Growth of the P388 Leukemia as an Ascites Tumor in Zinc-Deficient Mice (37574)

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Zinc is an essential element for the growth of protozoa (1), fungi (2), bacteria (3), and mammalian cells (4). Animals deprived of this element grow more slowly than normal and their tissues frequently show decreased DNA synthesis (5, 6). It has been reported that growth of a solid tumor is dramatically retarded when the host rats are maintained on a zinc-deficient diet (7, 8). Although this report did not include an analysis of cell kinetics, the literature suggests that mammalian cells are unable to enter S phase in the absence of zinc (4, 9).

These observations suggested that zinc deficiency might be a useful method for manipulating the growth fraction of tumors *in situ* for studies of the relationship between tumor growth fraction and sensitivity to ionizing radiation or drugs. To evaluate this possibility we tested the effect of dietary zinc deficiency on the growth and ^{3}H-thymidine labeling of the P388 murine lymphocytic leukemia growing as an ascites tumor. Our results show that dietary zinc deficiency exerts a significant growth-depressing effect on the P388, although not so dramatic a one as observed for solid tumors in rats (7).

Materials and Methods. Weanling male DBA/2 mice, purchased from Jackson Labs (Bar Harbor, ME), were 18–20 days old and weighed between 9 and 11 g at the beginning of the experiment. The mice were matched in weight and caged in groups of five in stainless steel cages covered with filter paper lids. The cages were fitted with stainless steel screens that allowed excreta to fall through to the bottom of the cage.

All animals were fed a zinc-deficient diet for 17–24 days before inoculation of tumor cells. Zinc-deficient animals were provided with triple distilled deionized water; pair-fed and control mice received water containing 670 ppm zinc as zinc acetate.

The zinc-deficient diet was prepared by General Biochemicals (Chagrin Falls, OH) and included reagent grade salts and egg white solids (modified catalog no. 170995). Atomic absorption assay of this diet (by Mr. J. Quick, University of California Agricultural Extension Service, Davis) indicated a zinc content of 0.8 ppm, which agreed closely with the value of 0.6 ppm given by the supplier.[1]

The P388 lymphocytic leukemia, originally obtained from Dr. J. Brennan (University of Pennsylvania), had been passaged weekly in adult DBA/2 mice for more than 1 yr before these experiments began. A complete cytokinetic analysis of this tumor and a detailed description of the methods used for determining the growth curves and the proportion of S cells are presented elsewhere (10, 11).

Results. Zinc deficiency depressed growth of the P388 in weanling mice after the third post-transplantation day (Fig. 1A). This effect was clearly additional to the modest depression caused by caloric deprivation (*e.g.*, in pair-fed animals). (Zinc-deficient mice gained an average of 4.0 g [carcass weight] between the beginning of the experiment and Day 5 of tumor growth, whereas pair-fed mice gained an average of 4.5 g and mice fed *ad libitum* gained 5.7 g during the same interval). By Day 7 the tumor cell population in the zinc-deficient mice was only 28% of

[1] A zinc-deficient diet purchased from Nutritional Biochemicals failed to produce clinical zinc deficiency in our mice. Analysis disclosed that this diet contained 9.0 ppm zinc.

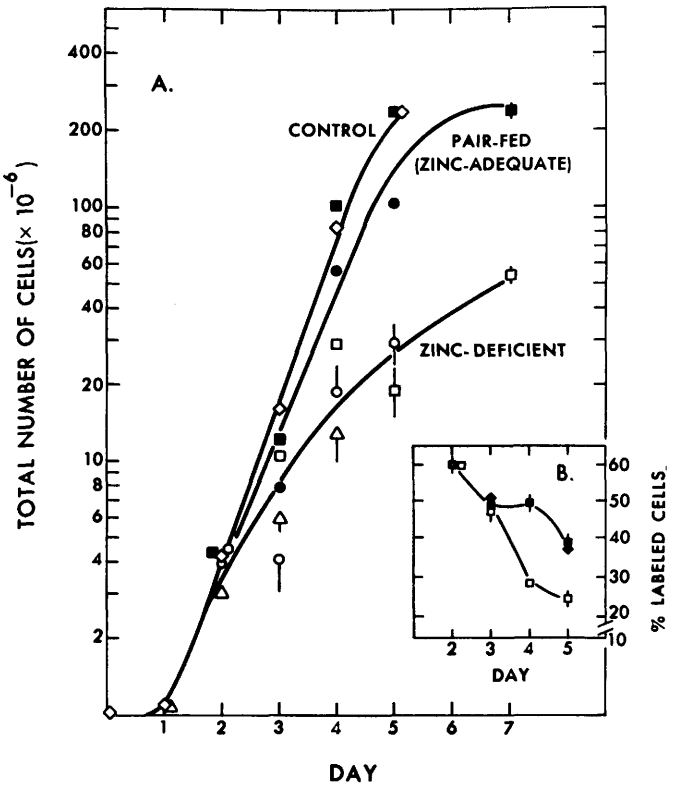

FIG. 1A. Population size and (B) proportion of cells labeled by a pulse of ^{3}H-thymidine during growth of the P388 leukemia in control, zinc-deficient, and pair-fed weanling DBA/2 mice. Animals were maintained on the appropriate diet (see text) 17 to 24 days before inoculation of 10^6 tumor cells. Tritiated thymidine (1 μCi/g, 17.3 Ci/mmole) was injected ip 30 min before sacrifice. Each symbol represents a separate experiment (three to six mice per point), with control (◇), pair-fed (●, ■), and zinc-deficient (○, △, □) animals from each experiment identified separately. The total number of cells was determined by washing the peritoneal cavity repeatedly with a known volume of heparinized saline and counting the wash fluid with a Model B Coulter counter. Nontumor cells were not included in the counts.

