Development of Hepatomas in Inbred Albino Mice Following Treatment with 20-Methylcholanthrene*

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Hepatomas have been induced in the liver of mice following administration of 4-dimethylaminoazobenzene, o-aminooazotoluene, 2-acetylaminofluorene, and other chemical agents (7, 15), but not with the carcinogenic hydrocarbons 20-methylcholanthrene (MCA), 3,4-benzpyrene, or related compounds. Thus, no hepatomas were obtained following the insertion of these compounds directly into the liver of mice (6, 8, 18, 21, 27) or rats (3, 9, 16, 18, 27), although on two occasions an equivocal effect was reported (8, 21). Strong (34) injected MCA subcutaneously into strain NHO mice and observed nineteen out of 1367 animals with hepatomas. A variety of tumors was obtained when carcinogenic hydrocarbons, e.g., MCA and 3,4-benzpyrene, were administered orally to young adult mice (12–14, 19, 20, 23). However, in none of these studies was mention made of an enhancing effect in hepatocarcinogenesis. In the present investigation, MCA was administered orally to suckling mice as part of a study in gastric carcinogenesis. At autopsy, many of the mice were observed with multiple hepatomas and other tumors. This finding was confirmed in a second experiment in which the age of the mouse at time of treatment with carcinogen also was investigated. Details of the initial and follow-up experiments are given in the present communication.

MATERIALS AND METHODS

Seven to 23-day and 3-month-old inbred male and female albino mice1 related to strain A/He were fed a methocel-Aerosol OT suspension containing 3 per cent 20-methylcholanthrene (MCA) adjusted to pH 9 with 0.1 N NaOH. The suckling mice received 0.05 ml. and the young adults 0.1 ml. of the same suspension by stomach tube 3 times weekly for 10–13 times. Other sucklings, and young adult control mice, received a suspension containing no MCA. Untreated male and female mice were employed as additional controls. The sucklings were weaned at 4–5 weeks of age, segregated according to sex, and observed as long as 390 days following the start of treatment. The young adults and the untreated controls were observed as long as 393 days and 550 days, respectively.

All mice used were born and reared in this laboratory, housed in metal cages in air-conditioned quarters, with access to an unlimited supply of Purina Laboratory Chow and tap water. At autopsy, all animals were examined initially for grossly visible tumors. All discrete tumors and those masses suspected of being tumors were excised and fixed in Tellyesniczky’s fluid. Tissues were prepared routinely and stained with hematoxylin and eosin. Tumor diagnoses were made following microscopic examination.

This investigation consisted of two separate experiments run consecutively. The first, Experiment 1, was in the nature of a preliminary study, in which suspensions with or without MCA were fed to groups of 9–23-day-old suckling mice for an average of 12 times. In the next, Experiment 2, 7–10-day and 3-month-old mice were treated as in Experiment 1 above, but each mouse now received a total of ten doses of the same suspensions.2

RESULTS AND DISCUSSION

Experiment 1.—The results are summarized in Table 1. Tumors of different types, including hepatomas, pulmonary adenomas, papillomas and squamous-cell carcinomas of the forestomach, and skin papillomas, were observed among the mice treated with MCA (Groups 1a, 1b). No tumors were observed in the gastric glandular mucosa. The two

1 Dosage of MCA was calculated on a body-weight basis: sucklings (av. age, 8 days)—av. wt., 6 gm.; 3-month-old mice—av. wt., 23 gm.
skin papillomas each measured less than 2 mm. in diameter and regressed within 2 months. It is probable that these resulted from accidental spread of MCA to the skin during the time of administration of carcinogen. Few tumors other than pulmonary adenomas were observed among the suckling mice exposed to suspension alone (Groups 2a, 2b), or among the untreated controls (Groups 3a, 3b). These included one hepatoma in Group 2a and three hepatomas in Group 3a. The increased incidence of lung and forestomach tumors among the mice exposed to MCA (Groups 1a, 1b) was anticipated, since a similar increase had been reported previously among mice treated with this or related carcinogens (7). However, an increased incidence of hepatomas in the same mice was not anticipated in view of the consistent failure of others (6, 8, 18, 21, 27) to induce tumors of this type in mice with MCA or related hydrocarbons. In the present investigation, the incidence of hepatomas varied from 0 to 8 per cent among the mice not exposed to MCA (Groups 3a-3b). Following treatment with MCA, the incidence rose to 47 and 17 per cent among the male and female sucklings, respectively (Groups 1a, 1b). These data indicate an enhancement in hepatoma development as a result of exposure to MCA.

