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Copper and zinc body levels in inflammation: an overview of the data obtained from animal and human studies

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Abstract

The development of acute and chronic inflammatory processes induces, in the laboratory animal, a net accumulation of both copper and zinc in many body compartments, the inflammed area included. In rheumatoid arthritis, as well as in animal models, only plasma zinc concentration seems to be significantly correlated with disease severity, while the increase in total plasma copper could be described as an "all or nothing" phenomenon. Moreover, in rheumatoid arthritis, it appears that the disease develops and progresses without being linked to either copper or zinc deficiency conditions.
Thus, it seems reasonable to suggest that a rationale for the use of copper and/or zinc in the treatment of inflammatory disorders can only be drawn from the intrinsic pharmacological properties of such trace elements, rather than from the need for their repletion.

Introduction

The anti-inflammatory/anti-arthritic potential of copper [1] and zinc [2–4] has been well documented in both laboratory animals and man, although at present it is not clinically exploited. On the other hand, the development of acute or chronic inflammatory processes induces substantial changes in the metabolism of copper and zinc in laboratory animals and man, and the prevailing opinion considers these changes to be part of the defence mechanism evoked by the organism to bring the inflammatory reaction under physiologic control [5].
A detailed understanding of inflammation-induced modifications of copper and zinc status may help to settle some important points. For instance, it may indicate whether copper and zinc plasma or tissue levels can be used as markers of the severity of inflammatory conditions. It may clarify whether, as recently proposed, a marginal copper [6, 7] or zinc [8] deficiency is a contributory factor to the etiology of rheumatoid arthritis. It may support or question the hypothesis according to which chronic inflammatory diseases, such as rheumatoid arthritis, induce a condition of trace element deficiency [9]. Finally, it may provide new rationales for the use of copper [10] and/or zinc [11] preparations in the therapy of inflammatory disorders.
Therefore the main purpose of this review is to summarize the major alterations in copper and zinc metabolism during inflammation and attempt to give some answers to the above questions.

Studies on laboratory animals

Inflammation-induced changes in copper and zinc metabolism in blood and urine

Copper. Studies carried out on many different animal species have shown unequivocally that almost

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all inflammatory processes, either acute or chronic, primed by non-septic, septic or immunologic challenges, are characterised by a significant and sometimes dramatic increase in total serum copper (Table 1; references quoted [2,11–33]). Usually the rise in serum copper is accompanied by a parallel increase in ceruloplasmin levels, these two parameters being highly and significantly correlated in normal as well as in acutely or chronically inflamed animals [12, 34–36]. Recently, Freeman and O'Callaghan [37], directly measuring (after column fractionation) the non-ceruloplasmin bound copper in serum of normal and inflamed rats, showed that this fraction of circulating copper undergoes only minor variations during adjuvant arthritis. Therefore, at least in animals fed on a diet containing normal amounts of copper, the total serum (or plasma) copper measured in inflammation seems to represent quite a good index of circulating ceruloplasmin levels.

Although the increase in copper in serum or plasma can be seen as an accurate marker of the existence of an inflammatory state (unless pregnancy is present), the data we have obtained in recent years on over 1200 inflamed rats seem to suggest that this parameter may not be closely related to the severity of the pathology studied. In fact, we found that acute non-septic inflammatory processes, which have developed in a very similar manner, caused an extremely variable increase in serum (or plasma) copper [36,38]. A great variation was also observed in arthritic rats in which the experimental pathology was fully expressed [15, 39]. Interestingly, the average increase in plasma copper in animals that developed only a mild arthritis (low score arthritic rats) was about 54%, and approximately 60% in animals with a significantly more severe pathology (high score arthritic rats) [15]. Thus, the hypercupremia induced by the inflammatory reactions appears to be a sort of "all or nothing" phenomenon that may indeed have little value as a marker of disease severity.

The evaluation of erythrocyte copper levels has been performed less frequently (Table 1). Some authors found that neither chronic nor acute inflammatory processes could modify blood cell copper status, whereas an increase in erythrocyte copper concentrations in rats with carrageenan-induced pleurisy [11] and adjuvant arthritis [15] was reported by others. However, the latter changes were small and have little, if any, biological significance for the acute process. On the other hand, a more pronounced increase, measured during the chronic inflammatory reaction, probably represented a secondary phenomenon essentially attributable, as was subsequently shown in rheumatoid arthritis patients [40], to a significant decrease in red cell volume. Hence, all evidence available so far seems to suggest that the amount of copper present in erythrocytes is not influenced by inflammation. The data on the urinary excretion of copper during the onset and development of acute or chronic inflammatory reactions in laboratory animals are, to our knowledge, insufficient to draw any reasonable conclusion. For instance, urinary copper excretion was found to be increased in rats subjected to femur fracture [24], but challenge with complete adjuvant did not change this urinary excretion [16]. However, this problem has been tackled better in man, and will be discussed in detail later in this paper.

Zinc. Unlike copper, serum or plasma zinc levels do not appear to respond in the same way to different inflammatory challenges and contradictory results have been published (Table 1). In rats, acute non-septic inflammation did not induce hypozincemia [11, 20], whereas septic inflammation or *E. coli* endotoxin injection caused a decrease in serum zinc [21]. In dogs, however, spontaneous otitis and dermatitis did not modify zinc levels in serum [27], while both adjuvant abscesses [26] and spontaneous anal gland fistulas [27] were found to increase the zincemia significantly. These latter two findings may be relevant to the hypothesis, proposed on the basis of data obtained in sham operated rats [41], that an increase (instead of a decrease) in circulating zinc is common to different stresses (including inflammatory conditions), but with some of them the liver uptake of zinc might be greater, leading to the observed hypozincemia. Studies carried out in chronic models of inflammation (adjuvant arthritis of the rat) generally report a characteristic decrease in serum or plasma zinc levels (Table 1). Interestingly, in this experimental disease, the zinc accumulation in the liver was three to four times higher than that observed in acutely inflamed animals [15]. These results, together with those obtained in man (as discussed in detail later), seem to suggest that, contrary to copper, plasma zinc concentration could be correlated with the severity of the inflammatory reaction studied.

