Effect of Chronic Zinc Intoxication on Copper Levels, Blood Formation and Polyamines

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Abstract

A twenty year old woman presented herself for routine examination after she had taken between 440 and 5700 mg elemental zinc or an average of 2300 mg every day for four months. The most striking laboratory findings were a serum zinc level of 7760 mcg/dl, a serum copper of 8 mcg/dl and undetectable ceruloplasmin. The patient was severly anemic and showed macrocytosis and neutropenia. Other abnormal parameters were a slightly elevated serum glucose, slightly depressed serum globulin and total protein, a very high alkaline phosphatase and low cholesterol. Polyamines were normal. Oral copper therapy and cessation of excess zinc produced remarkable reversal of all abnormal parameters beginning in the first week and virtual recovery of anemia and neutropenia by the fourth week post-treatment. Polyamine levels increased to between four and ten times normal during the period of accelerated blood formation, then reverted to normal. Macrocytosis rather than microcytosis in zinc toxicity or severe copper deficiency has been seen only in cases where generous supplements of vitamin C were supplied as in this case. There is a strong likelihood that the vitamin C fortuitously prevented even more severe anemia. The possible benefit of vitamin C and of zinc gluconate vs. zinc sulfate in zinc therapy is discussed. It appears that with proper monitoring, far larger doses of zinc than are now customary may be used if found to be therapeutically valuable.

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The need for zinc in human nutrition is now well established. In addition to its obvious use in zinc deficiency, zinc in larger than replacement levels is emerging as a potent therapeutic agent in many widely varying conditions (Prasad et al., 1963; Pories et al., 1967; Husain, 1969; Portnoy and Molokhia, 1972; Pfeiffer and Cott, 1974; Neldner and Hambidge, 1975; Brewer et al., 1977; Simkin, 1977). This ever expanding use of larger doses of zinc has created a need for more data on.human toxicity.

There have been numerous reports of acute toxicity from a single or short term exposure to high levels (as many as several grams) of zinc (Cowan, 1947; Brown et al., 1964; Murphy, 1970; Gallery et al., 1972; Chunn, 1973), and now patients being treated for sickle cell anemia (Prasad et al., 1978) or to promote wound healing (Pories et al., 1976) have provided data on the effects of extended exposure. However, the

maximum doses administered for a prolonged period have been 150 mg of elemental zinc daily.

Following is a case report of a 20 year old female seen by us after she had taken 400 to 5700 mg elemental zinc (from zinc gluconate) or an average 2300 mg every day for four months, an extraordinarily large and continued dose.

Case History

C.T. is a twenty year old female who, according to her parents, went through "an acute anxiety state" and brief period of psychosis at age 15, and spent three months in a psychiatric hospital at age 17. She was first brought to our Center in the middle of her freshman year at college and was diagnosed here as pyroluric, a vitamin B6 and zinc dependency condition indicated by kryptopyr-role in the urine (Pfeiffer et al., 1974). She responded very favorably to a multivitamin regimen which included daily 2000 mg B6 and 66 mg elemental zinc (30 mg from zinc gluconate and 36 mg from the sulfate). Much the same regimen was again recommended 10 months later when she was reexamined here on a follow-up visit. She faithfully adhered to the program another six months and then inexplicably stopped taking all pills 18 days before being brought home from college "completely psychotic and violent" according to her parents. Discouraged by the lack of results from their daughter's previous hospitalization, they decided to treat her at home. Since the small dose of zinc (66 mg) we had recommended before seemed beneficial, they tried increasing the dose, initially giving her between 600 and 900 mg/day of elemental zinc (Plus Products, 50 mg elemental zinc from zinc gluconate per tablet) in 200 mg doses every two hours. They did this for one week noting that she became "more manageable-improving 20 to 25 minutes after each zinc dose and worsening at the end of each two hours." The next two months they described as a "stormy period with high zinc doses" during which she was given between 1700 and 5600 mg (34 to 114 pills!) daily. She did vomit but only occasionally. With ignorance of zinc's well-known emetic effect, and also because she had been vomiting at the onset of this last psychotic episode, her parents did not implicate zinc. During the next 5 weeks they gradually reduced her zinc intake from 2400 mg to 400 mg/day at which time she was brought to our Center for a routine follow-up, 10 months after her previous examination. This visit was not prompted by any understanding that their daughter was suffering from zinc intoxication or indeed from anything unusual.

