Acta vet. scand. 1980, *21*, 578—586.

From the National Veterinary Institute, Oslo, and the Department of Pharmacology and Toxicology, Veterinary College of Norway, Oslo, Norway.

THE DISTRIBUTION OF SOLUBLE COPPER- AND ZINC-BINDING PROTEINS IN LIVER AND KIDNEY OF CHRONIC COPPER-POISONED GOATS

By

Milica Mjør-Grimsrud, Nils E. Søli and *Gunnar Norheim*

MJØR-GRIMSRUD, MILICA, NILS E. SØLI and GUNNAR NORHEIM: *The distribution of soluble copper- and zinc-binding proteins in liver and kidney of chronic copper-poisoned goats.* Acta vet. scand. 1980, *21*, 578—586. — The distribution of copper and zinc among the soluble proteins in the liver and kidney from chronic copper-poisoned goats was examined after gel filtration of the proteins. The concentrations of copper in the liver and kidney cortex from five experimentally copper-poisoned goats were: 550—810 μg/g liver and 190—420 μg/g kidney cortex (wet weight). In general the copper-binding proteins from both the liver and kidney samples were separated into two different fractions with approximate molecular weights (m.w.) of > 65,000 and 10,000, respectively. From the liver samples, varying amounts of copper were eluted in a fourth fraction with m.w. < 2,000. In the majority of kidney samples the dominating copper-binding protein fraction was the high molecular weight fraction. Absolute amounts of copper recovered in the metallothionein-like protein fraction were nearly the samt for all samples investigated. The distribution of zinc-binding proteins in both liver and kidney samples was nearly the same. The high molecular weight fraction dominated, and no zinc was bound to metallothionein-like proteins.

copper; zinc; protein binding; liver; kidney; copper-poisoned goat.

Chronic copper poisoning is rather common in sheep in Norway, but seems to be extremely rare in goats. Naturally occurring copper poisoning has not been reported in goats but investigations concerning copper metabolism and storage in goats may provide information which may help to understand copper intoxication in sheep. Experimental copper loading and intoxication have revealed that goats are considerably more resistant

to copper toxicosis than sheep (*Adam et al.* 1977, *Søli & Nafstad* 1978).

In general, it appears that the distribution of copper and zinc among soluble proteins in the liver of goats is dependent on the total hepatic zinc concentrations, and to a lesser extent on the total copper concentration (*Mjør-Grimsrud et al.* 1979). In sheep, however, the distribution varies with the degree of copper accumulation (*Norheim & Søli* 1977).

The aim of the present work was to investigate the total concentrations of copper and zinc, and the distributions of these metals, among soluble metal-binding proteins in the liver and kidney of experimentally copper-poisoned goats. The results are compared with previous reports on these distributions in experimentally copper-poisoned sheep.

MATERIALS AND METHODS

Animals' dosage

Five goats (Nos. 1—5) weighing between 30 and 47 kg were dosed orally with 0.2 % aqueous copper sulphate solution. The doses were 5.0 mg Cu/kg body weight twice daily, six days a week for 77 to 151 days. The animals were not dosed during the haemolytic crisis. The goats were fed hay and concentrates and kept in indoor pens. The animals died or were killed on different days of the experiment. Information on the experiment is summarized in Table 1. The liver and kidneys were removed immediately after death and stored at —20°C.

Analytical methods

Both kidney cortex and liver tissue were prepared and analysed according to the method of *Norheim & Steinnes* (1975, 1976). Gel filtration was performed using Sephadex G-75 superfine (Pharmacia) at 4°C. One part of sample was homogenized in a Sorvall Omni-Mixer with two parts of 0.01 N Tris buffer (pH = 8.0 at 4°C) containing 0.05 N sodium chloride. Sodium azide was added (100 mg/l) to prevent bacterial growth. The homogenates were centrifuged at 50,000 × g at 4°C for 2 h. Before application on the column, the protein extracts were filtered through 3.0 μ Millipore filters. Three ml of filtrates were applied on the column (2.6 × 40 cm), an ascending flow was used, and 5 ml fractions were collected. The flow rate was 12.5 ml/h. The

approximate mean molecular weight associated with each fraction was estimated using the following substances as references: albumin bovine (m.w. = 67,000), albumin chicken (m.w. = 45,000), chymotrypsinogen A (m.w. = 25,000), myoglobin horse (m.w. = 17,800), cytochrome C (m.w. = 12,400) and bacitracin (m.w. = 1,411). The appearance and separation of different copper- and zinc-binding fractions were judged visually from the actual distribution graphs. The fractions were numbered I—IV, with approximate mean molecular weights > 65,000 (I), 35,000 (II), 10,000 (III) and < 2,000 (IV). Molybdenum concentrations in liver and kidney were determined by a spectrophotometric dithiol method (*Norheim & Waasjø* 1977), and concentrations of copper and zinc were measured by flame atomic absorption spectroscopy by direct aspiration of each fraction or after decomposition of tissue with a mixture of nitric, perchloric and sulphuric acid. The results were expressed in μg/g wet weight.

