Influence of a Chronic Environmental Stress on the Incidence of Methylcholanthrene-induced Tumors

Donald G. Baker

Claire Zellerbach Saroni Tumor Institute, Mount Zion Hospital and Medical Center, San Francisco, California 94115

SUMMARY

The influence of a chronic environmental stress, living in a 2° environment, on the incidence of methylcholanthrene-induced tumors in albino female Simonsen rats, a Sprague-Dawley-derived strain, was studied. The results indicated that the metabolic rate was double for rats kept at 2°, compared with those kept at 25°. Exposure to 2° for life, with no other treatment, reduced median life expectancy to 560 days compared with 686 days for rats kept at 25°. Transfer to a 2° environment after 250 days at 25° reduced the incidence of spontaneous tumors, while transfer to 2° after 250 days at 2° increased the incidence of tumors compared to that for rats always kept at 25°. Exposure to an environmental temperature of 2° immediately following a carcinogenic stimulus (3-methylcholanthrene, 2 mg s.c.) significantly reduced the incidence of tumors compared to that in rats kept at 25° but did not change tumor induction time. The reduced tumor incidence may have resulted from inhibition of the carcinogenic transformation by chronic stress. The survival time of rats with 3-methylcholanthrene-induced tumors was not significantly less in a 2° environment than it was at 25°.

INTRODUCTION

A study by Rashkis (22) examined the relationship between the development of 3-MC-induced tumors and systemic stress in male Swiss albino mice. The results showed that a moderate degree of stress produced by forced swimming would inhibit tumor growth rate and that survival time was longer than that of mice in the nonstressed or severely stressed groups. Marsh et al. (19) observed that the stress of a short-term shuttle box confinement to 6-week-old female Swiss white mice produced some inhibition in the growth rate of transplanted Ehrlich carcinosas. Dechambre and Gosse (11) also observed that a moderate degree of stress on mice would inhibit the growth rate of several transplanted tumors. The transient stress of moving the mice from one cage to another, however, increased tumor growth rate. Andervont (2) studied the effect of crowding on tumor development in female C3H mice and concluded that this stress delayed the onset of spontaneous tumors.

Kaliss and Fuller (16) found no significant effect on the incidence of 3-MC-induced cancers in mice subjected to the stress of electroshock but did note a significantly higher incidence of 3-MC-induced tumors in mice subjected to audiogenic seizures. Riley (23) observed that 80 to 100% of female C3H/He mice carrying the Bittner oncogenic virus developed mammary tumors within 8 to 18 months under standard housing conditions. When the animals were placed in a protected environment (minimum environmental stress), tumor incidence fell to less than 10% at 400 days. Baker and Jahn (3), however, found that chronic cold stress (2° for the duration of life) reduced the incidence of radiation-induced tumors in rats. These and other reports (12) indicate that an environmental stress may modify the oncological potential for a variety of carcinogenic agents.

In this study, the influence of a chronic environmental stress, a 2° room temperature, on the incidence of methylcholanthrene-induced tumors has been assessed.

MATERIALS AND METHODS

The study used female Simonsen albino rats, a Sprague-Dawley-derived strain (Simonsen Laboratories, Inc., Gilroy, Calif.). They were housed 2 per stainless steel cage and offered food (Simonsen Maintenance Diet) and water ad libitum.

The animal room was maintained at 22-25°, with a relative humidity of approximately 40% and a light-dark cycle of 12 hr. Access was limited to essential personnel. The room was separately ventilated, and germicidal UV lamps were mounted above the doors. Strict hygienic conditions were maintained.

A temperature of 2° was selected as the environmental stress because, although it constituted a severe stress, it was one to which rats were able to adapt (6, 25). The temperature in the Forma Scientific Model C10-68 environmental chamber (Forma Scientific, Inc., Marietta, Ohio), which is approximately 10 x 7 x 7 feet, was 1-3°, with a relative humidity of approximately 80%.

For tumor induction, a single 2.0-mg dose of 3-MC (Sigma Chemical Co., St. Louis, Mo.), suspended in corn oil at 37°, was injected s.c. into the right thigh of the rat.

Oxygen consumption was measured in 10 non-tumor-bearing rats at the 2° environment and in 10 rats at the 25° environment between 50 and 100 days after the 3-MC...
injection. Oxygen consumption of the tumor-bearing rats was measured when the tumors were between 1 and 2 cm in diameter. All measurements were made with a closed system animal respirometer (Med-Science Electronics, Inc., St. Louis, Mo.). Metabolic rate was expressed in ml (at 0° and 760 mm Hg) per min per 100 g body weight. The temperature in the respirometer chamber was at 2 or 25° during the measurements of oxygen consumption and corresponded to the ambient temperature of the rat under study (Table 2).

