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Effect of Chronic Inflammation on Copper and Zinc Metabolism

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The effect of chronic inflammation on serum, blood and hepatic copper and zinc concentrations has been studied in arthritic female Sprague-Dawley rats, for a six week period. Metals were determined by atomic-absorption spectrophotometry and by inductively-coupled argon plasma spectrometry. Blood measurements evidenced hypercupremia and normal zinc levels. Serum and hepatic copper content were found to be increased. Serum zinc values were reduced whilst hepatic zinc concentration was higher. Serum alterations of both metals remained throughout the studied period. Good correlations were found between systemic alteration and changes in metal values.

Key words: Serum copper and zinc, Hepatic copper and zinc, Chronic inflammation.

The physiology of copper and zinc is currently the subject of considerable interest both because of the role these trace metals play in many biochemical reactions and because abnormalities in Cu and Zn metabolisms have been associated with severe clinical disorders in humans (15, 17).

A sharp rise of Cu concentration and caeruloplasmin activity in biological fluids and tissues has been measured in man and animals under a variety of acute and chronic inflammatory conditions. A strong positive correlation between Cu and caeruloplasmin in the sera of both normal and inflammed rats has been shown (3, 4). This latter observation confirms the results observed in rheumatoid patients by CONFORTI *et al.* (6) who proposses to take the measured of total circulating Cu as a good indicator of the amount of circulating caeruloplasmin.

Since Zn is a cofactor for protein and nucleic acid synthesis it is conceivable that in inflammation, a portion of the Zn accumulated in the liver is involved in the enhanced synthesis of the acute-phase proteins (18). Hypozincemia is observed during arthritis and following phagocytosis *in vivo*. Enhanced Zn accumulation during inflammation seems to occur within the liver and also in injured tissue such as synovial fluid of arthritic patients (17).

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It is well established that the liver appears to have important functions in the homeostatic regulation of trace metal metabolism (7). We decided to study serum, blood and liver Cu and Zn contents in an experimental model of chronic inflammatory process in the rat as is adjuvant arthritis, during a period of six weeks. Atomic absorption spectrophotometry (AAS) and inductively-coupled argon plasma spectrometry (ICP), were elected as techniques to quantify the Cu and Zn levels.

Materials and Methods

Female Sprague-Dawley rats, 9 weeks old were used. They were kept in standardized conditions, and water and standard rat chow (A-04® Panlab S. L., containing 29 mg Cu/kg and 85 mg Zn/kg) were given *ad libitum*.

Induction of arthritis. — Arthritis adjuvant was produced by a single intradermal injection (0.1 ml) in the right-hind-paw of *Mycobacterium butyricum* (Difco Laboraties, Detroit, Mi), suspended in mineral oil at a concentration of 5 mg/ml. The control animals only received 0.1 ml of mineral oil under the same conditions.

Evaluation of inflammation. — On day 0 (before induction) and subsequently 14, 21, 28, 35 and 42 days after adjuvant induction, the animals were weighed and left and right-hind-paw volumes were measured using a mercury plethysmograph (Ugo Basile, Milan). A mean volume for the uninjected paw of the control rats was established and a normal limit for this groups was fixed at 0.91 ml (0.41 + 0.50; mean + 2 s. d.). On day 14, those induced rats whose left-hind-paw volume increase exceeded 0.91 ml were selected as arthritic animals.

Samples. — Blood samples were obtained on day 0 and weekly between 14 and 42 days after induction. Blood was collected, after ether inhalation by heart puncture and was centrifuged. Sera were stored at —20°C. Livers were removed, washed, weighed and immediately frozen at —20°C until assay. The daily changes observed in copper concentrations indicate the need for a time control group that should be killed at the same time as each of the treated groups (6).

Reagents and Apparatus. — All chemicals used were of analytical grade and prepared with distilled and deionized water from a Milli-Q2 System (Millipore Corp., Bedford, Ma). Acid-washed glassware was used throughout the study.

From stock standard solutions of 200 mg metal/l, working standard solutions containing 0.6, 0.8, 1.0, 1.2 and 1.4 mg Cu or Zn/l for AAS and 0.05, 0.07, 0.1, 0.3 and 0.5 mg Cu or Zn/l for ICP, were prepared.

From AAS measurements an SP 1900 Pye Unicam Spectrophotometer was used under the following conditions: Wavelength 324.8 nm (Cu) and 213.9 nm (Zn); slit 0.10 nm (Cu) and 0.20 nm (Zn); acetylene-flow 0.8 l/min (Cu) and 0.9 l/min (Zn); lamp current 4 mA; air-flow 5 l/min and acetylene-pressure 0.7 kg/cm^2. For ICP determinations a Jobin Ivon 38 VHR Spectrometer was used under the following conditions: Wavelength 324.8 nm (Cu) and 213.9 nm (Zn); primary slit width 30 μm; secondary slit 40 μm; argon coolant gas flow rate 20 l/min and argon carrier gas flow rate 0.3 l/min.

