Acta vet. scand. 1980, *21*, 71—78.

From the National Veterinary Institute, Oslo, Norway.

FRACTIONATION OF SOLUBLE COPPER- AND ZINC-BINDING PROTEINS FROM CATTLE LIVER

By

Milica Mjør-Grimsrud and *Gunnar Norheim*

MJØR-GRIMSRUD, MILICA and GUNNAR NORHEIM: *Fractionation of soluble copper- and zinc-binding proteins from cattle liver.* Acta vet. scand. 1980, *21*, 71—78. — The distribution of copper and zinc among soluble proteins in liver from normal slaughter cattle was examined after gel filtration of the proteins. Copper- and zinc-binding proteins were mainly separated into three fractions. Varying amounts of zinc were eluted in a fourth fraction of molecular weight less than 2,000. A clear relationship was noted between the amount of copper bound to the low molecular weight fraction (m.w. ∞ 10,000) and the total liver zinc concentration. The high molecular weight protein fraction (m.w. $>$ 65,000) dominated in liver with zinc concentrations below 40 μg/g wet weight and total copper concentrations from 16 to 240 μg/g, while in liver with zinc concentrations above 40 μg/g and copper concentrations ranging from 20 to 107 μg/g, the low molecular weight metallothionein-like fraction dominated.

copper; zinc; liver; protein binding; cattle.

It has been known for a long time that sheep are particularly susceptible to chronic copper poisoning. It is also well known that cattle, goat and particularly swine and poultry are more resistant to copper toxicosis (*Hill* 1977 a, b).

In the study of similarities and differences between animal species concerning the pathophysiology of copper toxicosis, it seems appropriate to examine the liver. The liver is the most important organ as regards the accumulation of copper and is considered to play a key role in copper metabolism. Liver protein extracts from sheep and calf (*Bremner & Marshall* 1974 a, b, *Norheim & Søli* 1977), rat (*Bremner & Davies* 1976), swine (*Bremner* 1976, *Frøslie & Norheim* 1977) and goat (*Mjør-Grimsrud et al.* 1979) have previously been investigated for their copper- and zinc-binding proteins.

The aim of the present study was to investigate the distribution of copper and zinc among soluble metal-binding proteins in cattle liver and the relationship between these two metals, and to compare results with those from other species.

MATERIALS AND METHODS

Frøslie et al. (1980) determined copper and zinc concentration in 335 liver samples from normal slaughter cattle. Of these samples, 24 were chosen for the present investigation. The samples were chosen to cover the actual ranges of copper and zinc concentrations, and were divided into two groups on the basis of zinc concentration.

The first group, consisting of 14 samples, had zinc levels below 40 μg Zn/g wet weight, and is representative for most of the samples analysed both for copper and zinc. The mean zinc concentration in this group was 30 μg/g, a figure close to the mean concentration of all samples analysed by *Frøslie et al.* (32 μg Zn/g). Zinc and copper concentrations in this group ranged from 25 to 36 μg Zn/g and from 16 to 240 μg Cu/g, respectively. The second group, consisting of 10 samples had zinc levels above 40 μg/g, with a mean zinc concentration of 61 μg/g. Copper concentrations ranged from 20 to 107 μg/g. Only about 7 % of all samples analysed had hepatic zinc levels above 40 μg/g (*Frøslie et al.*).

As full details of the examination of liver extracts by gel filtration are described elsewhere (*Norheim & Steinnes* 1975, 1976, *Norheim & Søli* 1977), only a brief outline of the technique will be given here. Five g of liver tissue was homogenized with two parts of 0.01 N Tris buffer (pH = 8.0 at 4°C) and centrifuged at 50,000 × g. Three ml of the liver extract was applied on a Sephadex G-75 superfine (Pharmacia) column (2.6×40 cm). The flow rate was 12 ml/h, and 5 ml fractions were collected. The appearance and separation of different copper- and zinc-binding fractions were judged visually from the actual distribution graphs. Concentrations of copper and zinc in each fraction were measured by atomic absorption spectroscopy by direct aspiration of the samples.

RESULTS

The distribution of copper and zinc among soluble metal-binding proteins from three representative liver samples is pre-

sented in Fig. 1. Samples Nos. 1 and 2 are from the low Zn-group and No. 3 from the high Zn-group of samples. The approximate molecular weights of the observed metal-binding fractions were > 65,000 (Fraction I), 35,000 (Fraction II) and 10,000 (Fraction III). Varying amounts of zinc were eluted in Fraction IV with molecular weight less than 2,000.

