Inhibition of Walker 256 Carcinosarcoma Growth by Dietary Zinc Deficiency[1] (34978)

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(Introduced by Thomas C. Hall)

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Recently Dormandy and his co-workers have drawn attention to a possible relationship between growth of a neoplasm and zinc metabolism (1). They reported decreased plasma levels in patients with cancer, thus confirming earlier observations of decreased whole blood zinc levels in cancer patients (2). Since zinc is required for DNA (3–5) and protein (6) synthesis and tumors often have a high rate of DNA and protein synthesis, decreased plasma zinc levels in the human host may reflect high zinc requirements of a tumor. To investigate further the zinc requirements of neoplastic growth, the effect of dietary zinc deficiency on tumor growth was studied in rats using a transplantable tumor.

Materials and Methods. Weanling Sprague-Dawley male rats[3] ranging in weight from 37–47 g were housed in individual stainless-steel suspension cages. Laboratory chow, or a zinc-deficient synthetic diet based on dried egg white (7), was provided in glass feed cups with stainless-steel tops. Tap water, deionized distilled water, or deionized distilled water supplemented with 50 ppm zinc ion was supplied *ad libitum* in clear glass bottles with Neoprene stoppers and stainless-steel spouts.

The experimental animals were divided into 25 weight-matched sets of four and each member of a set was assigned to one of four experimental groups. A chow group received Purina Laboratory Chow *ad libitum* and tap water. A zinc-deficient group received the zinc-deficient synthetic diet *ad libitum* and deionized distilled water. A supplemented, pair-fed group received the zinc-deficient diet in the amounts eaten by the zinc-deficient member of each set plus water supplemented with zinc. A supplemented, weight-matched group received the zinc-deficient diet in amounts which resulted in weight change matching the zinc-deficient member of each set plus water supplemented with zinc.

On the eighth day of the assigned diet each rat was injected in the right hind leg muscles with 2×10^6 Walker 256 carcinosarcoma cells in 0.2 ml of Hanks' balanced salt solution prepared by mincing tumor fragments through a cytosieve (7). Daily each rat was weighed, the anteroposterior and mediolateral tumor diameters were measured with calipers, and food consumption was determined. Differences in survival were tested for significant using the Mann-Whitney U test.

Results. The measurements made on the four groups are presented graphically in Figs. 1–4 and numerically in Table I. As indicated in Fig. 1, a trend toward shortened survival was noted in the two groups receiving a restricted caloric intake but this difference was not statistically significant when compared with the Laboratory Chow group. In contrast, the zinc-deficient group showed a marked increase in survival (median survival 46.0 days vs 18.5 days for chow group) and this survival was significantly different from each of the other three groups ($p \leq 0.001$).

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[3] Purchased from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

TABLE I. Effect of Zinc Deficiency on the Number of Takes and Regressions.

Group	Takes[a]	Regressions	Deaths before day 60		Alive day 60	
			With tumor	Without tumor	With tumor	Without tumor
Laboratory chow	24/24	1	23	0	0	1
Zinc-deficient	17/25	3	12	6	2	5
Zinc-supplemented, pf[b]	25/25	2	23	0	0	2
Zinc-supplemented, wm[c]	25/25	0	25	0	0	0

[a] Numbers indicated as number of takes over number in group. One member of Laboratory Chow group was accidentally killed before tumor implantation.

[b] Pair-fed amount eaten by zinc-deficient rat within each set.

[c] Weight-matched group had food restricted to match the weight of zinc-deficient member of set.

The carcass growth rate (Fig. 2) of the zinc-deficient and the weight-matched groups was similar over the first 13 days of tumor growth. The tendency of the weight-matched group to deviate upward from the zinc-deficient group after day 13 was a reflection of earlier deaths for the most severely caloric-restricted rats in the weight-matched group. For example, in the zinc-deficient group on day 14, 16 rats weighed less than 75 g and 11 of their weight-matched partners were dead, while eight zinc-deficient rats weighed 75 g or more and only two of their weight-matched partners were dead. The reduced carcass growth in the pair-fed group was a consequence of the reduced caloric intake of pair feeding. The difference in growth rate between the pair-fed group and the zinc-