The concentration of zinc in erythrocytes has been the subject of very few studies (Table 1), but it seems

Table 1
Changes in Cu and Zn levels in some body compartments of different animal species affected by experimental or spontaneous(s) diseases bearing a relevant inflamatory component.

Species	Disease	Serum (plasma)		Blood cells		Liver		Kidney		Inflammed fluid or tissue		References
		Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	
Rat	Adjuvant arthritis	I	U	—	—	—	I	—	—	—	—	[2]
	Adjuvant arthritis	I	—	—	—	I	—	D	—	—	—	[12]
	Adjuvant arthritis	I	U	—	—	I	I	U	I	—	—	[13]
	Adjuvant arthritis	I	D	—	—	I	U	—	—	—	—	[14]
	Adjuvant arthritis	I	D	I	U	I	I	D	U	I**	I**	[15]
	Adjuvant arthritis	I	D	U	U	I	I	—	—	—	—	[16]
	Adjuvant arthritis	I	D	—	—	I	I	—	—	—	—	[17]
	Radiation injury	U*	—	—	—	—	—	—	—	—	—	[18]
	Talc granulomatosis	—	D	—	—	—	I	—	—	—	—	[19]
	Carrageenan pleurisy	I	U	I	U	U	I	U	U	P	P	[11]
	Carrageenan oedema	I	U	U	U	U	I	—	—	I**	I**	[20]
	Acute infections (*F. tularensis*)	—	D	—	—	—	I	—	—	—	—	[21]
	Endotoxemia (*E. coli endotoxin*)	—	D	—	—	—	I	—	—	—	—	[21]
	Turpentine abscesses	I	—	—	—	I	—	—	—	—	—	[22]
	Hypersensitivity reactions	—	D	—	—	—	I	—	—	—	—	[23]
	Femur fracture	—	—	—	—	D	D	—	—	—	—	[24]
	Turpentine oedema	I	—	U	—	U	—	—	—	—	—	[25]
	S. aureus abscesses	I	—	U	—	U	—	—	—	—	—	[25]
	Typhoid vaccine injection	I	—	U	—	U	—	—	—	—	—	[25]
Dog	Adjuvant abscesses	I	I	—	—	D	D	—	—	—	—	[26]
	Chronic otitis (s)	I	U	—	—	—	—	—	—	—	—	[27]
	Chronic dermatitis (s)	U	U	—	—	—	—	—	—	—	—	[27]
	Chronic anal gland fistula (s)	U	I	—	—	—	—	—	—	—	—	[27]
	Turpentine abscesses	I*	—	—	—	—	—	—	—	—	—	[28]
	S. aureus induced arthritis	I*	—	—	—	—	—	—	—	—	—	[28]
Guinea pig	Turpentine abscesses	I*	—	—	—	—	—	—	—	—	—	[29]
Rabbit	Ocular inflammation	I	—	—	—	—	—	—	—	I***	—	[30]
	Ocular inflammation	—	—	—	—	—	—	—	—	I***	—	[31]
	Turpentine oedema	I*	—	—	—	—	—	—	—	—	—	[32]
Mouse	Talc granuloma	I*	—	—	—	—	—	—	—	—	—	[33]
	Collagenase injection	I*	—	—	—	—	—	—	—	—	—	[33]

— not determined; D, statistically significant decrease; I, statistically significant increase; U, statistically unchanged; P, the trace element is present in the inflamed pleural effusion but no comparison with non-inflamed fluid was done; U*, I* measured as serum ceruloplasmin; I** measured in inflammed compared with normal tissue; I*** measured in inflamed compared with normal acqueous humor.

that no significant variations in zinc status result from either acute or chronic inflammatory processes.

The influence of inflammation on the urinary excretion of zinc has scarcely been investigated and conflicting results have been obtained. Urinary excretion of zinc in femur fractured rats was found to be increased [24], while it was unchanged in adjuvant arthritic rats [16]. Similarly to copper, the problem of urinary excretion of zinc has been widely studied in man and will be discussed subsequently.

Inflammation-induced changes in solid tissue copper and zinc metabolism

A preliminary note. While in normal rats of either sex hepatic copper concentration was found to increase significantly from morning (9.30 a.m.) to evening (6.30 p.m.), the total amount of this metal in the liver remained unchanged, the measured rise in concentration being exclusively due to a physiological decrease in organ weight [42]. Moreover, the presence of an inflammatory exudate in the paws of carrageenan or complete adjuvant challenged rats

caused a significant decrease in paw zinc concentrations but, owing to the increased paw weights, the total amount of zinc in the inflamed tissue was actually increased significantly [15, 20]. The above examples strongly suggest that the metal concentration *per se* is inadequate to give a correct picture of either copper or zinc status in a body compartment, while a more biologically significant assessment could be obtained when the total amount of the element was studied. Unfortunately, although in many solid tissues it is possible to measure both the concentration and the total amount of any trace element considered, this is not usually done by most researchers, and the metal concentration is the only parameter routinely reported. In our opinion, the lack of data on the total amount of copper or zinc present in the tissues and organs studied makes a correct interpretation of the published results difficult.

