Copper Deficiency Alters Rat Dopamine β-Monoxygenase mRNA and Activity

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ABSTRACT Dopamine β-monooxygenase (DBM), a cuproenzyme, converts dopamine to norepinephrine in selected cells. Studies were conducted in albino rats to resolve the known paradox of DBM after copper deficiency in which metabolite analyses of tissues suggest lower activity, whereas direct assay of homogenates suggests enhanced activity. After 4 wk of postweanling copper deficiency, male Holtzman rats exhibited 1.4-fold higher adrenal DBM activity and 1.8-fold higher adrenal DBM mRNA levels than copper-adequate rats. Mixing experiments did not support the existence of endogenous activators or inhibitors. Adrenal catecholamine content indicated lower norepinephrine, higher dopamine and unaffect ed epinephrine content in copper-deficient compared with copper-adequate rats. Studies in 22-d-old male Sprague-Dawley offspring of dams started on copper deficiency at d 7 of gestation indicated similar results for adrenal DBM mRNA, a 1.75-fold increase compared with copper-adequate pups. Adrenal dopamine content was higher in female copper-deficient offspring compared with controls, but norepinephrine was not lower. Medulla oblongata/pons DBM mRNA concentration was higher in 22-d-old copper-deficient female but not male rats compared with controls. Six weeks of copper repletion to the 22-d-old rats restored adrenal DBM mRNA levels to control values. Enzyme assay and RNA results are consistent with enhanced formation of DBM in adrenal gland and noradrenergic cell bodies of copper-deficient rats. The molecular signal may not be solely lower norepinephrine content because adrenal DBM mRNA changes were evident in both nutritional models, whereas the norepinephrine content was altered only in the postnatal model. J. Nutr. 129: 2147–2153, 1999.

KEY WORDS: copper deficiency rats mRNA dopamine β-monoxygenase

Copper (Cu) is an essential element for development and maintenance of most, if not all cells of the biological kingdom. Homeostasis of Cu is essential because its redox nature poses a potential threat if excessive amounts accumulate. This need for balance in humans is best illustrated by two genetic disorders, Menkes’ syndrome and Wilson’s disease, which lead to pathologic consequences from a relative deficiency and toxicity of Cu, respectively. The essential nature of Cu is hypothesized to be due to its cofactor role at the active site of a number of enzymes (Prohaska 1988). When Cu is inadequate for the cuproenzyme to catalyze its reaction at full capacity, an altered biochemical phenotype is observed (Prohaska 1990). For example, decreased melanin formation due to reduced tyrosinase activity leads to hypopigmentation. Decreased crosslinking of collagen and elastin due to reduced lysyl oxidase leads to a connective tissue disorder. For other cuproenzymes, the connection between lower enzyme activity and altered phenotype is less definitive.

Dopamine β-monoxygenase (DBM) is a cuproenzyme that catalyzes the final step in the biosynthesis of norepinephrine (NE) by hydroxylating dopamine (DA) in an ascorbate- and oxygen-dependent reaction (Friedman and Kaufman 1965). DBM is located in adrenal medulla, sympathetic neurons, and noradrenergic and adrenergic neurons of the brain. DBM is essential for embryonic development of the mouse as demonstrated in recent studies in which the DBM gene was ablated (Thomas et al. 1995). In humans, the lack of DBM results in severe hypotension (Gary and Robertson 1994). It has not been clearly established whether the attenuation of DBM activity that occurs when Cu is limiting results in abnormal physiology. In fact, the Cu story regarding DBM is quite puzzling.

