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Changes in Serum, Liver, and Tumor Zinc Levels during Plasmacytoma Growth in BALB/c Mice (41662)

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Abstract. The effect of a zinc-deficient diet on serum and liver zinc levels was studied in BALB/c mice in the presence and absence of the IgM-secreting plasmacytoma, TEPC-183. While serum zinc levels were significantly decreased in the zinc-deficient (ZD) mice by Week 2, there was no change in the level of zinc in the liver of this group compared to *ad libitum* (AL) or pair-fed (PF) controls even after 4 weeks on the respective diets. The presence of the tumor itself resulted in a significant decrease in serum zinc levels in mice maintained on a normal-chow diet. This decrease was not seen in the serum from tumor-bearing AL or PF control mice which were fed a synthetic diet containing 50 ppm zinc. There was, however, an increase in the liver weights of mice in these groups in the presence of TEPC-183 which was not seen in the ZD tumor-bearing mice. Although total zinc levels increased in the livers of AL and PF mice reflecting this increase in liver weight, there was no difference among the groups when the amount of zinc was determined per gram of liver tissue. There was also no difference in the amount of zinc per gram of tumor tissue in the tumors obtained from AL, PF, or ZD mice.

Alterations in serum and plasma zinc levels have been reported to occur as a consequence of tumor growth in the host. However, the changes which occur are often conflicting and may depend upon the zinc requirement of the particular tumor compared to normal tissue or whether the tissue in which the tumor develops has a relatively high zinc content (1). For example, a significant elevation of serum zinc was found in patients with basal-cell carcinoma (2), malignant tumor of the uterus (3), and primary osteosarcoma without metastases (4). Decreased zinc levels have been observed in the serum of patients with non-Hodgkin's lymphoma (5), lung cancer (6), and in the plasma of children with untreated leukemia (7). Zinc levels in the normal range have been reported in cases of multiple myeloma (8) and in bronchogenic carcinoma without metastases (9).

Changes seen in extracellular zinc levels could also be due to the effect of tumor growth on the metabolism of the liver of the host. Studies in experimental animals have shown that even the presence of small tumors lead to alterations in the metabolic capabilities of the host liver (10–12). Furthermore the liver has a major role in zinc metabolism. There is an influx of zinc into the liver during the early stages of the inflammatory response (13) and the liver is the site of synthesis of circulating zinc-binding proteins such as albumin and α2-macroglobulin a zinc-containing protein (14). Griffith *et al.* have reported an increase in liver zinc in patients with carcinoma (15). This increase was confined to portions of the liver which showed no signs of carcinomatous invasion; whereas malignant deposits contained lower amounts of zinc than normal liver tissue.

In tumor systems which have been studied in experimental animals, there are also conflicting reports. In tumor-bearing rats the serum level of zinc decreased and liver zinc increased compared to controls (16). Minkel *et al.* (17) found that liver zinc was increased in mice bearing Ehrlich ascites tumor when the diet contained sufficient zinc. Other studies have reported a hypozincemia in various mouse tumors when the tumors weighed 2–3 g (18). Liver zinc levels, however, were in the normal range. In the rat the presence of a Walker 256/M1 tumor had no effect on zinc levels in the liver, but plasma levels were decreased 40 to 60% as a function of tumor growth (19).

These conflicting reports indicate that the host response varies depending on the tumor system and point out the need to examine additional systems to gain further understanding of the relationship of zinc to tumor growth. In the present study we have examined

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how the growth of an IgM-secreting plasmacytoma, TEPC-183, affects serum and liver zinc levels in BALB/c mice. We have also determined zinc levels in the tumor itself at various stages of tumor growth. Finally, we have looked at the effect that a dietary zinc deficiency has on serum, liver, and tumor zinc levels.

Materials and Methods. *Mice.* Female BALB/c mice (15–18 g) were obtained from Dominion Labs, McLean, Virginia.

