incomplete serial sections at five microns were made and the slides alternately stained with hematoxylin-eosin, PAS, and also with the Masson staining technique.

\textit{Experiment II:} This experiment was set up much later and our subline of the BC$_{B}$ strain of mice was then exterminated from our colonies. Virgin female (C57BL $\times$ CBA)$_{F-1}$ mice were used as both donors and hosts of mammary isografts. This experiment consisted of two groups, A and B.

\textit{Experiment II-A.} The third pair of mammary glands of two 3-month-old mice were microdissected and 80 duct-segments were isografted into each of the right and left 4th mammary gland-free fat pads of forty sibling mice.

\textit{Experiment II-B.} Mammary glands were removed from a 3-month-old mouse which received intraperitoneally 20 mg of 3-methylcholanthrene in 1 ml of sesame oil 9 hours prior to the operation. Forty-two mammary duct-segments were microdissected and isografted into 21 sibling mice also at the fourth mammary gland-free fat pad.

All host mice in Experiment II received hormonal treatments as follows: a daily dosage of 0.03 $\mu$g of estradiol benzoate and 1 mg of progesterone dissolved in 0.02 ml of sesame oil was injected in the dorsal subcutaneous area for 3 days before transplantation, and every second day after transplantation. However, mice sacrificed at 3 and 5 days after transplantation received daily hormonal treatments.

The mice in Experiment II-A were sacrificed in groups of five at 3, 4, 5, 6, 7, 10, 14 and 21 days following transplantation. The mice in Experiment II-B were sacrificed also in groups of five at 3, 7, 14, and 21 days after isografting. All tissues at the sites of transplantation were removed, fixed in 10% buffered formalin solution, and embedded in paraffin. Serial sections at 5 microns were made, and alternate slides were stained with