[CANCER RESEARCH 29, 503–505, March 1969]

The Effects of Schedule and Dose of 7,12-Dimethylbenz(a)-anthracene on the Induction and Growth of Mammary Carcinomas in Sprague-Dawley Female Rats[1]

M. B. Shimkin, M. Gruenstein, D. R. Meranze, M. Acuff, and D. Thatcher

Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

SUMMARY

The induction and subsequent growth of mammary carcinomas in female Sprague-Dawley rats is significantly enhanced by dividing oral exposures to 7,12-dimethylbenz(a)anthracene into 5 weekly doses, and it is slightly depressed by 5 doses given every other day, as compared with single doses.

INTRODUCTION

During an investigation undertaken to compare the carcinogenic effect of 3-methylcholanthrene (MCA) and 7,12-dimethylbenz(a)anthracene (DMBA) in Wistar and in Sprague-Dawley female rats, we (1) found that the growth of mammary tumors was more rapid in rats that received repeated intragastric doses of MCA than in rats treated with single doses of DMBA. The conclusion was based on determinations of the diameters of the tumors during a 30-day period.

The observations were repeated (8), and again it was demonstrated that mammary cancers appearing in rats following multiple doses of MCA grew more rapidly than those appearing following single doses of DMBA. The findings were consistent and significant when derived from calculated volumes at 21 days following initial detection of the masses or when based upon actual weights of the tumors at 42 days. It was also shown (8) that these two types of hydrocarbon-induced tumors responded differently to ovariectomy and to exogenous estradiol, the DMBA-induced tumors being inhibited to a greater extent by these procedures than the MCA-induced tumors. A collaborative study with Dr. Russell Hilf (2) was initiated for the purpose of determining the comparative biochemical characteristics of these two populations of tumors. Such studies may yield biochemical correlates to the growth and responsiveness of the tumors that might be therapeutically exploitable.

The fact that the population of tumors induced by multiple MCA doses differed in growth potentials from tumors induced by single DMBA exposures could be attributed either to the chemical differences between the two hydrocarbons or to the different doses and schedules employed. A preliminary experiment (8) suggested that the schedule (that is, repeated multiple exposures *versus* single exposure) was a more important determinant of the subsequent growth of the tumors than the total dose or the chemical structure of the hydrocarbon (that is, MCA or DMBA).

The relationship between schedule and dose of the initiating stimulus, DMBA, and the growth characteristics of the induced mammary cancers was explored further in the investigation now being reported. The schedules for the multiple doses were selected on the basis of data on the incorporation of tritiated thymidine in rat tissues following a single feeding of DMBA (9). Between 1 and 3 days there is a striking decrease in the number of labeled cells of the mammary ducts, indicating inhibition of DNA synthesis, which is recovered by 1 week. DMBA was therefore given in single doses or in 5 doses spaced every other day or weekly.

MATERIALS AND METHODS

Young female rats of the Sprague-Dawley strain were purchased from Sprague-Dawley, Inc., Madison, Wisconsin. The animals were housed in plastic shoe box cages and fed Rockland complete mouse/rat diet and water *ad libitum*. At the age of 51 days, they were randomized among the 9 experimental groups.

DMBA was dissolved in sesame oil with the aid of a mechanized stirrer. All doses of 15 mg or less were dissolved in 1 ml of sesame oil; for doses of 25 and 45 mg DMBA, 2 ml had to be employed.

The carcinogen was administered to the rats through a No. 8 French soft rubber catheter introduced into the stomach. The following doses and schedules were set up for 9 groups: (A) 15 mg given in a single dose; (B) 15 mg given in 5 doses of 3 mg every other day; (C) 15 mg given in 5 weekly doses of 3 mg; (D) 25 mg given in a single dose; (E) 25 mg given in 5 doses of 5 mg every other day; (F) 25 mg given in 5 weekly doses of 5 mg; (G) 45 mg given in a single dose; (H) 45 mg given in 5 doses of 9 mg every other day; (I) 45 mg given in 5 weekly doses of 9 mg.

All animals were weighed at the initiation of the experiment and weekly thereafter. They were examined for subcutaneous masses once a week by palpation. When one (or more) mass appeared and continued to grow until it measured 0.5 to 1.0

[1]This work was aided by Grant No. CA-07771-04 from the National Cancer Institute.

Received September 11, 1968; accepted November 7, 1968.

cm in diameter, as determined by externally applied calipers, the animal was housed singly. Measurements were made twice weekly of the original mass or masses and any additional masses that became evident during a 42-day observation period. The rats were then killed, and all subcutaneous tumors were excised, measured, and weighed. The initial tumors were prepared for histologic sections.

The experiment was terminated at 34 weeks following the first administration of DMBA. Data on the growth of tumors exclude animals that died before 21 days of observation or animals that developed tumors later than 27 weeks following the first administration of DMBA.