More liver tumors were observed among the male sucklings (47 per cent) than among the females (17 per cent), evidence that the males were more susceptible than the females to the induction of hepatomas with MCA. In an investigation on the occurrence of spontaneous hepatomas in strain C3H mice, Andervont (1) observed a higher incidence of hepatomas among the males than among the females. Also, Leathem (11) exposed mice to 2-acetylaminofluorene and noted many hepatomas among the males but none in the females. On the other hand, female mice appear to be more susceptible than males to the hepatocarcinogenic action of o-aminooazotoluene (3, 4, 22) and n-n-dimethyl-p-aminooazaobenzene (10). Although a sex difference in the incidence of induced hepatomas was observed in the present study (Groups 1a, 1b), no such difference was noted among the same mice in regard to pulmonary adenomas (males, 95 per cent; females, 96 per cent) or forestomach papillomas (males, 42 per cent; females, 33 per cent).

The hepatomas observed in Groups 1a and 1b (Fig. 1) in most instances were multiple, some of the mice bearing as many as ten or more discrete tumors. For ease in diagnosis, no hepatoma was included in this study which did not measure at least 2 mm. in diameter. Some were observed with diameters as large as 20 mm. Grossly and microscopically, these tumors were like those described in detail by Andervont and Dunn (1) and were indistinguishable from spontaneous hepatomas, including those observed in Groups 3a and 3b. The tumors were conspicuous round masses, generally with a broad base but occasionally pedunculated. They varied in color from a brownish yellow to a pale red and contrasted sharply with the dark red background of the surrounding, apparently normal, liver tissue.

In those investigations in which no apparent increase was obtained in the incidence of hepatomas following administration of MCA or related carcinogens (6, 8, 18, 21, 27), the mice were invariably older at the start of treatment than the sucklings employed in the present study. Since it appeared from this that the increased yield of hepatomas in

TABLE 1

TUMORS INDUCED IN SUCKLING ALBINO MICE WITH 20-METHYLCHOLANTRENE (MCA)

<table>
<thead>
<tr>
<th>Group (no.)</th>
<th>Total mice and sex*</th>
<th>Treatment</th>
<th>Av. age at start</th>
<th>Av. observation (days)</th>
<th>No. mice with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (no.)</td>
<td>Agent</td>
<td>Av. applications</td>
<td>Period (days)</td>
<td>Hep.</td>
</tr>
<tr>
<td>1a</td>
<td>18M 24F</td>
<td>Susp. † MCA</td>
<td>12 11</td>
<td>14 12</td>
<td>337 11</td>
</tr>
<tr>
<td>1b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>320 11</td>
</tr>
<tr>
<td>2a</td>
<td>21M 23F</td>
<td>Susp.</td>
<td>13 13</td>
<td>13 13</td>
<td>340 11</td>
</tr>
<tr>
<td>2b</td>
<td></td>
<td>No MCA</td>
<td>12 12</td>
<td>12 12</td>
<td>335 11</td>
</tr>
<tr>
<td>3a</td>
<td>30M 36F</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>475 11</td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>480 11</td>
</tr>
</tbody>
</table>

* Mice alive at time first hepatoma was observed.
† Abbreviations: hep. = hepatoma; ad. = adenoma; pap. /ca. = papilloma /squamous-cell carcinoma; susp. = methcel-Aerosol OT suspension.