that in pair-fed controls, a reduction which prolonged the life of the animals by about 1 day. In a separate experiment, control mice inoculated with 10^6 cells died on Day 7 (10 of 10), whereas zinc-deficient animals died on Day 7 (2 of 10), Day 8 (6 of 10), and Day 9 (2 of 10).

Several attempts were made to elicit an earlier onset of the growth-retarding effect of zinc deficiency: (a) the tumor was grown for 7 days in zinc-deficient mice and then transplanted to other zinc-deficient mice, in an attempt to deplete the cellular zinc pool; (b) 4-day rather than 7-day tumors were used as donors, on the hypothesis that zinc deficiency affected only cycling cells and that the lack of effect in the first 3 days reflected the lag period before the increase in the tumor's growth fraction, as described elsewhere (11); (c) the period of zinc deficiency was increased from 17 to 24 days; and (d) the inoculum was reduced to 10^4 cells in order to prolong the life of the mice and allow more time for the zinc deficiency effect to develop. None of these treatments substantially altered the results shown in Fig. 1, suggesting that these mice have a small but nondepletable zinc pool on which the tumor cells can draw for the first few days of growth.

Autoradiographic analysis indicated that zinc deficiency causes a precipitous drop in

the proportion of P388 cells synthesizing DNA (*i.e.*, the proportion of cells in S phase) between Days 3 and 4 (Fig. 1B). This effect coincides with the onset of the depression in the growth curve. Analysis of grain counts over labeled tumor cells collected from zinc-deficient mice indicated that the rate of DNA synthesis is unaltered in these cells and that only the fraction of cells synthesizing DNA is depressed. Preliminary analysis of cellular DNA content supports the view that zinc deficiency causes an accumulation of tumor cells in G_1 *in vivo,* as is the case *in vitro* (4).

Attempts to reverse the growth-retarding effect of zinc deficiency by providing the animals with water containing 670 ppm zinc on Day 5 had limited success. Although this treatment does cause the ^{3}H-thymidine labeling index to rise within a few hours, both the extent and the time course of the increase were extremely variable.

In other experiments, we attempted to influence tumor growth by providing drinking water containing 3% EDTA, which increases the urinary loss of zinc in mammals. Although EDTA depressed tumor growth by 50% after the third day, pair-fed controls and controls drinking 3% EDTA plus an excess of zinc showed the same effect. Further investigation disclosed that the daily food and water consumption of mice given 3% EDTA was only one-third to one-half that of *ad libitum*-fed mice drinking tap water, causing a marked loss of carcass weight. We conclude that the effect of EDTA on P388 tumor growth is caused by caloric restriction or by some other nonspecific effect and is not ascribable to zinc deficiency.

Discussion. Dietary zinc deficiency retards the growth of the P388 ascites tumor beginning on about the third day after inoculation of 10^6 cells (Fig. 1). This effect, though highly significant, is not as dramatic as the almost complete stoppage of solid tumor growth observed in zinc-deficient rats (7). The available data indicate that zinc deficiency prevents initiation of DNA synthesis by some cells in the tumor but does not affect the rate of DNA synthesis by the cells that do succeed in entering S. This conclusion is consistent with the results of other studies (4–6, 9), although in some of these the data do not permit distinction between an effect on the proportion of cells in S phase and an affect on the rate of DNA synthesis *per se.*

The mechanism(s) by which zinc deficiency depresses tumor growth and causes cells to accumulate in G_1 are unknown. Zinc is necessary for the functioning of many metallo-enzymes (12) and zinc deficiency might impair the function of enzymes required for initiation of DNA synthesis. This possibility is supported by the recent discovery that DNA polymerase and RNA polymerase both contain zinc (13, 14) and by experiments that demonstrate directly that zinc plays an important role in the initiation of S phase (9, 15, 16). Other investigators favor the explanation that zinc deficiency leads to deficient RNA synthesis (1, 2) or to increased RNA catabolism (17).

Whatever the mechanism, zinc deficiency should be a useful means of manipulating tumor growth fractions *in vivo.* This manipulation would be of great value in studies of the relationship between tumor growth fractions and response to X-irradiation or chemotherapeutic drugs, for example. In view of our results, however, future studies should probably be conducted with rat tumors, since rats become zinc deficient when fed a diet containing less than 12 ppm zinc (18), whereas mice do not show clinical zinc deficiency until the level of dietary zinc is much lower, less than 2.5 ppm (19).

Summary. The growth of P388 leukemia cells as an ascites tumor is markedly depressed in mice fed a zinc-deficient diet. Tritiated-thymidine labeling data suggest that zinc-deficient tumor cells accumulate in G_1.

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