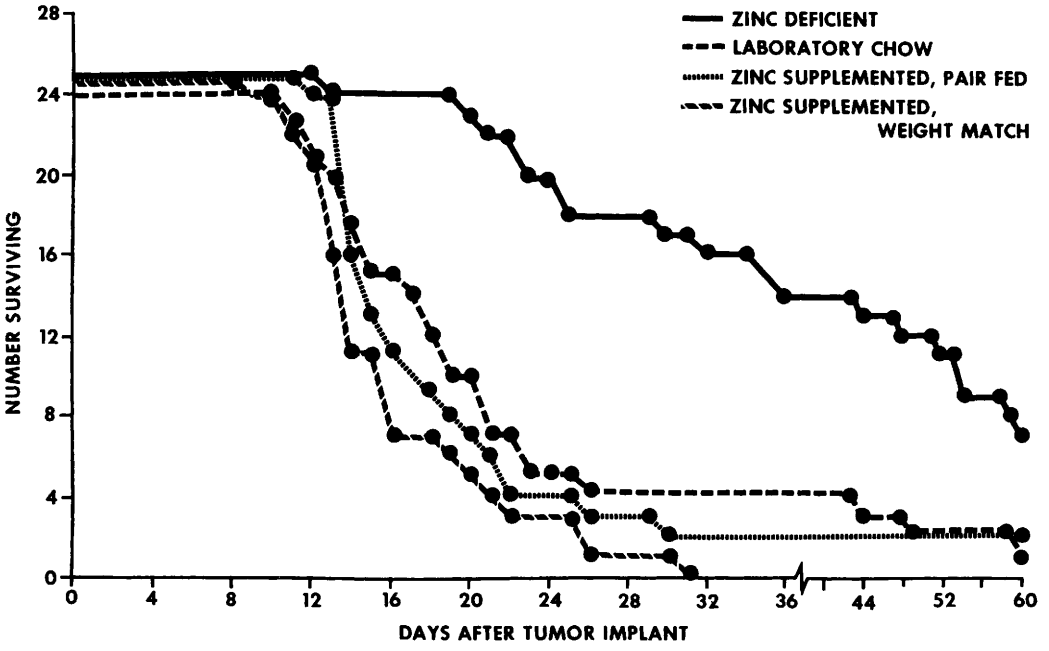

FIG. 1. Survival response for tumor-bearing rats receiving a zinc-deficient diet compared with several control groups. The difference in survival between the zinc-deficient group and the other groups is highly significant ($p \leqq 0.001$).

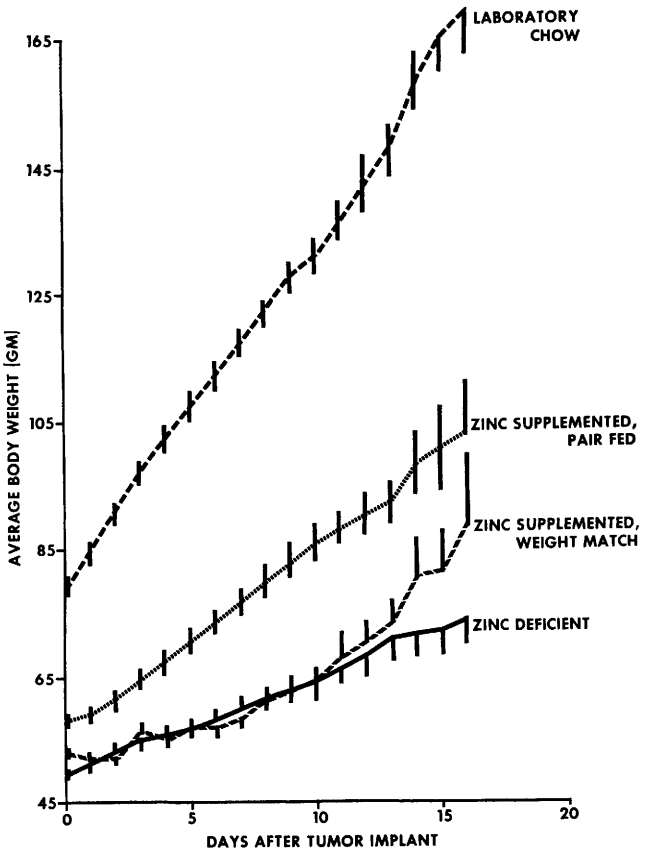

FIG. 2. Effects of zinc deficiency, pair feeding, and weight matching on carcass growth. Vertical bars in this figure and Fig. 3 represent ± 1 standard error of the mean. (Standard error bars projected in one direction only in certain instances for clarity of presentation.) Growth of the weight-matched group was essentially similar to that of the zinc-deficient group until after day 13 (see text).

deficient group probably reflects the inefficiency of food utilization with zinc deficiency (8). This inefficiency of food utilization was also noted by comparing the amount of food used for weight matching and that eaten by the zinc-deficient group. During the first 10 days of tumor growth the amount of food used for weight matching was 76% of that eaten by the zinc-deficient group.