Methods

All hematologic determinations and diagnostic profiles on serum (SMAC 22 or Sequential Multiple Analysis) were performed by Diagnostic Sciences, Inc., Morristown, N.J.

Analyses of the serum for copper, zinc and iron were performed by atomic absorption spectrophotometry according to the method of Olson and Hamlin (1969). Erythrocyte copper and zinc levels were determined by atomic absorption spectroscopy on trichloroacetic acid extracts of whole blood using the serum zinc and copper levels, the hematocrit, and red blood cell count to calculate copper and zinc per RBC. This was found to be more reliable than direct determination of washed red cells.

Hair samples were washed several times in acetone and distilled water, dried at 100°C, and weighed. Hydrolysis was in concentrated nitric acid at room temperature then in nitric and perchloric acid, 6:1, at 200°C until white fumes evolved and the solution became clear. Appropriate dilutions were analyzed by atomic absorption spectrophotometry.

Histamine, spermine and spermidine were determined on whole blood by the method of Iliev et al (1967). Ceruloplasmin was determined by Boston Medical Laboratory.

Pretreatment Clinical and Laboratory Findings

C.T.'s skin pallor was so marked that it elicited comment from laboratory personnel unaware of her toxicity and probable

anemia.

On examination the patient was pleasant and answered questions freely. She had no thought disorder and only a mild degree of paranoia because, as she stated, "her sex drive had decreased." Physical examination disclosed the liver palpable and 1 cm below the costal margin. The spleen was not palpable. Blood pressure was 100/60, pulse 105. Skin and mucous membranes were pale and the forehead was covered with fine come dones. The patient was seen weekly thereafter in order to assess her recovery from the chronic zinc overdosage.

The most striking laboratory findings were a serum zinc of 1160 mcg/dl (normal, 112±24), a serum copper of 8 mcg/dl (normal, 107±24), and undetectable cerulo-plasmin. Serum iron was, surprisingly, a normal 101 mcg/dl. The patient was severely anemic with a red cell count of 1.83 million, hemoglobin of 6.2 g/dl and hematocrit of 24.5 percent. She showed macrocytosis with a mean corpuscular volume (MCV) of 132JJ3, mean corpuscular hemoglobin (MCH) of 34.6 jupg and hypochromic RBCs with a mean corpuscular hemoglobin concentration (MCHC) of 25.6 g/dl. A 2+ anisocytosis was reported. The patient had leukopenia with a white-cell count of 3900/mm 3 and neutropenia with an absolute neutrophil count of 702. The other morphologic leukocyte types were within normal ranges when expressed as absolute counts.

The SMAC diagnostic profile was unexceptional for the most part with normal serum levels of sodium, potassium, bicarbonate, chloride, inorganic phosphorus, calcium, urea nitrogen, uric acid, creatine, glutamic oxaloacetic and pyruvic transaminases, and lactic dehydrogenase. Glucose was slightly elevated at 109 mg/dl, total bilirubin and albumin were at the lower limit of normal, and globulin and total protein were decreased at 2.0 and 5.5 g/dl respectively. The only values which deviated greatly from normal were her alkaline phosphatase, high at 187 U/L, and cholesterol, which was low at 107 mg/dl.

Her blood histamine was abnormally low at 21.2 ng/ml, (normal, 40 to 70) but unrelated to the high zinc intake; it had been even lower on her previous visit. Blood spermidine was slightly elevated but blood spermine was normal.

Upon learning of the patient's past four months of extraordinary zinc intake, we reduced it to 36 mg (from zinc sulfate contained in two capsules Vicon Plus). Since she was not now psychotic the careful and judicious use of small doses of copper and iron so as to fill her body needs but not precipitate psychotic behavior was a prime consideration. Accordingly, she was placed on a daily regimen of 1 capsule Theragran M/day containing 2 mg copper (as sulfate). She also received two capsules per day of Super Complex B50, 3 g of vitamin C, 3 g vitamin B6, 2 mg folic acid, and 39 mg elemental iron from Mol-lron. Mol-lron was used in the probability that molybdenum levels had been reduced by the zinc intoxication. 1 mg of vitamin B12, 3 times a week by injection, was also prescribed. In addition she received daily 400 mg niacinamide, 200U vitamin E, 25,000 U vitamin A, 50 mg Deaner, 3 g Dolomite and 10 mg Navane.

