Comparison of pathways of copper metabolism in aorta and liver

A functional test of metallothionein

James E. BALTHROP,* Charles T. DAMERON and Edward D. HARRIS†
*Department of Biochemistry and Biophysics and the Texas Agricultural Experiment Station,
Texas A&M University, College Station, TX 77843, U.S.A.*

(Received 30 October 1981/Accepted 15 February 1982)

Soluble fractions from chick liver and aorta were examined for copper-binding proteins. In liver a zinc-binding thionein appeared to be the major binding protein for copper. Aortic tissue contained only traces of this thionein protein. Unlike liver, moderate amounts of soluble copper in aorta showed no association with macromolecules. Chicks fed on copper-deficient diets for 8 days had one-third the liver copper concentrations of controls. Aortic copper concentration was decreased only slightly, but the activity of lysyl oxidase, a copper-dependent enzyme in aorta, was decreased significantly. Treating the deficient chicks with CuSO_4 (1 mg/kg) restored liver copper rapidly. The increase correlated with the binding of copper to a 10000-mol.wt. component in the soluble fraction. Aortic copper concentrations responded much less to the CuSO_4 treatment, but lysyl oxidase activity was again measurable in the tissue. Radioactive isotopes of copper bound almost exclusively to the 10000-mol.wt. component in liver and to components of mol.wt. 30000 or above in aorta. Hardly any of the administered radioactivity appeared with the 10000-mol.wt. components in aorta, and none was found with unbound copper. The 30000-mol.wt. components in aorta showed superoxide dismutase activity that was sensitive to NaCN. They also showed the highest specific activity of copper of any other aorta component. A clear distinction was seen between the metabolism of copper in liver and aortic tissues. Whereas a copper thionein, metallothionein, was a major component in the liver pathway, it is doubtful that this protein plays a major role in the intracellular metabolism of copper in aortic tissue.

On the basis of information derived from a number of systems, albumin-bound copper (Bearn & Kunkel, 1954) or smaller complexes of copper (Neumann & Sass-Kortsak, 1967) enter the liver and engage or induce synthesis of metallothioneins (Evans *et al.*, 1975; Riordan & Gower, 1975; Bremner & Davies, 1976; Bremner & Young, 1976; Webb *et al.*, 1979; Saylor *et al.*, 1980), now believed to be the major copper-binding proteins in liver (Porter, 1974; Rydén & Deutsch, 1978; Riordan & Richards, 1980). Liver tissue contains two isomeric forms of metallothionein differing slightly in amino acid composition, metal-binding capacity and binding kinetics (Bühler & Kägi, 1974; Suzuki & Yamamura, 1980). Metallothioneins have also been observed in other vertebrate organs (Terao & Owen,

1974; Norton & Heaton, 1980), and appear to be ubiquitous components of many living organisms (for a review see Kägi & Norberg, 1979).

Despite a widespread and increasing interest in metallothionein, no specific biochemical function has been ascribed to this protein other than as a detoxification agent or a temporary reservoir for important metals. That metallothionein serves as an intermediate in the transfer of zinc and perhaps copper to specific binding sites on apo-metalloenzymes has been speculated (Kojima & Kägi, 1978), and in more-recent studies has received experimental support (Udom & Brady, 1980; Li *et al.*, 1980; Lerch, 1980; Geller & Winge, 1980). However, the physiological significance of these findings has not been tested, and an obligatory role for metallothionein in the biosynthesis of metalloenzymes remains to be established.

In the present study we have attempted to determine whether metallothioneins intervene in the

* Present address: National Marine Fisheries Services, P.O. Box 12607, Charleston, SC 29412, U.S.A.

† To whom all correspondence and requests for reprints should be sent.

transfer of copper to lysyl oxidase in aortic tissue. Lysyl oxidase is a copper-dependent enzyme involved in the oxidation of procollagen and proelastin in the biosynthesis of cross-links (Pinnell & Martin, 1968). A marginal deficiency in dietary copper suppresses lysyl oxidase activity, whereas micro amounts of copper restore lysyl oxidase to nearly full function (Harris, 1976). Activation of the enzyme by copper has been firmly established, and studies suggest that this occurs in sequel to the binding of copper to the enzyme structure (Rayton & Harris, 1979). Lysyl oxidase activation may thus provide an excellent model for testing putative intermediates in copper-metalloenzyme biosynthesis, particularly those that may activate or transfer Cu^{2+} ions to the metal-free apoenzyme. Assuming metallothionein performs this role with lysyl oxidase, we have examined aortic tissue for the presence of such proteins. For comparative purposes we have also studied the soluble copper-binding proteins in liver tissue.

