3-Methylcholanthrene Concentration and Clearance in Some Adipose Tissues in Mice*

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SUMMARY

The 3-methylcholanthrene (3-MC) concentration following intraperitoneal administration was determined spectrophotofluorometrically in benzene extracts of the mammary fat pads and interscapular fat pads of female mice. The following consistent relationship in 3-MC concentration existed when varying dosages of the carcinogen were given in sesame oil: fourth mammary fat pad < third mammary fat pad < interscapular brown fat pad. As the 3-MC dosages increased lineally, the tissue concentration of 3-MC increased nonlinearly when examined 7 hours after injection. The peak concentration occurred 10 hours after the intraperitoneal injection of 30 mg. of 3-MC. Clearance of 3-MC from mammary fat pads, which had been isologously transplanted from donor mice 9 hours after injection of 30 mg. of 3-MC, was extremely slow. The tissue concentration 10 days after transplantation was still slightly higher than that of the controls. The carcinogen had a greater affinity for the mammary parenchyma than for mammary fat.

Mammary cancer in rats was induced after intragastric instillation of 3-methylcholanthrene (3-MC) by Shay and his co-workers (13). Huggins and his associates (9) and Dao and Sunderland (5) confirmed the original findings and furthermore were able to induce mammary cancer rapidly after a single feeding of 3-MC in rats. Shay et al. (14) also measured quantitatively in rats the mammary excretion of 3-MC administered orally, intravenously, and intraperitoneally. Selective concentration of 3-MC in rat breast and fat tissues after intragastric instillation of this compound was demonstrated quantitatively by Dao and his colleagues (3, 4). They observed that 3-MC concentration reached the peak 1 day after oral administration and remained in both fat and breast tissues in rats by the end of 7–10 days. Bock and Burnham (1), however, found maximum concentrations of hydrocarbons in mouse skin 2 hours after painting. The parallel between the dosages of carcinogen administered and the incidence of chemically induced tumors has been reported with cutaneous cancer in mice after skin painting (12). Bock and Dao (2) stated that the tissue concentration of 3-MC in rats after a single feeding of 10 and 30 mg. of carcinogen was proportional to the concentration of the compound in the sesame oil. Marchant has been successful, since 1958 (10), in inducing tumors in mice with ovaries grafted from donor mice which had been pretreated with skin painting of carcinogen.

The spectrophotofluorometric quantitative determination of 3-MC concentration in mammary fat pads has been studied only in rats. The work being done in our laboratory necessitated the study of this aspect of chemical carcinogenesis in the mammary glands of mice. The present experiments were designed to investigate 3-MC concentration in mammary tissues and interscapular brown fat pads of mice during the first 24 hours after intraperitoneal injection and to study the clearance of 3-MC from the mammary fat pads in an isologous host environment.

MATERIALS AND METHODS

In three of the four experiments reported in this paper, 135 female C57BL/6J mice, 6–7 weeks old, received intraperitoneally a single injection of 3-MC solution. The dosage was 1, 5, 10, 20, or 30 mg. of 3-MC in 1 ml. of sesame oil. Two sets of controls were used, one receiving no treatment, the other given injections intraperitoneally of 1 ml. of sesame oil and sacrificed 9 hours after injection. Mice receiving 1, 5, or 20 mg. of 3-MC were sacrificed 7 hours after injection, and those receiving 30 mg. of 3-MC were examined at 1, 3, 5, 6, 7, 8, 9, 12, 15, 18, and 24 hours after injection. The following tissues were removed: the interscapular brown fat pads, and the third and fourth mammary fat pads. The brown fat pads were cleared as closely as possible of the surrounding white fat. Segments of the third mammary fat pads approximately 1 cm. long were excised distally from the nipples. The fourth mammary fat pads were removed ventro-medially from the extreme dorsal end to the arbitrary border of the fifth mammary fat pad. Thirty-one of 47 fourth mammary fat pads, which were removed at 9 hours after injection, were

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transplanted into the subcutaneous areas of 31 intact female host mice of the same strain and age. Transplants were removed from hosts at 3, 6, 9, and 15 hours and 3, 7, and 10 days after the time of transplantation.