During the entire period she remained on essentially the same regimen except that she was taken off Theragran-M in the 18th week when her serum copper and hematological findings were in satisfactory ranges.

Results of Treatment

After one week of antidotal therapy her liver was no longer palpable and was at the costal margin. Her skin color improved and the acne of her forehead was better. She still complained of lack of any sex drive. After two weeks of therapy her lip, tongue and ear color was normal and the acne had cleared.

The serum zinc (Figure 1) dropped precipitously in the first week from 1160 to 440 mcg/dl; it fell another 70 points to 370 mcg/dl in the second week and thereafter only gradually, to 235 mcg/dl in the eighth and finally to within normal range by the thirteenth week. Serum copper (Figure 1) remained virtually unchanged at 8 mcg/dl until the third week when it rose to 17, then to 32 in the fourth week, reaching 67 mcg/dl

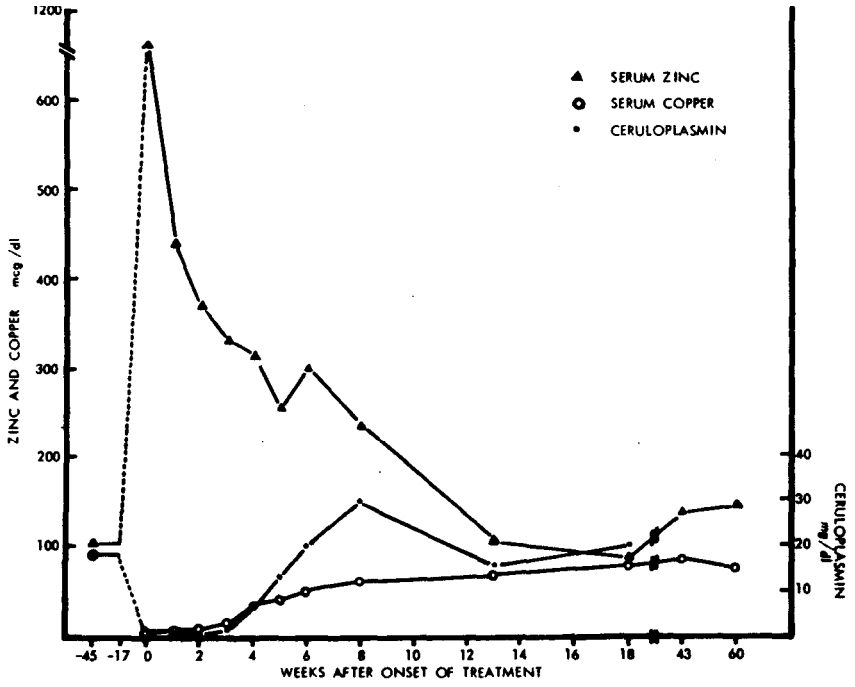

FIGURE 1

RESPONSE OF C.T.'S SERUM ZINC, COPPER AND CERULOPLASMIN TO TREATMENT. The point at 0 represents the last day of a 17 week period of high zinc intake (averaging 2300 mg/day) and the beginning of treatment. The point at −43 shows levels measured 43 weeks prior when the patient had been taking 66 mg zinc, a dose she maintained until −17 weeks, the onset of high zinc intake; the solid line between −43 and −17 weeks assumes that serum levels did not change substantially during this period although there were, in fact, no data at −17. Changes presumably occurring during the 17 week period of high zinc intake are indicated by the dotted lines.

by the thirteenth and 80 mcg/dl by the eighteenth week. Serum iron dropped from a normal 101 mcg/dl at the height of her zinc toxicity to 35 mcg/dl in the first week of treatment; it climbed rather steadily thereafter through the thirteenth week when it reached 128, then soared to 203 in the eighteenth week. Twenty months prior to this visit, before she had ever taken any supplemental zinc, C.T.'s serum zinc was 102, her serum copper, 90, and her serum iron 68 mcg/dl. Ten months prior, when she had been on 66 mg elemental zinc/day for ten months, her serum zinc was 90, her serum copper, 86, and serum iron, 140 mcg/dl. The ceruloplasmin (Figure 1) began to rise from below 2 mg/dl where it remained for the first three weeks to 6.4 in the fourth, to a high of 29 in the eighth. It remained within the normal range of 15-60 mg/dl thereafter.