Materials and methods

Animals, diets and copper administration

Chicks (Leghorn cockerels) were raised from hatching on copper-deficient (less than $1\mu\text{g}$ of copper/g) or control ($50\mu\text{g}$ of copper/g) diets, as previously described (Rayton & Harris, 1979). The animals were housed in galvanized metal brooders (Petersime Incubator Co., Gettysburg, OH, U.S.A.) with stainless-steel feeding and watering troughs. Temperature was maintained at 33°C . The animals were fed on the diets and given deionized distilled water *ad libitum*. Chicks were killed at 8–10 days, and liver and aortic tissues were removed and then stored at -10°C until used. In some experiments the animals were given a single injection of CuSO_4 (1mg/kg body wt.) in 0.9% NaCl and killed 12h later. Cycloheximide ($10\mu\text{g/g}$) was administered intraperitoneally 45 min before and 2h after CuSO_4 . Radioactive $^{64}\text{CuCl}_2$ (specific radioactivity $>10\text{mCi/mg}$; New England Nuclear, Boston, MA, U.S.A.), dissolved in 0.15 M-NaCl, was administered intraperitoneally at a dose of $10\mu\text{Ci}$ per animal. $^{67}\text{CuCl}_2$ (carrier-free) was obtained from Atomic Energy of Canada Ltd., Ottawa, Ont., Canada.

Preparation of soluble copper fractions from liver and aorta

Livers were homogenized in 3 vol. (by wt.) of 0.12 M-NaCl/15 mM-potassium phosphate buffer, pH 7.6 (buffer A), by using six strokes of a Potter–Elvehjem homogenizer with a motor-driven pestle. Three livers were combined for each sample, with five samples per group (a total of 15 livers in one group). The homogenates were centrifuged (type 30 rotor, Beckman L2-65B ultracentrifuge) twice at $78000g_{\text{av}}$ for 60 min at 4°C , with retention of the

supernatant fraction each time and discarding of the pellet. Exactly 5 ml of supernatant fluid was applied to a precalibrated Sephadex G-75 column ($2.5\text{cm} \times 30\text{cm}$) equilibrated against buffer A. Fractions of volume 4 ml were collected and analysed for 280 nm-absorbing material and copper.

Aortic tissue was cleaned of fascia and suspended in 4 vol. of buffer A supplemented with 10 mM-2-mercaptoethanol to prevent oxidation. The tissue was homogenized with 10–12 strokes of the motor-driven pestle. The homogenate was centrifuged ($100000g$ for 60 min at 4°C) in a type Ti 50 rotor. Exactly 5 ml of supernatant, clear but slightly tinted, was applied to a precalibrated Sephadex G-75 column ($2.5\text{cm} \times 40\text{cm}$) equilibrated against the extracting buffer, and 4 ml fractions were collected and analysed as before.

Incorporation of radioactive copper into aortic tissue in vitro

Cleaned and intact aortas, 20 in number, were suspended in Waymouth medium (MB752/1) (Waymouth, 1959) enriched with copper salts as described previously (Rayton & Harris, 1979). To the medium (final volume 20 ml) was added $50\mu\text{Ci}$ of $^{67}\text{CuCl}_2$ and $5.0\mu\text{g}$ of non-radioactive CuSO_4/ml . The flask was aerated with a stream of pure O_2 for 15 s, capped tightly and incubated with gentle shaking at 37°C for 16 h. After the incubation the tissues were separated from the medium, rinsed five times with 10 ml volumes of buffer A containing 10 mM-2-mercaptoethanol and homogenized in this same solution. After centrifugation, the soluble extracts were chromatographed on Sephadex G-75 as before.

Analytical procedures

Copper and zinc concentrations in column fractions were determined by atomic-absorption spectrometry (Perkin–Elmer model 303 instrument). Buffers were extracted with dithizone in CCl_4 to decrease background copper.

Radioactivity measurements were performed in a Beckman liquid-scintillation counter with the wide-spectrum setting. Small portions (0.1 ml) from each fraction were transferred to 10 ml minivials and immersed in 5 ml of Aquasol (New England Nuclear). Values recorded were corrected for decay from the time the radioisotope was administered to the animal. The ^{67}Cu had a measured half-life of 62 h. That of ^{64}Cu was 12.8 h.

