than were found for the muscles of the swimming duck and the free-range chicken, and especially of chickens restrained in an egg-laying battery were lowest of all. In contrast, the leg muscles of the swimming duck contained more glycogen than the leg muscles of pigeon and chicken.

Three examples of the histochemical determination of glycogen are shown in the figure. There is full agreement with the in vitro measurements. The most intensely stained was the pigeon pectoral muscle (fig., a, left), followed by the chicken pectoral muscle (fig., b, left), and in neither case did staining occur after treatment with amyloglucosidase (fig., a and b, right). There was no noticeable staining in the gizzard muscle proper; only the connective tissue septa contained PAS positive material (presumably collagen). This staining was not affected by amyloglucosidase treatment (fig., c, left and right).

The low levels of glycogen in the avian gizzard smooth muscle may not be representative of smooth muscle in general. The only other smooth muscle we examined, chicken uterus, had almost four times as much glycogen as the gizzard (2.41 μmoles/g); Lynch and Paul[6] found 2.82 ± 0.29 μmoles/g in porcine arterial muscle. It is possible that the gizzard muscle, distinguished from all other smooth muscle by its high content of myoglobin[11], derives the energy for its powerful contractions from sources other than glycogen. The differences in the isoform of phosphorylase kinase present[4,5], and the low levels of glycogen measured by us speak for this theory.

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Isolation, characterization, and determination of human liver (copper/zinc) metallothionein

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Summary. A copper-containing protein was purified from the liver of a patient with primary biliary cirrhosis by a combination of gel filtration and anion exchange chromatography. This copper-protein had UV absorption and emission spectra, an amino acid composition, and a molecular mass which were characteristic for metallothionein (MT).

From 8 livers (3 control, 1 fetal and 4 primary biliary cirrhosis) MT was extracted with non-reducing buffer and centrifuged, and the pellets were re-extracted with a 1 % 2-mercaptoethanol-containing buffer. The non-reducing buffer extracted a predominantly copper-containing MT from the livers of patients with primary biliary cirrhosis and a predominantly zinc-containing MT from control livers and the fetal liver. Only from the fetal liver was a copper/zinc containing MT solubilized during the re-extraction with 2-mercaptoethanol-containing buffer. These results indicate that human MT is a unique metalloprotein with age and disease-dependent characteristics.
Key words. Metallothionein; liver; primary biliary cirrhosis; copper; zinc.

Copper is an essential trace element which is, however, potentially toxic when present in large quantities. The liver plays a key role in the homeostasis of copper and prevents accumulation of the metal by excreting the surplus into the bile. Most of the liver copper is present in the cytoplasm of the hepatocytes, and it is associated with a number of proteins. The smallest of these proteins have been identified as metallothioneins (MTs) (for review see Winge[1]). MTs have been identified as the major copper binding proteins in the livers of fetusses and newborns[2-4] and in the livers of copper-loaded rats[5] and copper-loaded pigs[6].

MTs are a group of closely related proteins characterized by an unusually high cysteine content and the absence of aromatic amino acids. The molecular mass of MTs, as calculated from the amino acid sequence, is about 6,300 Da[7], but the proteins elute at a 10 kDa position from gel filtration columns[3,8,9], and in SDS-PAGE they

migrate as proteins of 10–15 kDa[4,10] depending on the type of gel system used. MTs have a high affinity for essential as well as toxic trace metals and are able to bind 7 g atoms of zinc, cadmium or mercury or to bind 12 g atoms of copper or silver per mol of protein[11]. Despite well-defined chemical properties the biological role of these proteins remains an enigma. It has been suggested that they detoxify noxious trace metals and unphysiologically increased amounts of essential trace metals. In addition, it has been hypothesized that they are involved in the regulation of copper and zinc metabolism and the storage of these metals[12–14].

Both in animals and man there are disorders in which the hepatic copper concentration is increased owing to impaired biliary excretion. It has been shown, for example, that in dogs with inherited copper toxicosis copper is bound to MTs[15,16]. In man, elevated hepatic copper levels have been detected in fetuses and neonates[3,4], in chronic cholestatic disorders like primary biliary cirrhosis (PBC)[17], and in Wilson's disease[18]. The accumulated copper in fetal and neonatal livers and in the liver of patients with PBC is supposed to be a consequence of impaired excretion[13,24]. Wilson's disease is an inherited disorder of copper metabolism characterized by the accumulation of copper in the cornea, brain, kidney and liver[18]. While the accumulated copper seems to play an important role in the pathogenesis of Wilson's disease, its role in the pathogenesis of PBC is still unclear, and in fetuses and newborns the elevated copper levels are physiological[13].