Statistical methods

Linear regressions and *t*-tests were calculated using a Compucorp 344 Statistician.

RESULTS

Table 1 gives the sex and body weight of the goats, the duration of the experiment and total copper dosed. Table 2 gives the total concentration of copper, zinc and molybdenum in liver and kidney cortex, percentage of copper and zinc extractable from the tissue homogenates and absolute and relative amounts of copper found in the different protein fractions after gel filtration. The average copper levels in the liver and kidney cortex of the copper-poisoned goats were 698 and 286 μg/g wet weight, respectively. The average zinc levels were 34 μg/g liver and 26 μg/g kidney cortex. The hepatic molybdenum level in these goats averaged 0.57 μg/g.

The soluble hepatic copper-binding proteins were mainly separated into two fractions (Fractions I and III) (Fig. 1). Varying amounts of copper were eluted in a fourth fraction (Fraction IV), having a molecular weight less than 2,000. The maximum copper concentration was found in the high molecular weight fraction (Fraction I). This fraction contained an average of 35 %

Table 1. Sex, body weight and dosing of the experimental goats.

Goat No.	Sex	Body weight	Experimental period (days)	Total Cu dosed (g)	Remarks
1	F	30	100	25	Kid after 57 days of experiment. Died on 4th day of crisis.
2	F	30	104	25	1st crisis after 90 days and the 2nd crisis after 102 days of experiment. Killed on the 2nd day of the 2nd crisis.
3	M	47	151	55	1st crisis after 100 days, the 2nd crisis after 111 days and the 3rd crisis after 140 days of experiment. Killed on the 2nd day of the 4th crisis.
4	M	45	77	27	Killed on the 5th day of crisis.
5	F	33	105	29	Kid after 49 days of experiment. Crisis after 51 days of experiment. Killed without a new visible crisis.

Table 2. The total concentrations of copper, zinc and molybdenum in the liver, and copper and zinc in the kidney cortex (μg/g wet weight), the percentage of copper and zinc in liver and kidney cortex extractable from the homogenates, and the absolute and relative amounts of copper found in the different soluble protein fractions after gel filtration.

Goat No.	Organ	Tissue concentration μg/g wet weight			% extractable		Fraction I		Fraction III		Fraction IV	
		Cu	Zn	Mo	Cu	Zn	μg Cu	% Cu	μg Cu	% Cu	μg Cu	% Cu
1	Liver	550	32	0.32	38	38	91	44	48	23	25	12
	Kidney	360	27		27	34	46	47	28	29		
2	Liver	650	28	0.48	53	54	114	33	69	20	117	34
	Kidney	420	24		15	41	30	46	21	32		
3	Liver	700	39	0.75	45	56	100	32	117	37	38	12
	Kidney	250	24		21	54	16	31	24	45		
4	Liver	780	31	0.72	60	90	150	32	117	25	136	29
	Kidney	190	33		29	42	9	17	29	53	10	18
5	Liver	810	42	0.60	39	52	104	33	98	31	57	18
	Kidney	210	20		54	46	59	53	29	26		

