Mammary Neoplasia in the Female Rat After Neonatal and/or Adult 7,12-Dimethylbenz(*a*)Anthracene Administration[1] (37534)

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The administration of 7,12-dimethylbenz (*a*)anthracene (DMBA) to young, adult, female Sprague–Dawley rats is almost invariably followed by the rapid development of mammary adenocarcinomas and over longer periods of time by the development of mammary fibroadenomas (1, 2). On the other hand, the mammary adenocarcinoma response to DMBA is very much diminished when the DMBA is given to newborn female Sprague–Dawley rats, although the mammary fibroadenoma response to DMBA is largely retained following neonatal DMBA administration (3). The objective of the following experiment was to study possible interactions of two doses of DMBA, one given to neonatal female Sprague–Dawley rats and the other given to young, adult females. In other words, it was hoped to learn if young, adult female Sprague–Dawley rats given DMBA would demonstrate a "memory response" to a previous, neonatal administration of DMBA.

Materials and Methods. Pregnant females, purchased from Sprague–Dawley, Inc., Madison, Wisconsin, received into this Laboratory, were allowed to deliver their young. The female offspring were marked with subcutaneous injections of India ink and then injected sc in the interscapular region, using a 30-gauge needle between 36 and 48 hr after birth with either 0.05 ml of sesame oil or the same amount of sesame oil containing 0.3 mg of DMBA. These females, along with their brothers, were cared for by their mother until they were weaned at 22 days of age. Females from each litter were distributed, as exactly as possible, so that each litter contributed to all experimental groups. Of the 70 females given DMBA at two days of age 66 were alive on the 52-day age. Thirty-six of these were given sesame oil by stomach tube and 30 were given 13.3 mg DMBA/100 g body weight, also by stomach tube. Thirty-nine of 40 rats given sesame oil at two days of age were alive at 52 days of age, 9 of these were given only sesame oil and 30 were given 13.3 mg DMBA/100 g body weight by stomach tube. Throughout the experiment, rats were examined for visible and/or palpable tumors and these tumors were removed by surgical techniques when they reached a size of approximately 2 cm. A written record was kept of the size and location of all tumors. At 175 days of age, all animals were killed and examined for neoplasia. Tissues suspected of being neoplastic were fixed, sectioned, and stained and classified histologically according to established criteria (4). Adrenal, ovarian, uterine, and body weights were obtained at the end of the experiment.

Results. The ovarian, uterine, adrenal, and body weights were obtained at the end of the experiment when the rats were 175 days of age, were not different between groups and are not here presented. Four sarcomas, at the injection site were found, 2 in each of the 2 groups that received DMBA at 2 days of age. Rats that received DMBA had a higher in-

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TABLE I. Mammary Neoplasms, Number of Rats with Mammary Neoplasia, Number of Rats, Treatment, and Age at Treatment and End of Experiment.

	Treatment and days of age of treatment			
	Oil at 2 Oil at 52	Oil at 2 DMBA at 52[a]	DMBA at 2[b] Oil at 52	DMBA at 2 DMBA at 52
Number of rats				
at 2 days of age	9	31	38	32
at 52 days of age	9	30	36	30
at 175 days of age	9	20	33	19
Mammary neoplasia				
No. of rats with neoplasms	1	19	30	26
No. of neoplasms	1	40	117	100
Mammary adenocarcinomas				
No. of rats with adenocarcinomas	1	19	6	21
No. of adenocarcinomas	1	37	9	44
Mammary fibroadenomas				
No. of rats with fibroadenomas	0	2	29	19
No. of fibroadenomas	0	3	108	56

[a] 13.3 mg of DMBA 100 g body weight by stomach tube.
[b] 0.3 mg of DMBA in 0.05 ml of sesame oil.

cidence of mammary neoplasia than did the rats that received no DMBA (Table I). DMBA given at 2 days of age produced a high yield of mammary fibroadenomas and a very low yield of mammary adenocarcinomas while DMBA at 52 days of age produced just the opposite, a high yield of mammary adenocarcinomas and a low yield of mammary fibroadenomas. When DMBA was given twice at 2 and 52 days of age, the mammary adenocarcinoma response was not different from DMBA given once at 52 days of age and higher than DMBA given once at 2 days of age. Again, when DMBA was given at both 2 and 52 days of age, the mammary adenofibroma response was considerably larger than DMBA given only at 52 days of age and somewhat smaller than DMBA given only at 2 days of age.