RESULTS

Table 1 summarizes the data on the induction of mammary tumors in the 9 experimental groups. Mortality was negligible except for the highest dose group, in which 45 mg of DMBA were given in a single dose or in every-other-day doses.

There is excellent consistency in the data. At each dose level, the carcinogenic response was relatively reduced when the DMBA was given every other day and accentuated when the carcinogen was given at weekly intervals, as compared with the response to a single dose. This relationship is evident in terms of the proportion of animals that developed tumors, the mean appearance time of the first tumors, and the mean number of tumors per rat that appeared during the subsequent 42-day period.

The differences in the mean responses of animals in the 3-dose levels are of approximately the same magnitudes as the differences encountered between the three schedules within each dose level.

Table 2 presents data on the growth of the tumors in the 9 experimental groups.

It is clear from Table 2 that there is a consistent relationship of tumor growth to the dose and schedule of the carcinogen. The mean tumor weight *per tumor* is uniformly greater in the groups that received weekly doses of DMBA than in the groups that were exposed to single or every-other-day doses. These relationships are retained if only the initially detected tumors are considered, as indicated in Table 2, or if only the largest tumor per animal is used. The relationships also persist if the weight of each tumor at discovery is estimated from the diameter values, and the weight increase of the tumor per day during the subsequent observation period is calculated.

Thus, despite the very wide variation in the weight of individual tumors and of tumors in individual animals, the *populations* of tumors segregate into some clear relationships to the initiating stimulus, especially to the schedule by which the hydrocarbon is given. The tumors induced by weekly doses of DMBA are consistently and significantly larger than the tumors appearing following single doses or doses given at two-day intervals (group CFI *vs* ADG or BEH, $P < 0.01$). The differences between single-dose and every-other-day doses are not statistically significant, but the trend is for the smaller values among the latter.

The total dose levels within the three-fold range employed in these experiments are not related to the mean tumor growth. The tumors induced with 15 mg are not significantly different from those induced with 45 mg of DMBA, if the schedules of the administrations are held constant.

DISCUSSION

Repeated, multiple exposures to chemical carcinogens or to radiation are usually more effective in eliciting neoplastic reactions than single exposures. Huggins *et al.* (3, 4) have shown this to be true for mammary cancer production in rats following multiple administrations of DMBA. Our investigation confirms this effect and emphasizes the importance of the time factors in the schedule of the exposures. Thus, 5 administrations of DMBA at 2-day intervals were somewhat less effective than single doses, but the neoplastic reaction was enhanced when the same doses were given at weekly intervals.

It is tempting to relate the findings to the depressed synthesis of DNA, as indicated by tritiated thymidine labeling, which occurs for several days following DMBA feeding, with recovery by 1 week. Further investigations are obviously necessary to substantiate this relationship and to explain the enhancement with the weekly schedule of exposures.

Table 1 — Article 9 — ms 202

Table 1

Group	Dose and schedule	Rats: Initial no.	Rats: No. at 4 weeks	Rats with mammary cancer: No.	Rats with mammary cancer: %	First tumor, mean appearance time (weeks) ± S.E.	Tumors per rat, 42 days after first tumor (mean no. ± S.E.)
15 mg total dose							
A	Single dose	20	19	13	68.4	16.3 ± 1.8	2.3 ± 0.78
B	3 mg q 2 days × 5	20	20	9	45.0	20.8 ± 2.5	1.4 ± 0.40
C	3 mg q weekly × 5	20	20	14	70.0	13.3 ± 1.3	2.4 ± 0.41
25 mg total dose							
D	Single dose	20	18	14	77.8	13.7 ± 1.1	2.5 ± 0.43
E	5 mg q 2 days × 5	20	20	14	70.0	14.5 ± 2.0	2.1 ± 0.35
F	5 mg q weekly × 5	20	20	20	100.0	10.1 ± 0.4	4.3 ± 0.50
45 mg total dose							
G	Single dose	30	17	17	100.0	10.5 ± 0.8	4.1 ± 0.55
H	9 mg q 2 days × 5	30	14	8	57.1	12.5 ± 3.2	3.3 ± 0.62
I	9 mg q weekly × 5	30	29	29	100.0	8.4 ± 0.3	8.3 ± 0.30

Induction of mammary cancers in Sprague-Dawley female rats following 3 dose levels and schedules of 7,12-dimethylbenz(a)anthracene.