The human fetal liver has been found to contain both copper- and zinc-rich forms of MT but the zinc-rich form was soluble whereas the copper-rich form was insoluble in absence of reducing agents[3,4]. In the liver of a Wilson's disease patient most of the copper was found to be associated with a soluble form of MT[18]. The copper binding protein in chronic cholestatic disorders has not yet been identified, but we have demonstrated that in PBC livers most of the copper is bound to a protein with an apparent molecular weight of about 10 kDa on gel filtration columns[9]. In the present study we isolated this protein from the liver of a patient with PBC and identified it as MT. Moreover, extractions of eight liver samples (four PBC, three control, and one fetal) were made by subsequent homogenization in a non-reducing and a reducing buffer and the copper, zinc, protein, and MT content of these extracts was determined.

Materials and methods

Chemicals. Trizma base and 2-mercaptoethanol (molecular biology reagent) were from Sigma (St. Louis, MO, USA); acrylamide and bisacrylamide were from BDH (Poole, England). The protein assay was from Bio-Rad (Munich, FRG); Sephadex G-75, Sephadex G-25 Superfine and DEAE Sephadex A-25 were from Pharmacia (Uppsala, Sweden). All other reagents were from Merck (Darmstadt, FRG) and were analytical grade or better.

Livers. PBC livers were obtained from subjects who underwent an orthotopic liver transplantation for end-stage disease. Control livers were obtained at autopsy from subjects who had died as a result of cardiovascular disease or traffic accidents. None of these subjects had a history of liver disease. A fetal liver was obtained from a child who died in utero after a gestation period of 6 months. Cadmium-loaded livers were obtained from male Wistar rats with a body weight of about 200 g, which received 3 i.p. injections of 1 mg/kg b.wt of cadmium (as cadmium chloride) at intervals of 48 h. Two days after the last injection the rats were sacrificed and the livers were removed. All livers were stored at −70 °C until use.

Extraction procedures. Liver samples were homogenized in 5 mM Tris-HCl buffer pH 8.5 (1:2 by weight) in an ice bath, using a Polytron (Kinematica, Switzerland) in a nitrogen-flushed glove bag. The homogenates were centrifuged at 70,000 × g for 1 h. The supernatants were directly applied to the columns or stored at −20 °C under a nitrogen atmosphere until used. Re-extractions were subsequently made according to the protocol of Rydén and Deutsch[3]. In brief: the pellets were washed with the same buffer and homogenized a second time in buffer (1:2 by weight) to which 1 % (w/v) 2-mercaptoethanol was added. After freezing and thawing 3 times and stirring for 1 h at 4 °C the homogenates were centrifuged. The supernatants were stored at −20 °C under a nitrogen atmosphere until used.

Column runs. A 5 mM Tris-HCl buffer pH 8.5 was used for all column runs. All buffers were degassed by sonication under vacuum and subsequently saturated with nitrogen gas. Columns were run in a cold room at 4 °C. Fractions were collected in a nitrogen atmosphere.

For analytical runs 6 ml supernatant was applied to a Sephadex G-75 column (1.6 × 90 cm). The column was eluted with buffer at a flow rate of 10 ml/h and 5-ml fractions were collected.

For preparative runs 60 ml supernatant was loaded on a 5 × 90 cm Sephadex G-75 column which was eluted with buffer at a flow rate of 60 ml/h. The copper-peak, containing proteins with a molecular mass of about 10 kDa, was led directly on to a 1.6 × 20 cm DEAE Sephadex A-25 column which had been carefully equilibrated at pH 8.5 with several liters of buffer. This column was eluted with an 800 ml linear gradient of buffer and buffer supplemented with 0.2 M NaCl at a flow rate of 60 ml/h, and 10-ml fractions were collected.