Dietary treatment. Four groups of mice were used in this study. Normal-chow (NC) controls were fed normal laboratory chow (Ralston Purina) and tap water *ad libitum.* The normal chow was assayed and found to contain 56 μg zinc/g. *Ad libitum* (AL), pair-fed (PF), and zinc-deficient (ZD) mice were housed in individual polyethylene cages fitted with stainless-steel covers and wood-chip bedding. ZD mice were fed a synthetic zinc-deficient diet containing (0.05 μg zinc/g). The composition of the diet is shown in Table I. They were given glass-distilled water from bottles fitted with polyethylene caps and stainless-steel sipper tubes. AL animals were given a synthetic control diet to which zinc carbonate was added to give 50 μg zinc/g and tap water *ad libitum.* PF mice were given the synthetic control diet in an amount equal to that of a corresponding zinc-deficient animal and tap water *ad libitum.* Animals were weighed weekly.

TABLE I. COMPOSITION OF ZINC-DEFICIENT DIET[a,b]

Component	Concentration (g/kg)
Egg white solids, spray dried	200.0
Dextrose, hydrate, technical	636.49838
Corn oil	100.0
Nonnutritive fiber (cellulose)	30.0
Mineral mix[c]	31.3
Vitamin mix[d]	0.308
Choline chloride	1.5
Vitamin B_{12}	0.02
Chlortetracycline HCl	0.39

[a] In the control diet, zinc carbonate ($ZnCO_3$) is added at a level of 0.0892857 g/kg of diet and the dextrose is reduced to 636.4090943 g/kg of diet (adds 50 ppm zinc).

[b] Reference: Luecke RW, Olman ME, Baltzer, BV. J. Nutr **94**:344, 1968.

[c] Composition of mineral mix (grams): NaCl, 5.6; K_2HPO_4, 10.7; $CaHPO_4$, 2.5; $MgSO_4$, 1.7; $CaCO_3$, 9.9; Fe-citrate, 0.91; KI, 0.03; $MnSO_4 \cdot H_2O$, 0.009; $CuSO_4 \cdot 5H_2O$, 0.01; $CoCl_2 \cdot 6H_2O$, 0.002.

[d] Composition of vitamin mix (grams): Biotin, 0.004; calcium pantothenate, 0.02; folic acid, 0.0005; niacin, 0.025; pyridoxine–HCl, 0.004; riboflavin, 0.006; thiamin–HCl, 0.01; Vitamin A palmitate (500,000 U/g), 0.02; Vitamin D_2 (500,000 U/g), 0.003; Vitamin E acetate, 0.22; menadione, 0.003.

Experimental design. In the first experiment mice were divided into three groups (NC, PF, or ZD) and maintained on the appropriate diet for 2, 3, or 4 weeks. Mice were bled by cardiac puncture and then sacrificed by cervical dislocation. Livers were excised and prepared for zinc analysis.

In the tumor studies, mice were maintained on either AL, PF, ZD, or NC diets for 3 weeks. Animals from each group then received 0.25 ml of a TEPC cell suspension subcutaneously on the back. The cells were suspended in minimal essential media (MEM) (GIBCO), pH 7.4, and adjusted to a concentration of 0.5×10^7 cells/ml.

Tumor transfer. TEPC-183, an IgM (k)-secreting plasmacytoma, was obtained from Dr. H. Francis Havas, Temple University School of Medicine. It was maintained by subcutaneous transfer into BALB/c mice fed normal chow. Tumors approximately 1.5 cm in size were excised, minced, and passed through a plastic strainer. Cells were suspended in minimal essential media (GIBCO), pH 7.4, and adjusted to a concentration of 0.5×10^7 cells/ml. Animals received 0.25 ml of the tumor cell suspension subcutaneously on the back.

Serum preparation. Blood obtained by cardiac puncture was allowed to stand for 30 min at room temperature followed by centrifugation at 900*g* for 20 min to obtain serum. Serum was assayed immediately for zinc concentration or frozen at −20°C. Hemolyzed samples were discarded.

