Effects of Selenium and of Arsenic on the Genesis of Spontaneous Mammary Tumors in Inbred C3H Mice*

GERHARD N. SCHRAUZER, Ph.D. AND DEBRA ISHMAEL, B.A.

Department of Chemistry, University of California, San Diego, Revelle College, La Jolla, CA 92037

ABSTRACT

Exposure of female virgin C3H/St mice to two ppm of selenite in the drinking water for 15 months lowered the incidence of spontaneous mammary tumors to 10 percent relative to the observed incidence of 82 percent in untreated controls. Arsenic in the drinking water in form of arsenite, at levels of 10 ppm, reduced the tumor incidence to 27 percent but caused a significant enhancement of the growth rate of spontaneous or transplanted mammary tumors. The present results provide a pertinent example for the cancer protecting effect of the essential trace element selenium. A comparison of the female breast cancer death rate in the Continental United States indicates that the breast-CA mortality is lower in areas with abundant or high dietary selenium supply than in regions deficient in this element.

Introduction

Until very recently, the essential human trace element selenium was included in the list of carcinogenic agents by the Food Additive Amendment, administered by the Food and Drug Administration. Evidence for its carcinogenic activity has been obtained only on the basis of animal experiments, however, and cases of selenium-induced neoplastic diseases in humans have not been reported. Even the evidence for carcinogenicity in animals is not undisputed; on the contrary, more recent work suggests that selenium in certain forms of application acts as a "cancer protecting agent." It also has been shown that the age-adjusted human cancer death rates in cities or states of the Continental United States are lower where the dietary selenium supply is abundant, as compared to areas in which the dietary intake of selenium is low. Furthermore, a historical "Cancer Test," first described in 1947 by Savignac and Black, consisting in the measurement of the methylene blue reduction time of human plasma, was recently shown to respond to the varying selenium levels in human plasma. What had been considered positive evidence for cancer on the basis of this
While it has been demonstrated that selenium delays or diminishes the appearance of chemically induced tumors in mice or rats,4,12,14 no study has been reported on the effect of selenium on the genesis or growth of spontaneous tumors. For this reason, investigations have been conducted in our laboratory on the effect of selenium on members of a strain of highly inbred, virgin C$_3$H/St mice, known to develop mammary adenocarcinoma with 85 percent incidence at the age of 12 to 16 months. The animals were continuously exposed to subtoxic levels of selenite in the drinking water and were otherwise kept under normal laboratory conditions. In addition to selenium, the effects of arsenic on the C$_3$H mice were explored. Although arsenic is no longer considered to be a universal carcinogen, claims of its ineffectiveness or alleged beneficial action on tumors are controversial. Presumably, arsenic may be oncogenic, innocuous or beneficial depending on the type of tumor, host, mode of application etc. However, arsenic may also be regarded as a potential or actual antagonist of selenium, since the administration of arsenic increases the biliary excretion of selenium in the rat.6 It has been shown also with rats that arsenic accumulates in the tissues, especially the aorta and red blood cells, with no signs of toxicity.10 Since arsenic could behave similarly in mice, a study of its effect on spontaneous mammary tumor genesis appeared to be of particular interest. In addition to work with spontaneous tumors, a number of experiments have been performed in our laboratory with transplanted mammary tumors of C$_3$H mice. The results were obtained after 16 months of observation of the animals under experimental conditions given.

* The mice were supplied from the Laboratory of Dr. L. C. Strong.
Materials and Methods

Selenium was supplied at levels of 2 ppm (Se) in form of SeO₂ in the (distilled) drinking water. At this concentration selenium is not toxic over long periods of administration. Arsenic was given at concentrations of 10 ppm (As) in form of NaAsO₂.† Whereas no signs of arsenic poisoning were noticeable, it was observed that a number of animals became hyper-

† "Ultrapure" from Alfa Inorganics-Ventron.
active. Hyperactivity in C₃H mice is rare under normal conditions of maintenance. Groups of 30 female virgin mice aged four to six weeks were placed into cages without overcrowding (three animals per cage) and were maintained under normal laboratory conditions and on the same diet.§ The animals in the controls and selenium group demonstrated normal weight-gains, as evidenced by monthly weighing. The As-exposed animals showed a slower weight gain during the first nine months of As-exposure. The average weight of the surviving As-treated animals was equal to that of the controls during the following six months of observation (table I). The underweight As-exposed animals were all hyperactive and developed tumors. All animals were checked for tumors once every week. Upon incidence, tumors were measured twice weekly using a caliper. Tumor size was computed from the product of the two largest tumor diameters. The experiments with transplanted tumors were also conducted with virgin female C₃H mice. Tumor growth was initiated by injecting malignant mammary cells into the mammary gland of tumor-free animals.

Results

Virgin female C₃H/St mice develop mammary tumors spontaneously at the age of between 12 and 16 months. The first tumors in our study appeared in the arsenic-fed group at the age of about seven months, i.e., after six months of continuous exposure to arsenic. However, after nine months and up to 16 months (figure 1), no

---

§ "Concord Maid" was used as the sole diet, providing vitamins and essential minerals in adequate amounts. The diet is essentially free of cholesterol and other potential tumor growth promoting lipophilic substances.
Figure 5. Growth curves of transplanted mammary tumors.