In those investigations in which no apparent increase was obtained in the incidence of hepatomas following administration of MCA or related carcinogens (6, 8, 18, 21, 27), the mice were invariably older at the start of treatment than the sucklings employed in the present study. Since it appeared from this that the increased yield of hepatomas in
Experiment 1 might have been due to the use of especially young animals, the experiment was repeated, but with the addition of 3-month-old mice. An indication that the age of the mouse may have influenced liver tumorigenesis was suggested by the finding in Group 1a that the males with hepatomas averaged 10 days, whereas those with no hepatomas averaged 17 days when first treated with MCA. A similar comparison was not made for the females in Group 1b, inasmuch as these mice varied but little in age at the start of treatment. On the assumption that hepatoma development would be increased with the use of sucklings younger than the 9-23-day-old animals treated in Experiment 1, 7-10-day-old sucklings (av., 8 days) were employed in the follow-up experiment (Experiment 2).

Experiment 2.—The results are summarized in Table 2.

As in the previous experiment, many of the suckling mice exposed to MCA in Experiment 2 developed tumors of the liver, lungs, forestomach, and skin (Groups 4a, 4b, Table 2). Only tumors of the liver and lungs, however, were observed among the sucklings not exposed to MCA (Groups 5a, 5b). As in Experiment 1, here, too, considerably more of the MCA-treated sucklings developed hepatomas (Groups 4a, 4b) than those treated with suspension alone (Groups 5a, 5b) or which received no treatment (Groups 3a, 3b). Thus, it may be concluded that exposure of suckling mice to MCA led to an increased incidence of hepatomas.

The mice in both Experiments 1 and 2 were treated with the same suspension of MCA. However, it will be noted that the incidence of hepatomas was higher for both the males and females in the second experiment as compared with the first. The increase in the case of the females was not significant (23 vs. 17 per cent), whereas in the males the incidence of hepatomas increased from

**TABLE 2**

INFLUENCE OF AGE ON TUMOR FORMATION IN ALBINO MICE EXPOSED TO 20-METHYLCHOLANTHRENE (MCA)

(Each mouse received a total of ten applications of suspension)

<table>
<thead>
<tr>
<th>GROUP (NO.)</th>
<th>TOTAL MICE AND SEX* (NO.)</th>
<th>TREATMENT AGENT</th>
<th>AVG. AGE AT START (DAYS)</th>
<th>AVG. OBSERVATION PERIOD (DAYS)</th>
<th>NO. MICc WITH TUMORS†</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>23M</td>
<td>Susp.†</td>
<td>8</td>
<td>311</td>
<td>17/0</td>
</tr>
<tr>
<td>4b</td>
<td>30F</td>
<td>MCA</td>
<td>8</td>
<td>392</td>
<td>0/0</td>
</tr>
<tr>
<td>5a</td>
<td>29M</td>
<td>Susp., no MCA</td>
<td>8</td>
<td>308</td>
<td>0/0</td>
</tr>
<tr>
<td>5b</td>
<td>27F</td>
<td>MCA</td>
<td>8</td>
<td>316</td>
<td>0/0</td>
</tr>
<tr>
<td>6a</td>
<td>26M</td>
<td>Susp.†</td>
<td>90</td>
<td>330</td>
<td>0/0</td>
</tr>
<tr>
<td>6b</td>
<td>29F</td>
<td>MCA</td>
<td>90</td>
<td>366</td>
<td>0/0</td>
</tr>
<tr>
<td>7a</td>
<td>27M</td>
<td>Susp., no MCA</td>
<td>90</td>
<td>340</td>
<td>0/0</td>
</tr>
<tr>
<td>7b</td>
<td>29F</td>
<td>MCA</td>
<td>90</td>
<td>344</td>
<td>0/0</td>
</tr>
<tr>
<td>3a†</td>
<td>39M</td>
<td>None</td>
<td>--</td>
<td>475</td>
<td>0/0</td>
</tr>
<tr>
<td>3b†</td>
<td>30F</td>
<td>MCA</td>
<td>480</td>
<td>316</td>
<td>0/0</td>
</tr>
</tbody>
</table>

* Mice alive at time first hepatoma was observed.
† Abbreviations: hep. = hepatoma; ad. = adenoma; pap. = papilloma; cu. = squamous-cell carcinoma; susp. = methocel-Aerosol OT suspension.
‡ Data from Table 1.