Fractions containing more than 10 µg protein/ml were concentrated by Amicon ultrafiltration using YM2 filters. Samples were desalted using Sephadex G-25 Superfine columns (0.5 × 10 cm) and stored at −20 °C under a nitrogen atmosphere until analysis.

Other methods and equipment. Polystyrene tubes were used to avoid metal absorption.

Column runs were monitored using an LKB Uvicord UV detector equipped with a 254 or a 280 nm filter and a 2-mm light path flow-through cell.

Conductivity of fractions was measured with a CDM 10 conductivity meter from Radiometer Copenhagen and expressed as Siemens/cm (Ω^{-1} cm^{-1}). UV absorbance spectra were recorded with a Zeiss model PMQ II spectrophotometer using a quartz cuvette with a 1-cm light path.

Fluorescence measurements were performed with a Perkin Elmer LS-3 spectrofluorometer in a 1×1 cm quartz cuvette.

Copper and zinc concentrations in supernatants of liver homogenates and column fractions were determined without further treatment in a Perkin Elmer model 3030 atomic absorption spectrophotometer with an air-acetylene flame.

Protein concentrations were measured using a Bio-Rad protein assay kit with bovine serum albumin as a standard. All samples were measured in 2-fold dilutions.

Amino acid analyses were performed with a LKB alpha plus analyser after hydrolysis in 6 M HCl for 24 h at 110 °C.

Slab gels containing 12.5 % acrylamide with 2.67 % cross-linking and 1 % SDS were prepared according to O'Farrel[19]. Samples were run with or without prior reduction with 1 % 2-mercaptoethanol or after prior peroxidation with performic acid according to Hirs[20]. The gels were stained with Coomassie Brilliant Blue G-250.

Results

The PBC liver with the highest copper concentration (PBC 2) was selected for preparative isolation. The supernatant of the first (non-reduced) extraction of this liver was fractioned on a Sephadex G-75 column. When a copper peak at about $V_e/V_o = 2.0$ eluted (fig. 1) the outlet of this column was directly coupled to a DEAE Sephadex A-25 column to minimize the exposure of the copper-protein to oxygen. About 90 % of the copper bound to the column. When this column was eluted with a salt gradient, one broad peak was obtained in which UV absorption at 250 and 280 nm, protein concentration and copper concentration closely paralleled each other (fig. 2).

By application of the same purification protocol to livers of cadmium-loaded rats two nearly fully separated MTs were isolated: MT_1 at a conductivity of about 7 mS/cm and MT_2 at a conductivity of about 9.5 mS/cm (fig. 2). The top of the human copper-containing protein peak was at about 8.8 mS/cm.

Characterization of the copper-containing human protein. The UV absorbance spectrum of the copper protein was characterized by a broad band extending from the far UV into the visible region with a very slight shoulder at about 270 nm (fig. 3). Concentrated preparations had a slightly yellow color. The fluorescence of the copper

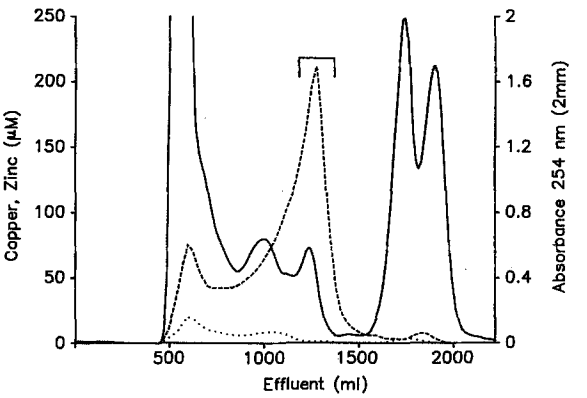

Figure 1. Sephadex G-75 fractionation of a non-reduced extract from a primary biliary cirrhosis liver. A human primary biliary cirrhosis liver (PBC 2) was homogenized in 5 mM Tris-HCl ph 8.5 (1:2 by weight) in a nitrogen atmosphere and centrifuged. Sixty ml of the supernatant was fractionated on a Sephadex G-75 column (5×90 cm) using deaerated, nitrogen gas saturated 5 mM Tris-HCl pH 8.5 at a flow of 60 ml/h. Absorbance at 254 nm (—) was continuously measured. Fractions (10 ml) were collected and analyzed for copper (– – –) and zinc (· · ·). Bracket indicates fractions loaded on DEAE Sephadex A-25.