The tumor growth data (Fig. 3) showed slight reduction in tumor growth in the pair-fed and weight-matched groups, and a marked reduction in tumor growth in the zinc-deficient group. The reduced tumor growth in the pair-fed and weight-matched groups was consistent with their respective degrees of caloric restriction. The pattern of tumor growth in the zinc-deficient group was clearly different from that in the group receiving an equal caloric intake or the group having an equal rate of carcass growth (Fig. 3). This difference was further illustrated in Fig. 4 which presents individual data of tumor size and body weight for the zinc-deficient and weight-matched groups on day 10 after tumor implant. Within each group there was a trend toward increased tumor size with increased body weight but the difference in tumor size between the two groups persists over the entire range of body weight.

An additional point of interest in this study was the possible evidence for immunologic reactivity in the zinc-deficient group as shown by the number of takes and regression

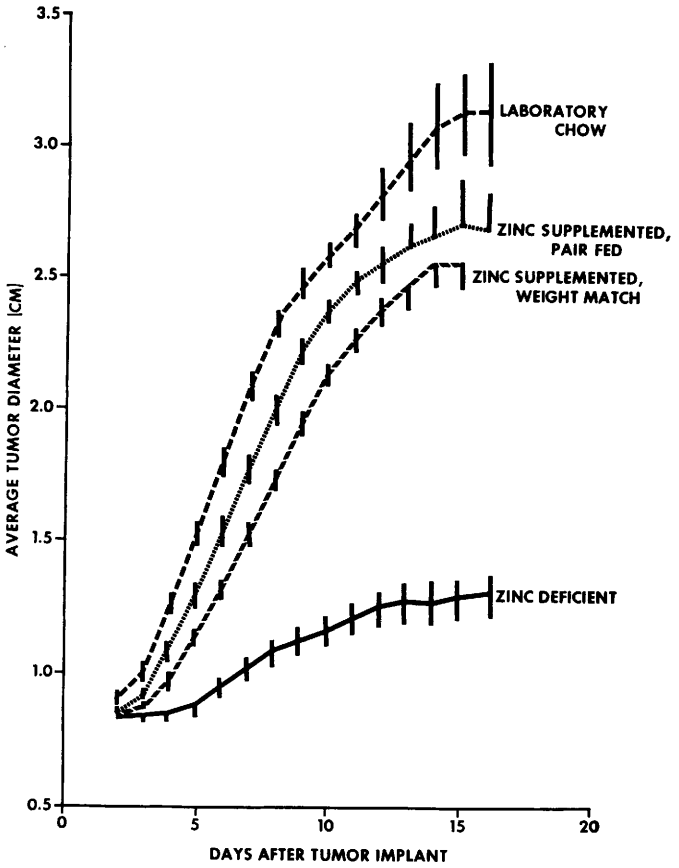

FIG. 3. Effect of zinc deficiency, pair feeding, or weight matching on tumor growth. A striking difference in tumor growth was noted between the zinc-deficient and the other three groups.

of tumor as presented in Table I. There were eight "no takes" in 25 rats in the zinc-deficient group compared with zero "no takes" in the 74 rats of the other three groups. Of the eight "no takes," all but one of the rats survived more than 23 days and three survived more than 60 days providing sufficient time for development of the tumor. Growth and then regression was noted in three of the 17 tumors that developed in the zinc-deficient rats compared with three regressions among 74 tumors in the other three groups. A total of 11 of the 25 rats in the zinc-deficient group were free of tumor at the time of the last observation, six at the time of death, and five at the time of sacrifice on day 60. In contrast, only three of the 74 rats in the other three groups were free of tumor at the time of the last observation.

Discussion. Tumor growth may be reduced by nonspecific factors, such as reduced caloric intake, or by specific factors, such as the effect of an antitumor agent. In a previous study, reduced tumor growth was observed in rats receiving a zinc-deficient diet (7). However, in that study the animals also showed a decreased carcass growth rate and reduced caloric intake and this reduced caloric intake would be a factor in the observed reduced tumor growth (9). The conclusion of reduced tumor growth as an effect of zinc deficiency in that study, therefore, depended on an indirect mathematical analysis of the relationship between reduced carcass growth and reduced tumor growth (7).