Copper. Liver is the tissue in which inflammation-induced changes in copper metabolism have been most extensively evaluated. From the data published so far it appears that, although significantly higher amounts of ceruloplasmin are synthesized and released into the general circulation by the liver, in non-septic, septic and immunologically promoted acute inflammation the level of hepatic copper does not decrease (Table 1). Moreover, in chronic inflammatory conditions (adjuvant arthritis of the rat), a remarkable increase in liver copper, both in concentration (Table 1) and total amount [15], was measured. Interestingly, the concentration as well as the total amount of hepatic copper were found to be related to disease severity, both being significantly higher in high-score compared with low-score arthritic rats [15]. However, two exceptions in which a decrease in liver copper concentrations was measured, have been reported in the literature i.e. the femur fracture model in the rat and the adjuvant-induced abscess in the dog (Table 1).

The kidneys, like erythrocytes and liver, are a body compartment in which a pool of "easily exchangeable copper" may exist [43], and hence the evaluation of their copper status during inflammation could be especially interesting. What has been found so far is that the acute process (carrageenan pleurisy of the rat) does not seem able to modify either the concentration or the total amount of copper [11], whereas significant decreases in kidney copper concentrations (Table 1) and total amounts [15] were reported in the adjuvant arthritic rat. The exact biological meaning of the chronic inflamation-induced decrease in kidney copper levels is unclear at present. However, from a more general point of view, we would like to stress the balance between total liver and kidney copper, which is largely positive, the average accumulation of the metal in liver being about 3 to 20 times higher than the decrease observed in kidneys [15].

Occasionally, during the course of adjuvant arthritis in the rat, the level of copper in tissues other than liver or kidney has been evaluated. Copper concentrations were found to be increased in brain, stomach, bone and pancreas [13], while they were unchanged in femur [16] , brain [44], heart and skeletal muscle [13]. An increase in copper concentration and total amount was observed in the spleen of adjuvant arthritic rats but these changes were interpreted as a secondary phenomenon reflecting the primary increase in blood copper level [15].

In conclusion, the most significant information emerging from all the evidence reported above is that, during inflammation, the copper needed to sustain the increased synthesis of complete ceruloplasmin is taken without depleting any of the body tissues examined, liver included.

Zinc. The status of zinc during inflammation has been evaluated mainly in the liver. In general, the induction of either acute or chronic inflammatory processes in the rat promotes an increase in liver zinc concentration which, as previously discussed, may or may not be accompanied by a parallel decrease in plasma zinc (Table 1). Examining the results obtained in detail it appears that the average increase in total hepatic zinc ranges from 13% to 24% in acute [11, 20] and from 40% to 69% in chronic [15] models of inflammation. Moreover, in the adjuvant arthritis model, the concentration as well as the total amount of hepatic zinc were found to be strictly related to disease severity, both being significantly higher in high-score compared with low-score arthritic rats [15]. As in the case of copper, however, a decrease in liver zinc concentration was observed in rats subjected to femur fracture [24], and in dogs with adjuvant-induced abscesses [26]; interestingly, in the latter model, the decrease in liver zinc concentration was accompanied by an increase in plasma metal level. Many fewer data are available on the status of zinc in kidneys of inflamed animals in which minor and

probably biologically meaningless changes have been reported in both chronic and acute models (Table 1).

Finally, zinc levels in the adjuvant arthritic rat were found to be unchanged in femur [16], brain, stomach, bone and muscle [13, 44], while increases in zinc concentration were found in heart and pancreas [13, 44], and in the total amount of zinc in spleen [15].

Therefore, the experimental evidence so far obtained seems to suggest that inflammation, particularly chronic inflammation, essentially promotes a redistribution of zinc between plasma and liver. However, it should be noted that, at least in adjuvant arthritic rats, the average decrease in zinc in the circulating plasma is approximately 7 μg, whereas over 95 μg of the metal accumulate in the liver [15]; interestingly, this accumulation occurs without zinc depletion in any of the body compartments examined.

Copper and zinc status in inflamed tissues and fluids

Copper and zinc in inflamed sites have not often been determined. Nevertheless, the results obtained so far deserve attention. Copper concentration was found to be higher, compared with the contralateral non-inflamed fluid or tissue, in the inflamed aqueous humor of the endotoxin-injected rabbit and in the inflamed paws of rats challenged with a carrageenan suspension (Table 1). Moreover, copper levels appeared to be significantly higher in the inflamed rear paws of adjuvant arthritic rats (Table 1) Conversely, the concentration of zinc was found to be remarkably decreased (up to 47%) in the inflamed paws resected from both adjuvant arthritic and carrageenan injected rats [15, 20]. At least in the case of solid tissues, the metal concentration changes observed are most likely to be related to the leakage of the inflammatory exudate within the paws, whose average copper and zinc concentrations, in non-inflamed animals, are about 40% lower and over 30 times higher respectively, than those normally observed in plasma [20]. However, it should be stressed that the copper and zinc concentration changes were still evident 144 h after the onset of the acute inflammatory process. By that time, the volume of the inflamed paws was drastically reduced in comparison with the peak hours of the experimental oedema, and the copper and zinc levels appeared to have been normalised in all the other body compartments examined [20]. Particularly interesting are the data obtained on the total amount of either copper or zinc available during the course of both acute and chronic processes in the inflamed area. A statistically significant increase in total copper in the acutely inflamed paw was already evident 1 h after the carrageenan injection (+40%), which peaked at 3 and 5 h (+102%), and at 144 h was still 54% higher than that measured in the controlateral non-inflamed tissues [20]. In the hind paws of adjuvant arthritic rats, total copper steadily and significantly increased from day 7 after the challenge asymptomatic rats) to day 30 (last day of the experimental observation) when a peak of 260% was reached [15]. It is also noteworthy that, during the chronic symptomatic phase, the total rise in copper in the paws of high-score arthritic rats was always significantly higher than that measured in the paws of low-score arthritic animals [15]. As previously mentioned, the concentration of zinc, in contrast to that of copper, was always decreased in the inflamed paws of both carrageenan and complete adjuvant injected rats. Nevertheless, the total amount of zinc in the acutely inflamed tissues was significantly increased compared to that in the contralateral non-inflammed paws [20]. Similarly, although through 7 (asymptomatic rats) to 30 days zinc concentrations were always significantly lower in the rear paws of arthritic compared with those of control animals, the total amounts of the metal were significantly higher in arthritic rats at 14 and 30 days (+23%, vs. a concentration decrease of 34%, and +22%, vs. a concentration decrease of 47%, respectively) [15].

