Influence of Environmental Temperature upon the Incidence and Course of Spontaneous Tumors in C3H Mice

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In a previous paper (4) we showed that tumors induced by subcutaneous injections of methylcholanthrene arise significantly earlier and grow faster in mice adapted to heat (91°F.) than in those kept at 68°F. Subcutaneous injections of emulsions of a spindle cell sarcoma also gave rise to tumors that grew rapidly in the heat, while in the 68°F. environment they grew very slowly or actually regressed. Such sarcoma emulsions injected intramuscularly, however, grew equally well in both hot-room and cold-room mice.

In 1941 Fuller, Brown, and Mills (3) reported a distinct difference of spontaneous tumor incidence in dba virgin females kept at 91°F. as compared to those living in a 68°F. environment. Not only was the number of tumors smaller in the heat, but they also appeared later in life and grew more slowly. That study was made on virgin dba females purchased at 6 weeks of age, with no litter-mate pairing between the hot and cold rooms. However, as this strain had a low tumor incidence, the number of tumors involved was not large, though the results were definitely significant.

In the present study our original breeders were obtained from Andervont's special subline of strain C3H mice (4) in January, 1941; careful inbreeding in our colony, however, has yielded only a 50 per cent incidence of mammary cancer in virgin females. Breeding of the mice was carried out in an air-conditioned room kept at 79°F., and at approximately 2 months of age the litters of young were segregated by sex and the females divided between the hot, cold, and control rooms. Purina dog chow checkers were used as the sole food throughout the study.

The mice were examined weekly for tumor appearance. Alternate tumor victims (considering each room separately) were sacrificed at the end of 1 month after the tumor was first noticed, in order that the rate of tumor growth might be estimated. All carcasses were slit open and preserved in 10 per cent formalin until time of examination and measurement of the mass. A number of the mice got a leg caught in the wire mesh floor of the cage and died or were killed; these were more numerous among the smaller hot-room mice and were not counted in the series. A minor typhoid or dysentery epidemic in one cage also caused a few early deaths, which were not included in the calculations. All possible efforts were made to base the final computations only upon proved tumor victims dying of the tumor (or killed at the end of one month) and nontumor mice not dying prematurely of accident or contagion.

All tumors were dissected out after formalin fixation of the carcass, and measurement was made of length, breadth, and thickness. These three dimensions (in millimeters) were multiplied together and the resulting numbers used as indices of tumor size. It is realized that such calculation does not represent the real tumor volume in any case, but its use seems justified for purely comparative purposes.

Table I presents the final data obtained on the series. Briefly stated, the significant findings are (a) a heightened tumor incidence in the cold-room animals, (b) a probably significant earlier age for tumor appearance in the cold, (c) a more rapid rate of tumor growth, and (d) longer duration of life after tumor appearance in animals exposed to the cold. Tumors appeared a month earlier in the cold than in the heat and grew almost twice as much in the first month after appearance. The difference in mouse age at tumor appearance (0.99 ± 0.42 months) is 2.3 times its own probable error and would occur by chance alone once in 8 times. The difference in indices of tumor size after 1 month’s growth (3198 ± 773) is 4.14 times its own probable error and would occur only once in 200 times by chance alone.

Although the tumors grew more rapidly in the cold room, they seemed to kill the hot-room mice.
more quickly. Duration of life after tumor appearance was 48.08 days in the heat and 62.37 days in the cold, the difference (14.29 ± 5.31 days) being 2.7 times its own probable error. Such a difference would occur only once in 14 times by chance alone. A similar difference in tumor size at death was found when the mice were left to die naturally, but in this case the larger tumors of the cold room had been allowed a longer time for growth before the death of the animals.

In addition to the higher incidence, earlier appearance, and faster growth of tumors in the cold room, there was also a more definite tendency for the development of multiple tumors in different breast segments of the same animal. In the hot room there were only 3 mice with 2 separate tumors each and one with 3; in the cold room, on the other hand, 14 mice developed 2 separate tumors each, 3 mice had 3, and 1 mouse showed 5 distinctly separate growths.

