Dietary Zinc Modulation of Moloney Sarcoma Virus Oncogenesis

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ABSTRACT

To explore the relationship between dietary zinc and the natural history of Moloney sarcoma virus (MSV) oncogenesis, BALB/c mice were fed diets which contained four levels of zinc: 100 ppm zinc (designated control); 9 ppm zinc (designated marginal deficiency); 5 ppm zinc (designated moderate deficiency); and 2.5 ppm zinc (designated severe deficiency). Moreover, because zinc deficiency is associated with some degree of inanition, a group of mice was fed the control diet but in amounts equal to the intake of animals fed 5 ppm zinc. All diets were initiated in 10-week-old mice at 0, 1, 3, and 6 weeks before injection of the MSV. When the diets were introduced simultaneously with the MSV, little significant impact upon natural history of the tumor was observed. With 1 week of prior dietary zinc deprivation, severely deprived mice experienced inhibition of tumor growth. However, with 3 weeks of limited zinc availability prior to MSV injection, alteration of tumor growth was observed and was directly dependent upon the specific level of zinc in the diet. Mice fed 9 and 5 ppm zinc showed an increase in sarcoma growth when compared with control animals while mice fed 2.5 ppm zinc demonstrated a decreased incidence of sarcomas and a reduction in the size of those tumors which developed. Feeding low-zinc diets for 6 weeks resulted in a marked reduction in sarcoma initiation and progression in mice fed 9, 5, and 2.5 ppm zinc. With sufficient severity and duration, animals maintained in low-zinc environments also experienced an increase in tumor latency period and tumor regression time. Caloric restriction, as evidenced by inanition controls, caused markedly altered tumor incidence and kinetics, making it difficult to quantify those effects due to inanition and those due to zinc deficiency. The present experiment underscores the critical influence that magnitude and duration play in determining the ultimate impact of various degrees of zinc deficiency upon tumor initiation and progression and further emphasizes the need for caloric restriction controls in all studies of trace element deprivation.

INTRODUCTION

Trace elements are recognized as having a major impact upon host metabolism and the response to injury (21), infection (5), and neoplasia (31). Indeed, the interaction of essential trace metals, such as zinc, and the immune response is a dynamic and complex process (11). Because of these relationships, the response to a variety of tumorigenic challenges has been examined in animals under conditions of deficient or excess zinc availability (9, 13, 24, 26, 35). Unfortunately, however, results have frequently proven equivocal and confounding due to methodological inconsistencies, inability to define levels of zinc intake adequately, and failure to provide controls for the inanition observed in zinc-deprived animals. The issue of zinc deprivation and altered natural history of the tumor is an important one because of the increasing recognition of marginal zinc status among substantial sectors of the population in developed nations (29). We have examined the effects of a range of clearly defined levels of dietary zinc upon the pathogenesis of MSV infection in mice. We report herein a marked influence of dietary zinc upon tumor incidence, latency period, maximum tumor circumference, and the time necessary for tumor regression. The alteration in tumor kinetics was in response to both limited dietary zinc per se as well as the inanition associated with such trace element deprivation. The precise nature of the effect of limited dietary zinc availability depended strictly upon (a) the specific level of dietary zinc and (b) the duration of the zinc deprivation prior to the MSV inoculation. These observations emphasize the fundamental importance of gaining a better understanding of the interactions between trace metal nutrition and tumorigenesis.

MATERIALS AND METHODS

Animals. Ten-week-old virgin-female BALB/c mice (The Jackson Laboratory, Bar Harbor, Maine) were housed in plastic cages suspended from stainless steel racks with each cage entirely covered with filter paper. Deionized distilled water was available ad libitum in nitric acid-washed glass containers with acid-washed neoprene stoppers. The purified diet was dispensed in polyethylene conical centrifuge tubes, which facilitated measurement of food intake. Individually wrapped, single-use, plastic film gloves were used to handle all experimental animals and materials. Throughout all experimental procedures, these strict controls were maintained to minimize contamination.

Diets. All animals were fed a complete purified diet containing isolated soybean protein; the complete composition of the diet has been reported previously (3). The isolated soybean protein had been treated with a chelating agent, tetrasodium EDTA to reduce the zinc content to negligible proportions. ZnCO₃ was subsequently added to the diet to attain desired levels of dietary zinc as verified by atomic absorption spectrophotometry. Animals were fed ad libitum diets containing 9.0 ppm zinc (designated marginal zinc deficiency), 5.0 ppm zinc (designated moderate zinc deficiency), or 2.5 ppm zinc (designated severe zinc deficiency) in addition to the control diet which contained 100 ppm zinc. Because zinc deprivation is known to cause inanition (7), a group of mice was fed the control diet (100 ppm zinc) but in amounts equal by weight to the recorded daily intake of the moderately deprived animals.