Assays of lysyl oxidase activity employed L-[4,5- ^3H]lysine-labelled substrate proteins from aortic tissue as previously described (Harris, 1976). Super-oxide dismutase activity was assayed in each chromatographic fraction by the method of Marklund & Marklund (1974).

Results

Changes in liver and aorta copper concentrations

With growth the chicks used in the present study showed slight but significant increases in liver copper concentrations (Table 1). Such was true only when the diets contained sufficient amounts of copper salts. When the chicks were fed on diets deficient in copper for 8 days, the liver copper concentrations fell to about one-third that of control chicks of the same age (Table 1). When the deficient chicks were given a single injection of CuSO_4 (1 mg/kg) intra-peritoneally, the liver copper concentrations rose about 6-fold in 24 h. Control chicks showed about a 2-fold increase with this treatment. In contrast with liver, aortic copper concentrations were observed to decrease with aging (Table 1). Copper deficiency appeared to have only a slight affect on the decline. Both the control and the deficient chicks showed very little change in aortic copper concentrations with treatment with copper salts. Clearly these two tissues show different responses to copper deficiency and administration.

Distribution of copper in the soluble fraction from liver

Changes in the copper concentrations in liver must reflect specific metabolic pathways for handling copper in this organ. The distribution of soluble liver copper before and after the administration of CuSO_4 to control chicks is shown in Figs. 1(a) and 1(b). A similar comparison for deficient chicks is shown in Figs. 2(a) and 2(b). Before treatment soluble copper in liver was distributed disproportionately into three fractions resolvable on Sephadex G-75 and corresponding to M_r 75 000, 30 000 and 10 000. Administering CuSO_4 resulted in a large increase in the copper in the 10 000- M_r fraction. No other fractions showed a strong increase. Soluble extracts from the livers of deficient

Fig. 1. Distribution of soluble copper-binding components in control livers before (a) and after (b) CuSO_4 treatment. Extracts (5.0 ml) from liver tissues were applied to a Sephadex G-75 column (2.5 cm $\times$ 30 cm) equilibrated against buffer A. Treated animals received one injection of CuSO_4 (1 mg/kg body wt.) 12 h before analysis. For full experimental details see the text. O, Copper; $\bullet$, A_{280} .

Table 1. Copper content of aorta and liver: response to copper deficiency and copper administration

CuSO_4 in 0.15 M-NaCl was administered at a dose of 1 mg/kg body wt. and tissues were examined 24 h later. For full experimental details see the text. Data are means $\pm$ S.D. for five samples, three livers or aortas per sample.

Treatment	Concn. of copper ($\mu\text{g/g}$ of tissue)	
	Liver	Aorta
Control, 1 day	3.4 ± 1.6	1.3 ± 0.2
Control, 8 days	5.4 ± 0.3	0.8 ± 0.1
+ CuSO_4	11.3 ± 3.0	0.7 ± 0.1
Deficient, 8 days	1.3 ± 0.4	0.5 ± 0.1
+ CuSO_4	8.4 ± 2.6	0.8 ± 0.1

chicks showed no definite copper-containing peaks (Fig. 2a). However, 12 h after the chicks had received CuSO_4 a substantial peak of copper was seen in the 10 000- M_r area of the chromatogram (Fig. 2b). Very little increase in copper was seen in any of the other fractions. Clearly both the control and copper-deficient chicks increase hepatic copper concentrations through a rather selective binding to low-molecular-weight proteins. A further characterization of the 10 000- M_r components revealed substantial amounts of zinc co-eluted precisely with the copper peak (results not shown), suggesting the identity of these components with metallothionein (Bremner & Davies, 1976). The protein has been purified to apparent homogeneity, and both copper and zinc have been found in its structure (Balthrop, 1980).

Fig. 2. Distribution of soluble copper-binding components in deficient livers before (a) and after (b) CuSO_4 treatment

Conditions were identical with those indicated in Fig. 1 legend. O, Copper; ●, A_{280} .

Uptake of radioactive copper into liver proteins

The previous experiment was repeated, only this time $10\mu\text{Ci}$ of $^{64}\text{CuCl}_2$ was administered along with the dose of non-radioactive copper salt. Groups of chicks were killed at 2h and 10h after the injection, and soluble components from the livers of individual chicks were examined as before. Components bearing the radioactive copper were observed to migrate with the $10000\text{-}M_r$ components (Fig. 3). There was very little change in the dispersity of radioactivity between the 2h and 10h samples. The increase observed with the later time period was seen only in the $10000\text{-}M_r$ fraction. These data confirm the results obtained with non-radioactive copper (Fig. 1b), suggesting that low-molecular-weight proteins are the major, if not the only, recipients of newly absorbed copper in liver in a 10h period.