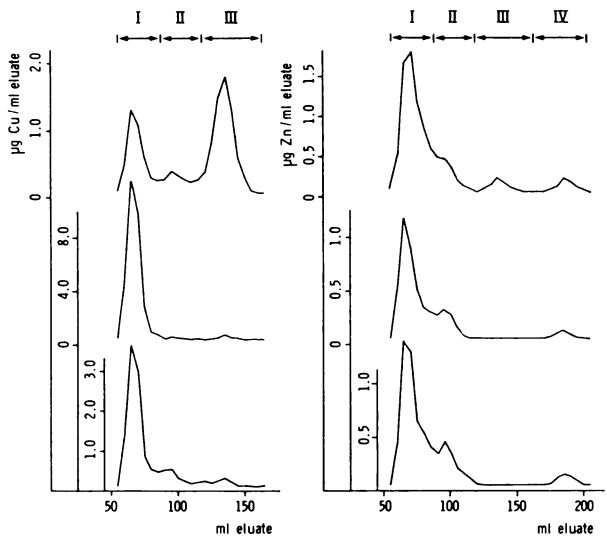

Figure 1. The distribution of copper (left) and zinc (right) bound to proteins from cattle livers after gel filtration on Sephadex G-75. The copper and zinc concentrations were: No. 1 (lower curves): 76 µg Cu/g and 28 µg Zn/g, No. 2 (middle curves): 240 µg Cu/g and 29 µg Zn/g, and No. 3 (upper curves): 66 µg Cu/g and 44 µg Zn/g. The ordinate axes are individually dimensioned. Roman numbers mark position and extention of the protein fractions.

Mean values and ranges of total concentrations of copper and zinc are shown in Table 1. The percentage of copper and zinc recovered in the soluble protein extract and the relative distribution of copper and zinc between the main fractions for the two groups are also tabulated.

In Fig. 2, the relative amounts of copper (per cent) in Fractions I—III are shown as a function of the liver copper concentration for each of the two groups, low and high in zinc.

In the samples examined there was no significant correlation between the hepatic copper concentration or its logarithm and the percentage of copper recovered in the soluble protein extract.

Table 1. Total copper and zinc concentrations, the percentages of copper and zinc recovered in the soluble protein extract, and the relative amounts of copper and zinc found in the different soluble protein fractions after gel filtration on Sephadex G-75 in liver samples from cattle grouped according to liver zinc concentration. The level of significance of differences (P) between the two groups is indicated in the table (ns = not significant).

	Low Zn-group (n=14) Zn<40 µg/g liver		High Zn-group (n=10) Zn>40 µg/g liver		
	mean	range	mean	range	P (*t*-test)
Total conc. of Cu, µg/g, wet tissue	87	16—240	55	20—107	ns
Extracted Cu, %	67	48—100	90	63—100	<0.001
Fract. I, %	62	31— 83	26	16— 50	<0.001
„ II, %	19	7— 37	16	12— 22	ns
„ III, %	20	10— 37	56	33— 70	<0.001
Total conc. of Zn, µg/g, wet tissue	30	25— 36	61	41—102	
Extracted Zn, %	85	72—100	88	72—100	ns
Fract. I, %	58	44— 65	51	30— 64	ns
„ II, %	29	24— 32	23	12— 27	<0.001
„ III, %			17	3— 54	
„ IV, %	12	6— 26	8	4— 16	ns

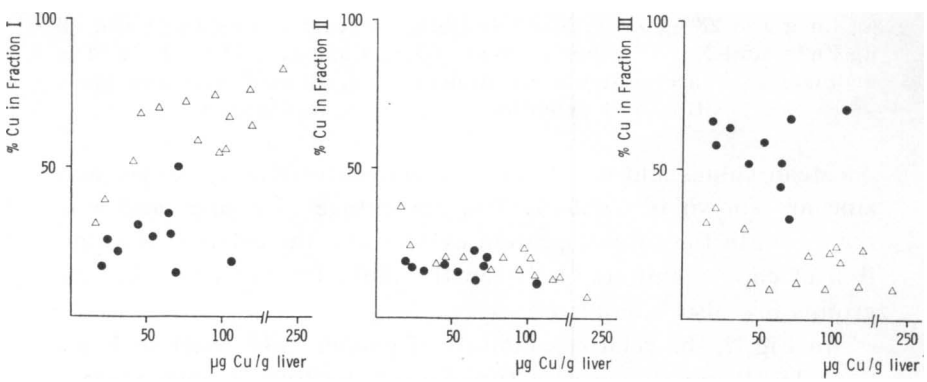

Figure 2. Relationship between percentage of copper in Fractions I, II and III and liver copper concentration in samples from normal cattle. The samples are divided into two groups according to zinc levels, one (n = 14) with zinc levels below 40 µg Zn/g liver (Δ) and the other (n = 10) with zinc levels above 40 µg/g (•).