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(5.0 ppm zinc). This allowed for the distinction between those alterations in neoplastic initiation and progression due to zinc deficiency per se and those attributed to caloric deprivation.

Experimental Protocol. Animals were divided into 4 groups of 50 mice each. Group 1 consisted of mice which consumed the purified control diet (100 ppm zinc) for 6 weeks and, simultaneously with MSV inoculation, began to consume diets containing specified levels of zinc (100, 9.0, 5.0, and 2.5 ppm zinc) (see “Diets”). Group 2 was comprised of animals which consumed the purified control diet for 5 weeks and then, 1 week prior to MSV challenge, began to consume diets containing the specified levels of zinc. Mice in Group 3 consumed the purified control diet for 3 weeks and then, 3 weeks prior to MSV injection, began consuming the diets containing specified levels of zinc. Group 4 consisted of mice which began consuming the diets containing specified levels of zinc 6 weeks prior to the MSV inoculation. After injection of MSV, all mice continued to consume the same diets containing specified levels of zinc. Body weights of all animals were obtained on a weekly basis. All data were analyzed by 2-way analysis of variance with Duncan’s multiple range test.

MSV. After the defined interval of dietary treatment, in all cases a total of 6 weeks of pretreatment, mice were inoculated i.m. in the hind limb with 0.1 ml of murine MSV (5 x 10⁷ focus-forming units), obtained by courtesy of Dr. Jack Gruber, National Cancer Institute. Mice were then observed daily, and the injection site was monitored for tumor latency period, maximum tumor circumference, and tumor regression time for 5 weeks (10). A calibrated vernier loop, developed for the measurement of hind limb sarcomas and assessed to be accurate to ±0.5 (S.E.) mm, was used to monitor hind limb circumference (12). It should be noted that all MSV utilized was free of lactic dehydrogenase-elevating virus. Finally, the ratio of the tumor circumference to body weight was calculated for each mouse at the time of maximum tumor circumference.

RESULTS

Group 1. Simultaneous imposition of zinc deficiency and MSV inoculation resulted in a decrease in body weight in animals fed 5.0 and 2.5 ppm zinc, but such differences were not significant (Chart 1). When deprivation of zinc was commenced at the time of the MSV inoculation, there was no significant impact upon tumor incidence (Table 1), latency period (Table 2), maximum tumor circumference (Table 3; Chart 2), or tumor regression time (Table 4) in mice fed 9.0 and 5.0 ppm zinc when compared to ad libitum-fed controls. In contrast, in mice fed 2.5 ppm zinc, a significantly increased latency period of 13.0 ± 0.5 days was observed as compared to 7.1 ± 0.3 days in the ad libitum-fed controls (p < 0.01). When commenced simultaneously with the MSV injection, caloric restriction consistent with the inanition observed in animals fed the 5.0-ppm zinc diet resulted in no significant alteration in tumor incidence, latency period, maximum tumor circumference, or tumor regression time. When maximum tumor circumference was compared to body weight (Table 5), the resultant ratios confirmed that no significant alteration in tumor size occurred in animals fed low-zinc diets.

Group 2. Mice consuming the deficient diets for 1 week prior to the MSV inoculation were affected to only a slightly greater extent than were mice deprived of zinc simultaneously with the MSV challenge. Body weights of animals in Group 2 were significantly lower in mice consuming the diets containing 5.0 and 2.5 ppm zinc than in ad libitum-fed controls (Chart 1); pair feeding of control animals did not have a similar impact upon body weight. With 1 week of dietary zinc deficiency, animals fed 9.0 ppm zinc showed no significant alteration in tumor incidence (Table 1), latency period (Table 2), maximum tumor circumference (Table 3; Chart 3), or tumor regression time (Table 4) when compared with the ad libitum-fed controls. Similarly, animals fed 5.0 ppm zinc showed no significant
alteration in tumor incidence, maximum tumor circumference, or sarcoma regression time. In contrast, consumption of the diet containing 2.5 ppm zinc for 1 week prior to MSV inoculation resulted in a significant decrease in tumor incidence, 66% as compared to 100% of the ad libitum-fed control mice ($p < 0.05$). Moreover, both moderately and severely deprived mice experienced a significant increase in tumor latency period with values of 9.2 ± 0.3 days ($p < 0.05$) and 12.7 ± 0.6 days ($p < 0.01$), respectively, when compared with a latency period of 7.1 ± 0.3 days in ad libitum-fed controls. Only mice fed the 2.5-ppm zinc diet showed a significant decrease in maximum tumor circumference with tumors reaching a peak size of 7.2 ± 2.3 mm, compared to 15.9 ± 2.2 mm in ad libitum-fed