Zinc analyses. Zinc determinations were done using a Varian AA-5 atomic absorption spectrophotometer with an air–acetylene flame. Serum samples were diluted 1/4 in 0.1 *N* HCl (Baker analyzed). Livers and tumors were excised, rinsed in sterile saline, patted dry with Kimwipes, and weighed. Samples ranged from 0.4 to 2.7 g. The tissues were placed in 25-ml Erlenmeyer flasks and ashed following the procedure of Kang *et al.* (20). To each flask was added 6 ml glass-distilled water, 3 ml nitric acid, and 3 ml perchloric acid. Samples were allowed to solubilize at

90°C and then the temperature was increased to 150°C until the acids boiled off and the volume was reduced to approximately 0.5 ml. Samples were diluted appropriately in glass-distilled water for analysis. A reagent blank was treated in the same manner.

Glassware and reagents. Disposable plasticware and pipets were used whenever possible. All glassware were soaked at least 3 hr in 10% nitric acid, rinsed in 1 m*M* EDTA, followed by extensive rinsing in glass-distilled water. All reagents were checked for zinc levels.

Statistics. Results are expressed as arithmetic means ± standard deviation (SD). Mean values were compared by a Student's *t* test.

Results. The effect of various dietary regimens on body weights, liver weights, and serum and liver zinc levels at 2, 3, or 4 weeks is seen in Table II. At Week 2 a decrease in serum zinc levels was observed in the ZD group which was significant when compared to values found in NC or PF controls. At Week 3 there was a further reduction in serum zinc levels in the ZD mice which showed no additional decrease at Week 4. Liver weights were also reduced in the ZD mice compared to NC controls at Week 3 ($P < 0.005$). By Week 4 the reduction in liver weight was significant when compared with both NC and PF control groups ($P < 0.05$). By Week 4 the total body weights were also decreased in ZD mice compared to NC controls. The total amount of zinc found in the liver of ZD animals was reduced compared to NC animals at Weeks 3 and 4. This is a reflection of the decreased liver weights. When zinc concentration was calculated per gram of liver tissue there was no difference in liver zinc content among the groups at any of the times tested.

The effect of TEPC-183 on liver and serum zinc content is seen in Table III. Mice maintained on normal chow were sacrificed on Day 7 after tumor injection when tumors were classified as small (<5% of body weight) or on Day 11 when tumors were classified as large (>15% of body weight). Compared to normal controls, there was a decrease in serum zinc levels in the presence of tumor which was significant even in the presence of tumors which were less than 5% of the body weight ($P < 0.01$). The total content of zinc in the liver was not affected by the presence of tumor. There was, however, a significant increase in liver zinc concentration in the group with small tumors when calculated per gram of liver tissue. The total zinc in the tumor increased with increasing tumor size but was not significantly different in large or small tumors when calculated on the basis of a gram of tumor tissue. Based on a gram of wet tissue weight the amount of zinc in the tumor was somewhat less than that found in the liver.

To determine the effect if any that a zinc-deficient diet would have on tumor zinc levels, animals were maintained on AL, PF, and ZD

TABLE II. EFFECT OF ZINC NUTRITION ON BODY WEIGHTS, LIVER WEIGHTS, AND SERUM LIVER ZINC LEVELS[a]

Group	Time on diet (weeks)	Body weight (g)	Serum zinc (μg/dl)	Liver weight (g)	Liver zinc	
					Total (μg)	μg Zn/g wet wt
NC (10)	2	18.8 ± 1.1	101.8 ± 23.7	1.22 ± 0.16	30.6 ± 3.5	26.7 ± 2.4
PF (10)	2	17.7 ± 1.9	147.0 ± 51.4	1.14 ± 0.18	26.2 ± 4.0	24.1 ± 4.6
ZD (10)	2	19.1 ± 1.2	77.2 ± 16.9[c]	1.11 ± 0.19	28.4 ± 7.0	24.8 ± 4.5
NC (10)	3	18.9 ± 2.9	87.0 ± 16.8	1.17 ± 0.18	29.9 ± 4.7	25.5 ± 3.4
PF (10)	3	18.9 ± 1.7	91.6 ± 2.4	1.04 ± 0.22	23.2 ± 4.0	22.3 ± 4.3
ZD (10)	3	19.2 ± 1.6	44.0 ± 8.5[c]	1.00 ± 0.10[d]	25.1 ± 3.7	24.0 ± 3.5
NC (10)	4	18.8 ± 1.7	94.4 ± 1.3	1.16 ± 0.13	30.3 ± 3.5	25.1 ± 3.4
PF (10)	4	17.6 ± 1.2	123.4 ± 30.2	1.00 ± 0.15	26.4 ± 3.5	27.2 ± 3.7
ZD (9)	4	16.4 ± 1.6[b]	41.7 ± 5.1[c]	0.82 ± 0.19[c]	24.4 ± 4.3	27.2 ± 3.5

[a] Results expressed as mean ± SD; number in parentheses represents number of animals per group.
[b] $P < 0.01$ compared to NC.
[c] $P < 0.05$ compared to PF and NC controls.
[d] $P < 0.005$ compared to NC.

