Induction of Lysyl Oxidase with Copper

PROPERTIES OF AN IN VITRO SYSTEM

(Received for publication, March 21, 1978)

John K. Rayton and Edward D. Harris
From the Department of Biochemistry and Biophysics and the Texas Agricultural Experiment Station, Texas A & M University, College Station, Texas 77843

The binding of copper to newly synthesized protein. The findings strongly support the possibility that induction involves enzyme synthesis rather than enzyme activation.

Aortic tissue from chicks raised 8 to 11 days on diets lacking copper contains diminished levels of the enzyme lysyl oxidase, a copper metalloenzyme. By incubating the deficient aortic tissue in fully oxygenated Waymouth growth medium (MB 751/2) supplemented with 3 to 5 mg/ml of CuSO4, it was possible to restore lysyl oxidase activity to the aorta. Enzyme activation required copper supplements to the serum-free medium. When a partially purified preparation of serum copper proteins supplied the copper, activation was also achieved but at a much lower copper concentration (0.2 mg/ml). Homogenizing the tissue or incubating it under N2 or in the cold blocked the appearance of the copper-induced enzyme activity. Incubating the tissue in a simple solution containing buffer salts and CuSO4 did not result in activation. A time course analysis showed that 3- to 5-h lag period preceded the appearance of enzyme activity in the tissue. Further studies with [3H]lysine and 65Cu revealed that the metal became bound to a newly synthesized protein component which affixed to a collagen-derivatized Sepharose 4B column and eluted with 6 M urea, typical of the behavior of lysyl oxidase. On polyacrylamide gel in the presence of sodium dodecyl sulfate, this component migrated with a molecular weight of about 60,000, approximately the molecular weight of chick aorta lysyl oxidase. Cycloheximide, but not actinomycin D, completely inhibited the incorporation of both [3H]lysine and 65Cu into the 60,000 molecular weight component. These data obtained with aortic tissue in a defined medium suggest that activation of lysyl oxidase with copper is a property of metabolically active tissues and proceeds with the binding of copper to newly synthesized protein. The findings strongly support the possibility that induction involves enzyme synthesis rather than enzyme activation.

The induction of metalloproteins by metal ions has been observed in a wide range of animal tissues and microbial organisms. The prototype for such studies has been the protein ferritin, a major iron storage protein in liver and other organs (1, 2). Since these earlier studies, numerous reports have appeared describing the regulation of nonferrous metalloenzymes this may not be the case. For instance, Keyhani and Keyhani (12) reported that yeast cells grown in a medium lacking copper accumulate proportionate numbers of the subunit components of cytochrome oxidase. Final assembly into a functioning enzyme, however, requires the presence of copper. Likewise, rats exposed to a moderate copper deficiency continue to synthesize and release a serum protein which resembles ceruloplasmin immunochemically but contains very little detectable copper (13). Such results suggest that the synthesis of apoceruloplasmin or cytochrome oxidase does not depend upon the presence of copper. They further suggest that insertion of copper into these metalloenzymes occurs after translation.

Lysyl oxidase is a copper metalloenzyme found primarily in connective tissue. The enzyme, a specific amine oxidase, catalyzes the oxidative deamination of peptidyl lysine residues in collagen and elastin preparatory to the formation of covalent cross-links in these proteins (14). Lysyl oxidase has been purified to homogeneity by different purification schemes (15-17). Chick aorta lysyl oxidase has a molecular weight of about 60,000 and like its counterpart in bovine aorta (18) shows evidence for multiple molecular forms (19), perhaps accounting for known differences in immunochemical (20) and inhibitory properties of lysyl oxidase (21). Low levels of dietary copper lower the lysyl oxidase activity in chick aorta (15) and

1 No Enzyme Commission (E.C.) number has been assigned to this enzyme.
cartilage (22), clearly demonstrating the importance of copper to enzyme function. However, enzyme activity is restored by injecting trace amounts of CuSO₄ (less than 0.5 μmol) intraperitoneally into deficient chicks (23). The activation process is blocked by cycloheximide but not actinomycin D, suggesting that copper somehow affects a post-transcriptional regulatory mechanism have led to the present work. Here we report that lysyl oxidase activity can be restored to deficient aortas in vitro when the largely intact tissues are incubated in a serum-free Waymouth medium. The copper concentration in the medium appears to control the response. A preliminary account of this work has appeared (24).