In the fourth experiment, 48 female (BALB/c × C3H/He) F1 hybrid mice, 3 weeks old, were preliminarily operated on to remove the mammary parenchyma from the right mammary gland tissues by type E operation (7). When they were 4–8 weeks of age they received, intraperitoneally, 10 mg. of 3-MC in 0.5 ml. of sesame oil and were sacrificed 7 hours after single injections. The right fourth mammary gland-free fat pads were totally removed for assay. The left fourth mammary fat pads were partially excised from areas adjacent to the nipples including the ventral inguinal lymph nodes. All specimens were weighed, minced, hydrolysed, and refluxed with alcoholic potassium hydroxide, and extracted with fluorescence-free benzene. Two to six fat pads were pooled before extraction. One or three benzene extracts for each group of determinations were examined with the Aminco-Bowman Spectrophotofluorometer using the 295 mμ excitation light and measuring the 410 mμ emission peak with slit width arrangement No. 5 having 1/8 inch of cell slit at positions of Nos. 2, 3, and 5. This procedure is a modification of the method described by Dao et al. (3).

The accuracy of the method was tested by adding 5 μg. of 3-MC in 0.05 ml. of sesame oil to the extraction system. Recovery in six determinations ranged from 4.8 to 5.2 μg. (average 5.1 μg.).

RESULTS

Detailed data are presented in four tables. The concentration of 3-MC for each group of determinations was

### Table 1

**3-MC Concentration in Mammary Fat Pads and Brown Fat Pads in Mice Receiving Single Intraperitoneal Injections of 30 mg. of 3-MC in Sesame Oil**

<table>
<thead>
<tr>
<th>HOURS AFTER INJECTION</th>
<th>FOURTH MAMMARY FAT PADS</th>
<th>INTERSCAPULAR BROWN FAT PADS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tissue assayed</td>
<td>3-MC (μg.) per gram tissue</td>
</tr>
<tr>
<td></td>
<td>No. pads</td>
<td>Total weight (mg.)</td>
</tr>
<tr>
<td>0 hour</td>
<td>16</td>
<td>1325</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>720</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>645</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>660</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>585</td>
</tr>
<tr>
<td>3 days</td>
<td>3</td>
<td>540</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>550</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>475</td>
</tr>
</tbody>
</table>

Note: Body weights of donor and host mice ranged from 16.2 to 18.0 gm. The average values are presented, followed by the individual values in parentheses.

### Table 2

**Comparison of 3-Methylcholanthrene Concentrations in Three Different Adipose Tissues in Mice 7 Hours after Intraperitoneal Injection of Various Dosages of 3-MC in Sesame Oil**

<table>
<thead>
<tr>
<th>3-MC (mg.)/ml SESAME OIL</th>
<th>TISSUE ASSAYED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/ml</td>
<td>(1) (μg/gm)</td>
</tr>
<tr>
<td>0/0</td>
<td>0.1</td>
</tr>
<tr>
<td>0/1 ml</td>
<td>0.2</td>
</tr>
<tr>
<td>5/1</td>
<td>0.3</td>
</tr>
<tr>
<td>10/1</td>
<td>0.5</td>
</tr>
<tr>
<td>20/1</td>
<td>1.0</td>
</tr>
<tr>
<td>30/1</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* In each determination six fat pads were pooled, extracted, and assayed. All determinations were done at the same time. Note: Range of weights of tissues assayed: (1) the fourth mammary gland fat pads, 635 ~ 1030 mg. (2) the third mammary gland fat pads, 200 ~ 505 mg. (3) the interscapular brown fat pads, 150 ~ 190 mg. Body weight of mice ranged from 16.0 to 20.5 gm.
always greater in the brown fat than in the white fat of mammary fat pads. The peak concentration for both tissues after injection of 30 mg of 3-MC occurred by 10 hours and leveled off after 12 hours (Table 1).

The concentration of 3-MC in the transplanted tissues ranged between 4.6 and 6.3 µg/gm tissue until 15 hours after transplantation, but by 10 days had gradually declined to 0.4 µg/gm tissue, which was still slightly higher than the control values (Table 2).

For dosages of 5, 10, or 20 mg of 3-MC the following consistent relationship in 3-MC concentrations among the three different adipose tissues was observed: fourth mammary fat pad < third mammary fat pad < brown fat pad (Table 3). No remarkable differences existed between the third mammary fat pad and brown fat pad for dosages of 1 and 5 mg. Both, however, were greater than the controls. Concentration of 3-MC in each of the three different adipose tissues increased proportionately with increasing dosage, but in a nonlinear fashion.