Fig. 3. Incorporation of $^{64}\text{CuCl}_2$ into soluble liver proteins *in vivo*

A Sephadex G-75 column ($2.5\text{ cm} \times 30\text{ cm}$) identical with that used for the experiments of Figs. 1 and 2 was used. Extracts from three livers (5ml) were examined 2h (●) and 10h (○) after the administration of $^{64}\text{CuCl}_2$ ($10\mu\text{Ci}$) along with the dose of non-radioactive CuSO_4 (1 mg/kg). For full experimental details see the text. Radioactivity was corrected for decay occurring between the time of administration and analysis.

Distribution of copper in the soluble fraction from aorta

Aorta contained less than one-third the copper concentration per weight as did liver (Table 1). The identity of the soluble copper components in this tissue was analysed as before, except that extracting and eluting buffers contained 10 mM -2-mercaptoethanol to minimize protein-protein interactions during chromatography (Minkel *et al.*, 1980). As with liver, the soluble copper-containing components in aortic extracts were resolvable into three macromolecular binding components (Fig. 4). Unlike liver, however, a moderate amount of the copper in the extracts migrated with fractions determined by experiment to be associated with free copper or very-low-molecular-weight complexes of the metal (namely fractions 55 and beyond). The overall concentration of copper in macromolecular components was considerably less than that seen in liver extracts from an equal weight of tissue. Nonetheless, it was possible to discern a small peak in the void volume (fractions 22–28), a second peak in the

Fig. 4. Soluble copper-binding components in aortic tissue from control chicks

Chicks were 8 days old at the time of analysis. Extracts (5.0 ml) from 20 aortas were prepared in buffer A supplemented with 10 mM-2-mercaptoethanol. The Sephadex G-75 column used to resolve the components was different from that used previously for liver samples. Its dimensions were 2.5 cm $\times$ 40 cm, and excluded components were observed to be eluted at fractions 21–28. Unbound CuSO_4 was eluted maximally at fraction 67. For full experimental details see the text. $\circ$, Copper; $\bullet$, A_{280} .

30000- M_r area (fractions 30–33) and a third in the 10000- M_r region of the chromatogram (fractions 42–46). The chemical identity of the components eluted beyond fraction 55 has not been determined at this time.

Uptake of radioactive copper into aortic proteins in vivo

The very small amount of copper appearing in column effluents contributed to the uncertainty of the location of copper-binding components. This made it necessary to characterize these components by other means. A 16 h pulse of $^{67}\text{CuCl}_2$ was used for this purpose. After the administration of the radioactive copper to copper-deficient chicks, an analysis of the soluble copper components showed substantial amounts of the radiolabel appearing with components of M_r 30000 or greater. Very little of the radioactivity was eluted with the 10000- M_r components (Fig. 5). This contrasts sharply with liver, where most if not all of the radioactive copper bound to 10000- M_r components (Fig. 3). In Table 2 the specific radioactivities of the copper ($\mu\text{Ci}/\mu\text{mol}$) in each of the three copper peaks of Fig. 5 have been determined. Note that the 30000- M_r components (fractions 28–38) had the highest specific radioactivity of copper, suggesting that these

were the first to bind the radioactive copper. A further characterization of these components revealed the presence of cyanide-sensitive superoxide dismutase activity with peak values corresponding to the peak of the radioactive copper (results not shown). It is noteworthy that none of the radioactivity was co-eluted with specific components in free copper fractions (Fig. 5). Further studies with the radioactive isotope revealed that aortic tissue from deficient chicks took up more of the label (urea-extractable) than did control aortas (Table 3). Moreover, the uptake of copper into the aortic components was sensitive to inhibition by cycloheximide. This antimetabolite, in agreement with previous results (Harris, 1976), also blocked the copper-induced activation of aortic lysyl oxidase (Table 3).