In the 1st experimental series, a total of 141 rats, ages 100 to 120 days, at the 25° environment, were given injections of 2 mg 3-MC. Immediately, 41 rats were placed at the 2° environment. After 25 days, 24 rats were transferred to the 25° environment. Other groups (Table 1A) were transferred at 50, 100, and 150 days after the 3-MC injection. One group remained at the 25° environment.

In a 2nd series of 121 rats, 100 to 120 days old, all were given injections of 2 mg 3-MC, and placed immediately at the 2° environment. After 25 days, 16 of the animals were removed to the 25° environment. At 50, 100, and 150 days after the 3-MC injection, other groups (Table 1B) were removed from the 2° to the 25° environment. One group remained at the 2° environment.

All animals were weighed and examined weekly for the presence of tumors at the site of the 3-MC injection throughout the duration of life. The times of initial appearance of a tumor, tumor incidence, and survival time were observed.

The growth rates of the tumors were calculated from caliber measurements of the 3 diameters (D) and the formula

\[ V = \frac{\pi D_1 D_2 D_3}{6} \]

From regression of tumor volume (V) against time, the time required for the tumor to increase in volume by a factor of 10 (decade time) was established. In any decade when volumes were not measured over the entire decade, i.e., 10^4 to 10^5 cu mm and 10^5 to 10^6 cu mm, the time was estimated by extrapolation. The relationship between decade time and tumor volume was used to assess the tumor growth rates (Table 4).

A 3rd experimental series involved a total of 213 rats with an average age of 10 weeks at the time of the experiment. The 1st group of 87 animals was placed in the 25° environment for 250 days. No other treatment was given. There were no deaths at this time. At 250 days, 26 rats were selected at random and moved to the 2° environment. No further treatment was given. All animals were examined weekly for life, and survival time and spontaneous tumor incidence were observed (Table 3).

The 2nd group of 126 rats was placed in the 2° environment for 250 days. No other treatment was given. Of the 83 survivors at 250 days, 26 were removed to the 25° environment and 57 remained in the 2° environment. Survival time and spontaneous tumor incidence were recorded for all animals (Table 3). Chart 1 shows that exposure to 2° resulted in an accelerated mortality rate compared to that of the rats at the 25° environment.

Data in Table 4 suggested that tumors may grow more slowly in the cold-exposed rats. For more precise information, 2- x 2- x 2-mm fragments of a gliosarcoma were implanted s.c. into the right thighs of 36 inbred male Fischer 344 rats (Simonsen Laboratories, Inc.). (This tumor was obtained from Marvin Barker, Naffziger Laboratories for Neurological Research, the University of California, San Francisco, San Francisco, Calif. It is syngeneic to the F-344 rats and has been maintained in our laboratory by serial transplant in this strain for 18 transplant generations.) One-half of the rats were placed immediately in the 2° environment, and the remainder were kept at 25°. Survival times and tumor growth rates were measured (Charts 2 and 3). It was expected that variations in growth rate due to tumor antigenicity would be minimal in this system.

The number of tumor cells necessary to induce tumors in 50% of the rats was determined. A suspension of gliosarcoma cells was prepared from small solid tumors, and the concentration of viable cells was estimated by the trypan blue exclusion method. Graded numbers of these cells were injected s.c. into the right thighs of 35 male Fischer 344 rats that had been in the 2° environment for 40 to 50 days and 36 controls rats kept in a 25° environment.

RESULTS

Table 1A summarizes the survival time and tumor incidence of rats placed in the 2° environment immediately following the injection of 3-MC, then transferred to the 25° environment either immediately or after periods up to 150 days. Values shown are means ± S.D. Immediate exposure to cold resulted in a significantly reduced incidence of tumors at the site of 3-MC injection (p < 0.05 by χ² test) compared to the incidence in animals never placed at 2° (Table 1B). The incidence of tumors in those rats placed at 2° 50 days after 3-MC injection was also significantly reduced. Transfer to the 25° environment at 25 or 100 days after 3-MC injection also resulted in a decreased tumor incidence. Although not significant at the 5% levels, the data, if combined, show that transfer to the cold up to 100 days after 3-MC injection did reduce the tumor incidence compared to that of rats always at 25°.