In the last experiment, 3-MC concentration was greater in the fourth mammary fat pads including parenchyma than in those which were gland-free (Table 4).

### DISCUSSION

The importance of the solvent in chemical carcinogenesis has been emphasized both with respect to tumor induction and concentration of the carcinogen in the target organs, with vegetable oils found to be the most efficient vehicle (2, 4, 6). Dao and his collaborators (4) failed, even with 100-mg doses of 3-MC in aqueous suspension, to produce tumors and to detect 3-MC in breast or fat tissues by the 3rd day after a single feeding in rats. The selective concentration and extended period of retention of unchanged 3-MC in adipose tissue, when administered in lipide solution, remains unexplained. The greater affinity of 3-MC for the interscapular brown fat as compared with the white fat is also unexplained and requires further study. As Huggins and his associates (9) first reported, a single feeding of carcinogen is enough to produce breast cancer in rats. Marchant (11) successfully produced tumors in ovarian grafts which were transplanted into normal mice 10 days after donor mice received a single skin painting of 1 mg of dimethylbenzanthracene in olive oil. Our experiments on chemically induced tumors in mammary glands transplanted from mice pretreated with 3-MC required such quantitative determination of tissue concentration of administered 3-MC as reported in this paper.

The patterns of clearance of 3-MC from the tissues in situ and from the transplanted tissues were different from one another. The 3-MC concentration 3 hours after transplantation was low in comparison with that in the 6- to 15-hour transplants which were, in turn, higher than in the corresponding nontransplanted tissues. Instead of being cleared from the grafts to the host's body which was free of 3-MC, the 3-MC was retained in the transplanted tissues in a relatively high concentration until 15 hours after transplantation. Subsequent clearance of 3-MC from the grafts was also slow. The reasons for the slow clearance should be further explored. However, pattern of clearance of 3-MC from the transplanted mammary tissues in mice was comparable to that from rat breast and adipose tissue in situ after a single feeding of 3-MC reported by Dao et al. (2–4). It remains to be seen whether the quantity of 3-MC retained for relatively long period of time in transplanted mouse mammary tissues as we found should be sufficient, or too much, to initiate carcinogenesis in these tissues when transplanted in host animals where no further supply of the carcinogen would be available.

Since the amount of mammary parenchyma removed with the third mammary fat pads was proportionately larger than that removed with the fourth by the technic employed in this experiment, and in view of the fact that 3-MC concentration in the third mammary fat pads was higher than in the fourth (Table 3), it was suspected that 3-MC has a greater affinity for mammary parenchyma than for mammary fat. The last experiment of this paper strongly supported this speculation. This finding, however, is opposed to the conclusion drawn by Bock and Dao (2), who presented data obtained 2 hours after intragastric instillation of 3-MC in a single rat. Their technic involved the isolation of mammary tissues from the mammary fat which they found to be difficult in nonpregnant rats, and the exact amount of fat in the mammary tissue removed was not determined. These facts were also reported with mice by Hoshino (7, 8). In view of these facts, and because some amounts of 3-MC must be lost during microdis-
section from the mammary tissues to be analyzed, we used the technic described above and obtained contradictory results. The rapid and selective development of mammary tumors after remote administration of polycyclic hydrocarbon (5, 9, 11) may be partly due to the great affinity of 3-MC for mammary parenchyma as well as for the surrounding fat pads.

In normal mammary tissues from control mice, treated with or without 1 ml. sesame oil alone, autofluorescent material was found in range from 0.1 to 0.3 µg/gm tissue in the present study. Bock and Dao (2) found fluorescence in rat mammary fat (0.03–2.5 µg/gm) and in rat perirenal fat (0.47–2.7 µg/gm). By using identical technics, the same investigators (3) found no fluorescence in benzene extracts of tissues from heart, spleen, uterus, ovaries, adrenals, and pituitaries in rats even after intragastric instillation of 3-MC, and found 12–70 µg/gm tissue in breast and fat tissues. Breast and fat tissues seem to have a greater affinity not only for carcinogenic hydrocarbon but also for autofluorescent materials.

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