Uptake of radioactive copper into aortic proteins in vitro

In an attempt to achieve a more extensive labelling of aortic proteins, aortic tissue from copper-deficient chicks was cultured in growth medium supplemented with $^{67}\text{CuCl}_2$. Previous studies have determined that a partial activation of lysyl oxidase occurs when the medium is supplemented with CuSO_4 (Rayton & Harris, 1979). After a

Table 2. Determination of radioactivity in extracts of deficient aortas labelled with $^{67}\text{CuCl}_2$ *in vivo*

The total and specific radioactivity of copper in the fractions shown in Fig. 5 are given in this Table.

Combined fractions	Total radioactivity (d.p.m.)	Specific radioactivity ($\mu\text{Ci}/\mu\text{mol}$)	Recovery (%)
20–27	1368	15.4	45.4
28–38	959	24.0	31.8
39–49	381	4.4	13.6
>50	303	—	10.1

16 h incubation *in vitro*, most of the radioactivity in the extracts of aortic tissue appeared with components that migrated behind the 10000- M_r fraction (Fig. 6). Smaller amounts proportionately appeared with the higher-molecular-weight fractions. A shoulder was observed on the descending side of the void-volume components corresponding to the 30000- M_r fraction. Although incubation of the aortic tissue with $^{67}\text{CuCl}_2$ *in vitro* resulted in much more radioactivity in the effluent fractions, the 10000- M_r fraction (fractions 42–46) did not show a propensity to bind the radioactive copper.

Fig. 5. Incorporation of $^{67}\text{CuCl}_2$ into soluble components from deficient aortic tissue

Twenty chicks raised for 8 days on a copper-deficient diet were administered with $^{67}\text{CuCl}_2$ ($10\mu\text{Ci}$ each) along with CuSO_4 (1 mg/kg body wt.) 16 h before analysis. The soluble radioactive components were resolved on Sephadex G-75 as indicated in Fig. 4 legend. $\circ$, Copper; $\bullet$, A_{280} ; $\square$, ^{67}Cu radioactivity.

Fig. 6. Incorporation of $^{67}\text{CuCl}_2$ into soluble components of deficient aortic tissue *in vitro*

Twenty aortas isolated from 8-day-old copper-deficient chicks were suspended in Waymouth's culture medium supplemented with $^{67}\text{CuCl}_2$ ($10\mu\text{Ci/ml}$) and CuSO_4 ($5\mu\text{g/ml}$). After 16 h the aortas were rinsed and homogenized, and soluble extracts were chromatographed on Sephadex G-75 as indicated in Fig. 4 legend. $\bullet$, A_{280} ; $\circ$, ^{67}Cu radioactivity.

Table 3. *Incorporation of copper into aortas of control and deficient chicks in vivo: effect of cycloheximide*
Cycloheximide (12.5 µg) was administered 45 min before and 2 h after CuSO₄ (1 mg/kg body wt.). Radioactive copper appearing in urea extracts of the tissue was determined 12 h later. Values are means ± s.d. for three samples, two aortas per sample.

Treatment	$10^{-6} \times {}^{64}\text{Cu}$ incorporated (d.p.m./g)	Lysyl oxidase activity (${}^3\text{H}_2\text{O}$ /h per g)
Control		49.8 ± 4.0
+ CuSO ₄	20.8 ± 8.1	55.4 ± 8.6
+ CuSO ₄ , cycloheximide	14.4 ± 2.9	42.9 ± 2.8
Deficient		Trace
+ CuSO ₄	41.5 ± 6.6	20.9 ± 2.9
+ CuSO ₄ , cycloheximide	25.4 ± 6.3	8.5 ± 3.6

Discussion

Aorta and liver show marked differences in the way each tissue handles copper, differences that may reflect the presence of specific copper-binding proteins and pathways for copper metabolism in these two tissues. In aorta non-radioactive copper was found associated mainly with components of M_r 30 000 or above. Liver, as expected, bound copper to low-molecular-weight proteins resembling metallothionein. Such metallothionein-like proteins were not the predominant copper-binding proteins in aortic cytosol. Aortic tissue from copper-deficient chicks showed a tendency to bind more radioactive copper than did aortas from control chicks, suggesting that mechanisms for detecting and rectifying low copper concentrations are operative in aorta.