In the present study the specific effect of zinc deficiency may be assessed by direct comparison of the zinc-deficient group with

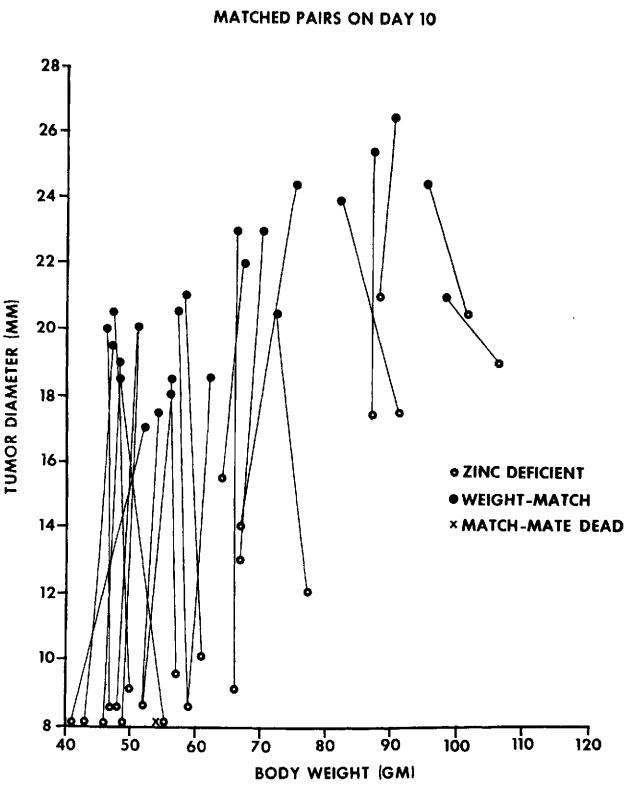

FIG. 4. Comparison of tumor size and body weight of the zinc-deficient and weight-matched rats. Points represent values of individual rats on day 10 with vertical lines connecting matched pairs. A definite difference between the tumor size of the two groups was noted.

the zinc-supplemented pair-fed and weight-matched groups. A striking difference in tumor growth was observed between the zinc-deficient group compared with the group receiving an equal caloric intake (pair-fed) or the group showing an equivalent rate of body growth (weight-matched). This was seen in comparison of group averages (Fig. 3) or in comparison of individual animal data (Fig. 4). This study, then, leads to the conclusion that tumor inhibition is a direct specific effect of zinc deficiency. The mechanism of this tumor inhibition by zinc deficiency is not clear. Zinc is known to be important in a wide range of cellular functions but the exact biochemical locus of importance in inhibition of cell growth in zinc deficiency has proved elusive (8).

Adverse effects of zinc deficiency were observed in many of the rats in the zinc-deficient group, including coarseness and loss of hair, dermatitis and scaling of skin, and erratic appetite (10). Since it is known that the severity of these adverse effects is linearly related to the degree of zinc deficiency (8), further study is needed to determine whether at an intermediate level of zinc deficiency antitumor effects can be separated from adverse effects.

These results of a requirement for zinc by tumor growth suggest a possible explanation for the reported decreased levels of zinc in plasma of cancer patients (1). Plasma zinc may be decreased because the element is selectively used by the neoplasm. The present results suggest that decreased levels of zinc may inhibit tumor growth and suggest the need for further clinical study to attempt to correlate tumor growth rate with plasma zinc levels.

The evidence for immunolgic reactivity against the Walker tumor by the zinc-

deficient group is of special interest. Although the present study did not provide a measure of the degree of immunologic reactivity, it does provide an indication that immunologic reactivity may persist in the presence of zinc deficiency. Further studies are in progress to measure the degree of immunologic reactivity in animals on a zinc-deficient diet.

Summary. The zinc requirements of neoplastic growth were evaluated by comparing growth of a transplanted tumor (Walker 256 carcinosarcoma) in rats on a zinc-deficient diet with growth of this tumor in pair-fed or weight-matched controls. Rats receiving a zinc-deficient diet showed marked reduction of tumor growth and this was accompanied by a striking increase in survival. Rats in the pair-fed or weight-matched groups showed a slight reduction in tumor growth compared with *ad libitum*-fed controls but this caloric restriction did not increase survival. These results are consistent with a zinc requirement for tumor growth and suggest the need for study of clinical correlations between tumor growth rate and plasma zinc levels.

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