<table>
<thead>
<tr>
<th>No. of mice with recognizable sarcomas/toal no. of mice at following timings of diet prior to the MSV injection</th>
<th>Simultaneous with MSV</th>
<th>1 wk prior</th>
<th>3 wk prior</th>
<th>6 wk prior</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppm zinc, ad libitum-fed controls</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>100 ppm zinc, inanition controls</td>
<td>8/10</td>
<td>8/10</td>
<td>9/10</td>
<td>6/10</td>
</tr>
<tr>
<td>9.0 ppm zinc, marginal deficiency</td>
<td>9/10</td>
<td>9/9</td>
<td>9/10</td>
<td>4/8</td>
</tr>
<tr>
<td>5.0 ppm zinc, moderate deficiency</td>
<td>10/10</td>
<td>8/9</td>
<td>4/10</td>
<td>2/9</td>
</tr>
<tr>
<td>2.5 ppm zinc, severe deficiency</td>
<td>8/10</td>
<td>6/9</td>
<td>3/9</td>
<td>2/8</td>
</tr>
</tbody>
</table>

* Significantly different from ad libitum-fed controls at $p < 0.05$.
* Significantly different from ad libitum-fed controls at $p < 0.01$.
* Significantly different from inanition controls at $p < 0.05$.
* Significantly different from inanition controls at $p < 0.01$.

<table>
<thead>
<tr>
<th>Latency period of sarcoma (days) at following timings of diet prior to MSV injection</th>
<th>Simultaneous with MSV</th>
<th>1 wk prior</th>
<th>3 wk prior</th>
<th>6 wk prior</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppm zinc, ad libitum-fed controls</td>
<td>7.1 ± 0.3</td>
<td>7.1 ± 0.3</td>
<td>7.1 ± 0.3</td>
<td>7.1 ± 0.3</td>
</tr>
<tr>
<td>100 ppm zinc, inanition controls</td>
<td>7.9 ± 0.2</td>
<td>10.3 ± 0.6</td>
<td>9.0 ± 0.1</td>
<td>7.3 ± 0.3</td>
</tr>
<tr>
<td>9.0 ppm zinc, marginal deficiency</td>
<td>6.8 ± 0.1</td>
<td>7.1 ± 0.4</td>
<td>8.5 ± 0.2</td>
<td>8.7 ± 0.2</td>
</tr>
<tr>
<td>5.0 ppm zinc, moderate deficiency</td>
<td>7.0 ± 0.2</td>
<td>9.2 ± 0.3</td>
<td>8.9 ± 0.2</td>
<td>13.4 ± 0.4</td>
</tr>
<tr>
<td>2.5 ppm zinc, severe deficiency</td>
<td>13.0 ± 0.5</td>
<td>12.7 ± 0.6</td>
<td>11.1 ± 0.3</td>
<td>10.3 ± 0.6</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* Significantly different from ad libitum-fed controls at $p < 0.05$.
* Significantly different from ad libitum-fed controls at $p < 0.01$.
* Significantly different from inanition controls at $p < 0.01$.

<table>
<thead>
<tr>
<th>Maximum hind limb circumference (mm) at following timings of diet prior to MSV injection</th>
<th>Simultaneous with MSV</th>
<th>1 wk prior</th>
<th>3 wk prior</th>
<th>6 wk prior</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppm zinc, ad libitum-fed controls</td>
<td>35.9 ± 2.2</td>
<td>35.9 ± 2.2</td>
<td>35.9 ± 2.2</td>
<td>35.9 ± 2.2</td>
</tr>
<tr>
<td>100 ppm zinc, inanition controls</td>
<td>33.5 ± 1.9</td>
<td>29.5 ± 0.9</td>
<td>30.3 ± 1.4</td>
<td>29.8 ± 0.7</td>
</tr>
<tr>
<td>9.0 ppm zinc, marginal deficiency</td>
<td>37.8 ± 2.0</td>
<td>36.0 ± 1.8</td>
<td>44.0 ± 2.8</td>
<td>22.0 ± 0.4</td>
</tr>
<tr>
<td>5.0 ppm zinc, moderate deficiency</td>
<td>37.2 ± 1.3</td>
<td>33.8 ± 1.7</td>
<td>35.9 ± 2.6</td>
<td>21.3 ± 0.4</td>
</tr>
<tr>
<td>2.5 ppm zinc, severe deficiency</td>
<td>33.7 ± 0.8</td>
<td>27.2 ± 2.3</td>
<td>31.2 ± 0.9</td>
<td>18.8 ± 0.5</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* Significantly different from ad libitum-fed controls at $p < 0.01$.
* Significantly different from ad libitum-fed controls at $p < 0.01$.
* Significantly different from inanition controls at $p < 0.01$.
**Table 4**