Details of the intracellular pathway for the incorporation of copper into metalloenzymes have not been clarified. There is, however, increasing evidence that the metabolic fate of copper intracellularly may depend on the serum protein bringing copper to the tissue (Terao & Owen, 1976). For instance, copper atoms derived from albumin show less tendency to accumulate in tissue than do copper atoms delivered by caeruloplasmin, the serum copper protein (Owen, 1965; Marceau & Aspin, 1972). Moreover, copper derived from albumin binds mostly to low-molecular-weight proteins in the cytosol of liver cells (Marceau & Aspin, 1973b), whereas copper derived from caeruloplasmin may engage newly synthesized cytochrome *c* oxidase (Marceau & Aspin, 1973a; Hsieh & Frieden, 1975). Recent studies in this laboratory have linked caeruloplasmin with the activation of lysyl oxidase *in vivo* (Harris & DiSilvestro, 1981). Other workers have obtained evidence that caeruloplasmin may be capable of binding and penetrating the cell membrane of many organs (Linder & Moor, 1977), and one of the proteins receiving copper from caeruloplasmin has been identified as superoxide dismutase (Marceau & Aspin, 1973b). In the present study a 30 000- M_r component showing cyanide-sensitive

superoxide dismutase activity was one apparent recipient of copper in aorta. No firm evidence linking newly incorporated copper with this enzyme is available at this time. However, at the time of incorporation of copper into the 30 000- M_r component little if any of the radioactive copper bound to a metallothionein-like protein. These findings speak against metallothionein as being in the pathway for copper in aorta. A critical appraisal of these results must take into account that metallothionein is not the major copper-binding protein in all tissues (Terao & Owen, 1974).

Aortic tissue was studied in the present work because the uptake of copper into aorta leads ultimately to accelerated activation of lysyl oxidase. The enzyme has an estimated half-life of 16–18 h (Harris *et al.*, 1977), and copper from the diet must be supplied continuously to sustain its activity in aorta and cartilage of growing chicks (Siegel *et al.*, 1970; Harris *et al.*, 1974). It thus becomes evident that the turnover of lysyl oxidase occurs with an irretrievable loss of copper from the enzyme structure, i.e. the once-bound copper cannot recycle back to be re-incorporated into the structure of new enzyme molecules. An explanation for that observation is not immediately apparent. The findings support the contention that the pathways for the incorporation of copper for continued metabolism and those associated with its removal do not mix freely in the tissues (Hazelrig *et al.*, 1966).

A property of metallothionein now receiving support from a number of laboratories is an ability to sequester and perhaps transfer bivalent metal ions such as Zn²⁺ and Cu²⁺ to newly synthesized apo-metalloenzymes (Udom & Brady, 1980; Li *et al.*, 1980; Lerch, 1980). In this respect it is important to note that aortic tissue, but not liver, appeared to have a moderate amount of copper in what may be considered a free copper pool. Because this copper pool was not labelled by exogenously administered copper, we suggest that the pool arises from copper discharged by the intracellular proteins during turnover. The pool is

apparently non-functional. One could further speculate that the pool exists because of the apparent absence of metallothionein from aorta or a failure of copper to induce its synthesis in this tissue.

In conclusion, the strongest argument against metallothionein being an intermediate in the activation pathway for lysyl oxidase rests with the failure to find radioactive copper in the metallothionein fraction after a 16 h pulse. Conceivably it is possible that the copper has been passed over to the enzyme before the time of examination. Such an event would be hard to determine experimentally because of the very small amounts of copper that enter aorta in a shorter time frame. Lysyl oxidase activation *in vivo* occurs in a non-linear progression over a 5–24 h period (Harris, 1976; Harris & DiSilvestro, 1981). If metallothionein is indeed a component in the activation pathway, it is difficult to reconcile why nearly all of the radioactive copper has left the metallothionein peak before 16 h. Should the protein be present in aorta, the findings suggest that the passage of copper through the protein is very rapid. However, since activation of lysyl oxidase is occurring at a time when no radioactivity is present in metallothionein, the findings cannot support the involvement of this protein in the activation of lysyl oxidase in aorta. Thus the copper donor for lysyl oxidase remains to be identified. In studying copper metabolism in both tissues we suggest that one function of metallothionein is to bind and recycle copper liberated by turnover of Cu-metalloenzymes back into the synthesis of new enzyme.

Funding for this work was provided by U.S. Public Health Service Grant AM-26604. We are grateful to Ms. Mary Haislet and Ms. Sandra Pehle for skilled technical assistance.