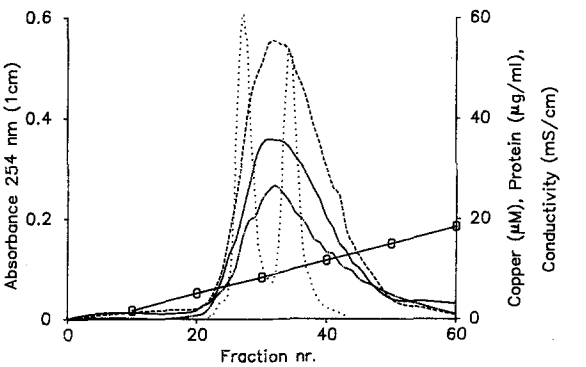

Figure 2. DEAE Sephadex A-25 chromatography of the major copper peak of figure 1. The major copper peak eluted from the gel filtration column (fig. 1) was directly led on a DEAE Sephadex A-25 column (1.6×20 cm) which had been equilibrated with 5 mM Tris-HCl pH 8.5. Almost all copper was bound by the column. Elution was accomplished with a 800 ml linear gradient of the equilibration buffer and the same buffer supplemented with 0.2 M NaCl. Both buffers were deaerated and nitrogen gas saturated. Absorbance at 254 nm (—) was continuously monitored. Fractions (10 ml) were collected and analyzed for copper (– – –), protein (– · –), and conductivity (-⊖-). Absorbance at 254 nm (· · ·) of rat liver cadmium/zinc MT using the same purification protocol.

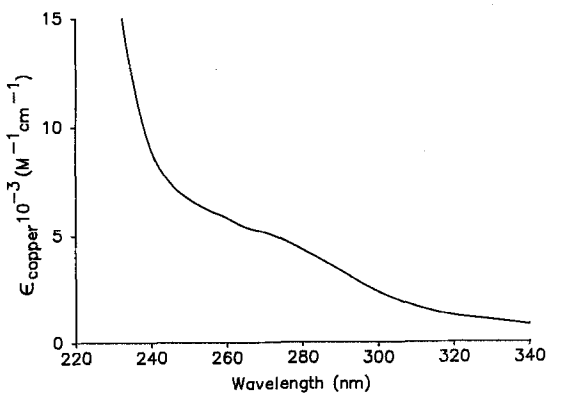

Figure 3. UV absorption spectrum of the isolated human copper-protein. The copper concentration in the sample was 8.75 µM (45 µg protein/ml) in 50 mM sodium phosphate buffer pH 8.5. The spectrum was recorded at 20 °C in a 1-cm cuvette. Data were expressed per mol of copper to avoid the problematics of protein determinations.

protein upon excitation at 310 nm showed a broad band with a maximum at 570 nm and a half band width of 70 nm.

SDS-PAGE of the human copper protein gave one narrow band at about 13 kDa which was indistinguishable from that of isolated rat cadmium/zinc-MT_2. Under all conditions tested, i.e. reduced, non-reduced or peroxidated, the human samples were similar to those of rat MT (fig. 4).

The amino acid composition of the isolated protein had the characteristics of MT and was almost identical to that of isolated rat cadmium/zinc-MT (table 1).

The metal content of the human protein, calculated on the basis of a Bio-Rad protein determination and an assumed molecular weight of 6,300 Da, was 12.3 g atoms of copper and 0.3 g atoms of zinc per mol of protein.

Protein, copper, and zinc extractions. The supernatants of the first (non-reduced) extraction of the PBC and control livers yielded much more protein, copper and zinc than the re-extractions in which 2-mercaptoethanol was used. The amount of protein, copper and zinc in the re-extraction supernatants were 2 %, 20 %, and 10 %, respectively, of the totally extracted quantities. In contrast, in the fetal liver most (59 %) of the copper could only be solubilized by re-extraction with buffer containing 2-mercaptoethanol, although the protein and zinc concentrations in this re-extraction supernatant were also low (table 2).