**MATERIALS AND METHODS**

**Animals**—Male, inbred, hybrid white leghorn chicks were obtained on the day of hatching and housed in all plastic (Plexiglas) cages (51 × 76 × 35 cm), maintained at 28 ± 2°C. Chicks were fed deionized distilled water and semipurified diets ad libitum prepared as described by O'Dell et al. (25). Diets of deficient animals contained by analysis (atomic absorption spectrophotometry) less than 1 μg of copper/g of diet. Control diets were identical in contents but were supplemented with 50 μg of anhydrous CuSO₄/g of diet.

**Preparation of Aortic Tissues for Culture**—Chicks were killed after the 8 to 14 days of the diets (beginning from the day of hatching). The skin over the cranial and spinal region was cut through the breast bone. The aortas with hearts attached were carefully removed and the intact tissue was placed in Waymouth MB 751/2 growth medium in a Petri dish kept at room temperature. Aortas from deficient chicks were quite fragile and care was taken not to tear the tissue during isolation.

**Incubation of Aortas**—Aortic tissue was transferred to screw-capped culture tubes (15 × 0.8 mm) (two aortas/tube) with 2.0 ml of fresh Waymouth medium (26). Penicillin G (50 units/ml) and streptomycin sulfate (50 μg/ml) were added to suppress bacterial contamination. Additional components to the medium are described in the tables and figure legends. Culture tubes were gassed with 109% oxygen in a buffer made up by mixing 19 ml of 0.625 M KH₂PO₄, 1 mg of bromphenol blue, 27 ml of glycerol, and 1 mg of bronopol. Samples in this buffer were heated for 2 min in a boiling water bath. After the electrophoresis run, one gel was stained with 25% (w/v) Coomasie brilliant blue (dissolved in an aqueous solution of 25% (v/v) isopropanol alcohol and 10% (v/v) acetic acid) for 30 min and destained by diffusion in 7.5% (v/v) acetic acid. The other was cut into 2-mm sections which were placed in scintillation vials and immersed in exactly 0.2 ml of 30% (v/v) H₂O₂. After the gels had dissolved (12 h at 4°C), 6 ml of Aquasol was added to each vial and both ¹³C and ³²P were quantitated in the Beckman counter.

**RESULTS**

**Requirement for Copper**—The aim of the experiments was to investigate the feasibility of activating aortic lysyl oxidase in vitro with copper. The simplest approach was to take aortas from 8- to 11-day copper-deficient chicks and transfer the largely intact tissue to Waymouth's growth medium. Aortas so treated when suspended in the medium for 18 h failed to show an increase in lysyl oxidase activity. Adding serum from copper-fed chicks to the medium also produced no response. However, when CuSO₄ was added to a final concentration of 5 μg of copper/ml, substantial amounts of enzyme activity appeared in the tissue after 18 h (Table I). The serum-free medium contained about 0.1 μg of copper/ml present as a contaminant from the other chemicals. This amount of copper apparently could not support the activation. Note, however, that raising the copper concentration from 0.1 pg/ml to 1.0 μg/ml brought about a two-fold increase in enzyme activity in the tissue (Fig. 1). Such copper-induced activation was maximum at 5.0 μg of copper/ml. Clearly the response was dependent on the medium copper concentrations. When ZnSO₄ and FeSO₄ were tested separately at the same effective metal ion concentration (5 μg/ml), these salts did not achieve the same degree of activation as did CuSO₄ (Table II).

**Assay of Lysyl Oxidase Activity**—The procedure followed that of Pinnell and Martin (27) with modifications as noted earlier (23). The protein substrates were prepared from 6-day-old chick aortas incubated for 24 h in a lysine-free Waymouth medium supplemented with 20 μCi/ml of L-[4,5-³²P]lysine (Schwarz/Mann, specific activity 35 Ci/μmol) and 25 μg/ml of unrelated protein (lactalbumin). Approximately 500,000 cpm of substrate proteins were mixed with 0.2 ml of extract containing the enzyme. Buffer A was added to a final volume of 1 ml. Incubations lasted for 2 h at 40°C. Exchangeable tritium (as tritiated water) was separated by vacuum distillation and 0.2 ml of the distillate in 5.0 ml of Aquasol scintillation fluid was measured for radioactivity in a Beckman model 205B liquid scintillation counter, with a counting efficiency of 30% for tritium. The enzyme activity is expressed in counts per min of tritiated water formed per hour per wet weight of tissue. The enzyme assays typically show a linear release of H₂O for at least 4 h.

