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Longterm Effects of Neonatal Anterior Hypophysial Isografts on the Mammary Gland of Mammary Tumor Virus-Expressed Mice (40487)

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It has been well established that ectopic transplantation of the anterior hypophysis results in a pronounced increase in secretion of prolactin (1). Hypophysial implants in sites remote from the hypothalamus in adult mice result in stimulation of the mammary gland to milk secretion, alteration of reproductive cycles (i.e., "pseudopregnancy-type" vaginal cycles), and increase in mammary tumor incidence (cf. 2–8). Anterior hypophysis isografted in female mice and rats on the day of birth induces irregular estrous cycles characterized by prolonged estrus and/or prolonged diestrus and some development of the mammary glands. At autopsy, the isografts implanted subcutaneously on the back were not found in any of the animals, even one month after the implantation (9–11). This study was designed to investigate further the effects of hypophysial implantation performed on the day of birth, and deals with longterm effects on the mammary glands.

Materials and methods. On the day of birth, female mice of the mammary tumor virus (MTV)-expressed BALB/cfC3H/Crgl and SHN strains were given implants of the anterior hypophysial glands obtained from 2-month-old male mice of their respective strain. The first group of BALB/cfC3H mice received one-half of an anterior hypophysis subcutaneously on the back and the second group received an entire anterior hypophysis in the right #4 mammary fat pad (MFP). Females of SHN mice also received an entire anterior hypophysis in the right #4 MFP and were separated into 2 groups: one group was maintained without further operation and in the second group the right #4 MFP with mammary gland tissue was removed at 21–23 days of age according to the method of DeOme *et al.* (12). Control mice of each group were given a piece of similarly-sized submaxillary gland at corresponding sites. In some control SHN mice, the right #4 MFP was also removed at 21–23 days of age. Some litters provided both experimental and control mice. Vaginal smears were recorded between 40 and 65 days of age in BALB/cfC3H mice. Animals were killed 1 week after the appearance of palpable mammary tumors or at 12 months of age in BALB/cfC3H mice and at 7 months of age in SHN mice if no tumors appeared. Mammary tumors, ovaries, uteri, vaginae and adrenals were fixed in Bouin's or Tellyesniczky's fluid and sectioned in paraplast at 7 μm. Sections were stained with hematoxylin and eosin. Mammary glands were also fixed in Tellyesniczky's fluid and stained as whole-mounts with iron-hematoxylin. In SHN mice, after examining the whole-mount preparation, the right #4 MFP were embedded in paraplast and cut at 7 μm. All serial sections were stained again with tetrachrome (13) or hematoxylin-eosin.

Data on the incidences of mammary tumors, hyperplastic nodules (HAN) and various types of vaginal cycles were analyzed by the chi-square test, and data on the average number of HAN per animal were analyzed by the Student t test.

Results. By careful examination of the whole-mount preparations and histological sections, the pituitary grafts were recovered from 87% of SHN mice (13/15) from which the right #4 MFP was removed at 21–23 days of age. Nine grafts were relatively large (Fig. 1) and were composed of active-appearing erythrosinophilic cells; four grafts were small (<0.1 mm in maximum diameter) and consisted of erythrosinophilic cells with pycnotic nuclei. All grafts were surrounded with dense connective tissue but not with adipose tissue (Fig. 1). By contrast, only one mouse of 28 animals killed at 4–7 months of age revealed a graft in the right #4 MFP. The mammary glands of SHN mice given hypophysial isografts showed more alveolar development than those of control mice at 21–23 days of