References

- Balthrop, J. E. (1980) Ph.D. Thesis, Texas A&M University
- Bearn, A. G. & Kunkel, H. G. (1954) *Proc. Soc. Exp. Biol. Med.* **85**, 44–49
- Bremner, I. & Davies, N. T. (1976) *Br. J. Nutr.* **36**, 101–112
- Bremner, I. & Young, B. W. (1976) *Biochem. J.* **157**, 517–520
- Bühler, R. H. O. & Kägi, J. H. R. (1974) *FEBS Lett.* **39**, 229–234
- Geller, B. L. & Winge, D. R. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 1768 (Abstr.)
- Evans, G. W., Wolenetz, M. L. & Grace, C. L. (1975) *Nutr. Rep. Int.* **12**, 261–269
- Harris, E. D. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 371–374
- Harris, E. D. & DiSilvestro, R. A. (1981) *Proc. Soc. Exp. Biol. Med.* **166**, 528–531
- Harris, E. D., Gonnerman, W. A., O'Dell, B. L. & Savage, J. E. (1974) *Biochim. Biophys. Acta* **341**, 332–344
- Harris, E. D., Rayton, J. K. & DeGroot, J. E. (1977) in *Elastin and Elastic Tissue* (Sandberg, L. B., Gray, W. R. & Franzblau, C., eds.), pp. 543–559, Plenum Press, New York
- Hazelig, J. B., Owen, C. A., Jr., & Ackerman, E. (1966) *Am. J. Physiol.* **211**, 1075–1081
- Hsieh, H. S. & Frieden, E. (1975) *Biochem. Biophys. Res. Commun.* **67**, 1326–1331
- Kägi, J. H. R. & Norberg, M. (1979) *Experientia Suppl.* **34**, 41–116
- Kojima, Y. & Kägi, J. H. R. (1978) *Trends Biochem. Sci.* **3**, 90–93
- Lerch, K. (1980) *Nature (London)* **284**, 368–370
- Li, T. Y., Kraker, A. J., Shaw, C. F. & Petering, D. H. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6334–6338
- Linder, M. C. & Moor, J. R. (1977) *Biochim. Biophys. Acta* **499**, 329–336
- Marceau, N. & Aspin, N. (1972) *Am. J. Physiol.* **222**, 106–110
- Marceau, N. & Aspin, N. (1973a) *Biochim. Biophys. Acta* **293**, 338–350
- Marceau, N. & Aspin, N. (1973b) *Biochim. Biophys. Acta* **328**, 351–358
- Marklund, S. & Marklund, G. (1974) *Eur. J. Biochem.* **47**, 469–474
- Minkel, D. T., Poulsen, K., Wielgus, S., Shaw, C. F. & Petering, D. H. (1980) *Biochem. J.* **191**, 475–485
- Neumann, P. Z. & Sass-Kortsak, A. (1967) *J. Clin. Invest.* **46**, 646–658
- Norton, D. S. & Heaton, F. W. (1980) *J. Inorg. Biochem.* **13**, 1–9
- Owen, C. A., Jr. (1965) *Am. J. Physiol.* **209**, 900–904
- Pinnell, S. R. & Martin, G. R. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **61**, 708–716
- Porter, H. (1974) *Biochem. Biophys. Res. Commun.* **56**, 661–668
- Rayton, J. K. & Harris, E. D. (1979) *J. Biol. Chem.* **254**, 621–626
- Riordan, J. R. & Gower, I. (1975) *Biochem. Biophys. Res. Commun.* **66**, 678–686
- Riordan, J. R. & Richards, V. (1980) *J. Biol. Chem.* **255**, 5380–5383
- Ryden, L. & Deutsch, H. F. (1978) *J. Biol. Chem.* **253**, 519–524
- Saylor, W. W., Morrow, F. D. & Leach, R. M., Jr. (1980) *J. Nutr.* **110**, 460–468
- Siegel, R. C., Pinnell, S. R. & Martin, G. R. (1970) *Biochemistry* **9**, 4486–4492
- Suzuki, K. T. & Yamamura, M. (1980) *Toxicol. Lett.* **6**, 301–307
- Terao, T. & Owen, C. A., Jr. (1974) *Mayo Clin. Proc.* **49**, 376–381
- Terao, T. & Owen, C. A., Jr. (1976) *Tohoku J. Exp. Med.* **120**, 209–217
- Udom, A. O. & Brady, F. O. (1980) *Biochem. J.* **187**, 329–335
- Waymouth, C. (1959) *J. Natl. Cancer Inst.* **72**, 1003–1015
- Webb, M., Plastow, S. R. & Magos, L. (1979) *Life Sci.* **24**, 1901–1906