Treatment of samples. — Liver samples were weighed (1.4-1.5 g) into 50 ml Pyrex-glass beakers and desiccated at 110-115°C for a period of 8 h. After addition of 5 ml concentrate HNO_3, the samples were placed overnight at 60°C. Then 5 ml of concentrate HNO_3 were added and the samples were completely digested on a sand-bath. The digested material was allowed to evaporate and

then was diluted to 10 ml. Blanks were also prepared following the same procedure. Copper was measured directly by AAS. For Zn determinations a 1:5 dilution was made. Results are expressed as μg Cu or Zn/g wet weight.

Blood treatment was similar to liver samples. Copper concentrations in the diluted digest were measured directly by ICP. For Zn determinations a 1:5 dilution was made. Results are expressed as μg Cu or Zn/g.

Serum measurements of Cu and Zn by ICP were realized directly after sera dilution (1:13). Results are expressed as μg Cu or Zn/ml.

To ensure that no copper or zinc would be lost from the samples during extraction by digestion, known amounts of these metals were added.

Statistics. — The level of significance was calculated using the Student's t-test. The regression lines were calculated by the method of least squares.

Results

In control animals, distributions of serum, blood and hepatic Cu and Zn con-

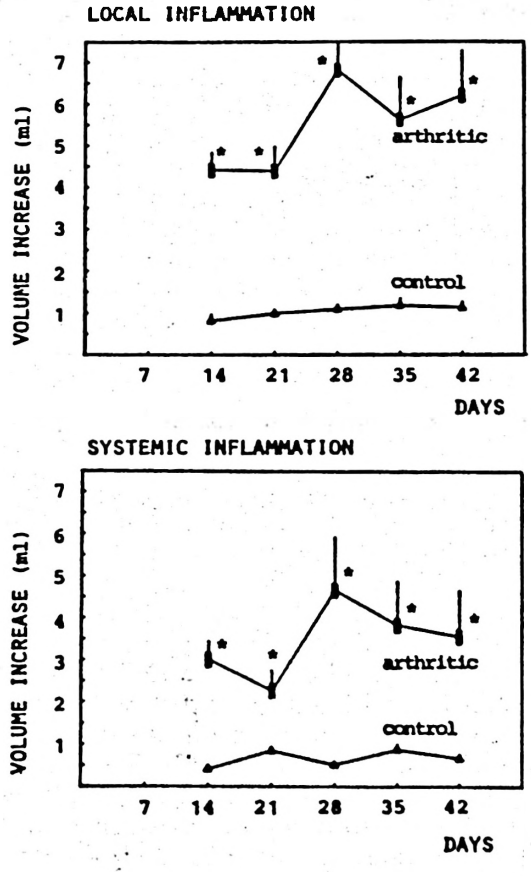

Fig. 1. *Time-course of injected (local inflammation) and uninjected (systemic inflammation) hind-paw volume in control and arthritic animals.*
Each point represents the mean + s.e.m. of 8 animals. *p < 0.05 in relation to control group.

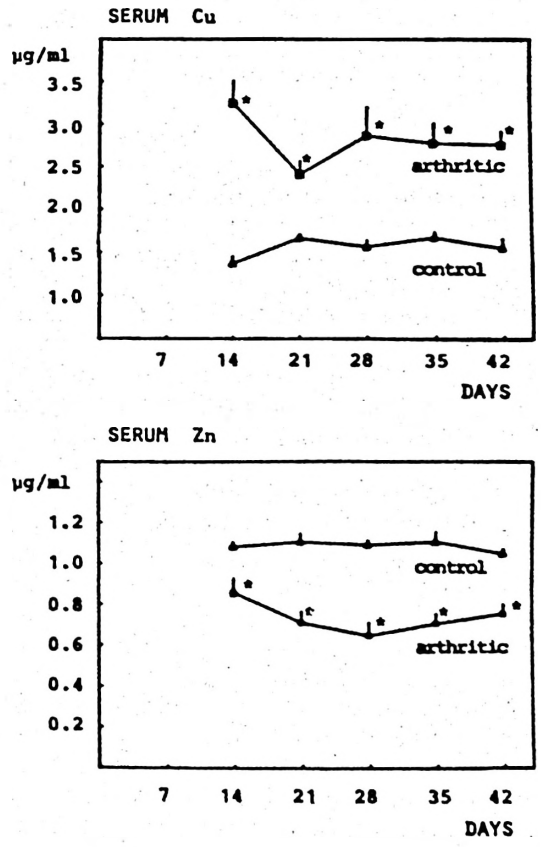

Fig. 2. *Serum copper and zinc levels in control and arthritic animals.*
Each point represents the mean + s.e.m. of 8 animals. *p < 0.01 in relation to control group.

