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Copper metabolism during acute inflammation: studies on liver and serum copper concentrations in normal and inflamed rats

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1 The concentration of copper in serum and liver was determined by atomic absorption spectrophotometry in a study performed on normal rats of either sex and in female rats with carrageenan-induced pleurisy.

2 In the normal animal, total serum copper concentration is significantly higher in female rats, and appears to be higher in mature animals in females.

3 In normal rats of either sex, liver copper concentration undergoes daily variations which are inversely related to the weight of the organ and which leave constant the total amount of metal in the liver. Moreover a day to day non-cyclic variability of liver copper concentration and liver weight was observed.

4 This first set of data showed that comparison with time control was essential.

5 In the inflamed rat, a significant rise of total serum copper at 22, 48 and 72 h after the induction of inflammation was observed. From 96 h up to 240 h post-injection no significant differences were evident.

6 Total liver copper content did not change in the inflamed rats.

7 During acute inflammation in the rat, the copper needed for the increased synthesis of caeruloplasmin is supplied without depletion of liver copper stores.

Introduction

In the last few years many experimental results obtained by us and others have contribued to emphasise the importance of the role played by endogenous copper in the modulation of the inflammatory process. We have shown that copper-deficient rats are more prone to the effects of standard acute inflammatory agents compared with animals on a normal copper diet (Milanino, Mazzoli, Passarella, Tarter & Velo, 1978; Milanino, Conforti, Fracasso, Franco, Leone, Passarella, Tarter & Velo, 1979). Moreover a sharp rise of copper concentration and caeruloplasmin activity in biological fluids and tissues has been measured in man and animals under a wide variety of acute and chronic inflammatory conditions (Milanino & Velo, 1981). In particular, we have recently found a statistically significant increase of serum copper concentration and caeruloplasmin activity in carrageenan-induced foot-oedema and pleurisy in rats. A strong positive correlation between copper and caeruloplasmin in the sera of both normal and inflamed rats was also shown (Conforti, Franco, Milanino & Velo, 1981; 1982). This latter observation confirms what has been described in normal humans and rheumatoid patients (Scudder, Al-Timini, McMurray, White, Zoob & Dormandy, 1978; Conforti, Franco, Milanino, Tarolli & Velo, 1982), and allows one to take the measure of total circulating copper as a sufficiently good indication of the amount of circulating caeruloplasmin.

Though all caeruloplasmin is synthesized by the liver, it is not clear whether the copper needed for the increased synthesis of this protein, which occurs during inflammation, is taken from the liver deposits or not and the literature is contradictory (Wintrobe, Cartwright & Gubler, 1953; Karabelas, 1972; Feldman, Keen, Kaneko & Farver, 1981).

We decided to study the liver copper content together with that of serum during the acute inflammatory process in the rat. However, a survey of the literature showed us that it is still uncertain whether serum and liver copper undergo any cyclic pattern of variation (during the day or from day to day) in the normal rat.

This paper describes a study in normal rats of either sex of both liver and total serum copper contents throughout the usual working day (from 09 h 00 min to 19 h 00 min) and for up to 10 days in the case of male and 28 days in the case of female rats. In addition, the results for total circulating and liver copper in female rats with carrageenan-induced pleurisy, are presented.

Methods

Sprague-Dawley rats (CD COBS from Charles River, Italy) of either sex were used in all experiments. They were kept housed in groups of 5–10 animals on a 12 h light-dark cycle at constant temperature (20° ± 1 C) and humidity (55 ± 5%), and fed *ad libitum* on a standard diet containing 30 mg/kg of copper and tap water.

All the chemicals used were of A.R. grade, free from copper contamination. The glassware was made copper-free by soaking overnight in a 1:1 solution of 70% HNO_3 and glass-distilled water.

Killing of animals and collection of samples

The rats were killed by exposure to diethyl ether. All the animals used at zero time and those used on days other than the first day of each experiment, were killed between 09 h 00 min and 10 h 00 min. Immediately after death, from each rat about 5 ml of blood was collected directly from the heart, allowed to clot at room temperature and the serum obtained was stored at 4°C. The liver was removed after blood collection, carefully weighed, minced with stainless steel scissors and stored at −20°C.