Table 2

Group	Dose and schedule	Rats (no.)	All tumors (no.)	Mean weight per tumor at 42 days[a] (gm ± S.E.)	First tumors[b] (no.)	Mean weight per tumor at 42 days (gm ± S.E.)
15 mg total dose						
A	Single dose	8	18	1.66 ± 0.58	11	2.63 ± 0.83
B	3 mg q̄ 2 days × 5	6	11	0.82 ± 0.45	7	1.17 ± 0.69
C	3 mg q̄ weekly × 5	12		3.86 ± 1.26	13	3.37 ± 1.37
25 mg total dose						
D	Single dose	12	29	1.24 ± 0.68	15	1.79 ± 1.32
E	5 mg q̄ 2 days × 5	11	22	1.00 ± 0.47	12	1.06 ± 0.65
F	5 mg q̄ weekly × 5	19	69	2.55 ± 0.58	26	4.58 ± 1.29
45 mg total dose						
G	Single dose	15	61	0.98 ± 0.27	19	1.59 ± 0.78
H	9 mg q̄ 2 days × 5	6	20	1.13 ± 0.64	6	4.68 ± 2.00
I	9 mg q̄ weekly × 5	27	215	2.53 ± 0.31	29	5.94 ± 1.06

Growth of mammary cancers in Sprague-Dawley female rats following 3 dose levels and schedules of 7,12-dimethylbenz-(a)anthracene.

[a] Following detection of first tumor on animal.

[b] Tumors initially detected on animal.

The main point of this report is the effect of dose and schedule, especially the latter, upon the subsequent *growth* of the induced mammary cancers.

It is usually assumed that the growth of neoplasms, as well as other biologic and biochemical characteristics, are independent of the initiating stimulus, at least once such neoplasms become fully established. This assumption appears valid for pulmonary tumors in mice (6) and for subcutaneous sarcomas induced by polycyclic hydrocarbons (7). That this assumption cannot be generalized, however, is well demonstrated by the induction of a wide variety of hepatomas by different doses and schedules of hepatocarcinogens (5). It appears that the assumption also does not hold for mammary cancers induced in rats exposed to MCA or to different schedules of DMBA.

Despite the great variability in the growth of individual tumors in Sprague-Dawley rats that receive DMBA (10), this investigation demonstrates that the populations of tumors so produced do segregate into slower and more rapidly growing neoplasms and that this segregation is related to the schedule of carcinogenic exposures.

As usual, the observations raise more questions than they answer, and they require further investigations in order to clarify some of the issues. Is the induction of more rapidly growing mammary cancers by multiple weekly doses due to the greater "stimulation" of the exposed cells at a particularly "susceptible" period of the cell cycle, the inclusion of additional cell types into carcinogenic transformation, or to some effect upon the host? Transplantation studies would be of obvious relevance, and they may allow the establishment of more stable tumor lines of different growth rates and biochemical characteristics.

REFERENCES

1. Gruenstein, M., Meranze, D. R., Thatcher, D., and Shimkin, M. B. Carcinogenic Effects of Intragastric 3-Methylcholanthrene and 7,12-Dimethylbenz(a)anthracene in Wistar and Sprague-Dawley Rats. J. Natl. Cancer Inst., *36:* 483–502, 1966.
2. Hilf, R. Biochemical Studies on Hormone-responsive Mammary Tumors. Cancer Res., *28:* 1888–1890, 1968.
3. Huggins, C. B., and Grand, L. Neoplasms Evoked in Male Sprague-Dawley Rat by Pulse Doses of 7,12-Dimethylbenz(a)anthracene. Cancer Res., *26:* 2255–2258, 1968.
4. Huggins, C. B., Grand, L., and Fukunishi, R. Aromatic Influences on the Yields of Mammary Cancers Following Administration of 7,12-Dimethylbenz(a)anthracene. Proc. Natl. Acad. Sci. U. S., *51:* 737–742, 1964.
5. Morris, H. P. Studies on the Development, Biochemistry, and Biology of Experimental Hepatomas. Advan. Cancer Res., *9:* 228–302, 1965.
6. Shimkin, M. B. Pulmonary Tumors in Experimental Animals. Advan. Cancer Res., *3:* 223–267, 1955.
7. Shimkin, M. B., and Bryan, W. R. Morphology and Growth of Subcutaneous Tumors Induced by Carcinogenic Hydrocarbons in Strain C3H Male Mice. J. Natl. Cancer Inst., *4:* 25–35, 1943.
8. Shimkin, M. B., Gropper, L., Thatcher, D., and Gruenstein, M. Hormonal Treatment of Mammary Tumors in Rats Induced by 3-Methylcholanthrene and 7,12-Dimethylbenz(a)anthracene. Cancer Res., *27* (Part 2): 1284–1288, 1967.
9. Shimkin, M. B., Gruenstein, M., Thatcher, D., and Baserga, R. Tritiated Thymidine Labeling of Cells in Rats Following Exposure to 7,12-Dimethylbenz(a)anthracene. Cancer Res., *27:* 1494–1495, 1967.
10. Teller, M. N., Stock, C. C., Stohr, G., Merker, P. C., Kaufman, R. J., Escher, G. C., and Bowie, M. Biologic Characteristics and Chemotherapy of 7,12-Dimethylbenz(a)anthracene-induced Tumors in Rats. Cancer Res., *26:* 245–252, 1966.