In conclusion, the induction of either an acute or a chronic inflammatory process in the rat promotes an accumulation of both copper and zinc in the inflamed site and, especially in the case of copper, the metal accumulation observed seems to be closely related to the severity of the experimental pathology examined.

Studies on human subjects

Inflammation-induced changes of copper and zinc metabolism in blood and urine

Copper. In man, the existence of infectious, immune disorders or illnesses with a significant inflammatory component is characteristically accompanied by an increase in serum (or plasma) copper

(Table 2; references quoted [3, 33, 40, 45–67]) and ceruloplasmin levels, these two parameters being highly and significantly correlated [40, 68–70].
As in the experimentally induced inflammatory pathologies, the distribution of copper between ceruloplasmin and the loosely binding ligands present in serum (albumin, amino acids, etc.) has not often been investigated in human diseases. The non-ceruloplasmin bound fraction of serum copper was indirectly determined (as the difference between total and ceruloplasmin bound metal, the latter fraction being calculated and not directly measured) in rheumatoid arthritis patients, and stated to be markedly higher than that in controls [49, 71]. However, using the same methodological approach, other authors did not find significant differences between the levels of non-ceruloplasmin bound serum and plasma copper present in rheumatoid patients and control subjects [40, 69]. Moreover, the direct measurement (atomic absorption spectrophotometry with graphite furnace) of this copper fraction, carried out, after pseudo-ligand chromatography followed by gel filtration, on the sera obtained from rheumatoid arthritis patients

Table 2
Changes in Cu and Zn levels in some body compartments of human patients affected by diseases bearing a relevant inflammatory component.

Disease	Serum (plasma)		Blood cells		24 urine		Inflamed fluid or tissue		References
	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	
Rheumatoid arthritis	I	U	—	—	I	U	—	—	[45]
Rheumatoid arthritis (juvenile)	I	D	—	—	—	—	—	—	[46]
Rheumatoid arthritis	U	—	—	—	—	—	—	—	[47]
Rheumatoid arthritis	I	D	—	—	—	—	—	—	[48]
Rheumatoid arthritis	I	D	U	U	—	—	—	—	[49]
Rheumatoid arthritis	—	—	—	—	—	—	—	I*	[50]
Rheumatoid arthritis	I	—	U	—	—	—	—	—	[51]
Rheumatoid arthritis	I	D	U	U	U	U	—	—	[40]
Rheumatoid arthritis	I	D	I	U	U	U	—	—	[52]
Rheumatoid arthritis	I	D	—	—	—	—	—	—	[53]
Rheumatoid arthritis	I	D	U	U	U	U	—	—	[54]
Rheumatoid arthritis	I	D	—	—	—	—	I*	I*	[55]
Rheumatoid arthritis	U	—	—	—	—	—	U*	—	[56]
Rheumatoid arthritis	—	—	—	—	—	—	I*	—	[57]
Rheumatoid arthritis	—	D	—	D	—	—	—	—	[58]
Ankylosing spondylitis	I	—	—	—	—	—	—	—	[59]
Ankylosing spondylitis	—	U	—	U	—	—	—	—	[60]
Collagen diseases	I	—	U	—	—	—	—	—	[51]
Mixed connective tissue diseases	I	I	—	—	—	—	—	—	[61]
Peridontal disease	I	U	—	—	—	—	—	—	[62]
Peridontal disease	—	—	—	—	—	—	I**	—	[33]
Crhon's disease	—	D	—	U	—	—	—	—	[63]
Psoriatic arthritis	I	U	U	U	U	U	—	—	[3]
Tonsillitis	I	—	—	—	—	—	—	—	[64]
Bronchiectasis	I	—	U	—	—	—	—	—	[51]
Empyema	I	—	U	—	—	—	—	—	[51]
Pneumonia	I	—	U	—	—	—	—	—	[51]
Rheumatic fever	I	—	U	—	—	—	—	—	[51]
Gout (clinically active)	—	D	—	U	—	U	—	—	[65]
Dermatitis herpetiformis	—	D	—	—	—	—	—	D*	[66]
Psoriasis	—	U	—	—	—	—	—	D*	[66]
Sandfly fever virus (on healthy volunteers)	I	D	—	—	D	D	—	—	[67]

— not determined; D, statistically significant decrease; I, statistically significant increase; U, statistically unchanged; I*, U* measured as total element (I*) or ceruloplasmin (U*) in synovial fluid of rheumatoid patients compared with synovial fluid of osteoarthritic patients; I**, measured as ceruloplasmin in the inflamed compared with normal peridontal tissue; D* measured in inflamed compared with normal epidermis.

and normal individuals, has recently revealed a slight but statistically significant decrease in non-ceruloplasmin bound copper in rheumatoid arthritic subjects [37]. Although this problem remains an open question and certainly deserves further research, ceruloplasmin in man probably represents the major carrier of the extra copper found in inflamed sera.