TABLE III. THE EFFECT OF TEPC-183 ON SERUM AND LIVER ZINC LEVELS IN MICE FED NORMAL CHOW[a]

Group	Relative tumor weight (%)[b]	Serum zinc (μg/dl)	Liver zinc		Tumor zinc	
			Total (μg)	μg/g liver	Total (μg)	μg/g tumor
Control (10)	—	101.8 ± 23.7	30.6 ± 3.5	26.7 ± 2.4	—	—
Small tumor (6)	3.3 ± 0.6	68.9 ± 27.4[c]	27.2 ± 4.0	35.1 ± 6.0[d]	11.0 ± 1.1	23.6 ± 4.2
Large tumor (6)	18.6 ± 1.9	31.0 ± 5.4[d]	28.5 ± 7.8	28.6 ± 5.7	48.2 ± 5.7	20.5 ± 1.8

[a] Mice in each group were fed normal chow. Results are expressed as mean ± SD. Numbers in parentheses represents number of animals per group.

[b] Relative tumor weight is calculated as percentage of body weight. Mean body weight for mice with small tumors was 14.58 ± 1.11 g; for mice with large tumors 13.06 ± 2.12 g.

[c] $P < 0.01$ compared to control.

[d] $P < 0.001$ compared to control.

diets for 3 weeks. At this time all mice were injected with a TEPC-183 cell suspension and sacrificed 1, 2, and 5 days later. Mice were also sacrificed when tumors were classified as small (<5% of body weight), medium (6–10% of body weight), and large (11–15% of body weight). On Days 1, 2, and 5 following tumor injection, serum and liver zinc levels did not change significantly from values found in mice maintained on the respective diets for 3 weeks. The total liver zinc content and zinc concentration per milligram of protein did not differ among the groups. The serum zinc level was decreased in the ZD mice corresponding to the decrease found after 3 weeks of dietary zinc restriction. The serum zinc level in this group then remained fairly constant throughout the remainder of the experiment (Fig. 1). The mean serum zinc levels of the AL and PF control mice were in the normal range and

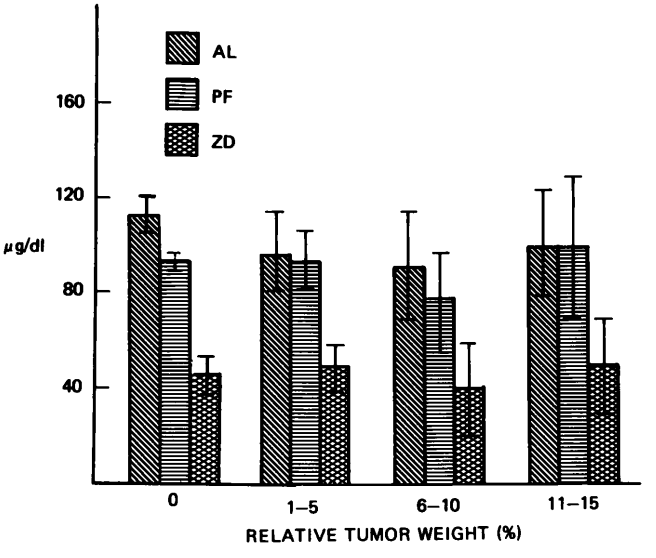

FIG. 1. Mean serum zinc levels expressed in micrograms per deciliter ± SD from mice at various stages of tumor growth. Mice maintained on *ad lib* (AL), pair fed (PF), or zinc-deficient (ZD) diets for 3 weeks were injected with TEPC-183 cell suspension sc. Mice were bled by cardiac puncture when tumors were 1–5%, 6–10%, or 11–15% of body weight. Values at 0% relative tumor weight are levels after 3 weeks on diet.

were relatively constant throughout the course of the experiment and did not decrease even in the presence of large tumors.