Copper determination in the serum

Copper determinations were made by atomic absorption spectrophotometry (Perkin-Elmer 360 atomic absorption spectrophotometer) on serum deproteinized by addition of an equal amount of a 10% trichloroacetic acid solution.

Copper determination in the liver

Minced liver (2.5 g) was placed in a 50 ml conical flask together with about 6 g of glass beads (3 mm diam.). Glass-distilled water (12.5 ml) was added, followed by 5 ml of a 1:1 mixture of 70% HNO_3 and 70% $HClO_4$. The sample was then boiled until the suspension became clear, cooled at room temperature, passed through a sintered glass funnel (Dure Jena Glass, n. 3), and brought to a final volume of 10 ml with glass-distilled water. The solution was then read directly in the atomic absorption spectrophotometer.

We made sure that no copper was lost during the extraction by digesting specimens from the same liver with or without the addition of a known amount of copper.

As previously shown by Karabelas (1972) we also found, after separate digestion of the lobes, that there was no difference in the copper concentration between the lobes of liver. This observation allowed us to calculate the total copper content of the liver from copper concentration (μg/g) and the total weight of the organ.

Acute inflammation

Pleurisy was induced in 160 female rats by intrapleural injections of 0.15 ml of 1% carrageenan (Rex 8231, Lambda carrageenan 12/15/78 Marine Colloids Inc.) suspended in sterile saline. The exudate was withdrawn 6 and 22 h after the irritant. At each time-interval (0 time and 6, 22, 48, 72, 96, 144, 192, 240 h after carrageenan) groups of 20 inflamed rats were paired with groups of 15 control rats selected by weight and age (time controls). The mean difference in weight was within 3 g.

Table 1 Serum and liver copper in male rats

Time	Body weight (g)	Liver weight (g)	Serum Cu (μg/100 ml)	Liver Cu (μg/g)	Liver Cu (Total μg)
0 h	189 ± 6	8.1 ± 1.6	120 ± 10	4.32 ± 0.44	34.42 ± 4.65
3 h	186 ± 8	7.7 ± 0.9	118 ± 14	4.54 ± 0.49	34.47 ± 2.18
6 h	180 ± 14	7.1 ± 1.0	128 ± 19	4.89** ± 0.39	34.41 ± 3.45
9 h	177** ± 8	6.9* ± 0.4	133* ± 13	4.96** ± 0.36	34.37 ± 2.30
2 d	203** ± 9	8.9 ± 0.7	122 ± 11	4.06 ± 0.30	36.09 ± 2.43
6 d	243** ± 12	11.3** ± 0.9	125 ± 14	3.80* ± 0.38	42.83** ± 5.54
10 d	278** ± 13	12.3** ± 0.9	124 ± 32	3.99 ± 0.45	48.87** ± 6.28

Mean results (± s.d.) of ten rats per group.
* $P < 0.05$; ** $P < 0.01$: comparison versus zero time (Dunnett's test).

Table 2 Serum and liver copper in female rats

Time	Body weight (g)	Liver weight (g)	Serum Cu (μg/100 ml)	Liver Cu (μg/g)	Liver Cu (Total μg)
0 h	213 ±10	9.5 ±0.9	174±33	4.11 ±0.25	38.87±3.56
3 h	209 ± 9	8.8 ±0.8	173±14	4.41 ±0.24	38.82±3.03
6 h	205 ± 8	8.1** ±0.6	170±16	4.73** ±0.28	38.06±1.55
9 h	203 ± 5	7.5** ±0.6	173±21	5.22** ±0.33	39.28±3.63
7 d	217 ± 6	8.6* ±0.4	191±19	4.46* ±0.22	38.32±2.65
14 d	224* ±11	8.7 ±0.9	193±24	4.39 ±0.27	37.91±4.03
21 d	238** ±10	9.2 ±0.6	153±18	4.47* ±0.25	41.24±3.16
28 d	231** ± 8	9.7 ±0.8	154±14	3.83 ±0.16	36.98±3.65

Mean results (±s.d.) of ten rats per group
*$P<0.05$; **$P<0.01$; comparison versus zero time (Dunnett's test).