Erythrocyte zinc and copper were determined in the 1st, 3rd, 5th and 6th treatment weeks and the values, expressed as meg per red blood cell, appear in Table 1. The patient's RBC zinc content was 40 percent greater in the first treatment week than the mean level found in a series of ten normal females not taking zinc, but her serum zinc was 400 percent greater than normal at this time. Although the serum zinc dropped by almost one-third between the 1st and the 6th treatment week, the RBC zinc remained essentially unchanged. Erythrocyte zinc is said to exist primarily as the enzyme, carbonic anhydrase, whereas in serum zinc is present as firmly bound to magroglobulin and loosely bound to albumin. Although serum copper was down to 1/10th the normal value, the copper per RBC was depressed to only about half normal. By the 6th week the RBCs had achieved about 75 percent of their normal copper content whereas the serum had just about half. Some 60 percent of the total red cell copper exists in the protein erythrocuprein which is known to remain constant under a wide range of conditions in man (Underwood, 1977). In swine made copper deficient, the

TABLE 1

ERTHROCYTE ZINC AND COPPER LEVELS DURING RECOVERY FROM ZINC TOXICITY

Week of Treatment	Zinc per RBC	Zinc in Serum		Copper per RBC	Copper in Serum
OigxIO"")	(ug/dl)			(ugxIO-[11])	(Mg/dl)
1	191	440		3.8	7
3	207	330		4.2	17
5	219	254	4.0	40	
6	204	298	6.6	48	
Control Level	147 ±13	112 ±24		8.8 ±.8	107 ± 24

decrease in erythrocyte copper is rapid initially but below a certain level suffers little further decline in spite of the severity of the copper deficiency (Bush et al., 1956). Thus, what is lost is a "labile fraction or pool". Furthermore, it was found that copper enters into the transfused copper deficient red cells from normal swine plasma, correcting the intracorpuscular defect. This explains the rapid response of the patient's red blood cell copper to treatment.

Figure 2 shows the zinc and copper content of hair taken one week and thirteen weeks after C.T. stopped her excessive zinc intake and began copper therapy. The hair was divided into 4 cm segments (corresponding roughly to 40 days of hair growth) for analysis, and the zinc and copper content along the strand categorized on the graph as "old hair" (hair grown before high intake of zinc), "hair grown during high zinc intake" and "hair grown during recovery". Obviously, this is a very rough approximation for we do not know C. T.'s rate of hair growth, or whether the rate is constant, and in taking 4 cm (or 40 day) segments we cannot be dissecting the hair precisely at these transition points as we would like. The copper content one week after onset of treatment decreases along the strand from distal (oldest hair) to the scalp end as expected but does not fall below generally accepted normal ranges. Zinc increases somewhat but never exceeds the upper limit of reported normal ranges (Underwood, 1977). In the sample analyzed thirteen weeks after onset of treatment, the copper seems to drop even further in the hair grown during the first 40 days or so of copper supplementation (4 to 8 cm on graph), falling to below normal for the first time, but then, in an apparent delayed response to treatment, it doubles in the next 40 days or very newest hair (0 to 4 cm from scalp). Zinc content is rather homogeneous along the length of the strand.

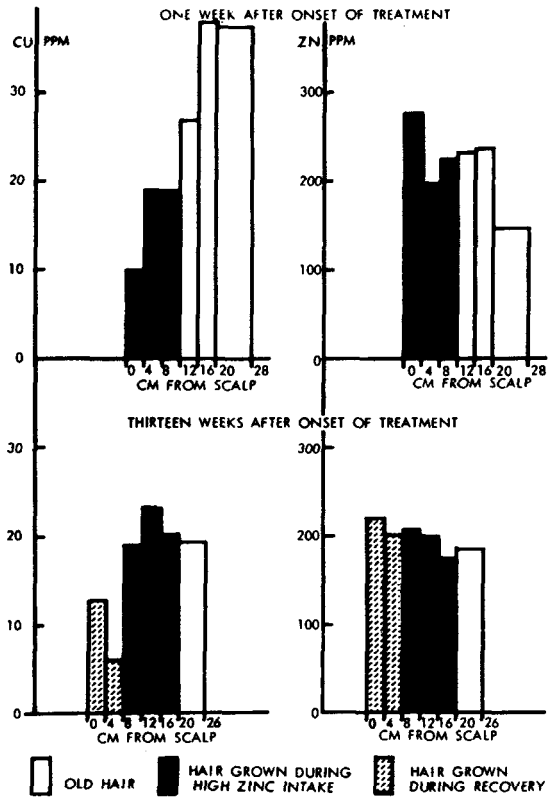

FIGURE 2

COPPER AND ZINC CONTENT OF PATIENT'S HAIR ONE WEEK AND THIRTEEN WEEKS AFTER ONSET OF TREATMENT. Normal ranges for hair are: 10-60 ppm Copper and 60 - 280 ppm Zinc (Underwood, 1977)