The results presented in Table IV represent the liver weights and body weights of the mice maintained on the various dietary regimens in the presence of small, medium, and large tumors. There is no significant change in body weights among the groups regardless of tumor size. There is, however, an increase in liver weights in the AL and PF controls which is not seen in the ZD mice. This change is significant when tumors are classified as medium or large. As a result there was also an increase in the total amount of zinc in the livers of both PF and AL control mice in the presence of small, medium and large tumors (Fig. 2). However, when zinc levels were calculated per gram of liver tissue there was no change among the groups and zinc levels remained quite constant regardless of the presence of tumor. The total amount of zinc increased in the tumors obtained from all groups as the tumors increased in size (Fig. 3). There was, however, no difference in tumor zinc content when calculated per gram of tumor tissue. The results in Table IV also illustrate that the ZD diet is able to retard tumor growth. There is a significant increase in the time required for tumors to reach medium and large size in the ZD mice compared to controls.

Discussion. In the present study the relationship of dietary zinc levels to serum, liver, and tumor zinc content was examined in female BALB/c mice. Young adult mice were used (7–8 weeks of age) as older mice do not suffer the severe affects of zinc deficiency that are seen in younger animals. Similar observations have been reported in tumor-bearing zinc-deficient rats (19). Approximately 2 weeks on a zinc-deficient diet were required before the serum zinc levels were significantly decreased compared to controls. In mice maintained on a normal-chow diet, the presence of tumor alone was sufficient to decrease serum zinc concentration to a level comparable to that seen in mice maintained on a zinc-deficient diet for 3 weeks. Similar decreases in serum zinc levels have been reported with other tumor systems in mice (18) as well as rats (19). Saito *et al.* (16) have reported a temporary increase in serum zinc levels 2 to 3 days following tumor inoculation which is followed by a marked decrease throughout the remaining period of observation. We observed that in the AL and PF mice the serum levels were in the normal range and remained so throughout the experiment. Decreased serum levels were not seen in the control groups even when the tumor burden was 11–15% of the body weight. In contrast when mice were fed normal chow, tumors of less than 5% of the body weight caused a significant depression in serum zinc levels. In a study cited previously (18), the decreases in plasma zinc observed in mice did not occur until the tumor burden was approximately 20% of the body weight.

In the absence of tumor the liver zinc con-

TABLE IV. EFFECT OF TEPC-183 GROWTH ON LIVER AND TUMOR WEIGHTS OF MICE MAINTAINED ON *Ad Lib*, PAIR-FED, OR ZINC-DEFICIENT DIETS[a]

Tumor size	Group	Time after tumor injection (days)	Body weight (grams)	Liver weight (grams)
Small (0–5%)[b]	AL (9)	8.5 ± 1.4	22.01 ± 1.29	1.18 ± 0.15
	PF (8)	8.6 ± 2.9	22.48 ± 1.96	1.32 ± 0.21
	ZD (8)	10.8 ± 3.3	20.02 ± 2.54	1.06 ± 0.16
Medium (6–10%)	AL (12)	10.6 ± 0.9	20.10 ± 3.30	1.42 ± 0.26
	PF (8)	10.3 ± 2.3	21.05 ± 2.64	1.36 ± 0.32
	ZD (8)	13.6 ± 3.8[c]	17.33 ± 2.73	0.95 ± 0.20[d]
Large (11–15%)	AL (6)	13.3 ± 1.0	23.59 ± 0.77	1.77 ± 0.20
	PF (5)	14.0 ± 0	23.19 ± 1.96	1.82 ± 0.23
	ZD (5)	18.5 ± 4.1[c]	20.54 ± 2.00	1.06 ± 0.20[d]

[a] Values are mean ± SD. Numbers in parentheses represents numbers of animals per group.
[b] Tumor size is expressed as a percentage of body weight.
[c] $P < 0.05$ compared to AL and PF controls.
[d] $P < 0.01$ compared to AL and PF controls.