Sarcoma regression time

Obtained from those mice which developed recognizable sarcomas; see Table 1 for sample sizes.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Simultaneous with MSV</th>
<th>1 wk prior</th>
<th>3 wk prior</th>
<th>6 wk prior</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppm zinc, ad libitum-fed controls</td>
<td>18.2 ± 0.8 <em>a</em></td>
<td>18.2 ± 0.8</td>
<td>18.2 ± 0.8</td>
<td>18.2 ± 0.8</td>
</tr>
<tr>
<td>100 ppm zinc, inanition controls</td>
<td>19.3 ± 0.5</td>
<td>19.7 ± 0.5</td>
<td>24.3 ± 0.9 <em>b</em></td>
<td>23.2 ± 0.6 <em>b</em></td>
</tr>
<tr>
<td>9.0 ppm zinc, marginal deficiency</td>
<td>17.6 ± 0.4</td>
<td>21.2 ± 0.7</td>
<td>24.9 ± 0.8 <em>b</em></td>
<td>26.2 ± 0.8 <em>b</em></td>
</tr>
<tr>
<td>5.0 ppm zinc, moderate deficiency</td>
<td>18.7 ± 0.6</td>
<td>20.4 ± 0.6</td>
<td>26.3 ± 0.8 <em>b</em></td>
<td>26.0 ± 0.4 <em>b</em></td>
</tr>
<tr>
<td>2.5 ppm zinc, severe deficiency</td>
<td>21.7 ± 0.9 <em>b</em></td>
<td>24.0 ± 0.8 <em>b</em></td>
<td>28.9 ± 1.1 <em>b</em></td>
<td>25.8 ± 0.7 <em>b</em></td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* a Significantly different from ad libitum-fed control animals at p < 0.01.
* b Significantly different from inanition controls at p < 0.05.

**Table 5**

Maximum tumor circumference related to body weight

Maximum tumor circumference/total body weight following timings of diet prior to MSV injection

<table>
<thead>
<tr>
<th>Diet</th>
<th>Simultaneous with MSV</th>
<th>1 wk prior</th>
<th>3 wk prior</th>
<th>6 wk prior</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppm zinc, ad libitum-fed controls</td>
<td>0.60 ± 0.05 <em>a</em></td>
<td>0.55 ± 0.06</td>
<td>0.55 ± 0.04</td>
<td>0.58 ± 0.04</td>
</tr>
<tr>
<td>100 ppm zinc, inanition controls</td>
<td>0.49 ± 0.06</td>
<td>0.36 ± 0.05 <em>c</em></td>
<td>0.39 ± 0.06 <em>c</em></td>
<td>0.44 ± 0.05 <em>b</em></td>
</tr>
<tr>
<td>9.0 ppm zinc, marginal deficiency</td>
<td>0.66 ± 0.04</td>
<td>0.52 ± 0.05</td>
<td>0.83 ± 0.07 <em>c</em></td>
<td>0.20 ± 0.03 <em>c</em></td>
</tr>
<tr>
<td>5.0 ppm zinc, moderate deficiency</td>
<td>0.70 ± 0.08</td>
<td>0.60 ± 0.08</td>
<td>0.63 ± 0.07 <em>d</em></td>
<td>0.26 ± 0.04 <em>c</em> <em>d</em></td>
</tr>
<tr>
<td>2.5 ppm zinc, severe deficiency</td>
<td>0.54 ± 0.04</td>
<td>0.30 ± 0.04 <em>c</em></td>
<td>0.46 ± 0.06</td>
<td>0.18 ± 0.03 <em>c</em></td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* a Significantly different from ad libitum-fed controls at p < 0.05.
* c Significantly different from ad libitum-fed controls at p < 0.01.
* d Significantly different from inanition controls at p < 0.05.

Chart 3. Group 2 tumor circumference: zinc-deficient diets commenced 1 week prior to the MSV inoculation. With 1 week of prior dietary zinc deficiency, only mice fed 2.5 ppm zinc showed an inhibition of tumor growth. Such mice ultimately developed tumors of 7.2 ± 2.3 mm maximum circumference as compared with 15.9 ± 2.2 mm in the ad libitum-fed (AL) controls (p < 0.01). Tumors in mice fed 2.5 ppm zinc began to develop on Day 14 as compared with Day 7 in the ad libitum-fed controls (p < 0.01). Tumors in mice severely deprived of zinc also regressed more slowly, requiring 24 days, as compared with 18 days in the ad libitum-fed controls (p < 0.01). Mice fed 5.0 ppm zinc did show a significant delay in the development of the tumor, but, when compared with pair-fed (PF) controls, such differences were not significant.