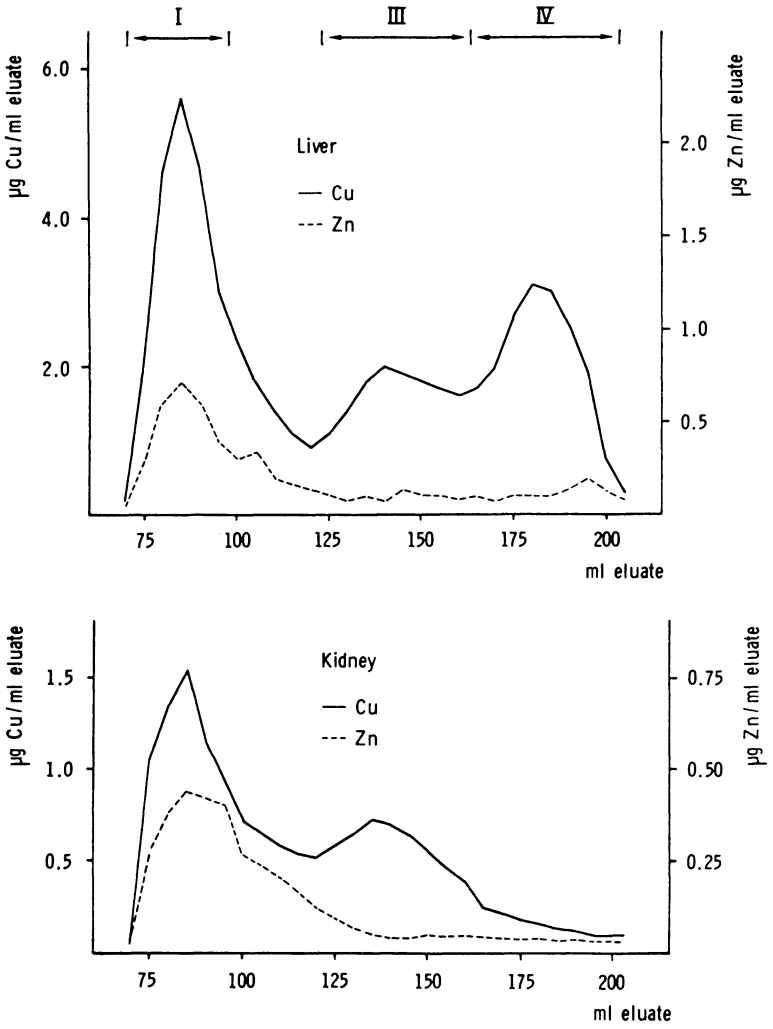

Figure 1. The distribution of copper- and zinc-binding proteins in liver and kidney cortex of copper-poisoned goat after gel filtration on Sephadex G-75 superfine. Goat No. 2 (liver concentrations: 650 µg Cu/g, 28 µg Zn/g, kidney concentrations: 420 µg Cu/g, 24 µg Zn/g). The roman numerals indicate Fractions I (m.w. > 65,000), III (m.w. = 10,000) and IV (m.w. < 2,000).

of the extracted copper. An average of 27 % of the copper was bound to low molecular weight proteins (Fraction III). The distribution pattern did not vary systematically with the total liver copper concentrations. On the other hand, the absolute amounts of copper in Fractions I, III and IV, were directly dependent on the concentration of extracted copper from the tissue homogenates. The correlation coefficients were 0.96, 0.71 and 0.87, respectively. The distribution of soluble hepatic zinc-binding proteins in all of the samples was nearly constant, i.e. with Fraction I dominating, and on average 69 % of zinc recovered in this fraction.

The soluble copper-binding proteins in the kidney cortex were also separated into two main fractions, I and III (Fig. 1). One exception, however, was observed in goat No. 4 which had a total copper concentration of 190 μg/g. In this goat the copper occurred mainly in Fraction III with small amounts recovered in Fraction I. In samples from goats Nos. 1, 2, 3 and 5, the high molecular weight fraction (Fraction I) dominated. The absolute amount of copper recovered in Fraction III was nearly the same for all samples investigated. The amount of copper recovered in Fraction I was more variable and did not depend on the total kidney concentration. Fraction I dominated the distribution pattern of the zinc-binding proteins in kidney cortex. Zinc bound to low molecular weight proteins (Fraction III) was found neither in the liver nor in kidney cortex.

DISCUSSION

In the present investigation, the mean copper concentration in the kidney cortex of copper-poisoned goats was found to be significantly higher than the levels reported in kidneys from copper-poisoned sheep (*Norheim & Søli* 1977, *Norheim* 1980), whereas no difference in hepatic copper levels was found between our results and those in sheep, reported by *Frøslie & Norheim* (1976) and *Norheim*. The zinc levels were the same as in normal goats (*Søli & Frøslie* 1979). The average molybdenum level in the liver of our copper-poisoned goats was found to be significantly lower than that in normal goats (*Søli & Frøslie*).

The present results indicate that the distribution of copper among soluble metal-binding proteins in the liver of copper-poisoned goats is very similar to the distribution of this metal in the liver of copper-poisoned sheep (*Norheim & Søli*). In the

livers of both copper-poisoned sheep and goats, the maximum copper concentrations were found in the high molecular weight fraction. However, a significant part of the copper was still bound to low molecular weight proteins. In addition, copper bound to substances with molecular weights less than 2,000 was observed in the livers of both copper-poisoned sheep (*Philip* 1973, *Norheim & Søli, Maribei* 1978), and goats. The distribution of zinc among soluble metal-binding proteins in the liver of copper-poisoned goats is different from that in copper-poisoned sheep. In copper-poisoned goats there were no zinc-binding substances with molecular weights less than 2,000 in any of the samples. Furthermore, no proteins corresponding to Fraction II were separated from the remaining proteins and no zinc was bound to Fraction III. The distribution of hepatic copper- and zinc-binding proteins in the copper-poisoned goats was compared to normal goats with zinc levels within 20 to 40 μg/g wet weight (*Mjør-Grimsrud et al.* 1979) and it was shown that the high molecular weight fraction (I) dominated in both the copper-poisoned and normal goats. At the same time no copper-binding substances with molecular weights less than 2,000 could be demonstrated in the normal goats, and no zinc-binding substances with molecular weights less than 2,000 could be shown in the copper-poisoned goats.