47 to 84 per cent (Group 1a vs. Group 4a, Tables 1, 2). Since the suckling males in Group 4a averaged 8 days while those in Group 1a averaged 14 days at the start of treatment, this suggests a direct relationship between age of suckling mouse and susceptibility to hepatoma induction with MCA.

The sex difference in incidence of hepatomas observed in Experiment 1 was again demonstrated in Experiment 2, this time being more marked than before (males, 84 per cent; females, 23 per cent, Groups 4a, 4b). Further evidence of an increased susceptibility to hepatoma development among the male sucklings was the observation that nineteen out of 21 males but only three out of seven females bore multiple hepatomas. Also, the average tumor diameter for these hepatomas
measured 5 mm. in the males but only 3 mm. for the females. In contrast to these findings on hepatoma development, no sex differences were observed either in incidence of pulmonary adenomas or in forestomach papillomas among the same mice (Groups 4a, 4b).

Several of the suckling mice fed MCA subsequently bore skin papillomas (Groups 4a, 4b) the result, perhaps, of an accidental spread of carcinogen to the outer skin. In addition to these tumors, three females in Group 4b were observed with solitary sebaceous adenomas in the nape of the neck region (Fig. 2) which ranged in size from 2 to 5 mm. The literature contains few reports concerning the induction of sebaceous gland tumors with the carcinogenic hydrocarbons despite repeated application of these agents to the skin of mice (7). Twort and Twort (26) obtained an occasional sebaceous adenoma among mice exposed to oleic acid and tar. Sunderland et al. (25) applied different fractions from petroleum products to the skin of 2700 mice and obtained one skin tumor which the authors considered to be similar to the sebaceous adenomas described by Twort and Twort (26). More recently, Rous (17) observed the development of multiple sebaceous adenomas in hairless mice treated with wood preservative or MCA. The sebaceous adenomas in the present investigation were visible grossly as smooth, rounded elevations in the skin. The tumors were not associated with papillomas or squamous cell carcinomas, and the overlying epithelium, at most, showed a moderate degree of hyperplasia. Mitotic figures were observed throughout different areas of the adenomas. Whether or not these tumors are ascribable to the treatment with MCA or were of spontaneous origin is not certain. However, in view of the rarity of spontaneous tumors of this type in the strain of mouse employed, it is probable that the adenomas were induced.

Although treatment with MCA led to a significant increase in incidence of hepatomas among the sucklings in both Experiments 1 and 2, no such increase was apparent among the 3-month-old mice treated with the same carcinogen (Groups 6a, 6b). Essentially the same low yield of hepatomas was observed for the latter groups as for the 3-month-old mice exposed to suspension alone (Groups 7a, 7b), or for the untreated controls (Groups 3a, 3b). A similar lack of hepatomas had been reported by other investigators when mice comparable in age to the 3-month-old animals in Groups 6a and 6b were treated with MCA or a related carcinogen (6, 8, 18, 21, 27). These findings point to a definite correlation between age of the mouse at the time of treatment with MCA and susceptibility to hepatoma induction. It remains to be determined what the conditions are which favor hepatoma development in the suckling mouse.

**SUMMARY**

Administration of a suspension of 20-methylcholanthrene (MCA) to suckling albino mice resulted in an increased incidence of hepatomas, no increase being observed among sucklings treated with suspension alone (no MCA), or among untreated control mice. A sex difference in the incidence of hepatomas was observed with MCA-treated mice, the male sucklings bearing considerably more of these tumors than the females. In contrast to the enhancement in liver tumorigenesis noted in the suckling mice, a similar exposure of older, 90-day mice to MCA produced little or no alteration in the incidence of hepatomas. These results indicate a definite association between the age of the mouse at the time of exposure to the carcinogen (MCA) and susceptibility to hepatoma development.

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