The Effect of Ultraviolet Radiation on the Production of Spontaneous Mammary Tumors in C3H Mice*

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Previous experiments carried out in a set of specially designed animal rooms with temperature and light control revealed a marked difference in tumor incidence between mice kept entirely in the dark and those exposed to artificial daylight, when the tumors were induced by painting with solutions of carcinogenic hydrocarbons. In both Swiss albino and C57 black mice there was a delay in the appearance of epidermoid carcinomas of the skin and a diminution in the number of animals which developed tumor in the groups exposed to light (7, 8). Light enhanced the leukemogenic properties of 3,4-benzpyrene and 2-o-methylcholanthrene in mice of the DBA strain (9). It was decided to study mice subject to spontaneous mammary tumors under the same environmental conditions used previously and also to expose part of the group to ultraviolet radiation below the level necessary to produce skin cancer (5). It seemed possible that ultraviolet radiation, which is itself a carcinogenic agent when intense enough, might activate the tumor agent and increase the rate of tumor incidence. Applerly and Cary (2, 3), using strain A mice in one experiment and DBA mice (subline I) in another, found that exposure to ultraviolet radiation had a deterrent effect upon the incidence of spontaneous mammary cancer; but these experiments did not seem entirely conclusive.

EXPERIMENTAL PROCEDURE

The animal rooms were fully described in a previous publication (7). The banks of fluorescent lamps gave an even illumination of 300 foot-candles for 12 hours a day. This illumination was originally tested as giving a total energy of the order of 0.1 gm/cal/cm²/min. The temperature was automatically controlled, and the rooms were kept at 22° C.

The mice used were individually identified virgin females of strain C3H, Andervont subline. Shiman (10) reported a mammary cancer incidence in such subjects of 97.4 per cent at a mean age of 10.4 months in 1945, while Andervont (1) in 1949 found carcinomas of the breast in 92.9 per cent at an average age of 11.3 months. All mice were kept in individual wire mesh cages and fed Purina Dog Chow and water ad libitum. Littermates were distributed evenly among the experimental groups, which were also carefully balanced for weight and age. Ultraviolet radiation including the carcinogenic wave lengths (5) was given by a quartz mercury arc (General Electric Uviarc) at 21 inches. At this distance an exposure of 2.5 minutes produced a minimal erythema on human skin. When radiated, the mice were placed in small individual wide wire mesh cages in which a wire mesh trap kept the animals at an approximately constant distance from the light source. They were able to move about, however, during the exposure.

All animals were weighed once a month. There was no significant difference in the weights in different groups. The mice from the light room ate less and were less active.

The first definite appearance of the tumors was noted. When a tumor had grown to a moderate size the animal was killed, and the tumor was removed, sectioned, and examined microscopically. As the mammary tissue is wide spread, tumors appeared in many different locations—axilla, groin, back of neck, jaw, base of tail, etc. The histologic appearance of the carcinomas conformed to the types described by Dunn (6), which occur regularly in mice of the C3H strain.

RESULTS

Three separate experiments were carried out. Experiment 1.—C3H mice of the Andervont subline, descendants of those obtained originally from Dr. H. B. Andervont and bred in Rochester, were used. These mice were divided into three groups. Group 1 was kept entirely in the dark. Group 2 was also kept entirely in the dark but was exposed to ultraviolet radiation, 2 erythema doses (E.D.) 3 times a week, which was increased to 5 E.D. 3 times a week, with a total dose ranging from 80 E.D. to 200 E.D. Group 3 was kept in the light room where the animals were exposed for 18 hours a day to visible light (300 foot-candles) which contained no ultraviolet rays.

There was a marked difference in the tumor incidence in the three groups (Table 1). At the age of 300 days, 60 per cent of the ultraviolet (U.V.) group had developed tumors, 35 per cent of the dark group, and none of the light group. The average age at which neoplasms appeared was 299 days in the U.V. group, 340 days in the dark group, and 394 days in the light group. Experiment 2.—The second experiment exactly repeated Experiment 1, except that the C3H mice of the Andervont subline were obtained from the Rockefeller Memorial Laboratory. The mice were all between 4 and 6 weeks old when received, and radiation was started at once in the ultraviolet group. The radiation was begun with one erythema dose 3 times a week and was increased to 5 E.D. 3 times a week. The total dose in all cases was 125 E.D. A few mice became ill from the radiation, which was perhaps begun too early, but 23 lived to tumor age (Table 2).