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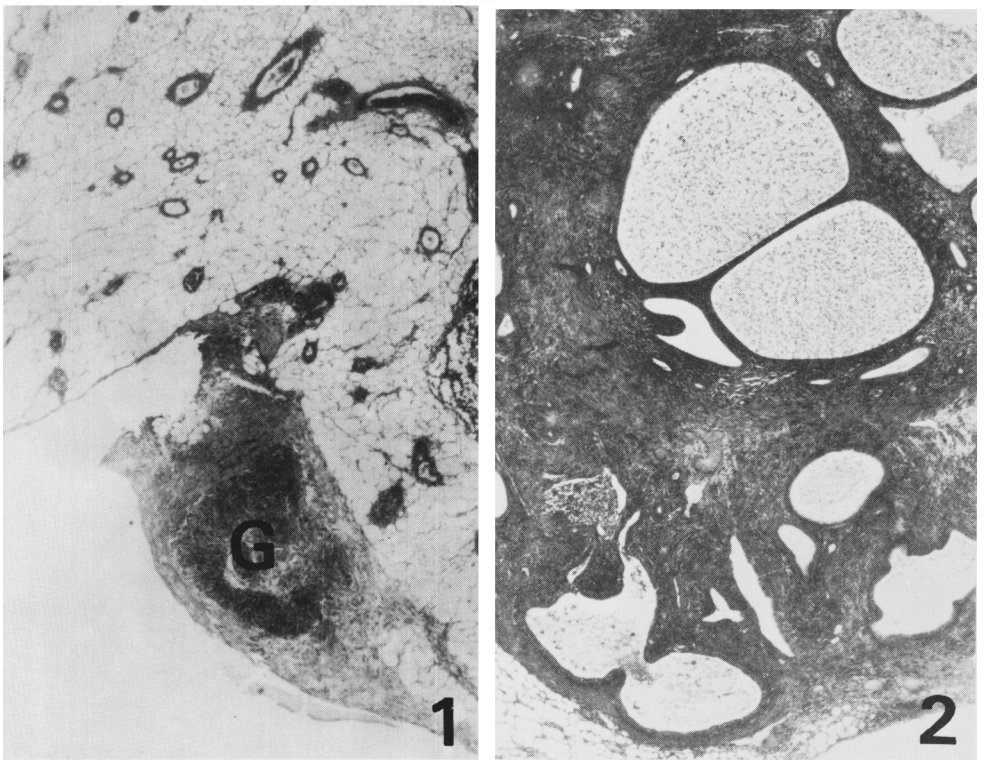

FIG. 1. Anterior hypophysial graft (G) encapsulated in connective tissue at periphery of right #4 MFP of 21-day-old SHN mouse. Tetrachrome. × 50.

FIG. 2. Cyst formation in uterus of 6.5-month-old SHN mouse which received implant of the anterior hypophysis on day of birth. Hematoxylin-eosin. × 50.

age. Grafts were not recovered from any mice of BALB/cfC3H strain killed at 6–12 months of age.

Based on vaginal smears, 7 of the 28 BALB/cfC3H mice (25%) given hypophysial isografts showed prolonged estrus, i.e., a 1- to 3-day diestrous period occurred only once or twice during days 40–65. Five mice (18%) exhibited prolonged diestrus, i.e., a 1- to 3-day estrous period occurred only once or twice during this period. The remaining 16 mice (57%) showed normal estrous cycles (Table I). Fifteen control mice given isografts of submaxillary gland exhibited normal cycles of 2 to 4 days' duration. Therefore, implantation of the hypophysis resulted in a significant occurrence of irregular estrous cycles ($P < 0.001$).

The incidences of mammary tumors and HAN are summarized in Tables II and III. In the experiments using BALB/cfC3H strain, mammary glands of control females contained few or no alveoli. Four of these 15 control mice had HAN and one mouse had a small tumor. In 18 of 28 females receiving hypophysial isografts, the mammary gland was characterized by extensive development of lobules of large alveoli and dilatation of the duct system; in 7 mice, the glands showed grossly dilated ducts and secretion-filled alveoli. Fourteen of 28 female mice of the experimental group had tumors by 6–12 months of age and 16 animals had HAN at one year of age. In the experimental series in which the hypophysis was implanted in the MFP, 4 of 12 mice showed HAN in the mammary gland in that fat pad only, and 8 of 10 mice had mammary tumors in that fat pad only. Mammary tumors occurred in the right #1 and #4 mammary glands in one mouse and in the right #5 in the remaining mouse.

In the experiments using SHN strain, all animals examined had HAN. Dilatation of

TABLE I. VAGINAL CYCLES IN BALB/cfC3H MICE IMPLANTED WITH ANTERIOR HYPOPHYSIS.

Group	Site of implantation	No. of mice	No. of mice showing prolonged estrus	No. of mice showing prolonged diestrus	No. of mice showing normal cycles
Hypophysial isografts	SC[a]	12	4	3	5
	MFP[b]	16	3	2	11
Total		28	7	5	16
Submaxillary isografts	SC	8	0	0	8
	MFP	7	0	0	7
Total		15	0	0	15

[a] SC; Tissues implanted subcutaneously on the back.
[b] MFP; Tissues implanted in the right #4 mammary fat pad.

TABLE II. MAMMARY GLAND RESPONSES OF FEMALE BALB/cfC3H MICE IMPLANTED WITH ANTERIOR HYPOPHYSIS.