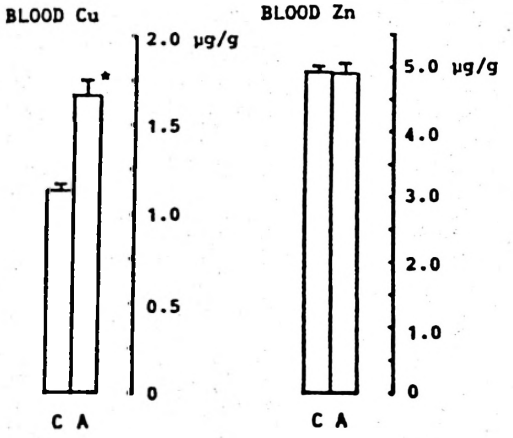

Fig. 3. *Blood copper and zinc values on day 21 after arthritis induction.*
(C = Control; A = Arthritic). Each bar represents the mean + s.e.m. of 8 animals. *p < 0.01 in relation to control rats.

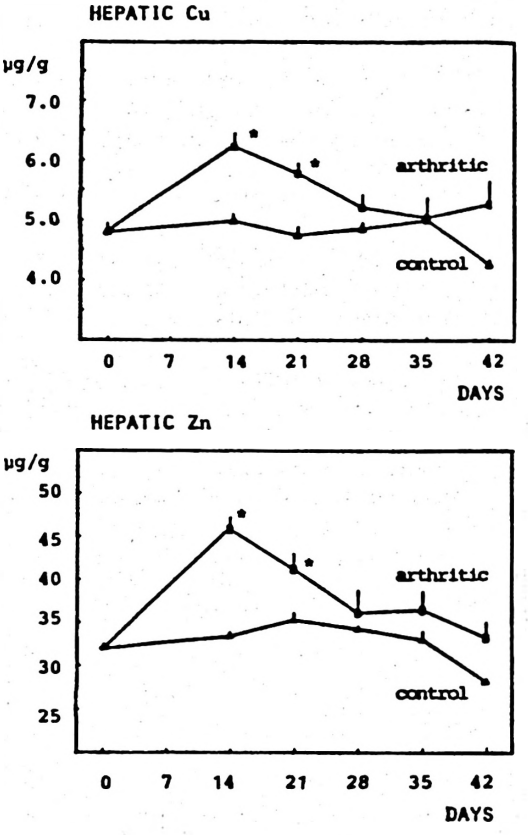

Fig. 4. *Hepatic copper and zinc content in healthy and inflamed animals.*
Each point represents the mean + s.e.m. of 8 animals. *p < 0.05 in relation to control rats.

centrations were gaussian. The mean value of normal serum Cu and Zn levels were 1.58 ± 0.19 µg/ml and 1.08 ± 0.08 µg/ml (mean ± S.D.), respectively. In healthy animals, hepatic concentrations of both metals were 4.81 ± 0.41 µg Cu/g fresh liver and 32.63 ± 5.41 µg Zn/g fresh liver (m ± S.D.). Blood levels in control rats were 1.13 ± 0.10 µg Cu/g and 4.92 ± 0.27 µg Zn/g (m ± S.D.).

Figure 1 shows the time-course of injected and uninjected hind-paw volume in control and arthritic animals. It can be seen that arthritic rats show a significant left and right hind-paw-volume increase (day 21: bottom 2.32 ± 0.79; top 4.38 ± 0.60), when compared to control group (bottom 0.85 ± 0.08; top 0.98 ± 0.08). Throughout the studied period (6 weeks) the arthritic animals remained with both hind-paws swollen.

The arthritic rats had increased serum Cu levels (fig. 2). This increase was observed during all the studied period and was on average 80% in relation to control rats. On the contrary, arthritic animals showed reduced serum Zn levels (fig. 2), a reduction of 32% on average throughout the study. Day 21 postinduction was chosen to study Cu and Zn levels in whole blood, because on this day the animals exhibited marked alterations in both serum and hepatic metal contents and also systemic inflammation. Blood metal concentrations only evidenced an increase in Cu levels in arthritic rats (fig. 3); zinc blood values remained at normal levels. Due to the minimum changes observed in blood metal levels and taking into account the laborious sample preparations, Cu and Zn measure-

Table I: *Linear correlations between the different parameters studied.* $p < 0.05$ for all coefficients; degrees of freedom = 51.

	Uninjected hind-paw volume	Serum Cu	Serum Zn	Hepatic Cu	Hepatic Zn
Injected hind-paw volume	0.8764	0.6882	—0.8014	0.5336	0.5568
Uninjected hind-paw volume		0.5813	—0.6934	0.3464	0.3781
Serum Cu			—0.7595	0.4631	0.5367
Serum Zn				—0.4471	—0.4370
Hepatic Cu					0.7399

ments in whole blood were not carried out throughout the study.