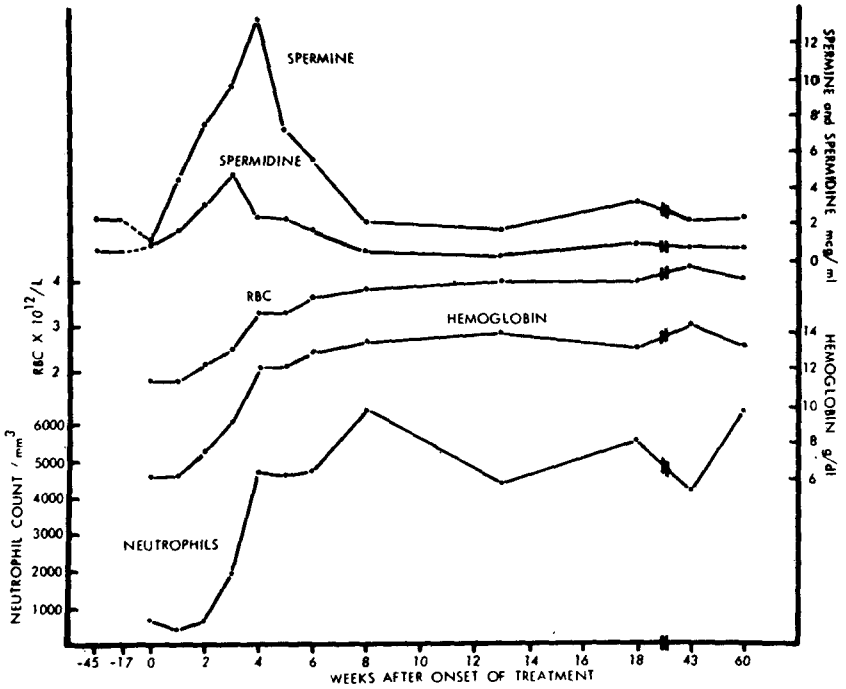

FIGURE 3
POLYAMINES, RBC, HEMOGLOBIN AND NEUTROPHILS DURING RECOVERY FROM ZINC TOXICITY.
See legend of Figure 1.

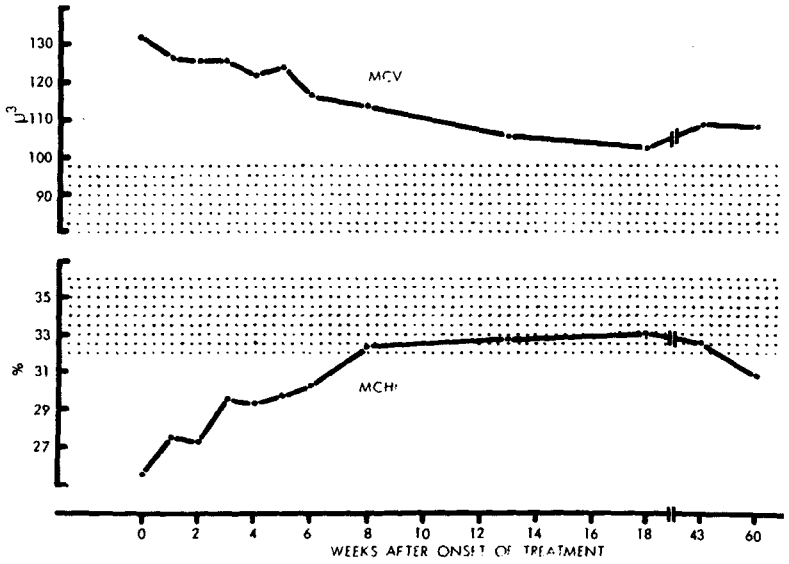

FIGURE 4
MEAN CORPUSCULAR VOLUME AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION DURING RECOVERY FROM ZINC TOXICITY.