The time for tumor induction was not affected by exposure to the 2° environment. For those rats with tumors, survival time after the tumor was detected was not influenced by exposure to the 2° environment. Animals without tumors survived longer in the 2° environment than did animals with tumors. Placing the animals in the cold at different times after the injection of 3-MC did not change the average tumor volume at death.

Table 1B summarizes the response of rats placed at 2° immediately after 3-MC injection and then removed to the 25° environment after periods of up to 150 days. In the group remaining at 2° for duration of life, tumor incidence was 34% compared to 92% for the rats that remained at 25° for their entire life-spans. The times for tumor induction did not differ. Remaining in the 2° environment for periods of up to 150 days after the 3-MC injection did not change significantly the incidence of tumors, the time for tumor
induction, or the survival time of the tumor-bearing rats. Several differences appeared when animals placed in the 2° environment after 3-MC were compared with those placed at the 25° environment after 3-MC. Animals with no tumor lived longer at 25° than at 2°. The survival time of rats with tumors was not significantly affected by the 2° environmental temperature. Rats that were given injections of 3-MC but did not develop tumors had a shorter average survival time than did rats who received no treatment (compare Tables 3 and 4). Since tumors not at the site of the 3-MC injection developed late in life and were not usually related directly to mortality, no distinction was made in survival time between rats with and without such tumors. These spontaneous neoplasms were seen only occasionally in any of the groups given injections of 3-MC. They had the gross characteristics of mammary tumors and were never detected before 400 days of age.

The metabolic rate doubled in rats exposed to the 2° environment as compared to that of rats kept at 25° (Table 2). The presence of a tumor tended to increase the metabolic rate, although this increase was not statistically significant.

Table 3 and Chart 1 summarize the tumor incidence and survival of rats taken from, or placed in, a 2° environment after 250 days. The incidence of spontaneous tumors was reduced in the group of rats transferred from the 25° to the 2° environment after 250 days (w/250/c) when compared with the incidence of tumors in rats always kept at 25° (w/250/w). By the same comparison, transfer from the 2° to the 25° environment after 250 days resulted in an increased incidence of tumors. The increased tumor incidence was significant (q<sup>2</sup>; p < 0.05), although the life expectancy of the rats in the 2 groups (c/250/w and w/250/w) was not different. The c/250/c group had the same tumor incidence as the rats in the w/250/w group. All tumors developing in this experimental group had the gross morphology and anatomical location of mammary tumors. Several rats had more than 1 such tumor.

The growth rate of the transplanted gliosarcoma tumors was less at the 2° than it was at the 25° environment, once the volume exceeded approximately 1 cu cm (Chart 2). The survival time of the tumor-bearing rats was less at 2° than at 25°, owing to an earlier onset of mortality (Chart 3). The mortality rates were the same.

**DISCUSSION**

Rats placed in the 25° environment immediately after the 3-MC injection, or at 2° for up to 150 days and then moved into the 25° environment, had a tumor incidence of 80 to

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**Table 1**

<table>
<thead>
<tr>
<th>Environment in (days)</th>
<th>No. of rats</th>
<th>Survival after 3-MC (days)</th>
<th>Age at death (days)</th>
<th>Tumor to death (days)</th>
<th>% Tumor Incidence</th>
<th>Tumor volume (cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No tumor</td>
<td>With tumor</td>
<td>No tumor</td>
<td>With tumor</td>
<td>3-MC to tumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>41</td>
<td>482 ± 160&lt;sup&gt;a&lt;/sup&gt;</td>
<td>317 ± 56</td>
<td>564 ± 148</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>24</td>
<td>551 ± 168</td>
<td>379 ± 111</td>
<td>628 ± 153</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>22</td>
<td>471 ± 173</td>
<td>353 ± 113</td>
<td>558 ± 163</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>27</td>
<td>530 ± 208</td>
<td>289 ± 114</td>
<td>606 ± 196</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150</td>
<td>27</td>
<td>613 ± 191</td>
<td>366 ± 157</td>
<td>677 ± 186</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.D.

**Table 2**

<table>
<thead>
<tr>
<th>Environmental temperature</th>
<th>Mean metabolic rate (μ mol O&lt;sub&gt;2&lt;/sub&gt;/min/100 g body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats with no tumors</td>
<td>MR&lt;sub&gt;25°&lt;/sub&gt; &amp;&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rats with tumors</td>
<td>MR&lt;sub&gt;25°&lt;/sub&gt; &amp;&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> MR<sub>25°</sub>, metabolic rate at 25°; MR<sub>2°</sub>, metabolic rate at 2°.