The correlations between total serum (or plasma) copper and the clinical markers characterising chronic inflammatory diseases have been studied by many authors, although almost exclusively in rheumatoid arthritis. Serum copper has repeatedly been found to be correlated with parametres such as C-reactive protein and/or erythrocyte sedimentation rate [40, 49, 54, 72–74], but the existence of significant correlations between copper serum levels and the clinical indices of rheumatoid arthritis seems to be much more controversial. For instance, serum copper was reported to be correlated with articular index [49] and with disease activity measured by a "Composite Activity Index" [73], an index that was devised by the authors of the paper themselves. However, other authors failed to find any correlation between many classical markers of rheumatoid arthritis severity (functional class, anatomical stage and physician assessment included) and the copper levels in plasma [40, 54]. Especially interesting, and perhaps conclusive, are the results obtained using a sophisticated statistical approach (forward stepwise multiple linear regression analysis) by Mussalo-Rauhamaa et al. [53], who also found that serum copper correlates very poorly with the overall disease severity as determined strictly according to the American Rheumatism Association criteria.

As stated in the introduction, a marginal deficiency of copper has been proposed to be either pre-existent and contributing to the development of rheumatoid arthritis and/or eventually determined by the progression of the disease itself. The determination of copper in plasma is, however, of little value in such circumstances since the metal concentration rises during inflammatory processes and this occurs dramatically even if the inflammation is elicited in severely copper-deprived animals [75]. The assay of erythrocyte metal levels is more useful in verifying the existence of a copper deficiency in man [76]. The data summarized in Table 2 seem to indicate clearly that the erythrocyte copper levels are not reduced in either acute or chronic inflammation (rheumatoid arthritis included) and that rheumatoid arthritis patients are basically not copper-deficient individuals. Moreover, studying the status of copper in erythrocytes of rheumatoid arthritis subjects divided according to their disease duration, it has not been possible to find any significant difference among the groups considered [40]. Hence, the persistence of the chronic pathology did not promote a progressive depletion of body copper stores.

Another parameter that could have some value in assessing the overall body copper status is the 24 h urinary excretion of the metal, which when high may suggest a possible exhaustion of body copper deposits, but when low, could indicate the existence of a fully developed copper deficiency status [76]. Examining the results obtained in man on the urinary excretion of this metal during inflammatory diseases (Table 2), it appears reasonable to conclude that such pathologies, especially rheumatoid arthritis, do not develop in individuals deficient in copper. Moreover, determining the copper urinary excretion in rheumatoid arthritis patients stratified according to their disease duration revealed that urinary copper did not change significantly with time [40]. The above observations therefore seem to confirm the conclusions drawn on the basis of the evaluation of erythrocyte copper status, and suggest that rheumatoid arthritis develops and progresses without an association with the body copper deficiency condition.

Zinc. Serum zinc is certainly reduced below normal levels in rheumatoid arthritis patients, but, as in laboratory animals and contrary to copper, the concentration of this metal in human serum or plasma may or may not be modified depending on the pathology considered (Table 2). However, since rheumatoid arthritis is the disease that has been most extensively studied, we shall focus our discussion primarily on this condition. Rheumatoid arthritis patients almost always show a significant decrease in plasma zinc levels (Table 2). Nevertheless, it is also well established that a decrease in serum albumin concentration typically characterises this chronic disease. Albumin is the carrier protein that regulates the portal transport of zinc from the sites of intestinal absorption to the liver [77]. Thus, it has been suggested that the circulating level of albumin may be an important determinant of the rate of zinc absorption [78], and that in patients with low albumin levels the plasma zinc concentrations may also be expected to be low [79]. In our opinion, however, the decrease in

plasma zinc in rheumatoid arthritis patients does not depend on the reduction of circulating albumin. This hypothesis is strongly supported by the data of Foote and Delves [80], who showed that in normal humans only 1.1–2.6% of the albumin present in serum is engaged in zinc transport, and zinc occupies less than 0.2% of the total binding capacity of the protein.

In contrast to copper, plasma zinc in rheumatoid arthritis patients significantly correlates not only with many markers of the disease, but also with disease severity as shown, for example, by the correlations found with functional class [54] and with swollen joints, grip strength, anatomical stage and physician assessment [40]. Moreover, it was also shown (by forward stepwise multiple regression analysis) that at least nine independent variables, including clinical indices of rheumatoid arthritis, together predicted 73% of the serum zinc variation [53]. Hence, these observations suggest that plasma zinc concentration could have some practical value in defining the overall severity of rheumatoid arthritis. However, this is put in doubt by the fact that since rheumatoid arthritis patients are often on steroids and/or non-steroidal anti-inflammatory agents (NSAIDs), these could interfere with zinc absorption and metabolism. In the next section we will discuss this important topic more in detail.