In the control mice (p < 0.01). Similarly, only mice fed 2.5 ppm zinc experienced a significant delay in sarcoma regression time; the tumors in such mice required 24.0 ± 0.8 days to regress, compared to 18.2 ± 0.8 days observed in the ad libitum-fed controls (p < 0.01). Caloric restriction, as exemplified by the inanition controls, had no significant impact upon tumor incidence or regression time. However, pair-fed controls experienced a tumor latency period of 10.3 ± 0.6 days and a maximum tumor circumference of 9.5 ± 0.9 mm, both of which were significantly different from values observed in ad libitum-fed control mice (p < 0.05). When compared with the moderately deprived mice, to which they were pair fed, there was no significant difference in tumor latency period. However, maximum tumor circumference in mice fed 5.0 ppm zinc was significantly greater than in their pair-fed counterparts, the moderately deprived animals attaining a maximum sarcoma circumference of 13.8 ± 1.7 mm, as compared to 9.5 ± 0.9 mm in the inanition controls (p < 0.05). As with Group 1, the ratio of maximum tumor circumference to total body weight (Table 5) confirmed the differences observed in the raw data with a significant decrease in tumor size in the inanition controls and in the mice fed 2.5 ppm zinc; in contrast, mice fed the 5.0-ppm zinc diet developed tumors of significantly greater size than did pair-fed controls.

**Group 3.** With 3 weeks of prior zinc deprivation, body weights of mice fed the diets containing 5.0 and 2.5 ppm zinc were significantly lower than in the ad libitum-fed control animals (Chart 1). After consuming the 5.0-ppm zinc diet for 5 weeks, body weights of the moderately deprived mice were also lower than in their pair-fed counterparts for the duration of the experiment. Zinc deprivation began 3 weeks prior to the injection of MSV had a profound influence upon tumor kinetics; the nature of the alterations was dependent upon the specific...
level of dietary zinc. Animals fed 9.0 ppm zinc experienced an incidence of tumors similar to that of ad libitum-fed controls (Table 1). However, tumor incidence in mice fed 5.0 and 2.5 ppm zinc was only 40 and 33% \( (p < 0.01) \), respectively, of that found in ad libitum-fed controls. Furthermore, when dietary manipulation of zinc status was commenced 3 weeks prior to MSV challenge, all mice fed low-zinc diets showed some delay in tumor latency period (Table 2). As compared to the value of 7.1 ± 0.3 days observed in the ad libitum-fed control animals, mice fed 9.0, 5.0, and 2.5 ppm zinc experienced latency periods of 8.5 ± 0.2 days \( (p < 0.05) \), 8.9 ± 0.2 days \( (p < 0.05) \), and 11.1 ± 0.3 days \( (p < 0.01) \), respectively.

Maximum tumor circumference (Chart 4) also reflected notable variation dependent upon dietary zinc; animals fed 9.0 ppm zinc had a significant increase in tumor size, mice fed 5.0 ppm zinc showed no significant difference, and mice fed 2.5 ppm zinc showed a significant decrease in sarcoma size, compared with ad libitum-fed control mice. For example, while the ad libitum-fed controls attained a maximum tumor circumference of 15.9 ± 2.2 mm, animals fed 9.0 ppm zinc achieved a maximum circumference of 24.0 ± 2.8 mm \( (p < 0.01) \), mice fed 5.0 ppm zinc attained a peak tumor size of 15.9 ± 2.6 mm \( (p < 0.05) \), and mice fed 2.5 ppm zinc attained a peak tumor size of 11.2 ± 0.9 mm \( (p < 0.01) \). Finally, as with tumor circumference, all animals fed low-zinc diets experienced a significant increase in tumor regression time with values of 18.2 ± 0.8 days for tumor regression in ad libitum-fed control mice, 24.9 ± 0.8 days in animals fed 9.0 ppm zinc, 26.3 ± 0.8 days in animals fed 5.0 ppm zinc, and 28.9 ± 1.1 days in animals fed 2.5 ppm zinc; all values for mice receiving limited amounts of zinc were significantly greater than the regression time observed in ad libitum-fed control mice \( (p < 0.01) \). Inanition control animals experienced significant alterations of latency period, maximum sarcoma circumference, and tumor regression time. Compared to the values observed in ad libitum-fed control mice, pair-fed controls demonstrated a latency period of 9.0 ± 0.1 days, a peak tumor circumference of 10.3 ± 1.4 mm, and a sarcoma regression time of 24.3 ± 0.9 days \( (p < 0.01) \). Comparison of animals fed 5.0 ppm zinc with their inanition control counterparts indicated a significant difference only with regard to maximum tumor circumference, the mice being fed the 5.0-ppm zinc diet developing significantly larger sarcomas than did the pair-fed controls \( (p < 0.01) \). In most instances, the ratio of maximum tumor circumference to total body weight (Table 5) affirmed the observations made regarding the absolute measurements. A significant increase in tumor size was seen in animals fed 9.0 ppm zinc while no significant difference was observed in animals fed 5.0 ppm zinc, when compared with the ad libitum-fed controls. However, when compared with their pair-fed counterparts, such moderately zinc-deprived animals demonstrated tumors of significantly greater size. The single difference was observed in tumors of mice fed 2.5 ppm zinc, which in absolute terms demonstrated a significantly smaller maximum tumor circumference, but when tumor size was adjusted for changes in body weight, no significant differences were observed.

