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Mouse Mammary Carcinoma Induced by Pituitary Isografts in Mammary Fat Pads (33554)

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The implantation of additional pituitary glands remote from the hypothalamus most effectively induced mammary carcinoma in female mice (1). The site of implantation of the glands is of secondary significance. The subcutaneous tissue, spleen, kidney, and ovary, have been successful implantation sites (2). The most effective was implantation under the capsule of the kidney.

Mammary glands adjacent to pituitary grafts were usually much better developed than the remote glands unless pituitary tumors were present (3). When these tumors were present, all mammary glands were large and lactating. Bardin *et al.* (4) showed that lobulo-alveolar and duct development occurred in close proximity to the implanted pituitary, indicating a local effect of a pituitary isograft in strain-A mice.

To determine whether this local effect of a pituitary transplant also accelerates the induction of a mammary carcinoma, pituitary glands were implanted into the fat pad of different mammary glands.

Material and Methods. Hybrid females (♀ C3H × ♂ 020)F_1 were used. The females of this strain have a high incidence of mammary carcinoma (5). The 020/A-strain carries no mammary tumor agent; its mammary tumor incidence with and without the mammary tumor agent has been determined (6). The hybrid females (♀ C3H × ♂ 020)F_1 carry the mammary tumor agent from their C3H-mothers and have a high incidence of mammary tumors.

For the isografting of the pituitary into the fat pad of the inguinal pair of mammary glands, a skin incision was made in the ventral midline up to the vaginal opening with two side-incisions to the middle of the hind legs. The mammary glands were then exposed. Care was taken not to damage the fat pads and to put the pituitary directly into the middle of the fat pad near to the main duct of the gland. For the isografts into the second mammary gland, a similar incision,

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TABLE I. Incidence of Mammary Tumors in Female (C3H × 020)F_1 Mice Bearing Pituitary Isografts in the Kidney and/or the Mammary Fat Pad.

Treatment	No. of animals	Percentage with tumors	Av tumor age (days)	Av age at death without tumors (days)
Group A, untreated controls	50	86	468	774
Group B, pituitary isografts into the kidney at the age of 4 weeks	50	100	202	—
Group C, pituitary isografts into second mammary gland at the age of 3 weeks	50	100	219	—
Group D, pituitary isografts into second mammary gland at the age of 2 months	50	100	242	—
Group E, pituitary isografts into fourth mammary gland at the age of 3 weeks	53	100	210	—

but without side-incision, was made in the middle of the thorax. The pituitary was taken out of the sella turcica and transplanted with a fine forceps into a canal prepared in the fat pad of the host's mammary gland. Donors of the isografts were (♀ C3H × ♂ 020)F_1 males 3–4 months of age.

The animals were kept in plastic boxes, 3-together, and given commercial food pellets and tap water *ad libitum*. Mammary tumors were noted when first palpable. Whenever multiple tumors were registered at the same time, all tumors found were included in the calculation. All tumors were studied histologically.

Five groups of mice were investigated: group A, untreated virgin females; group B, females with two pituitary glands implanted under the capsule of each kidney at the age of 4 weeks; group C, females with one gland implanted into the fat pad of each second left and second right mammary gland at the age of 3 weeks; group D, females with one gland implanted into the fat pad of each second left and second right mammary gland at the age of 2 months; and group E, females with one gland implanted into the fat pad of each fourth left and fourth right mammary gland at the age of 3 weeks.

Results. Mammary tumors occurred in all hosts bearing pituitary isografts in the kidney at an average age of 202 days (Table I). Mice of groups C and E receiving isografts into the second and/or fourth fat pad of the mammary gland at the age of 3 weeks had mammary tumors at the average age of 219 and 210 days, respectively. Mice receiving isografts at the age of 2 months (group D) had tumors appear at an average age of 242 days.

Because it is difficult to differentiate between a tumor arising in the second or third mammary gland, the tumors arising in these two pairs of glands were pooled. The tumors of the fourth and fifth pairs of glands also were pooled. In mice of group C, 44% of the tumors arose in graft areas and 36% in the fourth and fifth glands, (Table II). In group E, 33% of the tumors appeared in the second and third gland areas and 38% in the region of the fourth and fifth glands. The tumors were equally distributed in the right and in the left glands.