**Measurement of H and Cu in Protein Samples—Radioactive Copper (¹³C, 3.0 μCi/μg) was purchased from New England Nuclear Inc. Measurements of ¹³C and ³²P incorporation into aortic proteins were conducted after purifying the proteins on affinity columns (see above). About 3.5 ml of the column eluate in 6 μm urea buffer were dialyzed against 1000 volumes of deionized distilled water containing 10 -5 M cuprozine to remove loosely bound copper (two times over 6 h on each occasion) on water wash the column. Five microliters of this was mixed directly with 5 ml of Aquasol and measured for ³²P and ¹³C in the Beckman counter. Samples were counted twice: once for ³²P and ¹³C and, then, after sitting in the dark for 1 week to allow ¹³C to decay to background levels, a second time for ³²P alone. The remaining 3.0 ml from each of the four replicates were pooled and concentrated five times by vacuum distillation at room temperature. Two-times milliliters of the concentrated protein solution was further analyzed by the electrophoresis procedure described below.
higher than the concentration of copper in normal chick blood which is about 0.2 µg/ml (29). As to why the in vitro system required such high amounts of copper was not determined directly. It did suggest, however, that aqueous complexes of copper were not the physiological form of the metal, even though such complexes apparently could force the activation of the enzyme in vitro. Thus, it was important to determine whether the in vitro system could respond to a protein-bound copper at physiological levels of the metal. The serum copper proteins were tested at only one concentration in the medium. Copper-deficient aortas in pairs were incubated for 12 h at 37°C in Waymouth medium containing the indicated supplements of CuSO₄ as (CuSO₄). Each data point determination incubated at 37°C in Waymouth medium containing the indicated concentration in the medium. Copper-deficient aortas in pairs were incubated for 16 h at 37°C and then assayed for lysyl oxidase activity. Data ± S.E. n = 3.

![Graph](image)

**FIG. 1.** Activation of lysyl oxidase as a function of the copper concentration in the medium. Copper-deficient aortas in pairs were incubated at 37°C in Waymouth medium containing the indicated supplements of CuSO₄ as (CuSO₄). Each data point determination represents the average of three aorta pairs.

as effective as the free metal at the same concentration. Higher concentrations of protein-free copper, however, produced the strongest activation, confirming the earlier results. The serum copper proteins were tested at only one concentration so it is not known whether higher protein concentrations would elicit more activity. It seems clear, however, that the induced response can be achieved with much lower copper concentrations but at these lower concentrations a specific copper protein is needed.

**Kinetic Studies**—Kinetic studies were performed to 1) investigate the time of optimum activation and 2) the rapidity with which enzyme activity appeared after copper was added to the incubation medium. A close kinetic analysis showed that the copper-induced activation in vitro did not occur immediately but rather followed a delay period of about 3 to 5 h (Fig. 2). With no copper supplements there was no rise in enzyme activity at any time over a 25-h period. The delay period which characterizes the response in vitro is reminiscent of the activation of lysyl oxidase in the intact chick. There, a 1- to 2-h delay period after copper injections was typical and was assumed to be caused by the slow absorption and transport of copper to the aorta. That supposition may no longer be correct.

**Other Properties of the System**—A summary of other important variables which were observed to play a role in the activation of lysyl oxidase in the in vitro system is shown in Table IV. Briefly, disrupting the tissue by homogenization, incubating it under NZ or at 5°C, or substituting buffer salts for the Waymouth medium were treatments which abolished or lessened the copper-induced activation. The results suggest that activation of the enzyme in this manner is an energy-dependent process requiring intact, structurally sound tissue. They tend to eliminate the possibility that the activation is simply a fortuitous interaction of aqueous copper with the metal-free enzyme.

