Mammary Cancer Induction by 7,12-Dimethylbenz(a)anthracene: Relation to Age

Abstract. *Mammary glands were transplanted from donors treated with 7,12-dimethylbenz*(a)*anthracene to untreated isologous recipients. Incidence of mammary tumors in mammary grafts from 56-day-old donors was significantly higher than that in grafts from 120-day-old donors, regardless of the age of the recipient hosts. When mammary glands were transplanted from untreated donors to isologous recipients that subsequently received 7,12-dimethylbenz*(a)*anthracene, a similar difference in tumor incidence in the grafts was observed. In contrast to mammary glands of older females, mammary glands of young adult female rats are highly vulnerable to the carcinogenic effect of 7,12-dimethylbenz*(a)*anthracene.*

Age is very critical in the induction of mammary cancer by polycyclic aromatic hydrocarbons (*1*). Mammary cancer can be induced in intact rats by feeding a carcinogen to 23-day-old animals; the incidence of tumors rises progressively with age until the rats are 50 to 60 days old. The incidence declines markedly when the carcinogen is fed to rats 75 days old, and tumors develop rarely in animals fed the carcinogen at ages of more than 100 days (*1*). We confirmed this observation and in addition attempted to isolate and evaluate the effect of target tissue age and host age on the induction of mammary cancer by chemical carcinogen; we transplanted mammary glands from donors of various ages to recipients of the same age and different ages (*2, 3*). We now describe experiments carried out to determine whether the target tissue or the age of the recipient host is more important in relation to the occurrence of tumors. Our results suggest that the target tissue age is critical in tumor induction.

Female inbred Wistar-Furth rats were housed in an air-conditioned room, fed a commercial ration (Rockland diet), and given free access to water. At the time of transplantation, the animals either were 56 days old and weighed 115 to 130 g or were 120 days old and weighed 165 to 185 g.

In the first experiment, mammary glands from donors given a single intravenous dose of 7,12-dimethylbenz(*a*)anthracene (3 mg per 100 g of body weight) before grafting were transplanted to untreated recipient hosts. Mammary glands were transplanted from young donors (56 days old) to older recipients (120 days old), from older donors to young recipients, and from young and older donors to recipients of the same ages. Each group was divided into four subgroups, according to the times when mammary glands were transplanted after the carcinogen was given to the donor rats: 30 minutes, 4 or 24 hours, 10 days, and 20 days (Table 1).

When mammary glands from donors 56 and 120 days old were transplanted to 120-day-old recipients, the incidences of mammary tumors developing in the grafts were significantly different statistically. Although 14 mammary adenocarcinomas developed in a total of 34 surviving grafts from 56-day-old donors, only 3 were found in 27 surviving grafts from 120-day-old donors. When mammary glands from donors 56 and 120 days old were transplanted to 56-day-old recipients, the incidences of mammary tumors developing in the grafts were again significantly different. Mammary adenocarcinoma developed in 14 of the 35 surviving grafts from 56-day-old donors, whereas only 5 appeared in 32 surviving grafts from 120-day-old donors (Table 1).

The effect of 7,12-dimethylbenz(*a*)anthracene on the host may influence tumor development differently in grafted mammary glands of different ages, and also the presence of a mammary graft may alter the incidence of tumor induction in host's own mammary gland. To determine these effects, we removed mammary glands from untreated donors, transplanted the glands to the recipient hosts, and administered a single intravenous dose of 7,12-dimethylbenz(*a*)anthracene (3 mg per 100 g of body weight) to the recipient hosts 2 weeks after transplantation.

When mammary glands were transplanted from 56-day-old donors to both 56- and 120-day-old recipients, seven adenocarcinomas developed in 28 surviving grafts (25 percent). The tumor incidence was reduced if the donors were 120 days old. In a total of 28 surviving grafts in both 56- and 120-day-old recipients from 120-day-old donors, only one tumor developed (3 percent) (Table 2). Of particular interest is the observation of a phenomenon similar to that in the first experiment. If the effect of 7,12-dimethylbenz(*a*)anthracene on the host per se is of critical importance in carcinogenesis in the mammary gland, then grafts from 56-day-old donors in 120-day-old recipient hosts should have a tumor incidence similar to that in grafts from 120-day-old donors in 120-day-old recipient hosts. Similarly, the tumor incidence in grafts from 56-day-old donors to 56-day-old recipient hosts should be about the same as that in grafts from 120-day-old donors in 56-day-old recipient hosts. This is not the case.