Analytical Sephadex G-75 runs. The MT content of eight livers was estimated by calculating it from the copper and zinc contents of the Sephadex G-75 metal peak at $V_e/V_o = 2.0$ (table 3). Under the conditions used, most of

Table 1. Amino acid composition of rat cadmium/zinc-containing metallothionein-2 (MT-2) and the human copper-containing protein isolated from a primary biliary cirrhosis (PBC) liver.

	Rat liver MT-2	Human PBC liver copper-protein	Rat liver MT-2[7]	Human fetal liver copper/zinc-MT[3]
Asx	2.5	5.2	4	3.1
Thr	3.0	3.1	2	2.4
Ser	8.5	7.0	10	7.6
Glx	4.9	4.2	3	3.5
Pro	2.0	0.9	2	2.6
Gly	6.2	5.9	4	6.1
Ala	5.2	6.2	5	6.2
Val	2.8	2.6	1	1.8
Cys	11.6	8.9	20	14.1
Met	1.0	0.9	1	0.8
Ile	2.0	2.4	1	0.9
Leu	1.9	1.7	0	0.6
Phe	1.0	1.3	–	–
Lys	7.2	8.6	8	7.9
Arg	–	0.9	–	–
Total	59.8	59.8	61	57.6

Samples were hydrolyzed for 24 h 110 °C in 6 M HCl. Data were calculated assuming 61 residues/mol of protein. Amino acids present at less than 0.5 residues/mol were not included. For comparison, data from Winge et al.[7] for rat cadmium/zinc-MT and from Rydén and Deutsch[3] for human fetal liver copper/zinc-MT were included.

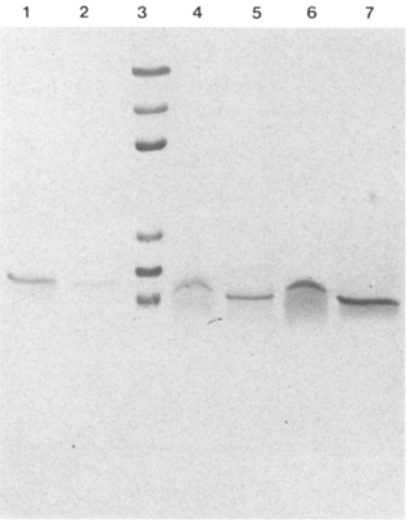

Figure 4. SDS-PAGE of the human copper-protein and rat cadmium/zinc-metallothionein. Slab gels contained 12.5% acrylamide with 2.67% cross-linking and 1% SDS. Staining was done with Coomassie Brilliant Blue G-250. Some samples were oxidized with performic acid according to Hirs[20] or reduced with 1% 2-mercaptoethanol prior to electrophoresis. In lanes from left to right: *1* Rat cadmium/zinc-metallothionein$_2$ oxidized. *2* Human copper-protein oxidized. *3* Marker proteins: bovine albumin (68 kDa), ovalbumin (45 kDa), lactate dehydrogenase (36 kDa), soybean trypsin inhibitor (21.5 kDa), myoglobulin (17.2 kDa), and cytochrome C (11.7 kDa). *4* Human copper-protein reduced. *5* Human copper-protein non-reduced. *6* Rat cadmium/zinc-metallothionein$_2$ reduced. *7* Rat cadmium/zinc-metallothionein$_2$ non-reduced.

Table 2. Concentrations of extracted protein, copper, and zinc obtained by two subsequent liver extractions.

Liver	Tris extraction			2-mercaptoethanol re-extraction		
	Protein	Copper	Zinc	Protein	Copper	Zinc
	µg/g wet weight of tissue					
PBC 1	51	144	53	1.5 (2.8)	37 (20.4)	4.8 (8.3)
PBC 2	48	249	24	1.5 (3.0)	127 (33.7)	5.8 (19.4)
PBC 3	39	44	19	1.5 (3.7)	18 (29.0)	3.0 (13.6)
PBC 4	87	88	58	0.9 (1.1)	13 (12.8)	3.5 (5.7)
Control 1	78	3	28	2.1 (1.0)	0.7 (18.9)	1.6 (5.4)
Control 2	84	8	35	1.5 (1.8)	1.4 (14.9)	3.3 (8.6)
Control 3	81	5	45	2.4 (2.9)	0.8 (13.7)	4.3 (8.7)
Fetal	72	14	111	3.3 (4.4)	20 (58.8)	13.8 (11.1)

Four primary biliary cirrhosis (PBC) livers, three control livers and one fetal liver were homogenized (1:2 by wet weight) in 5 mM Tris-HCl pH 8.5 and centrifuged. The pellets were washed and homogenized in the same buffer supplemented with 1 % 2-mercaptoethanol (1:2 by starting weight tissue). The concentrations of protein, copper, and zinc were determined in the supernatants and the concentrations extracted per g wet weight were calculated. In parentheses the amounts re-extracted with 2-mercaptoethanol are given as percentages of the total amounts extracted.