The anemia and neutropenia responded dramatically (Figures 3 and 4). After an initial lag phase w'th no change the 1st treatment week, hemoglobin, RBC count and hematocrit began a steep, steady, and almost linear ascent in the 2nd through the fourth treatment weeks and then plateaued, increasing only gradually thereafter. The neutrophils followed a similar pattern. The hemoglobin, hematocrit, leukocyte and neutrophil count entered normal ranges by the 4th week but the red cell count, though satisfactory, was

not yet normal even into the eighteenth week. Macrocytosis (Figure 4) was at a height on the initial visit and steadily declined during the patient's recovery; however, some macrocytosis persisted into the 43 rd and 60th weeks with MCV's of 108 arid 107. The RBCs were hypochromic, the low MCHC indicating that during toxicity the weight of hemoglobin did not increase correspondingly to the increase in size of the average red corpuscle. However, with the cell volume decreasing and hemoglobin increasing during treatment, the RBCs became normochromic by the 8th week (Figure 4).

An interesting finding, illustrated in Figure 3 was the steep rise from normal in the polyamine levels during the first few weeks, spermidine peaking in the third week of treatment to four times normal, and spermine in the fourth week to ten times normal. This coincides with the accelerated production of RBCs, hemoglobin and neutrophils in response to treatment. There was an equally rapid descent in the polyamines to normal levels again, coinciding precisely with the plateau phase of RBC, hemoglobin and neutrophil production as normal values for these were approached. Figure 5 plots the hemoglobin, RBCs, and log hemoglobin and RBC vs. spermine levels. Using the four points corresponding to 1st, 2nd, 3rd and 4th treatment weeks, straight lines with highly significant coefficients of correlation were obtained. These curves indicate that the accelerated erythropoiesis and possibly leukopoiesis are related to the increases in the polyamine levels.

The patient's low initial serum histamine, subnormal even in the pretoxicity period, dropped to zero in the first week of treatment and hovered in the low-normal range subsequently, showing no consistent pattern of change during recovery. Glucose, initially high at 109, rose to 116 in the 1st week and fell thereafter to normal levels; total bilirubin, albumin, globulin and, of course, the total protein steadily increased. The alkaline phosphatase showed the greatest decline in the first week, then continued to decrease slowly and the serum cholesterol rose steadily, both becoming normal by

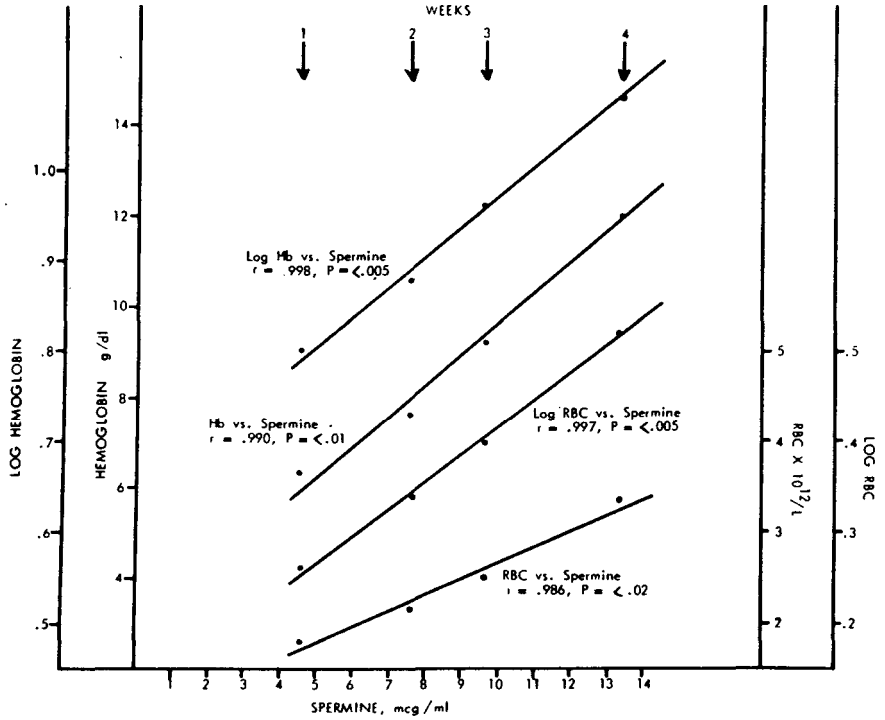

FIGURE 5
HEMOGLOBIN, LOG HEMOGLOBIN, RBC AND LOG RBC COUNT PLOTTED vs. SPERMINE LEVELS AFTER 1, 2, 3 AND 4 WEEKS OF TREATMENT.