Hepatic Cu and Zn concentrations in induced animals were increased 23% and 26%, respectively, in relation to control rats (fig. 4). These alterations remained only until 28 days after arthritis induction; the last two weeks of the study, the mean values reached normal levels.

Good correlations were found between both local and systemic inflammation and the observed changes in metal values (table I).

Discussion

Using AAS and ICP methods, Cu and Zn normal values of samples from female Sprague-Dawley rats are in agreement with results published by EVANS and WIEDERANDERS (11) who used spectrophotometry at 542 nm and by CONFORTI *et al.* (6) and HILL *et al.* (12) who used AAS tecnhiques.

Arthritis adjuvant markedly modifies the blood, sera and hepatic levels of Cu, whilst Zn is modified only in serum and liver, results that are in accordance with KISHORE *et al.* (13) on day 21 postinduction. In relation to control rats, arthritic animals show a serum Cu increase at all times in the studied period, whilst the increased hepatic Cu content reached normal values on day 35 postinduction. Serum Zn levels are diminished throughout the study period, whilst hepatic Zn content increases only up to 28 days after adjuvant induction.

Serum Cu increase can be due to the caeruloplasmin increase observed in chronic inflammation that has been reported by others (5, 8, 22). Caeruloplasmin is one of the acute phase proteins. There is good evidence that the increased synthesis by the liver of all acute phase reactants is related to the ability of interleukin-1 (IL-1) to stimulate hepatocytes. IL-1 would be produced by activated macrophages during the inflammatory process (21).

The role of caeruloplasmin in adjuvant arthritis is to neutralize oxygen free radicals, mainly superoxide anion (1, 10) in an attempt to stop the chronification of the process. This fact is, in part, supported by nutritional studies, in which, copper-deficient rats develop a higher degree of inflammation than normal fed rats (8).

The results from this study suggest that the copper used for the increased synthesis of caeruloplasmin is somehow taken without depleting the copper deposits of liver. Copper may be derived either from changes in the equilibrium between absorption and excretion of Cu in the gut or it may come from other areas of the body such as kidneys and red cells (6).

In relation to Zn alterations, although there is a decrease in sera Zn values, no alteration of this metal is evidenced if the measurements are carried out in whole blood; this could be due to the fact that

Zn content in sera represents only 10-20% of the total sanguineous Zn (20). Thus it seems that Zn measurements in whole blood are not recommended for inflammatory induced alteration studies, because it does not reflect the real Zn changes that, in fact, are produced in these processes. There is a 32% increase in whole blood Cu concentration which could correspond to the 31% of serum Cu increase, on day 21 postinduction.

The serum Zn reduction observed in arthritic animals (33% on day 21) could be produced by the enhanced leukopoiesis which is reflected in the leukocytosis that arthritic animals usually exhibit (16), but it also could be due to serum albumin decrease (32% on day 21) which is an important Zn-carrier (7). It should be noted that there is a good correlation between reduced serum Zn levels and decreased albumin serum values (2).

IL-1 produced by stimulated macrophages increases the hepatocyte Zn uptake and enhances metallothionein synthesis, a protein capable of avoiding toxic Zn cellular effects (9, 14). Zn bound to metallothionein constitutes a deposit of metal that is required for the acute-phase protein synthesis (19). It should be noted that a good correlation links serum decrease and hepatic increase of this trace metal, which could indicate that hepatic Zn increase results from serum depletion.

Inflammation is capable of inducing systemic alterations in trace metal metabolism that appear to be linked to the production of an acute-phase plasma protein response and to be part of a particular host-defense repair system which responds to factors released from stimulated phagocytes.

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Resumen

Se ha estudiado el efecto de la inflamación crónica sobre los niveles séricos, sanguíneos y hepáticos de cobre y zinc en ratas artríticas, Sprague-Dawley, hembras, durante un período de seis semanas. Los metales se han cuantificado por espectrofotometría de absorción atómica y espectrometría de plasma de argón acoplado por inducción. Las determinaciones sanguíneas muestran hipercupremia y normozinquemia. Se observa un aumento del cobre sérico y hepático. En relación al Zn existe reducción en suero y aumento de la concentración hepática. Las alteraciones séricas halladas en ambos metales se mantienen durante todo el período estudiado; por el contrario, los cambios en la concentración hepática de Cu y Zn se normalizan a los 35 días después de la inducción de la patología inflamatoria. Existe buena correlación entre la inflamación sistémica y los cambios observados en ambos metales.

Palabras clave: Cu y Zn séricos, Cu y Zn hepáticos, Inflamación crónica.

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