Shortly after the discovery that DBM was a cuproenzyme, radioisotopic studies in rats indicated that dietary Cu deficiency decreased the conversion of cardiac DA to NE (Missala et al. 1967). Indeed, the steady-state NE concentration is lower in hearts from Cu-deficient rats compared with controls (Prohsaka and Heller 1982). Later, it was shown that DA is elevated in hearts of Cu-deficient rats, supporting the hypothesis that DBM is altered by Cu deficiency (Prohaska et al. 1990). The original Cu-DBM history in the central nervous system (CNS) is due in large part to the seminal work of Hunt and Johnson (1972), who studied mottled mice. Mutations at the mottled locus in mice are homologous with mutations in humans with Menkes’ syndrome. Before the Cu-mottled mu-
tant mice converted less [3H]-tyrosine to NE and more [3H]-
tyrosine to DA than control littersmates. They concluded that
the former observation was due to decreased DBM and the
latter to enhanced tyrosine hydroxylase activity, which they
confirmed directly. In addition, they reported lower steady-
state NE levels in brain of the mutant mice compared with
littersmates (Hunt and Johnson 1972). Two years later Hunt
(1974) reported that Cu was lower in brains of the mutant
mice, and the Cu DBM connection in brain was formulated.
Concurrently, it was reported that brain NE was lower in 
Cu-deficient rats compared with controls (Prohaska and Wells
1974). The NE deficit was also observed in Cu-deficient lambs
(O’Dell et al. 1976). Regional analyses in rodent brain indi-
cated that Cu-deficient rats and mice have lower NE con-
centrations in all regions except hypothalamus (Feller and O’Dell
1980, Prohaska and Bailey 1993 and 1994). Cu-deficient ro-
dents exhibit elevated DA in brain regions enriched in
noradrenergic neurons (Prohaska and Bailey 1993 and 1994).
These data provide strong support for limiting DBM activity in
brain after Cu deficiency.

However, direct enzyme assay of brain homogenates dem-
strated higher DBM activity in mutant mouse brain (Hunt
1974, Prohaska and Smith 1982) and higher activity of DBM
after dietary Cu deficiency in mice (Prohaska and Smith 1982)
and rats (Prohaska and Bailey 1994). Regional analyses of six
rat brain areas confirmed and extended earlier work that DBM
activity, measured in vitro, was higher in brains of Cu-defi-
cient rats (Prohaska and Bailey 1995). Thus, a paradox exists.
On the basis of metabolite levels, DBM activity is lower; using
direct assay, however, DBM activity is higher after Cu defi-
ciency. Assay of DBM requires the addition of an agent,
usually Cu²⁺ or N-ethylmaleimide (NEM) to inactivate an
endogenous inhibitor. Perhaps there are different levels of
inhibitors or activators after Cu deficiency.

The adrenal gland is a rich source of DBM, and cate-
echolamines have been studied after Cu deficiency. Hesketh
(1981) reported lower NE, but not epinephrine levels in
adrenals of Cu-deficient rats and elevated DBM activity. In
cattle, the catecholamine data were confirmed, but not DBM
activity (Hesketh 1980). In Cu-deficient mutant mice, no
change in catecholamine content was detected, but higher
DBM activity in adrenal gland was measured (Hunt 1977).
After perinatal Cu deficiency in young male rats, adrenal DBM
activity was 44% higher than control values (Prohaska and
Bailey 1994). Adrenal NE content was reported to be lower in
Cu-deficient mice but not rats; in both species, DA was mark-
edly higher in adrenal gland of Cu-deficient rodents (Prohaska
et al. 1990). Epinephrine content was not altered in either
species. These facts suggest phenomena in adrenal gland sim-
ilar to those in brain, i.e., a paradox between metabolite and
enzyme assay data.

The purpose of these experiments was to extend earlier
observations on brain and adrenal DBM in two models of
dietary Cu deficiency, measuring metabolites, enzyme activity
and steady-state mRNA levels. A postnatal model studying
the effect of postweaning Cu deficiency and a perinatal model
studying the effect of gestational-lactational Cu deficiency
were compared. DBM mRNA was quantified to test the hy-
pothesis that higher DBM activity is due to increased DBM
levels and not the presence of activators or lower inhibitors.