Results

Normal male rats

On the first day of the experiment, liver and serum copper were determined every 3 h starting at 09 h 30 min (zero time) and ending at 18 h 30 min (9th hour). Liver and serum copper were measured on the 2nd, 6th and 10th day. The results obtained are summarized in Table 1. During the first day, from zero time to the 9th hour, a decrease of both body and liver weights was observed. An increase of liver copper concentration was measured which accompanied the decrease in liver weight. In particular by the 9th hour liver weight decreased by about 14% and liver copper concentration increased by about 15% compared with zero time, both differences being statistically significant ($P<0.01$). In spite of the variations of liver copper concentrations the total amount of copper measured in the liver from zero time to the 9th hour remained unchanged. By the end of the experiment (10th day) a significant increase in body weight was evident, accompanied by a proportional increase in liver weight. Liver copper concentration was scarcely changed, while the total copper content of the liver was considerably increased. Total serum copper concentration showed only minor changes throughout the experiment.

Normal female rats

As in the case of male rats, on the first day of the experiment liver and serum copper were determined every 3 h beginning from 09 h 30 min (zero time) and ending at 18 h 30 min (9th hour). Liver and serum copper were then measured on the 7th, 14th, 21st and 28th day. The results are summarized in Table 2. During the first day a gradual decrease of both body and liver weights was measured from zero time to the 9th hour. In parallel with the decrease in liver weight, a progressive increase in liver copper concentration was observed. In particular by the 9th hour liver weight decreased by about 21% and liver copper concentration increased by about 27% compared with zero time, both differences being statistically significant ($P<0.01$). However, the total amount of copper measured in the liver from zero time to the 9th hour did not show any appreciable variation. By the end of the experiment (28th day) a modest increase of body weight was noticed, while the weight of the liver remained almost unchanged. Liver copper concentration showed only minor changes from day to day, and the total copper content of liver, like organ weight, changed very little. Total serum copper concentration did not show statistically significant differences throughout the experiment. Since the first crucial survey in the carrageenan-induced pleur-

Table 3 One-day study of serum and liver copper in female rats

Time (h)	Body weight (g)	Liver weight (g)	Serum Cu (μg/100 ml)	Liver Cu (μg/g)	Liver Cu (Total μg)
0	167± 6	7.7 ±0.8	149±17	4.31 ±0.31	33.03±2.80
3	167± 4	7.4 ±0.6	164±18	4.54 ±0.46	33.29±2.58
6	164± 4	7.0* ±0.8	154±12	5.02** ±0.41	34.76±3.56
9	160±10	6.4** ±1.0	156±16	5.37** ±0.46	33.89±2.31

Mean results (±s.d.) of ten rats per group
*$P<0.05$; **$P<0.01$: comparison versus zero time (Dunnett's test).

Table 4 Serum and liver copper: comparison between male and female rats

	Serum		
Sex	n	*Body weight* (g) ± s.d.	*Cu* (μg/100 ml) ± s.d.
Male	90	212† ± 36	127 ± 19
Female	174	175**† ± 12	150** ± 23
Female	103	217‡ ± 14	170** ± 25

	Liver				
Sex	n	*Body weight* (g) ± s.d.	*Liver weight* (g) ± s.d.	*Cu* (μg/g) ± s.d..	*Cu* total (μg) ± s.d.
Male	40	228† ± 37	10.1 ± 2.0	4.04 ± 0.42	40.55 ± 7.51
Female	50	224‡ ± 13	9.1** ± 0.8	4.25* ± 0.34	38.66 ± 3.60

†: about 7 weeks old; ‡: about 11 weeks old
*$P<0.05$; **$P<0.01$: comparison versus males (Student's *t* test).