As in the case of copper, plasma zinc cannot be accepted as a true indicator of the body status of this metal since its level in circulating fluids is strongly influenced by many factors, particularly nutrition and disease [76, 81]. Thus, as for copper, the evaluation of erythrocyte and 24 h urine zinc levels may be more useful parameter of a deficiency condition [76, 81, 82] (note that the value of leucocyte zinc levels as an index of whole body zinc is rather controversial [83–86]). Svenson et al. [58], measuring erythrocyte zinc levels in 11 rheumatoid arthritis patients, reported an almost total disappearance of the metal from the cells (the reduction observed in comparison to healthy controls was about 95%). On the other hand, a transient decrease in 24 h zinc (and copper) urinary excretion was found in eight sandfly fever virus infected volunteers [67]. Apart from these exceptions, the studies performed on hundreds of patients, most suffering from rheumatoid arthritis, have never shown levels of either erythrocyte or 24 h urinary zinc different from those of control subjects (Table 2). Moreover, it was recently shown that the status of zinc in urine and erythrocytes of rheumatoid patients does not appear to be influenced by the duration of the disease [40]. Therefore, as suggested for copper and considering that the mononuclear leucocyte zinc levels appear to be higher than normal in rheumatic patients [86], rheumatoid arthritis seems to develop and progress without being linked to a body zinc deficiency.

The influence of drugs on copper and zinc levels: Studies on rheumatoid arthritis patients. Patients suffering from rheumatoid anthritis represent a population that often undergoes multiple drug therapy, and some of the agents currently used to treat these subjects are known to be able potentially to interfere with either copper or zinc absorption and metabolism or both. For instance, steroids and NSAIDs are thought to induce a decrease in plasma zinc levels in man [48, 54, 87] as well as in laboratory animals [88–90]. D-penicillamine, a potent cupriuretic agent in man [91] able to significantly modify the copper status in experimental animals [43, 92], is also suspected to be capable of interfering with the metabolism of zinc [93–95]. Finally, the administration of gold promotes the *in vivo* synthesis of metallothioneins [41, 96], a class of proteins known to be essential in the regulation of trace metal metabolism [41, 77].

The data on serum copper in rheumatoid patients in relation to drug treatment are somewhere contradictory, and while some authors found that a decrease in copper level is the result of therapy with NSAIDs, steroids, gold preparations or D-penicillamine, other authors failed to report any such effect (Table 3). Although unequivocal answers to this problem are not available at present, a significantly larger number of rheumatoid arthritis patients have shown no notable change in serum or plasma copper levels as a result of drug therapy D-penicillamine included).

In general, plasma zinc has not been found to be affected by the use of either gold salts or D-penicillamine, whereas a further decrease in serum or plasma concentration has frequently been observed in rheumatoid patients treated with NSAIDs or steroids compared with patients not taking these drugs (Table 3). However, the situation is complicated by the fact that these two drugs, which are both potentially capable of decreasing plasma zinc levels, are often taken simultaneously by rheumatoid arthritis patients [40, 53]. Moreover, the use of NSAIDs, or steroids, or both, tends to be significantly more frequent when the disease is more severe [40]. Therefore, it is difficult to establish whether the lower levels of serum or plasma zinc observed in the more severely affected rheuma-

Table 3
Influence of drug therapy on copper and zinc in serum (or plasma), erythrocytes (or total blood cells) (BC) and 24 h urinary excretion in rheumatoid arthritis patients.

Drug therapy	Serum (plasma)		Blood Cells		24 h Urine		References
	Cu	Zn	Cu	Zn	Cu	Zn	
NSAIDs	U	—	—	—	—	—	[72]
	D	—	—	—	—	—	[97]
	U	—	—	—	—	—	[98]
	—	D	—	—	—	I*	[87]
	—	U	—	—	—	I*	[99]
	—	D	—	—	—	—	[48]
	—	U	—	U	—	—	[60]
	U	D	U	U	U	U	[40]
Steroids	—	U	—	I	—	—	[58]
	D	—	—	—	—	—	[72]
	U	—	—	—	—	—	[98]
	U	D	—	—	—	—	[100]
	U	D	U	U	U	U	[54]
	—	U	—	U	—	—	[60]
	U	D	U	U	U	U	[40]
Gold preparations	D	—	—	—	—	—	[68]
	U	—	—	—	—	—	[98]
	U	U	—	—	—	—	[100]
	—	U	—	U	—	—	[60]
	U	U	U	U	U	U	[40]
D-penicillamine	D	—	—	—	—	—	[72]
	D	—	—	—	—	—	[97]
	D	—	—	—	—	—	[68]
	U	—	—	—	—	—	[98]
	—	U	—	U	—	—	[60]
	—	I	—	I	—	I	[94]
	U	U	U	U	I	U	[40]

For each drug, comparison was made with rheumatoid arthritis patients on different medication or untreated — not determined; U, statistically unchanged; D, statistically significant decrease; I, statistically significant increase; I*, a statistically significant increase; was observed only during the first day of treatment followed by a recovery.

toid patients and in those taking NSAIDs or steroids are brought about by the severity of one arthritic condition or by the use of steroids or NSAIDs. Indeed they may be the result of a combined effect of both disease severity and drug therapy. However, there is little evidence supporting the proposal that the decrease in plasma zinc in rheumatoid arthritis patients is determined by the severity of the illness, independently of any treatment. First, it has been shown, in both normal and adjuvant arthritic rats, that glucocorticoid administration causes an initial and transient decrease in serum zinc concentration, promptly followed by a return of this parameter to the levels measured before the beginning of the treatment [101]. On the other hand, oral administration of indomethacin (50 mg/day, for 3 days) did not modify the intestinal absorption of orally given zinc sulphate in healthy human volunteers [102]. Second, rheumatoid arthritis patients who take both steroids and NSAIDs do not have lower plasma zinc concentrations than patients taking only steroids or NSAIDs, i.e. the combined use of these drugs does not seem to have any cumulative effect on plasma zinc [40]. Moreover, rheumatoid arthritis is characterised by an increased synthesis and secretion of cytokines [103] which, as discussed later, are known to promote a redistribution of zinc in the organism, inducing especially the accumulation of the metal in the liver and a concomitant decrease in plasma [77].