MATERIALS AND METHODS

Animal care and diets. Sperm-positive Sprague-Dawley rats and
male weanling Holtzman rats were purchased commercially (Harlan
Sprague Dawley, Indianapolis, IN). Rats were fed one of two dietary
treatments, copper deficient or copper adequate, consisting of a
Cu-deficient purified diet (Teklad Laboratories, Madison, WI) and
either low Cu drinking water or Cu-supplemented drinking water,
respectively. The purified diet was similar to the AIN-76A diet (AIN
1977 and 1980) and contained the following major components (g/kg
diet): sucrose, 500; casein, 200; cornstarch, 150; corn oil, 50; cellulose,
50; modified AIN-76 mineral mix, 35; AIN-76A vitamin mix, 10;
DL-methionine, 3; choline bitartrate, 2; and ethoxyquin 0.01.
Cupric carbonate was omitted from the AIN-76 mineral mix. The
purified diet contained 0.30 mg Cu/kg and 44 mg Fe/kg by chemical
analysis. Holtzman males, Sprague-Dawley offspring and dams con-
suming the Cu-deficient treatment drank deionized water, whereas
Cu-adequate treatment groups drank water that contained 20 mg
Cu/L by the addition of CuSO₄ to the drinking water. Rats were
given free access to diet and drinking water. All rats were maintained
at 24°C with 55% relative humidity on a 12-h light-dark cycle (lights
on 0700–1900 h). All protocols were approved formally by the
University of Minnesota Animal Care Committee.

In Experiment 1, male weaning Holtzman rats (n = 16) were
divided equally and randomly assigned to either Cu-deficient or
Cu-adequate treatments. Rats were maintained on their respective
treatments for 4 wk in stainless steel cages.

In Experiment 2, Sprague-Dawley pregnant dams were given the
Cu-deficient treatment 7 d after they were identified as sperm-posi-
tive. Two days after parturition, litter size was adjusted to eight pups.
Offspring were weaned when 3 wk old and were given the same
treatment as their respective dams for an additional 24 h. A total of
eight litters [four Cu adequate (+Cu) and four Cu deficient (−Cu)]
were studied. This paradigm is similar to that described previously
(Prohaska and Bailey 1994). Remaining offspring (both +Cu and
−Cu) were offered a nonpurified commercial diet, Purina LRC 5007
(Ralston Purina, St. Louis, MO), and tap water. Six weeks after Cu
treatment, the LRC diet contained 14 mg Cu/kg, four rats from each
control treatment group, Cu-adequate and Cu-repleted female and male
were sampled to evaluate recovery.

Rats were killed by decapitation. Livers, adrenal glands and brains
were removed, weighed and processed for biochemical analysis. Males
and females were killed on consecutive days in Experiment 2. Brains
were dissected on a chilled glass plate according to established guide-
lines (Glowinski and Iversen 1966). The cerebellum, medulla oblon-
gata + pons, cerebral cortex (cerebrum) and remainder, referred to as
“midbrain” for the purposes of these experiments, were dissected and frozen in liquid nitrogen.
Some adrenal glands were homogenized for 30 s in 24 vol of
0.05 mol/L potassium phosphate (pH 7.0) using a Tissumizer and
microprobe (SDT-080 EN, Tekmar, Cincinnati, OH).

Biochemical analyses. Hemoglobin was determined spectropho-
tometrically as metmyhemoglobin. Adrenal homogenate protein
levels were measured by a modified Lowry procedure using bovine
albumin as reference (Markwell et al. 1978). Adrenal catecholamines
were determined from urine and adrenal gland extracts by electrochemical detection as described previously (Prohaska et al.
1990).

Copper analyses. Portions of liver and diet (−1 g each) and the
entire cerebrum were weighed to the nearest milligram and wet-digested
with 4 mL concentrated HNO₃ (AR select grade, Mallinkrodt, St.
Louis, MO); the residue was brought to 4.0 mL with 0.1 mol/L HNO₃.
Samples were then analyzed for total Cu and Fe by flame atomic absorp-
tion spectrophotometry (Model 2380, Perkin-Elmer, Norwalk, CT). The
method was checked with a certified standard, U.S. National Bureau of
Standards 1577 bovine liver (Gaithersburg, MD).

DBM activity. Activity of adrenal gland DBM (EC 1.14.17.1) was
determined spectrophotometrically as described previously (Prohaska and
Smith 1982). The endogenous inhibitor of DBM activity was inactivated by 25 mmol/L NEM rather than copper. Homogenates
were diluted in 0.005 mol/L potassium phosphate (pH 7.0) containing
0.2% Triton X-100 and centrifuged at 6,500 × g for 10 min. This
phosphate-Triton buffer was stored in an acid-washed bottle contain-
ing 1 g of Chelex 100 (Bio-Rad Laboratories, Hercules CA) sus-
pended within dialysis tubing.