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The experiment was terminated when 100 per cent of the U.V. group had developed tumors and the surviving mice in the other two groups were 585 days old. The average tumor age did not show a significant difference, probably because the experiment was not continued until all animals developed tumors. The average age at onset of cancer was 347 days for the U.V. group, 851 days for the dark group, and 377 days for the light group.

Up to the age of 300 days there was no difference in tumor incidence in the three groups, but after that the rate of incidence in the U.V. group increased compared to the other two groups (Table 2).

As in Experiment 1, the mice given ultraviolet radiation developed tumors earlier. The difference between the dark and light groups which seemed significant in Experiment 1 was not apparent in Experiment 2.

Experiment 3.—In this experiment, CSH mice of the Ander- vont strain bred in Rochester were used, but the groups were set up differently. Group 1 was kept in the dark, and all other groups were exposed to visible light 12 hours a day. Group 2 had light plus exposure to ultraviolet radiation given 3 times a week beginning with 2 E.D. a day and increasing to 4 E.D. a day with the total dose ranging from 100 to 250 E.D. Group 3 was exposed to visible light only.

As the ultraviolet-irradiated animals were living in the light 12 hours a day instead of in the dark as in Experiment 1 and Experiment 2, the results of the three experiments may not be entirely comparable. The average age at the onset of the tumor was 540 days for the light plus U.V. group, 408 days for the light group, and 423 days for the dark group (Table 3).

A comparison of the results for the animals from all three experiments, at the age of 565 days, is given in Table 4. It is evident that the mice used in Experiment 3 differed from those in Experiments 1 and 2, as the tumors developed more slowly.

### DISCUSSION

No consistent difference could be demonstrated at any given time in the relative frequency of mammary tumors between groups of mice housed in a light or dark environment in the absence of ultraviolet rays. Application of the chi-square test to the pooled experience in light and dark failed to disclose any statistically significant differences in relative frequency of carcinoma of the breast at any interval between 300 and 600 days.

Mice that received ultraviolet irradiation developed their mammary cancers earlier than did the other groups in each experiment. The data obtained from all groups exposed to ultraviolet rays were compared to the total experience obtained in visible light and in the dark and to the two latter groups together, since no valid difference in the relative frequency of mammary tumors was demonstrated between them. Each of the three procedures gave the same result when subjected to χ² tests. The difference between the ultraviolet-irradiated and those with no exposure to ultraviolet was significant at a level of less than 0.05 and usually far less than 0.01 at each 50-day interval from 300 through 500 days. The hypothesis that mammary tumors appeared earlier among mice exposed to ultraviolet rays than those with no such exposure was tested further by a different application of the χ² method referred to by Snedecor (11) as "Test of independence in an R × C table." The technic may be applied when one or both of two attributes are recorded in more than two classes. This statistical treatment of the data indicated that, if the three groups of mice came from the same population, the probability of obtaining re-
suits like those found in our experiments would be less than 0.05.

The earlier appearance of the mammary carcinomas among mice exposed to ultraviolet rays may have been due to a difference in the rate of tumor production or to a difference in latent interval, the rate of production being relatively constant. Blanding et al. (4) described a close correlation between tumor development and time expressed logarithmically among mice painted with some carcinogenic substances obtained from petroleum refineries. Similar treatment of the spontaneous tumor data from our experiments indicates a relationship of the order:

\[ y = a + b \log x \]

in which \( y \) is the relative frequency of tumor production expressed as per cent and \( x \) the time of appearance of the mammary tumors expressed in days. Correlation coefficients of this relationship varied from 0.90 to 0.97. The slopes of the curves \( b \) for each group varied from 0.15 to 0.29. The differences were not statistically significant. The mean latent periods of tumor production and the standard errors of the means were:

- Ultraviolet group: 299 ± 10 days
- Light group: 391 ± 11 days
- Dark group: 369 ± 12 days

Differences among these means were not statistically significant.

**SUMMARY**

We are left, therefore, with good evidence that C3H mice exposed to ultraviolet rays developed spontaneous mammary cancers more rapidly than did those reared in either light or dark environments in which they received no ultraviolet irradiation. The differences are not due solely to a more rapid rate of tumor production or to a decreased latent interval, but to a combination of both.

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