Table I: Mammary Cancer Incidence in C3H Virgin Females Exposed to Heat and Cold

<table>
<thead>
<tr>
<th></th>
<th>At 68°F</th>
<th>At 90-91°F, rel. hum.</th>
<th>Difference (68°F vs. 90°F)</th>
<th>Controls at 79°F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mice placed in rooms</td>
<td>125</td>
<td>122</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Included in final calculations</td>
<td>114</td>
<td>96</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Developing mammary tumors</td>
<td>82</td>
<td>48</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Percentage incidence of mammary tumors</td>
<td>72</td>
<td>50</td>
<td>45% higher in cold</td>
<td></td>
</tr>
<tr>
<td>Mice with multiple tumors</td>
<td>18</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Mean age (in months) at tumor appearance</td>
<td>13.34 ± 0.26</td>
<td>14.33 ± 0.34</td>
<td>0.99 ± 0.42</td>
<td>15.00 ± 0.62</td>
</tr>
<tr>
<td>Duration of life (in days) after tumor appearance</td>
<td>62.37 ± 4.25</td>
<td>48.08 ± 3.19</td>
<td>14.29 ± 5.31</td>
<td></td>
</tr>
<tr>
<td>Mean index of tumor size in mice killed 1 month after tumor appeared</td>
<td>7650 ± 514</td>
<td>4452 ± 577</td>
<td>3198 ± 773</td>
<td>4167 ± 685</td>
</tr>
<tr>
<td>Mean index of tumor size in mice left to die naturally</td>
<td>13710 ± 1107</td>
<td>8913 ± 1273</td>
<td>4797 ± 1687</td>
<td></td>
</tr>
<tr>
<td>Mean death age (in months) of nontumor mice</td>
<td>16.43 ± 0.55</td>
<td>16.52 ± 0.41</td>
<td>0.09 ± 0.68</td>
<td>20.75 ± 0.69</td>
</tr>
</tbody>
</table>

There was only one internal tumor found at autopsy, and that arose from the ovary of a hot-room mouse showing no mammary tumor.

Mean age at death of the nontumor mice was practically the same in the rooms kept at 68°F and 91°F, but was later in the 79°F breeding room.

DISCUSSION

In general this series of strain C3H mice yielded findings that fully verify the results previously obtained with the smaller group of dba virgin females. It seems evident that a cool environment stimulates spontaneous mammary neoplasia as compared to the effect of depressing heat upon this tendency in mice of special cancer strains. In a study still in progress, results already at hand indicate that this same difference exists in spayed females, in which case any ovarian influence upon mammary neoplasia is eliminated.

Our findings, previously published (4), also throw possible light upon climatic effects in skin cancer genesis. Actinic radiations from the tropical sun have been suspected by many as being one irritating factor concerned, but our hot-room mice show an increased susceptibility to skin cancer, either chemically induced or transplanted, in the absence of such actinic radiations. The answer would seem more likely to lie in an increased blood supply for heat-loss purposes and a more active cutaneous metabolism. In only one article, that by Bain, Rusch, and Kline (2), has the action of ultraviolet radiation been considered separately from the more general effects of radiant heat of all wave lengths. In their studies they found that filtered ultraviolet light had a definitely greater carcinogenic effect when environmental air temperatures were high (35° to 38°C) than under ordinary laboratory conditions.

With cancer of the deeper tissues the situation is reversed, for such neoplasia shows definitely stronger tendencies in our cold-room mice. Although the mammary glands are located immediately under the skin, their blood supply and hormonal and nervous control caused them to be classed with the deeper tissues of the body in a metabolic sense. They are little affected, for instance, by the cutaneous vasconstriction that takes place when the animal is chilled; rather, they are more likely to take part in the heightened metabolism that such chilling induces in the body as a whole.

Transplanted tumor cells develop poorly when injected into the subcutaneous regions of scant blood supply in cold-room mice, while very rapid growth results from their emplacement in the deeper and more active muscle tissues of the same animals. It would thus seem that one factor in cancer genesis is probably the blood supply and metabolic activity permitted under a given set of conditions. Environmental temperatures thus play their part in two ways: (a) by very definitely altering the blood supply to the skin for heat-loss purposes, and (b) by their slower effects upon metabolic activity in the deeper
tissues—stimulation in cool surroundings and depression in the heat. It should be borne in mind that the hot-room temperatures used here were just below the level required to produce fever in the animals. The development of thermic fever would alter the picture in many ways.

SUMMARY

1. Spontaneous mammary cancer in virgin C3H female mice shows the same increased incidence in cool environments that was previously found in virgin dba females.

2. These tumors appear 1 month earlier in life and grow faster at 68°F than at 91°F, although they kill the hot-room victims more quickly.

3. Multiple tumors in this C3H mouse series were 4 times more frequent among the cold-room mice than among those kept in the heat.

REFERENCES


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