Table 5 Serum copper concentration in rats with carrageenan-induced pleurisy

Time	*Group* (n)†		*Body weight‡* (g) ± s.d.	*Exudate volume* (ml) ± s.d.	*Serum Cu* (μg/100 ml) ± s.d.	*P* versus 0 h controls	*P* versus time controls
0 h	Controls	(20)	173 ± 6	—	145 ± 19	—	—
6 h	Cont.	(15)	165 ± 5	—	150 ± 21	NS	—
	Infl.	(20)	168 ± 11	0.89 ± 0.33	150 ± 21	NS	NS
22 h	Cont.	(15)	173 ± 10	—	130 ± 18	NS	—
	Infl.	(20)	172 ± 11	1.28 ± 0.69	220 ± 26	<0.01	<0.01
48 h	Cont.	(15)	180 ± 10	—	145 ± 16	NS	—
	Infl.	(20)	184 ± 7	0	189 ± 13	<0.01	<0.01
72 h	Cont.	(15)	183 ± 9	—	142 ± 23	NS	—
	Infl.	(20)	184 ± 9	0	176 ± 33	<0.01	<0.01
96 h	Cont.	(15)	185 ± 6	—	162 ± 20	NS	—
	Infl.	(20)	186 ± 9	0	150 ± 18	NS	NS
144 h	Cont.	(15)	189 ± 9	—	155 ± 29	NS	—
	Infl.	(20)	191 ± 6	0	149 ± 32	NS	NS
192 h	Cont.	(15)	194 ± 12	—	147 ± 29	NS	—
	Infl.	(20)	199 ± 12	0	150 ± 26	NS	NS
240 h	Cont.	(15)	199 ± 15	—	154 ± 16	NS	—
	Infl.	(20)	201 ± 12	0	152 ± 25	NS	NS

†*n* = Number of rats. ‡At the time of death. NS = Not significant.

Table 6 Liver copper in rats with carrageenan-induced pleurisy

Time	*Group*	*Body weight*† (g)	*Liver weight* (g)	*P* versus 0 h	*P* versus time cont.	*Liver Cu* (μg/g)	*P* versus 0 h	*P* versus time cont.	*Liver Cu* (total μg)	*P* versus 0 h	*P* versus time cont.
0 h	Cont.	178 ± 3	7.7 ± 0.5	—	—	4.22 ± 0.16	—	—	31.61 ± 1.91	—	—
6 h	Cont.	166** ± 5	6.4 ± 0.7	<.01	—	4.87 ± 0.20	<.01	—	31.27 ± 3.20	NS	—
	Infl.	165* ± 15	7.3 ± 1.0	NS	<.05	4.42 ± 0.42	NS	<.01	31.96 ± 4.32	NS	NS
22 h	Cont.	174* ± 11	8.4 ± 0.9	NS	—	3.74 ± 0.37	<.01	—	31.49 ± 4.97	NS	—
	Infl.	168* ± 14	8.4 ± 0.7	<.05	NS	4.09 ± 0.69	NS	NS	34.42 ± 6.83	NS	NS
48 h	Cont.	183** ± 10	8.5 ± 0.8	<.05	—	3.57 ± 0.13	<.01	—	31.95 ± 3.47	NS	—
	Infl.	183** ± 9	8.1 ± 0.8	NS	NS	3.86 ± 0.32	<.01	<.05	31.26 ± 2.71	NS	NS
72 h	Cont.	188** ± 6	8.7 ± 0.8	<.01	—	3.89 ± 0.35	<.05	—	33.91 ± 4.39	NS	—
	Infl.	186** ± 9	9.0 ± 0.8	<.01	NS	3.47 ± 0.31	<.01	<.05	31.10 ± 2.74	NS	NS
96 h	Cont.	186** ± 4	9.0 ± 0.6	<.01	—	4.19 ± 0.21	NS	—	37.60 ± 3.82	<.01	—
	Infl.	188** ± 11	9.2 ± 0.9	<.01	NS	3.80 ± 0.59	<.05	NS	34.67 ± 3.32	NS	NS
144 h	Cont.	193** ± 6	8.9 ± 0.7	<.01	—	4.10 ± 0.32	NS	—	36.19 ± 2.46	<.01	—
	Infl.	192** ± 7	8.9 ± 0.8	<.01	NS	3.93 ± 0.29	<.05	NS	35.08 ± 4.25	NS	NS
192 h	Cont.	200** ± 10	9.6 ± 0.8	<.01	—	3.86 ± 0.25	<.01	—	36.87 ± 3.29	<.01	—
	Infl.	200** ± 13	9.7 ± 0.8	<.01	NS	4.01 ± 0.26	<.05	NS	38.84 ± 2.37	<.01	NS
240 h	Cont.	204** ± 15	9.0 ± 1.1	<.01	—	4.01 ± 0.36	NS	—	35.98 ± 2.40	<.01	—
	Infl.	207** ± 9	9.2 ± 0.7	<.01	NS	4.13 ± 0.14	NS	NS	37.88 ± 3.09	<.01	NS

†At the time of death.
Mean results (± s.d.) of ten rats per group.