**Studies of the Activation Mechanism**—With the attainment of a simple reproducible system above, studies were

### Table I

<table>
<thead>
<tr>
<th>Nutritional status of chicks</th>
<th>Additions to Waymouth medium¹</th>
<th>Lysyl oxidase activity (x10⁻⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper-fed</td>
<td>Serum (Cu⁺)</td>
<td>35.7 ± 6.1</td>
</tr>
<tr>
<td>Copper-deficient</td>
<td>Serum (Cu⁺)</td>
<td>6.4 ± 1.7</td>
</tr>
<tr>
<td>Copper-deficient</td>
<td>CuSO₄</td>
<td>24.4 ± 4.3</td>
</tr>
<tr>
<td>Copper-deficient</td>
<td>Serum (Cu⁺) + CuSO₄</td>
<td>20.7 ± 2.2</td>
</tr>
<tr>
<td>Copper-deficient</td>
<td>Serum (Cu⁻) + CuSO₄</td>
<td>21.4 ± 3.1</td>
</tr>
</tbody>
</table>

¹ Source of serum was either deficient (Cu⁻) or control (Cu⁺) chicks.

### Table II

**Effects of Cu²⁺, Zn²⁺, and Fe²⁺ salts on the in vitro activation of aortic lysyl oxidase**

<table>
<thead>
<tr>
<th>Salt added</th>
<th>Lysyl oxidase activity (x10⁻⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>9.82 ± 1.7</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>23.70 ± 1.32</td>
</tr>
<tr>
<td>FeSO₄</td>
<td>11.76 ± 5.72</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>8.33 ± 7.91</td>
</tr>
</tbody>
</table>

### Table III

**The in vitro activation of aortic lysyl oxidase by serum copper proteins**

10-day copper-deficient aortas were suspended in Waymouth's medium supplemented with either partially purified copper-binding proteins from chick serum, a copper-albumin complex, or CuSO₄. Incubations lasted 12 h at 37°C. Lysyl oxidase was then extracted and assayed. Data ± S.E. n = 3.

<table>
<thead>
<tr>
<th>Agent added</th>
<th>Copper level</th>
<th>Protein level</th>
<th>Lysyl oxidase activity (x10⁻⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (control)</td>
<td>0.0</td>
<td>0.0</td>
<td>4.2 ± 0.48</td>
</tr>
<tr>
<td>Copper-binding proteins</td>
<td>0.2</td>
<td>1.0</td>
<td>14.6 ± 1.02</td>
</tr>
<tr>
<td>Copper-albumin</td>
<td>0.2</td>
<td>1.0</td>
<td>7.9 ± 0.51</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>0.2</td>
<td>0.0</td>
<td>6.8 ± 0.84</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>5.0</td>
<td>5.0</td>
<td>21.4 ± 1.39</td>
</tr>
</tbody>
</table>
Activation of Lysyl Oxidase in Vitro

Fig. 2. Time course of in vitro activation. Deficient aortas in pairs were incubated for the indicated amounts of time shown. Lysyl oxidase activity was measured in 6 M urea extracts from the tissue after dialysis against Buffer A. Copper concentration was 5 &mu;g/ml. Each data point determination represents the average of three aorta pairs.

Table IV
Conditions for activating aortic lysyl oxidase in vitro with copper

All aortas were obtained from 8-day deficient animals. The aortas were incubated for 12 h under the conditions shown. Those which were ground were homogenized under sterile conditions in the incubation medium until the structural state of the aorta was destroyed. Tubes were gassed with either O_2 or N_2 before sealing. Data ± S.E. n = 3.

<table>
<thead>
<tr>
<th>Waymouth medium</th>
<th>Added copper (5 μg/ml)</th>
<th>Buffer salts</th>
<th>Temperature</th>
<th>State of tissue</th>
<th>Surrounding gas</th>
<th>Lysyl oxidase activity (x10^{-5})</th>
<th>cpm H_2O/h/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waymouth</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>37 Intact</td>
<td>100% O_2</td>
<td>4.25 ± 0.5</td>
<td>0.85</td>
</tr>
<tr>
<td>Waymouth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>37 Intact</td>
<td>100% O_2</td>
<td>17.47 ± 0.08</td>
<td>3.08</td>
</tr>
<tr>
<td>Waymouth</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>5 Intact</td>
<td>100% O_2</td>
<td>8.46 ± 0.79</td>
<td>0.79</td>
</tr>
<tr>
<td>Waymouth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>37 Ground</td>
<td>100% O_2</td>
<td>9.91 ± 1.86</td>
<td>1.86</td>
</tr>
<tr>
<td>Waymouth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>37 Ground</td>
<td>100% O_2</td>
<td>10.04 ± 1.99</td>
<td>1.99</td>
</tr>
<tr>
<td>Waymouth</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>5 Intact</td>
<td>100% O_2</td>
<td>4.5 ± 0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Waymouth</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>37 Intact</td>
<td>100% N_2</td>
<td>11.09 ± 1.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