Table 3. Estimation of liver metallothionein content based on the metal determinations of the 10 kDa peak obtained by Sephadex G-75 gel filtration chromatography.

Liver	Copper in MT peak (nmol/g wet wt)	Zinc in MT peak (nmol/g wet wt)	Estimated MT content (µg/g wet wt)
PBC 1	865	115	560
PBC 2	1883	31	1020
PBC 3	480	55	300
PBC 4	823	189	600
Control 1	65	83	110
Control 2	68	182	200
Control 3	19	245	230
Fetal	165	901	900

Four primary biliary cirrhosis (PBC) livers, three control livers and one fetal liver were homogenized in 5 mM Tris-HCl buffer pH 8.5 (1:2 by wet wt). The supernatants were separated by Sephadex G-75 gel filtration (1.6 × 90 cm column) and the amounts of metallothionein were estimated from the concentrations of copper and zinc in the 10 kDa peak, assuming 7 g atoms zinc or 12 g atoms copper per mol protein and a molecular mass of 6,300 Da.

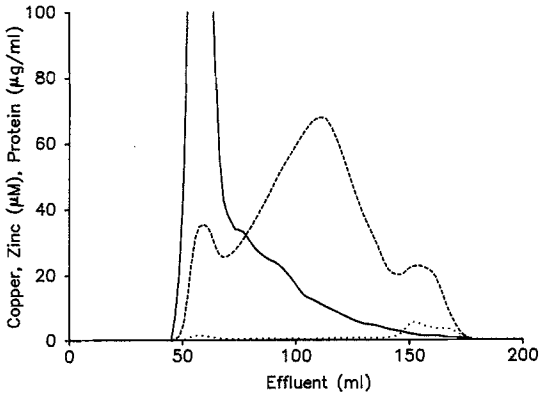

Figure 5. Sephadex G-75 fractionation of a reduced re-extraction from a primary biliary cirrhosis liver. A primary biliary cirrhosis liver (PBC 2) was homogenized in 5 mM Tris-HCl pH 8.5 (1:2 by weight) and centrifuged, and the resulting pellet was washed by repeating the procedure and then homogenized a third time in the same buffer supplemented with 1% 2-mercaptoethanol. After centrifugation 6 ml of the supernatant was fractionated on a Sephadex G-75 column (1.6 × 90 cm) equilibrated with 5 mM Tris-HCl pH 8.5 and 5-ml fractions were collected and analyzed for copper (– – –), zinc (· · ·), and protein (——).

the copper and zinc in this peak eluted between 110 and 130 ml when the supernatants of the first extractions were analyzed. When the supernatants of the re-extraction of the PBC livers were fractionated, a very broad copper peak was found with a maximum at about 120 ml (fig. 5), and most of the zinc eluted near the total volume of the column. Although these re-extractions contained very little protein (table 2), no band was detected at 13 kDa on SDS-PAGE. Such a band was easily detectable in all the supernatants of the first extractions.

Discussion

We report the isolation and characterization of a low molecular weight copper-binding protein from the liver of a PBC patient with a high hepatic copper concentration.

The protein had the light-spectroscopic characteristics of copper-MT: the UV absorbance with a broad shoulder at about 270 nm[21] and the emission spectrum depicted a maximum at 570 nm[6,22]. Although MTs have a molecular mass of about 6.3 kDa, these proteins migrate on SDS-PAGE at the position of a globular protein of about 13 kDa, and the apparent molecular mass increases upon addition of reducing agents[10] or the peroxidation of the protein. The typical behavior of MT on SDS-PAGE has been suggested as a characteristic for its identification[10]. The isolated copper protein in our study migrated similarly to rat liver cadmium/zinc metallothionein, which also indicated that it is an MT.