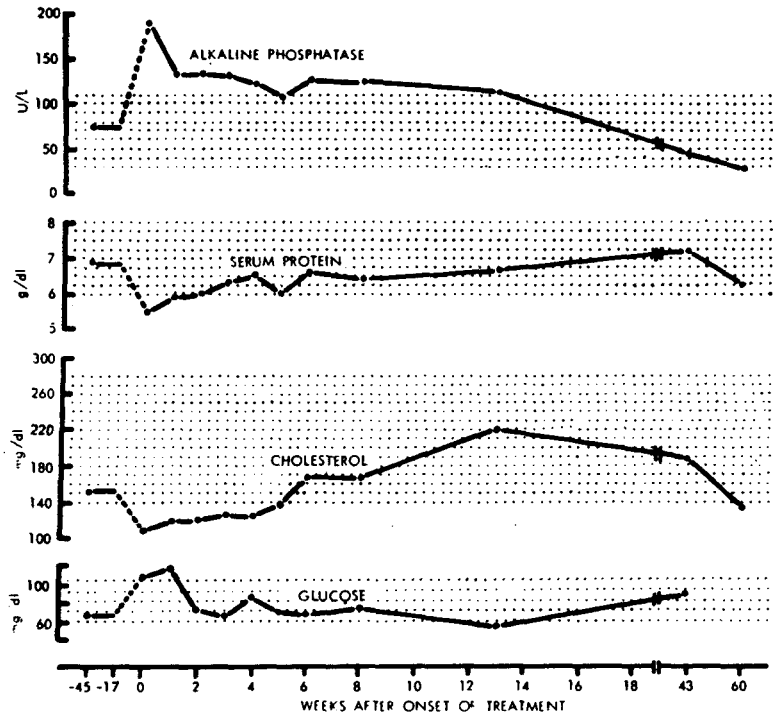

FIGURE 6
RESPONSE TO TREATMENT OF CERTAIN SERUM PARAMETERS ALTERED DUE TO ZINC EXCES

the 5th week. Figure 6 shows some of the preceding parameters which seemed to be affected by the toxicity and subsequent treatment.

Discussion

C.T.'s precarious experience with high levels of zinc over such a prolonged period has provided us with valuable data. Within the four months of high zinc where her intake averaged 2300 mg per day and ranged between 400 mg and 5700 mg, there was one thirty-five day stretch during which she ingested over 4 g elemental zinc (from the gluconate) per day twenty-two times, and over 5 g, six times, with little respite since on the remaining days her average intake was 2700 mg. Though she did experience some vomiting, she did not manifest any other distress, such as dizziness, diarrhea, fever and stomach cramps, reported as occurring with only 225-450 mg zinc from the sulfate, the emetic concentration according to Sollman (1957). One woman who had ingested 6 g elemental zinc from zinc sulfate suffered vomiting, cramps, renal damage, hemorrhagic pancreatitis and died four days later (Cowan, 1947). C.T. took her 4 to 6 g in two hour intervals throughout the day rather than in a single dose.

We must consider whether the more deleterious effects observed with zinc sulfate should be attributed to the sulfate anion rather than to the metal. Indeed Brewer et al. (1977) in treating sickle cell anemia, found that zinc acetate was absorbed as well as the sulfate and was better tolerated by the gut. It would be interesting to determine whether the gluconate may be even better tolerated.

It is indeed interesting that C.T.'s parents found that zinc soothed her psychosis, and recalls the report of the Iranian boy who plunged into a state of lethargy after ingesting 12 g of metallic zinc (and incidentally suffered no gastrointestinal symptoms). One could of course argue that anyone with severe anemia, zinc-induced or not, would be rather placid, however the parents observed "calming" effects of zinc 20 minutes after each dose, wearing off at the end of each two hours.