Finally, the data reported in Table 3 show that the drugs most frequently used in the treatment of rheumatoid arthritis do not significantly modify the

status of copper and zinc in either erythrocytes or 24 h urine. The only exception is D-penicillamine, which is known to increase the urinary excretion of copper and, perhaps, zinc.
Therefore, copper and zinc status parameters in rheumatoid arthritis may not be significantly influenced by drug treatment.

Copper and zinc in inflamed fluids and tissues

To our knowledge, the assay of copper and/or zinc in human inflamed fluids or tissues has been carried out in only a few cases, but the results obtained seem to confirm the observations made on laboratory animals. Although ceruloplasmin concentrations were reported to be the same in the synovial effusions taken from rheumatoid arthritis and osteoarthritic patients [56], a significant increase in ceruloplasmin levels in rheumatoid fluids was subsequently reported to characterise arthritic subjects [57]. Moreover, total copper and zinc concentrations in the joint effusions of rheumatoid patients were found to be higher than those measured in the fluids withdrawn from osteoarthritic or traumatic knees (Table 2). Interestingly, Peretz et al. [55] have recently shown that a dramatic accumulation of both copper and zinc occurs in the joint exudates taken from rheumatoid patients in comparison with those derived from osteoarthritic and traumatic subjects.
As far as solid tissues are concerned, a 15-fold increase in ceruloplasmin levels was found in inflamed as compared to normal periodontal tissues [33]. Conversely, zinc concentration was decreased in the epidermis and papillary dermis of dermatitis herpetiformis and psoriasis patients (Table 2). It was suggested that these patients were zinc-deficient subjects even though they had normal serum zinc values [66]. However, this conclusion may represent a false interpretation since papillary dermis could, in such pathological conditions, be infiltrated by an inflammatory exudate. As previously observed in laboratory animals, a local decrease in metal concentration may hind the fact that the total amount of zinc in the inflamed area could actually have increased [15, 20].

Final remarks

Recent research draws attention to the endogenous mediators and functional proteins that may be involved in both inflammation and trace metal metabolism. The pro-inflammatory cytokines, in particular interleukin 1 (IL-1) and interleukin 6 (IL-6), are now recognised to participate in the pathogenesis and development of acute and chronic inflammatory processes, where they seem to play a pivotal role [104]. For example, IL-1 production has been found to occur in mice during calcium pyrophosphate-induced pleurisy [105] and in rat zymosan pleurisy and mouse peritonitis [106]. Moreover, it has been shown that the intercellular adhesion molecule 1 (ICAM-1), whose expression could be dependent on the synthesis and secretion of pro-inflammatory cytokines [107], is critically involved in the pathogenesis of adjuvant arthritis in rats [108]. Also, in humans suffering from inflammatory or autoimmune diseases (rheumatoid arthritis included) cytokine production, especially that of IL-1 and IL-6, is significantly enhanced [103]. On the other hand, copper and zinc absorption and metabolism are strictly regulated by metallothioneins [41, 77] that are increased in the liver of both turpentine-injected [109] and adjuvant arthritic [2] rats. The synthesis of these inducible proteins is typically promoted in many different tissues such as liver, intestinal walls and kidney [41, 77], or even in isolated monocytes and lymphocytes [110, 111], by either copper or zinc themselves. Nevertheless, IL-6 [112] and IL-1 are potent inducers of metallothionein synthesis in liver, bone marrow and thymus, causing marked accumulation of zinc [113], probably in co-operation with glucocorticoids and glucagon [77]. Either alone or perhaps co-operatively with glucocorticoids and glucagon, IL-1 induced the *in vitro* synthesis and secretion of ceruloplasmin by rat liver parenchymal cells [77]. Finally, the *in vivo* administration of both IL-1 [113, 114] and IL-6 [114] to rats was found to promote the synthesis of acute phase proteins, ceruloplasmin included. Thus, the changes in copper and zinc metabolism occuring during acute and chronic inflammation in man as well as in laboratory animals are most likely to be initiated and sustained by the endogenous synthesis of pro-inflammatory cytokines and metallothioneins.
By now it is clear that profound variations in copper and zinc status occur during the course of inflammation. However, from the evidence outlined in this paper it appears that, although a direct relationship between the severity of the inflammatory condition and the levels of copper and zinc may