Because the localization of the mammary tumors did not depend on the mammary gland bearing the grafts, the results of groups C, D, and E were combined and compared with the untreated controls (group A) and with group B, with hypophyses under the capsule of the kidney (Table III). Untreated virgin females had 63% mammary tumors in the second and third pair of glands and only 16% developed in the fourth and fifth pairs. Mice with pituitary isografts either in the kidney or in the mammary glands had relatively many more tumors developed in the fourth and fifth pairs of glands: 47 and 35%, respectively. The percentages in the first pair of glands were the same in all groups.

Morphologic classification of all tumors

TABLE II. Distribution of Mammary Tumors in Mice Bearing Pituitary Isografts in the Mammary Fat Pad.

Treatment	Total no. of tumors	Percentage of tumors		
		First gland	Second and third glands	Fourth and fifth glands
Group C, pituitary grafts in the fat pad of the second glands at the age of 3 weeks	59	20	44	36
Group E, pituitary grafts in the fat pad of the fourth glands at the age of 3 weeks	69	29	33	38

made according to the criteria of Dunn (7) revealed a slightly higher occurrence of "atypical" tumors in untreated virgin females, but the data were not sufficiently extensive to permit an analysis of the relationship between tumor age and the distribution of atypical tumors.

Discussion. Pituitary isographs greatly enhanced the appearance of mammary tumors in female mice. Pituitary isografts in the kidney—the most effective site found so far, induces mammary tumors in half the time as they develop in untreated virgin females of the hybrid stock used. Isografts into the mammary fat pad is as effective as isografts into the kidney.

The average age at which mammary tumors appear depends on the age at which the isografts were made: at the age of 2 months, tumors appeared 23 days later than in animals isografted at the age of 3 weeks.

Because mammary fat pad bearing isografts had no more tumors or tumors at earlier ages, there is no local influence of the grafted pituitary on mammary tumorigenesis.

If the hormonal stimulation of the mammary gland is of primary importance for the development of mammary tumors, one would expect a stronger hormonal stimulation in the gland bearing the graft and, as a consequence, a higher tumor-rate in this gland. Very probably, the hormone production of the grafted pituitary in the hybrid used is so abundant that all mammary glands are stimulated maximally so that a local influence cannot be more effective.

Strain differences with respect to production of prolactin by isografted pituitaries and/or strain differences with respect to the susceptibility for prolactin of the mammary gland may, however, exist.

The results of Bardin *et al.* (4) suggest that in strain-A mice there is a low production of prolactin by isografted pituitaries and, therefore, a local effect of the implanted hypophyses can be demonstrated. One could conclude from the results of Gardner (3) that there is a relation between the amount of prolactin produced and the demonstration of a local effect of this hormone on the mam-

TABLE III. Localization of Mammary Tumors in Mice Bearing Pituitary Isografts.

Treatment	Total no. of tumors	Percentage of tumors		
		First gland	Second and third glands	Fourth and fifth glands
Group A, untreated controls	49	27	63	16
Group B, pituitary isografts into the kidney at the age of 4 weeks	64	20	33	47
Groups C, D, and E, pituitary isografts into the fat pad of the mammary glands	190	24	41	35

mary gland. As soon as the isografted pituitaries transformed into tumors and the prolactin production is much higher, the local effect is no longer visible.

The presence of the mammary tumor virus (Bittner virus) in the animals has surely a strong accelerating effect on the development of the mammary tumors. This makes the recognition of minor differences more difficult. The hypophyseal isograft produces a continuous high level of prolactin. Although prolactin is to be considered the hormone mainly responsible for the induction of mammary tumors, other hormones have to be taken into account as well, especially progesterone. Under the influence of prolactin, the ovaries of the graft-bearing mice produce a large amount of progesterone. Mühlbock and Boot (8) found that progesterone greatly accelerates the development of mammary tumors by prolactin. This could mean that in our experiments a local influence of prolactin on the mammary gland could be overrun by the influence of the high doses of progesterone. Experiments with castrated animals given a low dose of estrogenic hormones and an isograft of a hypophysis could clarify this point.