**Group 4**. As with 1 or 3 weeks of prior dietary zinc deprivation, with 6 weeks of prior deficiency, mice fed 5.0 and 2.5 ppm zinc demonstrated significantly lower body weights than did ad libitum-fed control mice (Chart 1). Such decreased body weights continued after the MSV injection. Pair-fed controls did not show a similar decrease in body weight although they

![Chart 4. Group 3 tumor circumference: zinc-deficient diets commenced 3 weeks prior to MSV inoculation. With 3 weeks of prior limited zinc availability, variable influences of low-zinc diets upon tumor initiation and progression were observed, being directly dependent upon the specific level of dietary zinc. Maximum tumor circumference reflected these differences as animals fed 9.0 ppm zinc developed tumors of significantly greater maximum circumference than did ad libitum-fed (AL) controls with tumors measuring 24.0 ± 2.8 mm as compared with 15.9 ± 2.2 mm in the control mice \( (p < 0.01) \). In animals fed 5.0 ppm zinc, maximum tumor circumference did not differ from the values observed in ad libitum-fed controls, such tumors measuring 15.9 ± 2.6 mm. However, when such tumors were compared with those seen in their pair-fed (PF) controls, the mice fed 5.0 ppm zinc developed tumors of significantly larger maximum circumference. Mice fed 2.5 ppm zinc developed tumors of smaller maximum circumference, measuring 11.2 ± 0.9 mm \( (p < 0.01) \). When 3 weeks of prior dietary zinc deprivation were introduced, there were also alterations in the initiation and regression of tumors. Tumors began to develop at approximately 8.5, 9, and 11 days in the mice fed 9.0, 5.0, and 2.5 ppm zinc, respectively, as compared with 7 days in the ad libitum-fed control mice \( (p < 0.05) \). Tumors required approximately 25, 26, and 29 days to regress in the mice fed 9.0, 5.0, and 2.5 ppm zinc, respectively, as compared with 18 days in the ad libitum-fed controls \( (p < 0.01) \).
did show lower body weights than did the ad libitum-fed control mice. In addition, all mice deprived of zinc for 6 weeks experienced marked alterations in tumor kinetics. As compared to 100% tumor incidence in ad libitum-fed controls, the total incidence of tumors was 50% in mice fed 9.0 ppm zinc (p < 0.05), 22% in mice fed 5.0 ppm zinc (p < 0.01), and 25% in mice fed 2.5 ppm zinc (p < 0.01; Table 1). Similarly, all mice fed limited amounts of zinc for 6 weeks prior to MSV inoculation had significant alterations in tumor latency period (Table 2). While ad libitum-fed control mice experienced a tumor latency period of 7.1 ± 0.3 days, mice fed 9.0, 5.0, and 2.5 ppm zinc had significantly increased latency periods of 8.7 ± 0.2 days (p < 0.05), 13.4 ± 0.4 days (p < 0.01), and 10.3 ± 0.6 days (p < 0.05), respectively. While ad libitum-fed controls attained a peak tumor size of 15.9 ± 2.2 mm, all animals deprived of zinc for 6 weeks prior to the MSV, which developed recognizable sarcomas, showed a significant decrease in tumor growth with animals fed 9.0 ppm zinc reaching a maximum of 6.7 ± 0.4 mm, animals fed 5.0 ppm zinc reaching a maximum of 6.9 ± 0.4 mm, and animals fed 2.5 ppm zinc reaching a maximum of 3.7 ± 0.5 mm, all decreases being notably significant (p < 0.01; Chart 5). Whether they were fed 9.0, 5.0, or 2.5 ppm zinc, all mice experienced a significant increase in tumor regression time (Table 4) with 26.2 ± 0.8 days, 26.0 ± 0.4 days, and 25.8 ± 0.7 days, respectively, required for tumor regression, compared to 18.2 ± 0.8 days required in ad libitum-fed controls (p < 0.01).