An unusual feature of this case was that C.T. manifested a macrocytic anemia. In a search of the literature dealing with the effects of either zinc toxicity or copper deficiency per se in animals or in humans, the resultant anemia was always microcytic with the exception of two studies. The first involves four infants who developed anemia while recovering from marasmus (Cordano et al., 1964). Unaware, until much later, that the anemia was due to copper deficiency, the infants were treated first with high doses of vitamins C and B12 with some success. Modest to impressive reticulocyte responses were seen with each of these agents. Macrocytosis was then manifested which the authors attributed to this vitamin induced production of red cells. Other sources (Wintrobe, 1974) also attribute certain macrocytic anemia to intense bone marrow activity, i.e. especially active RBC regeneration. In the second study, (Vilter et al., 1974) a woman who suffered copper deficiency anemia due to long term parenteral hyperalimentation, exhibited some macrocytosis (MCV was 111P3), unimpressive except that, again, microcytosis is the rule in copper deficiency anemia. But, like the infants, C.T. as.we shall see, was receiving a relatively large supplement of vitamin C. C.T. had been taking 2 g of vitamin C per day through the period of high zinc intake which probably accounts for the initial macrocytosis. It is also very likely that without this supplemental vitamin C she would have suffered more severely from her bout with zinc-induced copper deficiency anemia. The anemia of copper deficiency is said to be due in part to a reduction in the life span of the RBC's, and if vitamin C favors production of new red cells as Cordano et al. (1964) as well as other investigators suggest (Vilter, 1967), its protective role in this case cannot be overlooked.

The significant correlation between the sharp increase in the polyamines and the increase in the production of RBC's and hemoglobin indicates that they are related events. This is not surprising since increased synthesis of polyamines is found to occur in a number of systems characterized by rapid erythropoiesis. It probably occurs during the rapid recovery from any form of anemia. We have previously reported (Papaioannou et al., 1978) on higher than normal spermine levels in lead intoxicated battery workers who are in a constant state of accelerated erythropoiesis to compensate for the fragility and low life span of their red corpuscles. Another result seemingly unusual in the face of severe copper deficiency anemia was C.T.'s normal serum iron when zinc intoxicated. However, in a study of long term copper deficiency in swine (Lee et al., 1968), there is only a brief hypoferremic phase initially, followed by a "terminal phase" during which the plasma iron returns to normal or even higher levels while the hemoglobin continues to decrease.

The zinc in C.T.'s hair never exceeds normal levels. Although low hair zinc values are reported for zinc deficient Egyptian dwarfs, it would appear from our data that excessive zinc cannot be detected. Perhaps there is an upper limit on the number of binding sites in hair available for zinc. Although the hair copper decreases steadily, it does not fall below normal levels until well into her treatment phase. For those who regard hair as an index of trace metal status, we find that such extremes of zinc excess and copper depletion as we witnessed here are barely reflected in the patient's hair.

That the activity of C.T.'s alkaline phosphatase, a zinc metalloenzyme was significantly increased and responded to treatment by dropping to normal levels is not at all surprising since Zn++ is listed as an activating ion by Dixon and Webb (1964). However, her lactic dehydrogenase, also a zinc metalloenzyme, was unaffected. Consistent with our observations, serum alkaline phosphatase is reported to show a significant reduction of its activity in zinc depletion whereas lactic dehydrogenase suffers no loss of activity even in a severe stage of depletion (Roth and Kirchgessner, 1974).

Even though a protein losing enteropathy and hypoproteinemia has been demonstrated in copper deficiency anemia in infants, (The Merck Manual of Diagnosis and Therapy, 1977) the patient's serum albumin and globulin were decreased only slightly.

Petering (1974) cites studies which found a direct increase in serum cholesterol at low levels of copper with increases in dietary zinc. C.T.'s serum cholesterol, on the other hand, was low and slowly increased with treatment. The low cholesterol with a tremendous zinc/copper ratio is not in line with the hypothesis of Klevay based upon rat studies (Klevay, 1974).

Our patient's rapid recovery from long term zinc toxicity, unparalleled either in dose or duration, reinforces the general belief that zinc is a relatively non-toxic metal with a wide margin of safety. Her hematologic and other parameters rebounded to satisfactory levels within four weeks and examinations 1 and *VA* years later confirmed that there is no apparent permanent damage. It appears that so long as a small amount of copper is provided when serum copper or ceruloplasmin levels begin to be affected, even larger doses of zinc than are now customary may be administered if found to be therapeutically valuable.

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