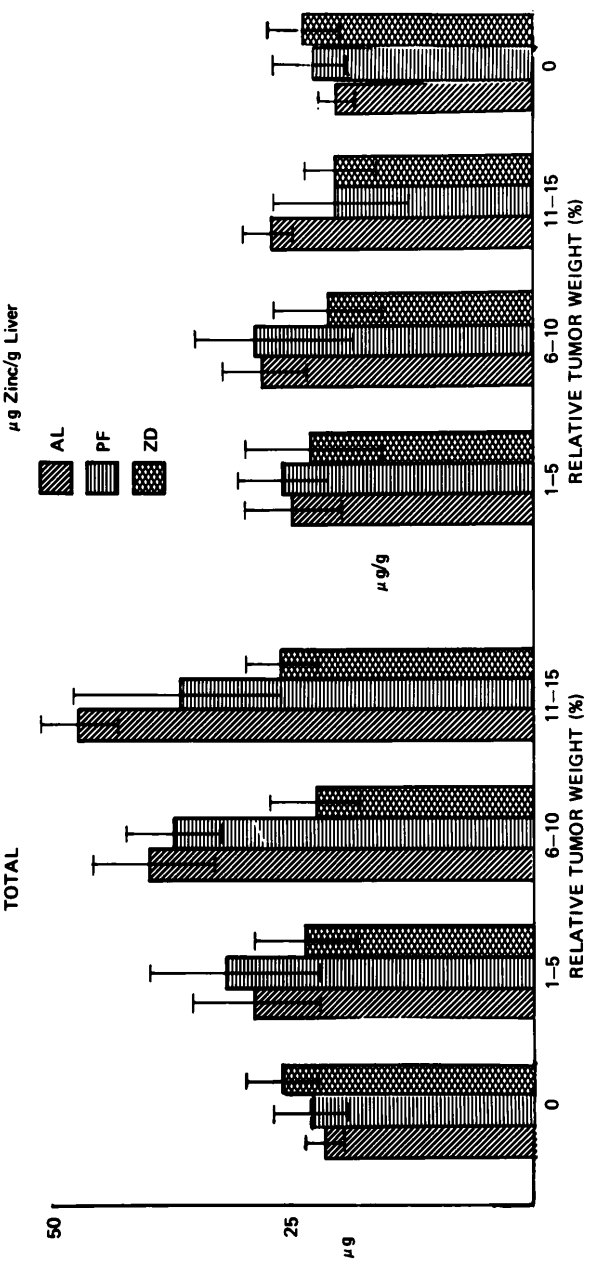

FIG. 2. Liver zinc levels were determined when tumors were 1–5%, 6–10%, or 11–15% of body weight. Results are shown as total liver zinc in micrograms and micrograms of zinc per gram of liver tissue (wet weight). Values at 0% relative tumor weight are levels after 3 weeks on diet prior to tumor injection.