With 6 weeks of dietary pretreatment, inanition control mice showed no difference in tumor latency period when compared with ad libitum-fed controls. However, such food-restricted control animals did show significant alterations in tumor incidence, maximum tumor circumference, and tumor regression time. Recognizable sarcomas developed in 60% of the inanition controls, and they achieved a maximum tumor circumference of 29.8 ± 0.7 mm and required 23.2 ± 0.6 days for tumor regression; these differences were significantly different from the ad libitum-fed controls (p < 0.05). When compared with their counterparts fed the 5.0-ppm zinc diet, with 6 weeks of dietary pretreatment, the moderately zinc-deprived mice showed a higher incidence of sarcomas (p < 0.05), a shorter latency period (p < 0.01), a larger maximum tumor circumference (p < 0.01), and an increase in tumor regression time (p < 0.05). After 6 weeks of dietary pretreatment, these differences between mice fed 5.0 ppm zinc and their isocalorically restricted control counterparts were notably larger than were the differences which occurred after shorter periods of dietary limitation (Groups 1 to 3). Observation of the ratio of the maximum tumor circumference to body weight confirmed the conclusions obtained from actual tumor circumference. Significant decreases in tumor size were seen in all groups of mice fed limited amounts of zinc, i.e., 9.0, 5.0, and 2.5 ppm zinc. In addition, mice fed 5.0 ppm zinc demonstrated tumors of significantly smaller size than did the isocalorically restricted inanition controls.

**DISCUSSION**

Trace metals essential for mammalian survival and reproduction have only recently begun to gain recognition commensurate with the important role that they play in metabolism. While human zinc deficiency was first described in lesser developed nations (27) and has recently been found to be a factor in protein energy malnutrition and the immunodeficiency associated with it (16), there is now concern regarding marginal zinc deficiency in developed nations (29). Increased requirements for zinc during periods such as pregnancy, lactation, growth, development, infections, or chronic disorders may contribute to marginal zinc status among specific subpopulations (17, 30). Consumption of highly refined and heavily processed food items, the trace element content of which may be significantly reduced, may contribute the marginal zinc status in such countries as the United States (29). Observations of marginal zinc status in human populations, coupled with experimental findings of altered immunocompetence and impaired response to...
pathogenic challenge obtained in experimental animals (11), underscore the importance of studies designed to ascertain the effects of marginal or moderate zinc deficiency.

Deprivation of zinc has been shown to alter many facets of immunocompetence in both experimental animals and humans (11, 16). Inadequate provision of zinc has been clearly implicated in the altered function of many host cell types charged with the responsibility of tumor rejection, including lymphocytes, eosinophils, monocytes, macrophages, whether specifically or nonspecifically activated, nonphagocytic cells bearing Fc receptors, and mast cells (8, 11, 15, 19, 20, 23, 28). Antibody responses, which have been shown to be of notable importance in neutralizing oncogenic viruses, may be similarly impaired (4). Lack of zinc may also affect the function of various effector cells which have been implicated in the killing of antibody-coated tumor cells, e.g., B-lymphocytes, cells bearing Fc receptors, macrophages, and null (K) cells (8, 11, 19, 20, 23, 28). Animals deprived of zinc from birth have markedly reduced responses to T-cell mitogens and thymic-dependent antigens (2, 4). Such functional changes occurred simultaneously with severe atrophy of the thymus in these animals (2). Finally, serum immunoglobulin profiles were also altered in zinc-deprived animals with notably low levels of IgA, IgM, and IgG2A and markedly elevated IgG1 levels.

While alterations in host immunological function due to zinc deprivation are, in part, responsible for changes in host-tumor interactions, deprivation of this essential trace metal is also known to alter many aspects of host and tumor metabolism. Zinc is required for the catalytic activity of over 100 enzymes, including those involved in nucleic acid and protein synthesis (33). While the anabolic reactions of all tissues are slowed by a deficiency of zinc, rapidly growing tissues are most severely affected (34). Experimental animal tumor tissue is known to undergo a period of extremely rapid cellular proliferation, and insufficient zinc would be expected to sharply impair this marked hyperplasia. Indeed, altered DNA synthesis, as reflected by decreased rates of [3H]thymidine uptake by neoplastic tissue, has been demonstrated as a contributory factor in the inhibition of tumor growth in zinc-deficient animals (9). It is difficult to assess, in precise terms, the relative role of changes in host and tumor metabolism, as opposed to changes in host immune responsiveness to the tumor. However, the observed results are, at least in part, a reflection of the interaction of these two forces.