The absolute amounts of copper in Fractions I and II increased with increasing hepatic copper concentration in the low Zn-group as well as the high Zn-group. The absolute amounts of copper in Fraction III from the low Zn-group increased slightly with increasing hepatic copper concentration, while copper in Fraction III from the second group showed no systematic trend. The absolute amounts of zinc in Fractions I and II were directly dependent on the total zinc concentration in both sample groups. Zinc-binding proteins in Fraction III were only demonstrated in samples from the high Zn-group.

Referring again to Table 1, there was a marked difference in the distribution of the copper- and zinc-binding proteins between groups low and high in zinc. The high molecular weight Fraction I, containing on average 62 % of extracted copper, dominated in samples from the low Zn-group. The relative amounts of copper in this fraction increased with increasing hepatic copper concentration. The relative amount of extracted copper in Fraction II showed a slightly falling trend with increasing hepatic copper concentration. The relative amount of extracted copper recovered in Fraction III showed no obvious trend comprising on average 20 % of the extracted copper. The soluble protein distribution of zinc in the low Zn-group of samples was nearly constant, with Fraction I dominating. Fraction II which was not well separated from Fraction I, contained 24—32 % of extracted zinc. No zinc was detected in Fraction III, while Fraction IV contained 6—26 % of extracted zinc.

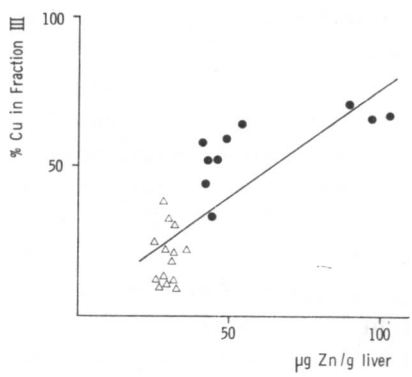

Figure 3. Relationship between extracted copper (per cent) recovered in Fraction III and liver zinc concentration in cattle ($r = 0.79$, $P < 0.001$). Symbols: See Fig. 2.

The distribution of both copper- and zinc-binding proteins differed in the samples from the high Zn-group. The percentage of total copper extracted was significantly different from that in the first group ($P < 0.001$). The relative amounts of copper in Fraction I showed no systematic tendency, and was almost constant in Fraction II. In this group, most of the copper was bound to the low molecular weight Fraction III, which contained 56 % on average. Significant proportions of zinc were found in all four fractions in the high Zn-group, but any trend was uncertain.

The relationship between the proportion (per cent) of extracted copper in Fraction III and liver zinc concentration is shown in Fig. 3. The relative amount of copper present in this fraction was found to be a function of the liver zinc content, the correlation coefficient being 0.79 ($P < 0.001$).

DISCUSSION

The distribution of copper and zinc among soluble proteins in cattle liver was found to depend mainly on the total hepatic zinc concentration and to a little extent on the total copper concentration. At zinc concentrations below 40 μg Zn/g liver, most copper and zinc is bound to high molecular weight proteins. No zinc was found in the low molecular weight fraction in any of these samples. At higher zinc concentrations significantly higher percentages of both copper and zinc are bound to low molecular weight proteins.

The results are in general agreement with those found in the calf (*Bremner & Marshall* 1974 a, *Kincaid et al.* 1976) and in the goat (*Mjør-Grimsrud et al.* 1979), but not with those in sheep (*Norheim & Søli* 1977) or swine (*Frøslie & Norheim* 1977). In sheep, the distribution of copper- and zinc-binding proteins was dependent on the hepatic copper level, while in swine the distributions of the two elements were not interdependent.

In the livers examined no significant correlation was found between the amount of copper recovered in the protein extract and the total concentration of copper. This is at variance with earlier results from sheep (*Norheim & Søli*) and swine (*Frøslie & Norheim*), but is in accordance with results from goat (*Mjør-Grimsrud et al.*).

The observed relationship between the distribution of copper- and zinc-binding proteins in Fractions I and III and total con-

centration, prompted an examination of the relationship between the total zinc concentration and the percentage of extracted copper present in Fraction III in all samples examined. It appears that the amount of copper present in Fraction III in cattle liver is related to liver zinc concentration. This finding is in accordance with previous results from goat (*Mjør-Grimsrud et al.*), calf (*Bremner & Marshall* 1974 a) and rat (*Bremner & Davies* 1976) though such a relationship was not demonstrated in swine (*Bremner* 1976, *Frøslie & Norheim*).

A relationship between copper and zinc similar to that found in cattle was also recorded by *Bremner & Marshall* (1974 a) in zinc-deficient sheep with an average zinc level of 19 μg Zn/g liver. There are, however, no reports of such low zinc concentrations being found in normal grazing Norwegian sheep. Most cattle in the present investigation, normal slaughter animals, had a distribution pattern which seems to correspond to that found in zinc-deficient sheep.