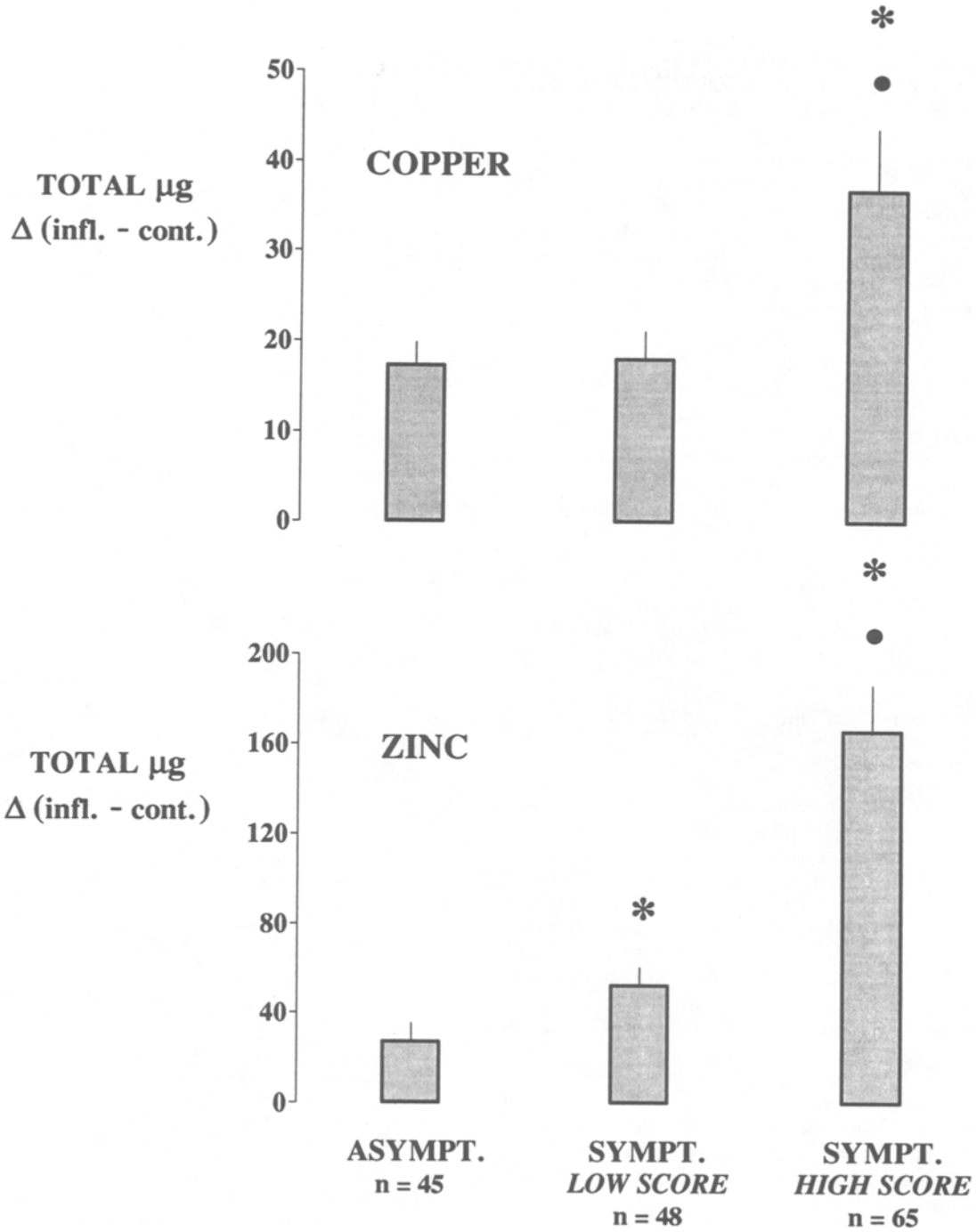

Figure 1
Comprehensive accumulation of copper and zinc induced by adjuvant arthritis in whole blood, liver, kidneys, spleen and inflamed hind paws of rats both during the asymptomatic (days 3 and 7) and the symptomatic (days 14, 21 and 30) phases of the disease. Data recalculated from those reported in ref. [15]. * $P<0.001$ Student's t-test vs. asymptomatic rats. ● $P<0.001$ Student's t-test vs. symptomatic, low-score rats.

exist in solid tissues such as the liver and inflamed area, the assay of these trace metals in serum or plasma only has value as a diagnostic tool in the case of zinc.

A further point emerging from the studies we have summarized is that the development of rheumatoid arthritis is probably not supported by marginal copper or zinc deficiency conditions. Moreover, the progress and persistence of the chronic disease does not appear to promote any trace metal depletion. This implies that a rationale for treating rheumatoid arthritis with copper and/or zinc preparations cannot be established on the basis of a need to restore deposits of these trace elements in rheumatoid arthritis patients.

Nevertheless, inflammation is clearly a condition in which more copper and zinc are demanded by the organism, this increased requirement being fulfilled by enhanced intestinal absorption and/or decreased intestinal excretion [11]. In fact, on the basis of the cumulative amounts of copper and zinc present in plasma, blood cells, liver, kidneys, spleen and inflamed hind paws of adjuvant arthritic rats, both copper and zinc were found to accumulate significantly in the inflamed animals (Fig. 1). Interestingly, this accumulation was shown to precede the appearance of any visible pathological symptom while during the symptomatic phase it was found to be significantly different in the rats that developed only a mild arthritis (low-score animals) from those in which the disease was fully expressed (high-score animals) (Fig. 1). Moreover, turpentine oedema seems to increase the amount of dietary copper required to maintain hepatic Cu–Zn superoxide dismutase levels equal to those of non-stressed rats [115], and the challenge with either *Francisella tulariensis* live vaccine or *E. coli* endotoxin appears to produce both increased absorption and retention of zinc in the rat [21].

Since exogenously administered copper and zinc have been shown to possess anti-inflammatory/anti-arthritic activity in both laboratory animals and man, the data on copper and zinc levels in inflammation indicate that the use of these metals, alone or in combination, in the therapy of chronic inflammatory diseases certainly deserves further research.

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