The calculated metal content was 12.3 g atoms of copper and 0.3 g atoms of zinc per mol MT. These values are near the amount of 11 g atoms of copper and 2–3 g atoms of zinc per mol MT which has been reported for MT isolated from a liver of a patient with Wilson's disease[18], and close to the 12 g atoms of copper which can maximally be bound by MT[11]. The amino acid analysis gave results which were similar to those published by others[3,4,23], and were characteristic of MTs (high cysteine content, no aromatic amino acids).

Most mammalian MTs are separated into two isoforms (MT_1 and MT_2) during anion exchange chromatography. Similarly, our rat liver cadmium/zinc MTs eluted as two sharp peaks from the anion exchange column; however, the copper MT from the PBC liver eluted in one broad peak, indicating that there was a heterogeneous charge on this metal-protein complex. Human copper/zinc MTs isolated from fetal livers have been reported to elute as four[3], three[4] or two[23] peaks from anion exchange columns. Probably oxidation of the protein and mixed copper/zinc charges can lead to a heterogeneous MT population, in particular in PBC.

In our procedures the use of sulfur-containing reducing agents in the eluting buffers was avoided, and oxidation was prevented by using deaerated, nitrogen gas-saturated buffers and working in a nitrogen atmosphere. However, 2-mercaptoethanol was used to solubilize the particulate, lysosomal type of copper-MT[2] which can only be isolated after prior reduction[3].

The extraction of fetal liver gave results identical to those reported by Riordan and Richards[4]: a mainly zinc-containing MT was extracted by non-reducing buffers and a mainly copper-containing MT was extracted from the pellet of the non-reduced extraction with 1 % 2-mercaptoethanol containing buffers. In contrast, PBC livers provided different results. In all non-reducing extracts the major metal bound to MT was copper. The MT from the PBC 2 liver contained almost no zinc. The second extractions with buffers containing 1 % 2-mercaptoethanol solubilized considerable amounts of copper, but no copper-binding protein could be detected in these supernatants. A smear of copper eluted from the Sephadex G-75 column with a maximum at the position where MT normally eluted; however, the PBC 2 liver gave 67 µmol copper/l

and 10 µg protein/ml at that elution volume; even if all the protein had consisted of MT, one mol of MT would have had to contain 40 g atoms of copper at this protein/copper ratio. On SDS-PAGE no MT band was detected even after peroxidation of the samples, although in the primary, non-reduced extracts such a band could readily be seen in spite of the much higher protein/copper ratio.

The four PBC livers were found to contain no major amount of the so-called particulate lysosomal type[2] of copper-MT which could only be extracted with reducing buffers, as in the livers of human newborns and fetuses[3,4], dogs with an inherited copper toxicosis[15,16], and copper-loaded pigs[6]. This is in agreement with the relatively low lysosomal copper concentration found in PBC livers with X-ray microanalysis[24]. Nartey et al.[18] reported similar results using a liver from a patient with Wilson's disease in which 73 % of the accumulated liver copper was solubilized using a non-reducing buffer. Thus the characteristics of the copper MT extracted from the livers of patients with Wilson's disease and PBC are similar and indicate that there is no difference between the two diseases with respect to the induction or the metabolism of liver copper MT.

The MT concentration per gram of wet weight tissue of the 8 livers were calculated on the basis of the metal content of the Sephadex G-75 $V_e/V_o = 2.0$ peak. Our control values (average 180 µg/g, range 110–230 µg/g) were similar to the values previously obtained with cadmium saturation methods: 286 µg/g, as found by Chung et al.[26] or 164 µg/g, as found by Drasch et al.[27]. The PBC livers had markedly elevated MT levels (average 620 µg/g, range 300–1020 µg/g) when compared to our control values or to the cadmium saturation data.

The amount of zinc bound to MT is decreased in PBC livers (97.5 nmol/g as compared to 170 nmol/g for the controls) so copper seems to displace zinc from MT in this disease. The displaced zinc could induce MT synthesis but the very slow accumulation of copper (and thus the very low concentrations of displaced zinc) in PBC argues against such an indirect mechanism. Probably the high hepatic copper levels found in PBC are able to induce the extra MT synthesis, and these high MT levels are able to detoxify most of the accumulated copper.

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