The low molecular weight metallothionein-like proteins present in Fraction III are thought to be involved in the storage, metabolism and detoxification of copper and zinc. In Norwegian sheep, the amount of copper and zinc present in this fraction was dependent on the hepatic copper level. This is not the case in swine. In goats and cattle, the binding of copper and zinc to the metallothionein-like proteins is primarily dependent on hepatic zinc levels. A comparison between the copper- and zinc-protein profiles in the above mentioned four species, seems clearly to indicate that sheep differ not only from swine, but also from other ruminants.

REFERENCES

Bremner, I.: The relationship between the zinc status of pigs and the occurrence of copper- and Zn-binding proteins in liver. Brit. J. Nutr. 1976, *35*, 245—252.

Bremner, I. & N. T. Davies: Studies on the appearance of a hepatic copper-binding protein in normal and zinc-deficient rats. Brit. J. Nutr. 1976, *36*, 101—112.

Bremner, I. & R. B. Marshall: Hepatic copper- and zinc-binding proteins in ruminants. I. Distribution of Cu and Zn among soluble proteins of livers of varying Cu and Zn content. Brit. J. Nutr. 1974 a, *32*, 283—291.

Bremner, I. & R. B. Marshall: Hepatic copper- and zinc-binding proteins in ruminants. 2. Relationship between Cu and Zn concentrations and the occurrence of a metallothionen-like fraction. Brit. J. Nutr. 1974 b, *32*, 293—300.

Frøslie, A. & G. Norheim: The concentrations of copper, zinc and molybdenum in swine liver and the relationship to the distribution of soluble copper- and zinc-binding proteins. Acta vet. scand. 1977, *18*, 471—479.

Frøslie, A., G. Norheim & E. Waasjø: Copper, zinc and molybdenum in livers of Norwegian cattle at slaughter. Acta vet. scand. 1980, *21*, 62—70.

Hill, R.: Copper toxicity. I. Brit. vet. J. 1977 a, *133*, 219—224.

Hill, R.: Copper toxicity. II. Brit. vet. J. 1977 b, *133*, 365—373.

Kincaid, R. L., W. J. Miller, P. R. Fowler, R. P. Gentry, D. L. Hampton & M. W. Neathery: Effect of high dietary zinc upon zinc metabolism and intracellular distribution in cows and calves. J. Dairy Sci. 1976, *59*, 1580—1584.

Mjør-Grimsrud, M., N. E. Søli & T. Sivertsen: The distribution of soluble copper- and zinc-binding proteins in goat liver. Acta pharmacol. (Kbh.) 1979, *44*, 319—323.

Norheim, G. & E. Steinnes: Determination of protein-bound trace elements in biological material by gel filtration and neutron activation analysis. Analyt. Chem. 1975, *47*, 1688—1690.

Norheim, G. & E. Steinnes: Distribution of some protein-bound trace elements among soluble protein fractions from human liver. Acta pharmacol. (Kbh.) 1976, *38*, 137—144.

Nordheim, G. & N. E. Søli: Chronic copper poisoning in sheep. II. The distribution of soluble copper-, molybdenum-, and zinc-binding proteins from liver and kidney. Acta pharmacol. (Kbh.) 1977, *40*, 178—187.

SAMMENDRAG

Fraksjonering av løslige kobber- og sinkbindende proteiner fra storfelever.

Løslige kobber- og sinkholdige leverproteiner fra storfe ble fraksjonert ved hjelp av gelfiltrering. Prøvene kom fra normale slaktedyr og ble plukket ut for å dekke et størst mulig konsentrasjonsområde av de to metallene. De løslige kobber- og sinkholdige proteinene ble separert i 3 fraksjoner med følgende omtrentlige molekylvekter: fraksjon I >65.000, fraksjon II 35.000 og fraksjon III 10.000. Varierende mengder sink ble eluert i en fjerde fraksjon med molekylvekt <2.000. Det var en tydelig relasjon mellom innholdet av kobber i fraksjon III og konsentrasjonen av sink i leveren. Prøvene ble derfor delt inn i 2 grupper, med sinkkonsentrasjoner i leveren over og under 40 μg/g våtvekt. I prøver med sinknivåer under 40 μg/g og kobbernivåer fra 16 til 240 μg/g dominerte fraksjon I. I disse prøvene ble ikke sink påvist i fraksjon III. I prøver med sinknivåer over 40 μg/g og kobbernivåer fra 20 til 107 μg/g ble det funnet signifikant høyere innhold av kobber og sink bundet til de metallothionin-lignende proteinene i fraksjon III.

(Received September 26, 1979).

Reprints may be requested from: The National Veterinary Institute, P. O. Box 8156, Dep., Oslo 1, Norway.