Discussion. It seems clear from this experiment, and in agreement with others (1, 3), that when DMBA is given to neonatal Sprague–Dawley female rats, a rapid mammary fibroadenoma response occurs but when DMBA is given to young, adult female Sprague–Dawley rats a rapid mammary adenocarcinoma response occurs. The reason for this differential mammary neoplastic response to DMBA is not presently known but it is unlikely that the differential mammary neoplastic response can be attributed to the route of DMBA administration. This statement is supported by the report of Huggins and Fukuniski (3) who found also a predominantly fibroadenoma response following ip DMBA administration to neonatal rats. Similarly, when these investigators (3) used the ip route in adult animals they found also that a predominantly adenocarcinoma response ensued. It is interesting to note also that the neonatal administration of either estradiol benzoate or testosterone propionate has been shown to increase the rat mammary fibroadenoma response to DMBA (5). However, the animals given neonatal sex steroids were found to have depressed ovarian weight and an absence of corpora lutea while the animals who were given neonatal DMBA did not have depressed ovarian weights and corpora lutea were visible grossly. Thus, while both neonatally administered sex steroids and DMBA increase the mammary fibroadenoma response to subsequent DMBA administration, the sex steroids and DMBA must act by different mechanisms.

The results following DMBA administered

at both 2 and 52 days of age tend to show that each dose seemed to provoke its own specific result with little interaction between the two doses. That is to say, the mammary fibroadenoma response was large regardless of whether DMBA was given once at 2 days of age or two doses of DMBA were given at both 2 and 52 days of age. Similarly the mammary adenocarcinoma response was large regardless of whether a single dose of DMBA was given at 52 days of age or two doses of DMBA were given at both 2 and 52 days of age. The somewhat smaller mammary fibroadenoma response noted in the rats that received 2 doses of DMBA, as compared to those that received only a single dose of DM BA at 2 days of age, may be more apparent than real and related to the survival rate and to the fact that fibroadenomas tended to occur later than adenocarcinomas. Fewer rats survived when 2 doses were given, largely because of the adenocarcinoma response which tends to call for more surgical removals, and thus there were fewer rats alive to show the fibroadenoma response. No statements can be made about the final or ultimate neoplastic response to either the neonatal or the adult DMBA administration because these rats were studied only until they were 175 days of age, and because it is known (6) that spontaneous mammary adenocarcinomas and fibromas occur largely as a function of age.

These results are interpreted to mean that DMBA given at two days of age produces largely a mammary fibroadenoma response, that DMBA given at 52 days of age produces largely a mammary adenocarcinoma response, and that the combination of both treatments has little influence on the mammary neoplastic response to each of the individual administrations. It is concluded that female Sprague–Dawley rats given DMBA at 52 days of age, and studied until 175 days of age, do not "remember" a previous DMBA administration given at 2 days of age.

Summary. At 175 days of age it was found that DMBA given to female Sprague–Dawley rats at 2 days of age was followed by a high incidence of mammary fibroadenoma. DMBA given at 52 days of age was followed by a high incidence of mammary adenocarcinoma. When both DMBA treatments were given to the same animals, the mammary adenofibroma incidence was much the same as in the rats given only DMBA at 2 days of age and mammary adenocarcinoma incidence was much the same as in animals given only DMBA at 52 days of age. No significant interaction between two doses of DMBA given at both 2 and 52 days of age on mammary neoplasia was noted.

1. Huggins, C., Moon, R. C., and Morii, S., Proc. Nat. Acad. Sci. U.S.A. **48**, 379 (1962).

2. Sydnor, K. L., Butenandt, O., Brillantes, F. P., and Huggins, C., J. Nat. Cancer Inst. **39**, 805 (1962).

3. Huggins, C., and Fukunishi, R., Cancer Res. **23**, 785 (1963).

4. Shellabarger, C. J., Cronkite, E. P., Bond, V. P., and Lippincott, S. W., Radiat. Res. **6**, 501 (1957).

5. Shellabarger, C. J., and Soo, V. A., "IV International Congress of Endocrinology," Abstract 374, Excerpta Medica, 1972.

6. Thompson, S. W., Huseby, R. A., Fox, M. A., Davis, C. L., and Hunt, R. D., J. Nat. Cancer Inst. **27**, 1037 (1961).

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