NS = Not significant.
*$P<0.05$; **$P<0.01$; Comparison versus 0 time.

isy is usually carried out 6 h after the injection of the irritant, i.e. in the early afternoon, we became interested in the daily variations observed in liver weight and liver copper concentration. We decided to repeat the one day monitoring experiment with a second group of female rats and we obtained results (Table 3) that fully confirmed the trend observed with the previously studied animals.

Comparison between normal male and female rats

The serum copper concentration data used for this comparison have been obtained in our laboratory since 1977 and refer to three groups of rats (one of males and two of females) that were killed in the morning, between 09 h 00 min and 10 h 00 min. The liver copper data also have been obtained in our laboratory from rats killed in the morning (between 09 h 00 min and 10 h 00 min) during 1981. Serum and liver copper data are summarized in Table 4. The results show that total serum copper concentration is always higher in females than in males, no matter whether the comparison is made between groups matched by body weight or matched by age. Only groups matched by body weight were studied for the liver copper analysis. Liver weight is significantly lower and liver copper concentration is significantly higher in females compared with males. However, when the total amount of copper in liver is considered the difference between male and female rats was not statistically significant.

We should like to point out that when comparison

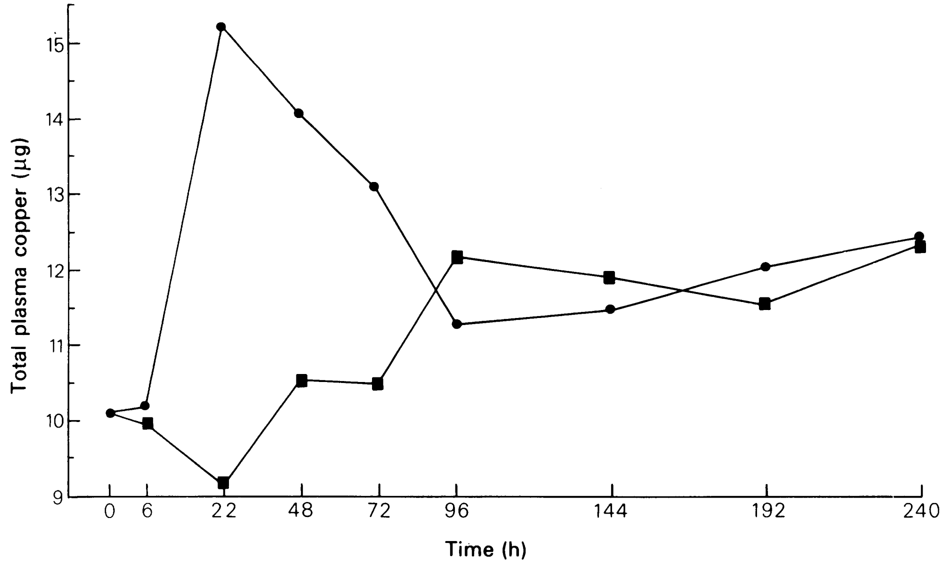

Figure 1 Total plasma copper in control rats (■) and rats with carrageenan-induced pleurisy (●). Each point represents the mean of 20 rats.

was made between the two groups of females (7 week and 11 week old respectively) the difference in total serum copper concentration was statistically significant ($P<0.01$, Student's *t* test). Unlike serum, liver copper concentration does not seem to be significantly affected by age as shown by the zero time values reported in Tables 2 and 3. The total amount of copper in liver is higher in the older female group (38.87 μg versus 33.03 μg; $P<0.01$, Student's *t* test) being higher the mean weight of the organ (9.48 g versus 7.69 g; $P<0.01$, Student's *t* test).

Female rats with carrageenan-induced pleurisy

In our experimental conditions the rats inflamed by intrapleural injection of a carrageenan suspension developed a severe acute inflammatory reaction that peaked at 22 h as shown by the amount of exudate withdrawn (Table 5). Total serum copper concentration was higher ($P<0.01$) compared with both 0 and time controls, 22, 48 and 72 h after the injection of the irritant. From 96 h post-injection onwards, total serum copper concentration in inflamed animals returned to normal values (Table 5).