The localization of the mammary tumors shifts remarkably after implantation of the hypophysis (Table III). Untreated controls had few mammary tumors in the fourth and fifth pairs of the glands and most in the second and third pairs of glands. Whereas, mice with pituitary isografts in either the kidney or fat pads had more tumors in the fourth and fifth pair of glands. Earlier experiments with partly mammectomized mice (9) shows the reduction of the number of mammary glands per female delayed the appearance of tumors considerably.

Pullinger (10) first reported that the second mammary gland is a gland of predelection for developing hyperplastic nodules in the RIIIb-strain of mice deprived of the milk agent. Prehn *et al.* (11) in his studies on the distribution of tumors among the mammary glands of four inbred strains and one group of F_1-hybrids, based on a very large sampling, found that an uneven distribution of mammary tumors (increased anteriorly) among the glands occurred in the absence of the mammary tumor agent, whereas a comparatively even distribution occurred when the agent was present. These authors concluded that the degree of unevenness of tumor distribution was largely a function of tumor age. The greater the tumor age, the greater was the percentage of anteriorly occurring tumors. The only influence of the mammary tumor agent on the distribution of tumors was probably by its effect on the average tumor age.

In our experiments, all our mice harbored the mammary tumor agent. In the control group with the highest tumor age, most tumors occurred anteriorly in the second and third mammary gland. When the induction of mammary tumors is accelerated by hypophyseal grafts, and the average tumor age is considerably lower, the tumors are evenly distributed. Therefore, in our experiments, too, the distribution of mammary tumors seems to be related to the average tumor age.

Summary. Although in short-term experiments pituitary isografts have shown a direct local effect on the growth of the mammary gland, isografts of a hypophysis into the fat pads of the mammary glands showed no local effect on the incidence or an earlier appearance of mammary tumors near the site of implantation. The hormonal inciting influence is assumed to be so great that a local effect cannot be recognized. After isografting of hypophysis, a remarkable shift in the localization of the mammary tumors occurs: more tumors develop in the fourth and fifth pairs of glands than in untreated animals.

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The Effect of Hyperthermia on Dengue Virus Infection of Mice* (33555)

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The relationship between the expression of viral virulence and an optimal environmental temperature was demonstrated in cell culture systems (1) and *in ovo* (2). Equally convincing studies showed that the temperature of the intact experimental animal plays an important role in determining the degree of its susceptibility to viral infection. Walker and Boring (3) found that when mice inoculated with a lethal dose of Coxsackie virus were held at 36°, the resulting 2–3° increase in their body temperature prevented virus replication and no deaths occurred. In similar experiments by other workers, passively induced hyperthermia could prevent or delay the lethal effects in mice of several different viruses (4–6).

The present work deals with the effect of hyperthermia on the course of experimental central nervous system (CNS) infections of weanling mice produced by a low (MP-33) and high mouse brain-passaged line (MP-125) of type 1 dengue virus of the Hawaiian strain. Both of these lines were uniformly lethal for weanling mice after intracerebral (i.c.) inoculation but differences in their neurovirulence could be shown in adult animals which, although highly susceptible to MP-125, were relatively resistant to MP-33. Since virulence of other viruses has been correlated with an ability to replicate at elevated temperatures (4), it was of interest to determine for each of the two dengue lines: (i) the possible relationship between temperature sensitivity *in vivo* and the differences in their virulence; and (ii) whether any demonstrable protection by elevated temperature was due to its direct effect on virus multiplication in the CNS or was mediated by an enhancement of the immune or interferon response of the host.

Materials and Methods. Virus. Both lines of type 1 dengue (Hawaiian strain) were prepared as clarified 20% brain suspensions from infected moribund suckling mice. Low passaged MP-33 was identical to the MD-1 human attenuated vaccine strain developed by Wisseman and co-workers (7) and had initially undergone 18 i.c. passages in young weanling mice followed by 15 additional passages in 3–5 day-old sucklings. Highly neurovirulent MP-125 had a history of 125 serial brain passages. Initially adapted to 10–12-day-old suckling mice, weanlings were employed through passage 92 and 3–4 day-old animals were used for remaining passages.

The mean i.c. LD_{50} in 4-week-old weanling mice of the low and high passaged lines were, respectively, $10^{7.5}$ and $10^{8.40}$/g of brain.

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