There are other factors which influence the rate of tumor growth in these animals experiencing limited zinc availability in addition to altered immunological function and altered host and tumor metabolism. The effects of zinc deprivation upon the tumor virus itself must also be considered. Zinc deficiency has been shown to affect the activity and viability of a number of viruses under a variety of conditions (14, 25). Central to this concept of zinc deficiency and altered viral viability in tumor viruses, such as MSV, is the dependence of enzymes critical for viral replication upon zinc for effective biological activity (1). Any alterations in viral replicative ability could greatly affect its pathogenicity. In addition, deficiency of zinc affects the metabolism of a number of other essential nutrients which have also been shown to affect the initiation and progression of tumors, e.g., protein (18), vitamin A (32), and copper (6). While these factors may have an involvement in the alteration of tumor growth through interaction with host zinc status, it is not possible at present to assess the relative importance of these factors.

In the present experiment, by utilizing diets containing 4 different levels of dietary zinc, we were able to delineate the specific effects of a precise level of zinc intake upon tumor initiation and progression. The levels selected included 100, 9.0, 5.0, and 2.5 ppm zinc, which were designated control, marginally deficient, moderately deficient, and severely deficient, respectively. A zinc-free diet was avoided inasmuch as this does not correspond to any situation known to occur presently in humans (29). Because the zinc requirements of mice, including interspecies variation, have not been well characterized, these designations were somewhat arbitrary. They were based upon previous studies (2-4), many of which utilized pregnant, lactating, and growing mice. Growth rates and plasma zinc values in these mice indicated that such levels of dietary zinc resulted in conditions which resembled marginal, moderate, and severe zinc deprivation. Actual plasma zinc values ranged from 123.6 µg/ml in ad libitum-fed control animals to 91.6, 47.6, and 32.8 µg/dl in the animals fed 9.0, 5.0, and 2.5 ppm zinc, respectively. While some of these animals may have experienced increased zinc requirements due to the additional stress of pregnancy, lactation, or growth, it is also quite probable that challenge with an oncogenic virus also represented an additional stress, which may have contributed to an elevated zinc requirement in these young adult animals. A further notable consideration was the fact that an isolated soybean protein-based diet was used in the present study; many previous studies, for instance that of Luecke and Fraker (22), have utilized a spray-dried egg white-based diet. Different basal diets may have contributed to differential availability of that zinc which was present in the diet. The high phytate content of the soybean-based diet used in the present experiment may have limited the availability of that zinc which was present in the various diets, thereby, at least in part, accounting for differences between this and previous studies (22).

Consideration of body weight data indicated that, in general, given sufficient duration, feeding the diets containing 5.0 and 2.5 ppm zinc resulted in significantly lower body weight than was observed in ad libitum-fed control mice fed diets containing 100 ppm zinc. In contrast, animals fed the diet containing 9.0 ppm zinc did not demonstrate any appreciable impact upon body weight as compared with ad libitum-fed controls. While this may indicate that this group of animals was not, in fact, marginally zinc deficient, it may also be that significant loss of body weight may be an indicator of more moderate or severe zinc deprivation while marginal deficiency of zinc could be monitored by more sensitive indices, such as plasma zinc and immunological function, as was adequately demonstrated in our previous work (2-4). Whatever the diet containing 9.0 ppm zinc is labeled, the important consideration was that animals fed 9.0 ppm zinc for specified periods of time, i.e., 3 and 6 weeks prior to MSV challenge, in fact, did demonstrate patterns of tumor initiation and progression significantly different from those seen in mice fed a diet containing 100 ppm zinc.

These observations must be tempered by several shortcomings of this experiment. Animals deprived of zinc have been shown to exhibit considerable inanition (7). In addition, caloric restriction has been shown to significantly alter tumor incidence and kinetics (18). In the observations presented here, caloric
restraint through pair feeding was utilized only as a control for animals fed 5.0 ppm zinc. However, in light of the marked impact of caloric restriction upon sarcoma initiation and progression, as exemplified by inanition controls, it would have been preferable to also include pair-fed groups for all levels of dietary zinc. For example, lack of such data limits interpretation of data obtained in mice fed 2.5 ppm zinc. In addition, it must be pointed out that reduction of tumor incidence in select groups, dependent upon the level of zinc and the length of prior deprivation, produced relatively smaller group sizes at specific points. Nonetheless, despite this relative reduction in data points, differences presented were in many cases still statistically significant. Finally, one must not lose sight of the descriptive nature of these results. The current results emphasize the importance of defining the precise relationship between micronutrients, such as zinc, and the process of carcinogenesis and/or tumorigenesis. It is hoped that future experiments, using more defined levels of zinc and specific quantitation of immunological responsiveness, will permit extrapolation to the more important mechanistic basis of these observations.

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