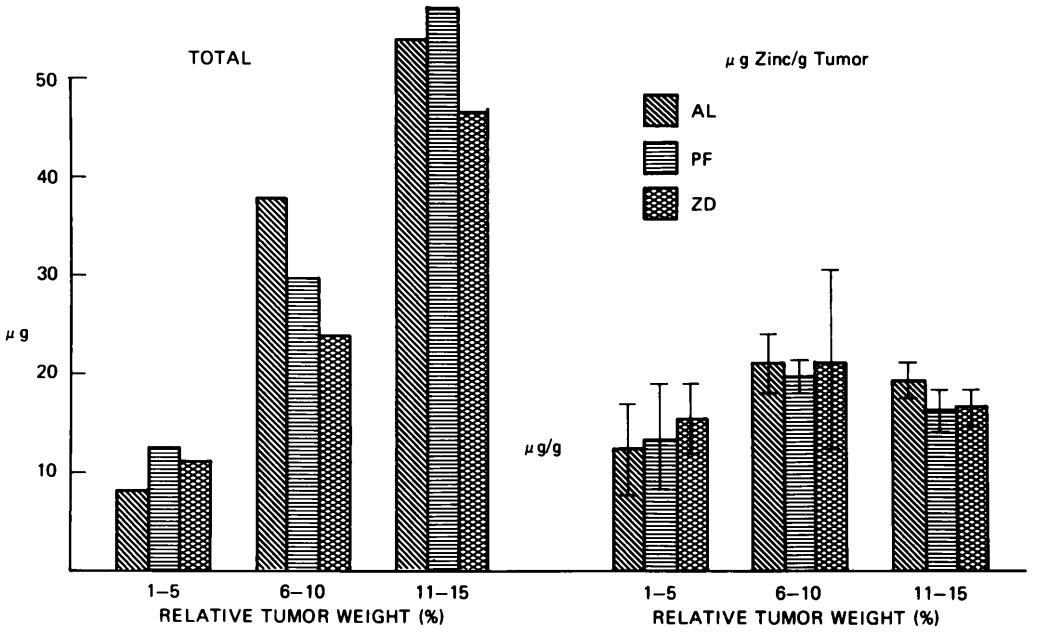

FIG. 3. Zinc levels were determined in TEPC-183 plasmacytomas at various stages of tumor growth. Results are shown as total zinc in the tumor in micrograms and micrograms zinc per gram of tumor (wet weight).

tent remained unchanged regardless of dietary regimen or length of time on the diet. This is in agreement with previous studies which have shown that soft tissue zinc levels remain unchanged even when serum zinc levels drop precipitously (21). In tumor-bearing mice fed a normal-chow diet there was no change in total liver zinc compared to controls. In studies in which the control mice were fed the synthetic diet and then injected with tumor the total liver zinc levels increased reflecting an increase in liver weight in these groups. Previous studies have shown that solid tumors but not ascites forms cause an increase in liver weight of tumor-bearing mice (11). Mills *et al.* (19) observed small but insignificant increases in liver weights of tumor-bearing rats on control diets. The liver zinc content in this group showed a similar increase. The liver zinc content in rats bearing Yoshida-Sarcoma when calculated per gram of liver dry weight increased compared to controls (16). Minkel *et al.* (17) found a significant decrease in liver zinc concentration of zinc-deficient mice in the presence of Ehrlich ascites tumor. These variable results are probably due not only to the fact that different tumor systems were used in each of these studies but also to the fact that there was a great deal of variability in the diets which were used. These results tend to indicate, however, that changes seen in serum zinc levels do not correlate with fluctuations in liver zinc levels. The decreased serum levels seen in tumor-bearing mice may merely reflect the requirements of the individual tumor systems for zinc. Zinc is required for cell growth and rapidly growing systems may have an increased requirement. In this experiment the level of zinc in the tumor remained quite constant even when the diet was deficient in zinc and serum zinc levels had dropped significantly. The concentration of zinc in Ehrlich ascites cells was quite constant regardless of dietary zinc levels (17). In contrast in tumor-bearing rats maintained on zinc-deficient diet the tumor zinc concentrations were decreased 23 to 37% (19). Again these differences may reflect differences in the diets which the animals were fed or the nature of the particular tumor system. It should be pointed out that in our studies the presence of tumor did not affect food consumption in the various groups. The changes seen in serum and liver zinc levels were due to the presence of the tumor itself and not alterations in dietary zinc intake.

In previous studies we have shown that the

growth of TEPC-183 is inhibited in mice maintained on a zinc-deficient diet (22). The zinc-deficient diet did not delay the time of tumor appearance or prolong the survival time of the ZD mice compared to controls. However, a significant difference was found in the number of animals that developed tumors. Of 43 ZD mice, 20 remained free of tumor at a dose that resulted in 40 to 45 takes in PF animals and 29 of 30 takes in the AL controls. It must be noted that in the present study the tumor cell suspension dose was adjusted to a concentration so that all of the mice in each group developed tumor. Under these conditions there was an increase in the time required for tumors to reach medium and large size in the ZD mice. We have also obtained preliminary evidence that a zinc-deficient diet results in an increase in *in vitro* lipid peroxidation in mitochondrial and microsomal membranes compared to controls (23). Based on the results reported here it appears that these changes are not the result of a decrease in the total zinc content in livers or tumors of zinc-deficient mice.

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