Further "arsenic tumors" were observed. The remaining animals were well and showed no sign of chronic arsenic poisoning. The total tumor incidence after 16 months of exposure to arsenic was 27 percent. The tumors grew very rapidly and the average lifetime of affected animals was one to two months. It is of interest to note that the animals which developed tumors in the arsenic group were all underweight and hyperactive; normally behaving animals in this group did not develop tumors during the time of observation. This is in contrast to empirical observations with C₃H/St mice which, if hyperactive under normal maintenance, do not develop spontaneous mammary tumors.* Only three (10 percent) of the selenium exposed mice developed tumors, all in the ninth month. The tumors grew and histologically appeared less malignant than those in the As-group (figures 2 and 3). The average survival time of affected animals was five to seven months. In the control group, the first tumors appeared 11 months after the beginning of the experiment, i.e., at age of 12.5 months. The tumors grew more rapidly than in the selenium and more slowly than in the arsenic group (figure 4); the average survival time was four months. After 16 months the total tumor incidence was 82 percent.

Mammary tumors transplanted from selenium exposed C₃H mice to selenium-exposed tumor-free female virgin C₃H mice grew slowly, only about as fast as transplanted control tumors (figure 5). The growth rate of transplanted tumors in arsenic exposed animals was very high; the tumors also had the greatest tendency to metastasize.

Discussion

The female virgin C₃H mice raised under normal laboratory conditions developed spontaneous tumors at the expected age and with the statistically normal incidence. A substantial diminution of the tumor incidence occurred both in the selenium- and the arsenic-exposed animals. The presence of these additives in the

<table>
<thead>
<tr>
<th>Median Selenium Concentration ppm</th>
<th>Breast Cancer Death Rate 1959-1961*</th>
<th>S.D. †</th>
<th>Number of States</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02 - 0.03</td>
<td>1.11</td>
<td>0.16</td>
<td>5</td>
</tr>
<tr>
<td>0.05</td>
<td>1.09</td>
<td>0.14</td>
<td>14</td>
</tr>
<tr>
<td>0.05 - 0.10</td>
<td>0.80</td>
<td>0.20</td>
<td>13</td>
</tr>
<tr>
<td>0.26</td>
<td>0.75</td>
<td>0.15</td>
<td>16</td>
</tr>
</tbody>
</table>

*Ratio to U.S. rate per 100,000 population.
†Standard deviation from average death rate.
drinking water also affected the growth rates of the tumors. The "selenium tumors" grew more slowly than the controls and appeared less malignant, causing death of the animals only after five to six months as compared to four months in the control group. The tumors in the arsenic group grew very rapidly and were more malignant, killing the affected animals within six weeks. It has been concluded from our experiment that in C3H mice selenium and, to a lesser extent, arsenic are capable of inhibiting the growth primarily of precarcinomatous mammary cell populations. Arsenic, however, stimulates the growth of more mature spontaneous or transplanted tumors. Selenium, on the other hand, neither accelerated nor inhibited the growth of advanced spontaneous or transplanted mammary tumors significantly. Our results also suggest that neither arsenic nor selenium were genuinely carcinogenic in the animals during the period of observation and under the experimental conditions chosen.† Since the human cancer mortality in the Continental United States is lower in areas with adequate or high dietary selenium supply, it appeared to be of interest to compare published death rates of female breast cancer in the U.S. as a function of the amount of selenium present in grains and forage crops of the respective geographical areas. Using the data of Kubota et al,5 for the Se-distribution and mortality rates quoted by Seidman11 the results obtained are summarized in table II. Since the female breast cancer death rate is lower in areas with adequate or high dietary selenium supply than in states which are low or deficient in this essential trace element, it is possible that human breast cancer incidence and mortality could be lowered by appropriate dietary selenium supplementation.

Acknowledgments

Thanks are extended to Dr. Leonell C. Strong for supplying the authors with C3H mice and for his interest and support of our work and to Messrs, W. J. Rhead, Curtis Tom and Henry Matsanaga for valuable technical assistance.

References


† Note added with proof: Between the submission of this manuscript and the receipt of the proof, of the remaining 10 animals in the selenium group, all have since died of causes other than malignancy at the age of 26 ± 2 months, which is corresponding to the normal life expectancy. Autopsies revealed no abnormalities compared to tumor-free mice dying of old age.
PROFICIENCY TEST SERVICE

DIRECTORS OF CLINICAL LABORATORIES, Clinical Pathologists, Clinical Chemists, and Associates are invited to become participants in the Proficiency Test Service. This monthly self-audit program in clinical chemistry has been in continuous operation since 1949.

The Program

The Proficiency Test Service is conducted as follows: Two ampules containing two different serums or solutions are mailed to participants on the first day of each month for measurements of one or more of the chemical components usually analyzed in clinical laboratories. Participants are requested to undertake their analyses on the day on which the samples are received, and then to report their results on a form furnished by the Service. On the fifteenth day of the month each participant receives a Monthly Report which includes:

1) The results of a statistical analysis of the values reported by all the participating laboratories,
2) An up-to-date review of pertinent methodology,
3) A comprehensive bibliography, and
4) A validation of each laboratory's proficiency based upon the results which the laboratory reported.

The Proficiency Test Service enables each participating laboratory to obtain an unbiased assessment of its proficiency in relation to that of 1000 other clinical laboratories throughout the country.

Subscription

Subscription to the Proficiency Test Service is $100 per year. This amount covers the actual costs. At the subscriber's written request and on payment of a $5 fee, the Proficiency Test Service will furnish a transcript of the subscriber's annual performance to state health departments and other accrediting agencies.

Additional subscriptions available for resident and technologist training programs.

Make checks payable to "PROFICIENCY TEST SERVICE" and mail to

INSTITUTE FOR CLINICAL SCIENCE
1833 Delancey Place, Philadelphia, Pa. 19103