The incidences of mammary tumors in the host glands in two groups of 56-day-old recipients were 46 and 57 percent, whereas those in two groups of

Table 1. Effect of ages of donors and of recipient hosts on tumor development in transplanted mammary gland. 7,12-Dimethylbenz(*a*)anthracene was given intravenously into the tail vein in a single dose to the donor rats, and inguinal mammary glands were excised and transplanted to the dorsum of the recipient rats. All recipient rats were killed at the end of 6 months. Both grafts and hosts' own mammary glands were removed for whole-mount preparations. A Yates-corrected chi-square test of the difference between groups a and b gives a calculated value of 6.71, which is statistically significant at $P = .01$; the difference between groups c and d gives a calculated value of 3.87 which is statistically significant at $P = .05$. HAN, hyperplastic alveolar nodule in grafts without tumors.

Age (days)		Time between carcinogen administration and grafting	Rats (No.)	Surviving grafts (No.)	Grafts with lesion (No.)	
Donor	Recipient (group)				HAN	Tumor
56	120 (a)	30 minutes	10	9	0	5
56	120 (a)	4 hours	9	7	1	3
56	120 (a)	10 days	10	10	5	4
56	120 (a)	20 days	10	8	4	3
120	120 (b)	4 hours	8	6	0	2
120	120 (b)	24 hours	8	8	5	0
120	120 (b)	10 days	7	7	3	0
120	120 (b)	20 days	7	6	5	1
56	56 (c)	4 hours	10	7	0	4
56	56 (c)	24 hours	10	8	1	3
56	56 (c)	10 days	10	10	4	2
56	56 (c)	20 days	10	10	4	5
120	56 (d)	30 minutes	10	7	3	2
120	56 (d)	4 hours	10	8	1	1
120	56 (d)	10 days	10	8	5	0
120	56 (d)	20 days	10	9	5	2

Table 2. Effect of ages of donors and of recipients on tumor development in transplanted and host mammary gland. The mammary glands removed from the untreated donors were transplanted into recipient hosts. Two weeks after transplantation, a single dose of 7,12-dimethylbenz(*a*)anthracene was given intravenously into the tail vein of the recipient host. Recipient hosts were killed 6 months later. Grafts and hosts' own mammary glands were removed for whole-mount preparations. The difference in tumor incidences in grafts from 56-day-old donors to 56- and 120-day-old recipients and from 120-day-old donors to 56- and 120-day-old recipients is statistically of borderline significance (Yates-corrected chi-square test, $\chi^2 = 3.64$). The tumor incidence in mammary glands of both 56-day-old recipient hosts is significantly higher than that in 120-day-old recipient hosts. HAN, hyperplastic alveolar nodules. The number of rats indicated in column two is the original number of rats in each experimental group.

Age (days)		Rats (No.)	Surviving grafts (No.)	Rats with "preneoplastic" lesions or tumors or both (No.)			
				In surviving grafts		In host glands	
Donor	Recipient			HAN	Tumor	HAN	Tumor
56	56	14	13	9	3 (23%)	5	6 (46%)
56	120	16	15	3	4 (26%)	2	4 (25%)
120	56	16	14	1	1 (7%)	6	8 (57%)
120	120	14	14	3	0 (0%)	1	2 (14%)

120-day-old recipients were 14 and 25 percent. The tumor incidences in the control groups of 56- and 120-day-old rats were 80 and 20 percent, respectively (Fig. 1). It thus appears that the mammary glands *in situ* of the recipient hosts bearing mammary grafts have a lower tumor incidence than do those of control rats that receive no mammary grafts.