The similarity of the distribution of the renal copper-binding proteins between the poisoned goats and poisoned sheep (*Norheim & Søli, Bremner & Young* 1977) was less apparent than that found in liver. It is concluded that metallothionein (Fraction III) constitutes the major copper-binding protein in the kidney of copper-poisoned sheep (*Norheim & Søli, Bremner & Young*) whereas in the kidney of copper-poisoned goats, the dominant copper-binding fraction was, in most of the samples, the high molecular weight fraction (I). However, in goat No. 4, the greatest copper concentration occurred in Fraction III. Also, the close relationship observed between the concentration of copper in Fraction III and the total amount in kidney cortex, which was very obvious in the kidney of poisoned sheep (*Bremner & Young*), could not be demonstrated in the goats. In copper-poisoned goats, the absolute amount of copper recovered in Fraction III showed little variation between the samples. These results indicate that the upper limit of the copper-binding capacity of the metallothionein-like proteins in the kidneys of copper-

poisoned goats had been reached. Unlike in sheep kidney, most of the remaining copper is bound to high molecular weight proteins (Fraction I).

REFERENCES

Adam, S. E. I., I. A. Wasfi & M. Magzoub: Chronic copper toxicity in Nubian goats. J. comp. Path. 1977, *87*, 623—627.

Bremner, I. & B. W. Young: Copper thionein in the kidneys of copper-poisoned sheep. Chem.-Biol. Interactions 1977, *19*, 13—23.

Frøslie, A. & G. Norheim: The concentrations of molybdenum and zinc in liver in relation to copper accumulation in normal and copper poisoned sheep. Acta vet. scand. 1976, *17*, 307—315.

Maribei, J. M.: Experimental chronic copper poisoning in sheep. Thesis, Cornell University 1978, 263 pp.

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.: Copper, zinc and molybdenum in sheep, swine and cattle in Norway. 1980. In press.

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 fraction from human liver. Acta pharmacol. (Kbh.) 1976, *38*, 137—144.

Norheim, 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.

Norheim, G. & E. Waasjø: On the determination of molybdenum with dithiol in biological materials with high levels of copper and iron. Fresenius Z. Anal. Chem. 1977, *286*, 229—231.

Philip, E.: Intracellular distribution of copper in sheep liver and its response to the administration of copper sulphate. Thesis, University of Aberdeen 1973, 28 pp.

Søli, N. E. & I. Nafstad: Effects of daily oral administration of copper to goats. Acta vet. scand. 1978, *19*, 561—568.

Søli, N. E. & A. Frøslie: Copper, zinc and molybdenum in goat liver. Acta vet. scand. 1979, *20*, 45—50.

SAMMENDRAG

Fordelingen av løslige kobber- og sinkbindende proteiner i lever og nyre fra kronisk kobberforgiftede geiter.

Fordelingen av kobber og sink blandt de løslige proteiner i lever og nyre fra kronisk kobberforgiftede geiter ble undersøkt ved hjelp

av gelfiltrering. Konsentrasjonen av kobber i lever og nyre fra de 5 eksperimentelt kobberforgiftede geitene var henholdsvis 550—810 og 190—420 μg/g våtvekt. Nyrekonsentrasjonen av kobber var signifikant høyere enn hos sau død av kronisk kobberforgiftning. De kobberbindende proteinene fra både lever og nyre ble hovedsakelig separert i 2 fraksjoner med en tilnærmet molekylvekt på henholdsvis > 65.000 og 10.000. I lever ble det også påvist varierende mengder kobber i en fjerde fraksjon med molekylvekt mindre enn 2.000. Fordelingen av de sinkbindende proteinene fra lever og nyre var nær like. Mesteparten av sinken ble funnet i den høymolekylære fraksjonen, mens det ikke ble påvist noe sink i den lavmolekylære metallothionein-lignende fraksjonen. I nyrene fra 4 av dyrene dominerte den høymolekylære kobberbindende fraksjonen. Absoluttmengden av kobber i den metallothionein-lignende fraksjonen i nyre var nær den samme i alle geitene.

(Received September 9, 1980).

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