<sup>b</sup> Mean ± S.D.
exist because several independent processes probably are involved (8, 10, 15, 20).

The environmental stress did not affect the time required for the tumors to reach a volume that permitted detection by palpation. Kaliss and Fuller (16), working with mice, also observed the absence of a stress effect on induction time after 3-MC injection. In their study, this could have occurred because the stress situations were not maintained for periods extending beyond the expected tumor induction times. The stress of fighting among leukemia-prone AK mice has been proposed as the reason for reduced leukemia incidence among male mice of this line (18). This stress situation existed for duration of life and, consequently, may be analogous to the chronic temperature stress.

The reduced tumor incidence for the cold-stressed rats could signify that the neoplastic transformation may never have reached completion. Carter (9) reported that the tumor-initiating effect of small doses (100 μg) of DMBA was lost if a promoting stimulus was not applied within 50 weeks of the DMBA initiation. In this study, the neoplastic transformation might have been inhibited because of the high rate of cellular metabolism, the altered chemical composition of the extracellular milieu of the stressed rats, or both (17, 25). At 2°C, the metabolic rate doubled, with concomitant changes in a variety of physiological functions (25).

In rats made hyperthyroid by exogenous thyroid feeding, the incidence of methylcholanthrene-induced tumors decreased from 92 to 36% in euthyroid rats (4). The number of injected cells required to induce tumors in 50% of the rats was no different at 2°C than it was at 25°C, an observation also consistent with the possibility that the neoplastic transformation may have been incomplete in the chronically
Tumor growth rates
Rats were removed from one environment to the other immediately after 3-MC injection. Tumor volume (cu mm) at following decade time is shown below.

<table>
<thead>
<tr>
<th>Environ. (days)</th>
<th>Tumor volume (cu mm) at following decade time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^1-10^2</td>
</tr>
<tr>
<td>A. Removal from 25° to 2°</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>61 ± 10</td>
</tr>
<tr>
<td>25</td>
<td>57 ± 10</td>
</tr>
<tr>
<td>50</td>
<td>24 ± 10</td>
</tr>
<tr>
<td>100</td>
<td>31 ± 10</td>
</tr>
<tr>
<td>150</td>
<td>49 ± 10</td>
</tr>
</tbody>
</table>

B. Removal from 2° to 25°

|                | 10^1-10^2 | 10^2-10^3 | 10^3-10^4 | 10^4-10^5 | 10^5-10^6 | 10^6-10^7 |
| 0              | 40 ± 10   | 22 ± 10   | 35 ± 10   | 25 ± 10   | 37 ± 10   | 80 ± 10   |
| 25             | 9 ± 10    | 22 ± 10   | 29 ± 10   | 30 ± 10   | 34 ± 10   | 41 ± 10   |
| 50             | 30 ± 10   | 27 ± 10   | 28 ± 10   | 24 ± 10   | 30 ± 10   | 36 ± 10   |
| 100            | 20 ± 10   | 49 ± 10   | 36 ± 10   | 23 ± 10   | 43 ± 10   | 55 ± 10   |
| 150            | 20 ± 10   | 40 ± 10   | 33 ± 10   | 24 ± 10   | 44 ± 10   | 57 ± 10   |

*Mean ± S.D.
production of mitotic inhibitors (5) or in other changes in the extracellular milieu incompatible with the proliferation of transformed cells. Removal from the cold with the decrease in the secretion of inhibitors could permit transformed cells to develop into tumors. Since the possibilities also apply to the chemically induced tumors, experiments have been initiated to explore these further.

Van den Brenk et al. (27) noted that an acute stress, adrenaline injection, or inflammatory agent increased the efficiency with which i.v.-injected Walker 256 tumor cells produced lung colonies. These acute responses are not comparable with the situation in our study wherein the animals adapted to the chronic cold stress by developing a new physiological steady state characterized by a high metabolic rate.

The data suggest that the response to carcinogens, and possibly the risk of tumor dissemination, may be affected in opposite ways in animals subjected to an acute severe stress and those living with a chronic stress to which they adapt. In those situations in which tumor induction has been inhibited, the duration of the stressful stimuli has been extremely protracted, and a high metabolic rate has been maintained for the duration of, or for a large portion of, the life of the animal. It would be of interest to know whether individuals whose life styles result in high metabolic rates are at a lower risk for developing cancer than are others who led more sedentary lives.

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REFERENCES

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