The most important factor in the development of tumors in transplants seems to be the age of the transplant. The mammary glands from 120-day-old rats are clearly less susceptible to carcinogenic stimuli than their 56-day-old sisters. Why the age of the target tissue is so critical to carcinogenesis in the mammary gland is a question that cannot be answered here. The exact biological mechanism that determines susceptibility to cancer induction on the basis of age has yet to be elucidated. Our data nevertheless demonstrate that the critical factor that influences the susceptibility of mammary cancer induction by 7,12-dimethylbenz(*a*)anthracene in rats resides in the target tissue. In every instance, the tumor incidence is significantly higher in younger grafts, regardless of the age of the hosts. Could there be a difference in the state of differentiation of the mammary gland of the 56-day-old and 120-day-old rats? Examination of the mammary glands of these two age groups by means of whole-mount preparations revealed no significant morphological difference. However, in most instances, the lobulo-alveolar growth in the 56-day-old mammary glands is more extensive than that seen in the 120-day-old rats.

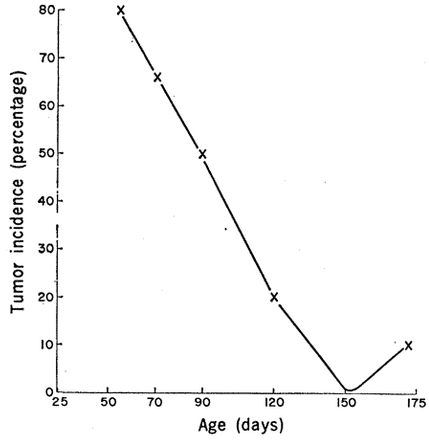

Fig. 1. Mammary tumor incidence related to age in Wistar-Furth female rats. The experiment consisted of six groups of ten rats each. The ages of the rats in the six groups are: 56, 70, 90, 120, 150, and 175 days old, respectively. They were given a single dose of 3 mg per 100 grams of body weight of 7,12-dimethylbenz(*a*)anthracene intravenously into the tail vein and were killed at the end of 6 months.

DeOme *et al.* (*4*) used the fat-pad transplantation technique to show that outgrowth of hyperplastic alveolar nodules from mice infected with mammary tumor virus gave rise to tumors more frequently than did normal tissue from the same animals. Recently Beuving (*5*) demonstrated that when hyperplastic alveolar nodules and ducts from mammary glands of rats treated with a carcinogen were transplanted into gland-free fat pads of isologous animals, 8 of the 37 resulting nodular outgrowths gave rise to mammary tumors. Our data (Table 2) seem to suggest that mammary transplants from 120-day-old donors are less apt to develop hyperplastic alveolar nodules, but that once produced the hyperplastic alveolar nodules are as apt to develop into tumors as are the hyperplastic alveolar nodules from 56-day-old donors. It suggests that the age factor influences normal mammary glands to form "preneoplastic" lesions rather than the subsequent development into tumors. This explanation, however, is not in agreement with the results from the first experiment (Table 1) demonstrating that the development of hyperplastic alveolar nodules in the grafts does not exhibit a significant difference in relation to the age of the grafts. It appears that not all hyperplastic alveolar nodules will ultimately become neoplastic. The study suggests that neoplastic cell variants are lacking in cell population of the hyperplastic alveolar nodules that develop in the mammary glands of 120-day-old female rats. The susceptibility of the mammary gland to neoplastic transformation may be an "intrinsic factor" residing in the mammary tissue, and that the latency of tumor appearance and the progression of tumor growth are dependent on the host factor or factors (*6*).

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References and Notes

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Rats Enriched with Odd-Carbon Fatty Acids: Maintenance of Liver Glycogen during Starvation

Abstract. *In young rats a diet containing triundecanoin as the major source of fat produces substantial enrichment of adipose tissue triglycerides with undecanoate and higher fatty acids with odd-numbered carbons. The terminal three-carbon residues arising from beta-oxidation of these acids are glucogenic and help to counteract the decreases in liver glycogen and serum glucose ordinarily induced by prolonged fasting.*

The propionate residue arising from β-oxidation of odd-carbon fatty acids is known to be glucogenic (*1*). Studies have been described in which triglyc-