In order to determine liver copper, groups of 10 rats were used (Table 6). When inflamed animals and their time controls were compared with the zero time group, by the end of the experiment for both inflamed and time control rats it was noticed that: (1) a gradual increase of body and liver weight occurred; (2) liver copper concentration (μg/g) showed little day to day variability; (3) parallel and most probably depending on the increase of liver weight, an increase of total copper content was also measured.

However, when the comparison inflamed versus normal was made between groups matched by body weight, i.e. between inflamed rats and their time controls, at each time interval it was evident that: (1) no major differences in the weight of liver were seen; (2) liver copper concentration (μg/g) remained almost unchanged; (3) total copper content of liver showed no statistically significant differences.

Figure 1 shows the whole amount of total plasma copper calculated according to Altman & Dittmer (1964). Although approximate and only theoretical, this calculation underlines the remarkable difference existing between control and inflamed rats. In control animals the whole amount of total plasma copper at 22 h (9.09 ± 1.28 μg) was appreciably lower than that of inflamed rats (15.24 ± 1.71 μg). A noticeable difference also existed 48 and 72 h after irritant injection. The highest difference in the whole amount of total circulating copper was calculated 22 h post-injection and was about 6 μg.

Discussion

Few distinct points emerge clearly from the results obtained studying liver copper in normal male and female rats. Our data, in particular those referring to the one day monitoring studies, indicate unequivocally that the parameter 'copper concentration' is *per se* insufficient to give a correct picture of the copper status of liver. The latter can be fully described only by considering together the metal concentration value and the weight of the organ. The daily changes observed in liver copper concentration and liver weight (which are possibly due to different states of liver hydration and/or glycogen content) strongly suggest the use of a control group ('time controls') that should be killed at the same time as each treated group. Particular care should be taken that time control rats and treated animals have the same average body weight and age. Moreover the day to day variability that may occur in both liver weight and liver copper concentration is further support for the use of time controls throughout the experiment. We have also shown that total serum copper concentration is significantly different not only between sexes but also when female rats of different ages were compared. This latter finding may be explained by the fact that, according to the Charles River breeder, the 7 week old females were not fully mature. The age-dependency of total serum copper concentration observed in female rats suggests, once more, the use of time control animals whenever dealing with middle- or long-term studies on copper metabolism.

However, the main aim of our work was to clarify whether the liver exploits its metal reservoir to meet the demand for copper due to the increased synthesis of caeruloplasmin which occurs during inflammation. This seems to be the most widely held opinion. In fact Underwood (1977) reports in his classical text-book that: 'in rats, depletion of liver copper has been demonstrated in acute and chronic infections, due to increased hepatic synthesis and secretion of ceruloplasmin by that organ' and the same hypothesis is sustained by Sorenson (1978) for rheumatoid arthritis.

In our opinion, the above hypothesis is not supported by the data so far published, though different experimental models have been used. For instance Karabelas (1972) has shown that the copper content of liver of the adjuvant-induced arthritic rat markedly increased at 21 days, while total serum copper concentration was still significantly higher than control values. Feldman *et al.* (1981) described the opposite, i.e. a decrease in hepatic copper concentration associated with a related increase in total serum copper content in dogs injected with Freund's complete adjuvant. Finally, Wintrobe *et al.* (1953) found that copper concentration in liver has little relation to the hypercupraemia measured during the inflammation produced by injection of turpentine in rats. Our own experiments indicate that the copper utilized for the increased synthesis of caeruloplasmin, which occurs in inflammation, is somehow taken without depleting the copper deposits of liver. We wish to emphasize that we found an increase of about 6 μg in the whole amount of total circulating copper in inflamed animals. This value is a remarkable one when compared with the total liver content; as one can see from Table 6 a change of total hepatic copper of that extent is not seen at any time.

Our data on the liver copper content in normal rats show that the parameter 'copper concentration' does not offer a correct picture of the copper status of liver when taken without considering the total amount of metal contained in that organ. Since all the above mentioned authors have discussed their experimental results without considering the total level of hepatic copper, this may be a possible explanation for the contradictory results so far reported. Alternatively, the differences found may be simply due to the different experimental models used.