Group	Site of implantation	No. of mice	No. of mice bearing mammary tumors	No. of mice bearing HAN
Hypophysial isografts	SC	12	4	4
	MFP	16	10	12
Total		28	14[a]	16
Submaxillary isografts	SC	8	0	2
	MFP	7	1	2
Total		15	1	4

[a] Differs from control, $P < 0.02$.

the mammary duct system was observed in 12 of 43 experimental mice and in 3 of 37 control mice. The intact control mice showed approximately the same number of nodules in each mammary gland. Six of 23 control mice (26%) had tumors by 5–7 months of age. Two of the 6 mice had mammary tumors in the right #4 mammary gland only. The control mice with their right #4 mammary gland removed showed incidences of mammary tumors and HAN similar to those in intact controls (Table III). Four of 14 mice (29%) had tumors by 6–7 months of age. On the other hand, 21 of 28 females (75%) of the experimental group had tumors by 4–7 months of age. Incidence of mammary tumor occurrence in the right #4 mammary gland only was 29% (6/21). However, the average number of HAN was significantly higher in the right #4 mammary gland than in the left #4 mammary gland. HAN were observed at high incidence in all mammary glands of the intact experimental group examined as compared with those in intact control group. After removal of the right #4 mammary gland, the incidence of mammary tumors and HAN was not significantly lower when compared with intact experimental mice. On the other hand, the average number of HAN was significantly higher in the experimental mice with their right #4 mammary gland removed than in the corresponding control mice.

Cystic dilatation of the lumen and glands in the upper uterus was observed in 18 out of 43 experimental SHN mice (Fig. 2). Fifteen of the 18 instances occurred in the mice bearing mammary tumors. This abnormality was also observed in 2 of 37 control mice without mammary tumors, significantly less than in experimental mice ($P < 0.001$).

In all groups, almost all ovaries contained both follicles and corpora lutea. In general, no changes were detected in other organs examined.

Discussion. Neonatal implantation of the anterior hypophysis resulted in irregular estrous cycles. In the previous reports (9, 10), the primary factor involved in the alteration of vaginal cycles by neonatal hypophysial implants was considered to be prolactin, inasmuch as neonatal prolactin injections induce prolonged estrus in both mice and rats (14, 15).

Neonatal implantation of the anterior hypophysis is effective in stimulating mammary development and tumorigenesis. Isografts implanted neonatally may not remain for long periods, although they can survive for at least 21–23 days. Even if the right #4 MFP bearing isografts of anterior hypophysis was removed at 21–23 days of age, the development of the mammary gland and HAN was greater in the experimental group than in the control group. These facts suggest that a relatively short-term exposure to neonatal hypophysial grafts

TABLE III. MAMMARY GLAND RESPONSES OF FEMALE SHN MICE IMPLANTED WITH ANTERIOR HYPOPHYSIS.

Group	Removal of right #4 mammary gland	No. of mice	No. of mice bearing mammary tumors	Average number of HAN per mammary gland		
				left and right #3	left #4	right #4
Hypophysial isografts	No	28	21[a]	39.8 ± 2.9[b]	17.6 ± 1.8[c, d]	29.8 ± 3.8[e]
	Yes	15	9	29.5 ± 4.8[f]	16.5 ± 3.1[g]	—
Submaxillary isografts	No	23	6	23.0 ± 3.8	10.0 ± 1.6	10.0 ± 1.6
	Yes	14	4	18.6 ± 1.9	9.9 ± 1.1	—

[a] Differs from intact control, $P < 0.01$.
[b] Differs from intact control, $P < 0.001$.
[c] Differs from intact control, $P < 0.01$.
[d] Differs from the value of the right #4 of the same group, $P < 0.01$.
[e] Differs from intact control, $P < 0.001$.
[f] Differs from controls with right #4 mammary gland removed, $P < 0.05$.
[g] Differs from controls with right #4 mammary gland removed, $P < 0.02$.

(presumably secreting prolactin) during early postnatal life results in permanent alterations in development of mammary glands, although the effect may be mediated by the endocrine system.

In the BALB/cfC3H mice with anterior hypophysis transplants, a strong local effect was exerted on tumorigenesis in the right #4 MFP. Transplanted nodules give rise to a carcinoma earlier when a hypophysial isograft is placed next to the nodule transplant (16). However, this local effect was not observed in SHN mice. SHN mouse mammary glands appear to be more sensitive to neonatal hypophysial grafts than BALB/cfC3H mouse mammary glands, inasmuch as they show a higher incidence of mammary tumors.

The hormonal milieu conducive to mammary tumorigenesis also resulted in cystic changes in the uterus. Prolactin synergizes with estrogen to bring about relaxation of the uterine cervix and with progesterone to promote decidual growth (17, 18). Prolactin increases the estradiol-binding capacity of rat uterine explants *in vitro* (19). However, the cystic change may be strain specific in view of its occurrence in SHN mice but not in BALB/cfC3H mice.

Summary. Hypophysial isografts stimulated mammary HAN formation and tumorigenesis in MTV-expressed female mice, even when the grafts were removed at 21–23 days of age. Exposure to the grafts (presumably secreting prolactin) during shortterm postnatal periods may induce proliferation of mammary tissue and result in early onset of mammary tumorigenesis, either directly or through the animal's endocrine system. This exposure also results in increased occurrence of uterine cyst formation in SHN mice.

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