a) Contained 2.1 mM Na_2HPO_4, 0.6 mM KH_2PO_4, and 26.7 mM NaHCO_3, pH 7.4.

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of eluates from affinity gel. Copper-deficient aortas were incubated for 12 h in Waymouth medium. 6 M urea extracts were dialyzed against Buffer A and passed through collagen-derivatized Sepharose 4B columns. The eluate from the column which contained the enzyme was concentrated and examined on polyacrylamide gels run in the presence of sodium dodecyl sulfate. A series of protein standards was also run in separate gels to estimate the molecular weight. A, L-[4,5-^3H]lysine, no copper; B, L-[4,5-^3H]lysine, ^64Cu; C, same as B but with 10 &mu;g/ml of cycloheximide; D, same as B but with 25 &mu;g/ml of actinomycin D.

Table V
Incorporation of ^3H]lysine and ^64Cu into aortic proteins: effect of cycloheximide and actinomycin on incorporation and lysyl oxidase activation

After the incubation, dialyzed extracts containing the enzyme were passed through affinity columns as a means to further purify the enzyme. Data ± S.E. n = 4.

<table>
<thead>
<tr>
<th>Source of tissue</th>
<th>Copper added (5 μg/ml)</th>
<th>Antimetabolite</th>
<th>^3H]lysine incorporated (x10^{-5})</th>
<th>cpm/g aorta</th>
<th>^64Cu incorporated (x10^{-4})</th>
<th>cpm/h/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper-fed</td>
<td>+</td>
<td>None</td>
<td>64.20 ± 6.5</td>
<td>67.88 ± 4.0</td>
<td>0.23 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Copper-deficient</td>
<td>+</td>
<td>None</td>
<td>50.20 ± 3.8</td>
<td>74.75 ± 4.8</td>
<td>4.02 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Copper-deficient</td>
<td>-</td>
<td>None</td>
<td>98.40 ± 6.3</td>
<td>1.06 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper-deficient</td>
<td>+</td>
<td>Cycloheximide</td>
<td>1.44 ± 0.0</td>
<td>42.45 ± 0.0</td>
<td>2.08 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Copper-deficient</td>
<td>+</td>
<td>Actinomycin D</td>
<td>49.2 ± 2.9</td>
<td>77.44 ± 7.9</td>
<td>4.82 ± 1.1</td>
<td></td>
</tr>
</tbody>
</table>

* Corrected for disintegrations apparent at zero time. The actual counts observed were between 240 and 465 cpm.

^ + 10 &mu;g/ml.

^ + 25 &mu;g/ml.
TABLE VI

Additions to medium | Lysyl oxidase activity (×10^4 cpm H_2O/h/g)
--- | ---
First incubation | Second incubation
--- | ---
- | CuSO_4 | 23.6 ± 1.3
BAPN | - | 11.5 ± 0.6
BAPN | No additions | 5.9 ± 1.0
BAPN | Cycloheximide | 18.3 ± 1.3
BAPN | CuSO_4 + cycloheximide | 11.6 ± 0.0

* No incubation.

Aortic tissue was obtained from 6-day copper-fed chicks. The first incubation was in Waymouth medium (60 min, 37°C) containing 50 μg/ml of β-aminopropionitrile fumarate. The tissue was transferred to fresh medium containing supplements as shown (cycloheximide, 10 μg/ml; CuSO_4, 5 μg of copper/ml) and incubated at 37°C for 18 h. Data ± S.E. n = 5.

DISCUSSION

Copper exerts close control over lysyl oxidase, a copper metalloenzyme in aortic tissue. The phenomenon, observed first in vitro (23), has now been observed in intact isolated aortic tissue suspended in a chemically defined medium. The activation of lysyl oxidase with copper is clearly dependent on the concentration of copper added to the medium, high concentrations of the metal ions appearing capable of overcoming a need for serum proteins. However, the isolated tissue showed partial activation when homologous serum copper proteins were added, suggesting that the simple in vitro system was capable of reproducing events which occurred physiologically.