In conclusion, during carrageenan-induced pleurisy, the copper needed for the increased synthesis of caeruloplasmin does not deplete existing liver copper stores. It may derive either from changes in the equilibrium between absorption and excretion of copper in the gut or it may come from other compartments of the body such as kidneys and red cells. Albergoni, Cassini, Favero & Rocco (1975) have shown that in kidneys and red cells, copper is easily removable after treatment with D-penicillamine. This suggests the presence in these compartments of a 'weakly bound copper pool' that may actually supply the 'extra copper' needed by inflamed animals.

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References

ALBERGONI, V., CASSINI, A., FAVERO, N. & ROCCO, G.P. (1975). Effect of penicillamine on some metals and metalloproteins in the rat. *Biochem. Pharmac.*, **24**, 1131–1133.

ALTMAN, P.L. & DITTMER, D.S. (1964). Blood volumes. In *Biology Data Book*, pp. 263–266. Washington: Federation of American Societies for Experimental Biology.

CONFORTI, A., FRANCO, L., MILANINO, R. & VELO, G.P. (1981). Serum copper concentration and ceruloplasmin activity during carrageenan foot oedema in rat. *Br. J. Pharmac.*, **72**, 137–138P.

CONFORTI, A., FRANCO, L., MILANINO, R. & VELO, G.P. (1982). Copper and ceruloplasmin (Cp) concentrations during the acute inflammatory process in the rat. *Agents and Actions*, **12**, 303–307.

CONFORTI, A., FRANCO, L., MILANINO, R., TAROLLI, G. & VELO, G.P. (1982). Copper and ceruloplasmin activity in rheumatoid arthritis. In *Advances in Inflammation Research Vol. 3. Rheumatoid Arthritis.* ed. Ziff, M., Velo, G.P. & Gorini, S. pp. 237–244. New York: Raven Press.

FELDMAN, B.F., KEEN, C.L., KANEKO, J.J. & FARVER, T.B.

(1981). Anemia of inflammatory disease in the dog: measurement of hepatic superoxide dismutase, hepatic nonheme iron, copper, zinc, and ceruloplasmin and serum iron, copper and zinc. *Am. J. Vet. Res.*, **42,** 1114–1117.

KARABELAS, D.S. (1972). Copper metabolism in the adjuvant induced arthritic rat. *Univ. Microfilms*, Ann Arbor, Mich., Order No 72–31,092. From Diss. Abstr. Int. B. 1972–33(6), 2776.

MILANINO, R., CONFORTI, A., FRACASSO, M.E., FRANCO, L., LEONE, R., PASSARELLA, E., TARTER, G. & VELO, G.P. (1979). Concerning the role of endogenous copper in the acute inflammatory process. *Agents and Actions*, **9,** 581–588.

MILANINO, R., MAZZOLI, S., PASSARELLA, E., TARTER, G. & VELO, G.P. (1978). Carrageenan oedema in copper-deficient rats. *Agents and Actions*, **8,** 618–622.

MILANINO, R. & VELO, G.P. (1981). Multiple actions of copper in control of inflammation: studies in copper-deficient rats. In *Trace Elements in the Pathogensis and Treatment of Inflammation. Agents and Actions Supplements* Vol. 8, ed. Rainsford, K.D., Brune, K. & Whitehouse, M.W. pp. 209–230. Basel, Boston and Stuttgart: Birkhäuser Verlag.

SCUDDER, P.R., AL-TIMINI, D., McMURRAY, W., WHITE, A.G., ZOOB, B.C. & DORMANDY, T.L. (1978). Serum copper and related variables in rheumatoid arthritis. *Ann. Rheum. Dis.*, **37,** 67–70.

SORENSON, J.R.J. (1978). Copper complexes. A unique class of anti-arthritic drugs. In *Progress in Medicinal Chemistry* Vol. 15, ed. Ellis, G.P. & West, G.B. pp. 211–260. Amsterdam: Elsevier/North Holland.

UNDERWOOD, E.J. (1977). In *Trace Elements in Human and Animal Nutrition.* Fourth Edition, pp. 56–108. New York, San Francisco and London: Academic Press.

WINTROBE, N.N., CARTWRIGHT, G.E. & GUBLER, C.J. (1953). Studies on the function and metabolism of copper. *J. Nutr.*, **50,** 395–419.

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