The apparent requirement for a fully supplemented growth medium is a significant feature. The growth medium supplies amino acids, some of which are known to bind copper and hasten its uptake into suspended liver slices (32). However, enzyme activation was never immediate in the in vitro system; a 3- to 5-h delay was typical and reminiscent of copper-induced activation in vitro (23). Since low temperatures, homogenization, and anaerobic conditions diminished the activation response, it seems clear that activation of lysyl oxidase in vitro is a property of intact metabolically active tissue. The Waymouth growth medium, unlike simple buffer salts, supplies those ingredients which sustain growth as well as chelate copper. Moreover, the sensitivity to very low concentrations of cycloheximide suggests that full activation occurs only when protein synthesis is uninterrupted. The fact that cycloheximide did not completely shut off lysyl oxidase activation by copper could be due to the reversible nature of cycloheximide inhibition (30). Such activity could reflect protein synthesized late in the incubation period when inhibitory effects had been overcome.

In the present study, the copper-induced activation was accompanied by the binding of radioactive copper to a 60,000-dalton protein which attached to collagen-derivatized Sepharose. The protein-copper complex was of sufficient stability to permit analysis on electrophoresis gels in the presence of detergents. As yet, it is not known how many copper atoms became bound to 1 molecule of the enzyme. However, when protein synthesis was blocked with cycloheximide, no radioactive copper became bound to protein. Thus, a diminution in activation correlated with the diminished binding of ^65Cu to protein and both events showed a dependence on de novo synthesis of protein. Since the identity of the 60,000-dalton component has not been established conclusively, there is the possibility that this component is an activator or transport protein, or both, whose synthesis is a key event in the activation mechanism. The procedure for preparing the protein makes this possibility remote.

That copper did not bind to any protein when protein synthesis was blocked strongly suggests that the insertion of the metal into the protein structure must occur at translation or before the newly assembled protein has equilibrated with its intracellular pool. Failure to bind with inhibitor present could also mean that apoenzyme components are not present or, if so, are incapable of binding copper. The data do not permit a distinction. Nonetheless the lysyl oxidase system appears to contrast with that of cytochrome oxidase in yeast. There copper becomes incorporated into preassembled subunits which accumulate although sufficient supplies of copper...
are not available (12). If copper incorporation into lysyl oxidase is synchronized with specific stages of protein assembly, this could explain why activation cannot be achieved in homogenized tissues (Table IV) or in extracts from copper-deficient aortas. The findings in fact have relevance to the observation that the protein moiety of ceruloplasmin, once synthesized, will not bind nor exchange serum copper atoms (33) unless its structure is modified chemically (34).

In the rapidly growing chick, aortic lysyl oxidase has an estimated half-life of about 16 h (35). Thus, lysyl oxidase does not escape the usual turnover processes that control protein levels. Assuming minimum recycling of copper, the stress of depletion of copper from specific precursor pools can be expected to have a rapid effect on lysyl oxidase activity. Reactivation of lysyl oxidase with copper occurs via pathways yet to be identified and a mechanism largely unknown. Although intracellular copper-binding proteins have been identified in many tissues (8, 10, 36-40), as yet no one has reported their presence in aortic tissue. This, then, is a major gap in the information. Since the suspended tissue appears to respond to a homologous serum protein fraction which may deliver copper to lysyl oxidase, it should be possible to study the transfer of copper from serum proteins to the metal-free enzyme, thus identifying the key intermediates in the process. Progress in metalloenzyme activation by metal ions has been hampered by the lack of a well defined system for studying the mechanism. The in vitro system described in this report should overcome some of these difficulties and hopefully eventually attach a physiological significance to the intracellular proteins which bind copper.

Acknowledgments—We are indebted to Mr. James E. Balthrop and Miss Margarita Garcia de Quevedo for providing expert technical assistance.

REFERENCES
Induction of lysyl oxidase with copper. Properties of an in vitro system.
J K Rayton and E D Harris


Access the most updated version of this article at http://www.jbc.org/content/254/3/621.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/254/3/621.citation.full.html#ref-list-1