Northern blot analysis. Total adrenal and brain RNA was iso-
lated from quick-frozen samples using a modified guanidium thiocy-

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Characteristics of 48-d-old male Holtzman rats after postnatal copper deficiency

<table>
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<tr>
<th>Characteristic</th>
<th>Copper-adequate</th>
<th>Copper-deficient</th>
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<tr>
<td>Body weight, g</td>
<td>254 ± 9.4</td>
<td>199 ± 8.8*</td>
</tr>
<tr>
<td>Adrenal weight, mg</td>
<td>47.1 ± 3.7</td>
<td>53.9 ± 2.2</td>
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<tr>
<td>Hemoglobin, g/L</td>
<td>146 ± 5.0</td>
<td>101 ± 8.6*</td>
</tr>
<tr>
<td>Liver copper, nmol/g</td>
<td>58.4 ± 2.2</td>
<td>5.82 ± 0.79*</td>
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<tr>
<td>Liver iron, μmol/g</td>
<td>1.10 ± 0.08</td>
<td>2.45 ± 0.31*</td>
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</table>

1 Values are means ± SEM (n = 8); *Student’s t test, P < 0.05.

RESULTS

Studies in Experiment 1, in which Cu deficiency was initiated in weanling male rats, indicated that after 28 d of treatment, +Cu and −Cu rats exhibited several clear differences (Table 1). Compared with +Cu rats, −Cu rats were smaller and had higher heart/body weight ratios (data not shown). Liver Cu levels in −Cu rats were 10% of those in +Cu rats, whereas liver Fe levels were 123% higher than those in +Cu rats. Hemoglobin concentration in −Cu rats was 69% higher than that in +Cu rats. These features of −Cu rats indicate a state of severe Cu deficiency.

One of the pair of adrenal glands from six +Cu and six −Cu rats were analyzed for DBM activity in the presence of NEM (Fig. 1). The mean value for −Cu rats was 140% higher than the mean +Cu value. Protein concentration was not altered in adrenal homogenates by Cu deficiency. A mixing experiment was conducted in which samples from three +Cu and three −Cu rats were run individually and then mixed in equal parts to determine whether endogenous inhibitors (+Cu samples) or activators (−Cu samples) might explain the 1.4-fold difference in enzyme activity. The value determined was 102 ± 4.5% (mean ± 50% of the theoretical value, consistent with the lack of any obvious endogenous factors affecting the adrenal DBM activity in the two sample pools.

The remaining six adrenal glands from each treatment group were used to isolate total RNA and conduct Northern
hybridization analyses. The density of the signal for adrenal DBM and adrenal 18S was proportional to the amount of RNA loaded onto the gel, between 1 and 20 μg for DBM (r = 0.99) and 1-15 μg for 18S (r = 0.95). The steady-state concentration of DBM mRNA, normalized to the 18S ribosomal RNA signal, was significantly higher in the samples from −Cu rats than in those from +Cu rats (Fig. 2). The −Cu values were 180% higher than those of the +Cu rats, in agreement with the DBM enzyme activity data. The 18S signal was not different between groups. The mean density ratio −Cu/+Cu was 1.05.

The remaining two adrenal samples from each dietary group were analyzed for catecholamines by HPLC. NE content in the two −Cu samples, 23.7 and 23.2 nmol, was lower than the corresponding values for two +Cu samples, 44.9 and 45.0 nmol. The corresponding values for epinephrine did not differ between treatment groups. The −Cu samples were 50.1 and 60.4 nmol and for the +Cu samples, 46.7 and 63.0 nmol. The DA content of the two −Cu samples was higher at 5.16 and 2.7 nmol compared with the +Cu values of 0.82 and 0.64 nmol. However, the sample size was small for adequate statistical evaluation. To confirm and extend these observations, fast-frozen pairs of adrenal glands from five −Cu and five +Cu male Holtzman rats with severe copper deficiency studied in a previous experiment [Experiment 1, (Lear and Prohaska 1997)] were extracted and analyzed for catecholamines (Fig. 3). Compared with +Cu rats, the −Cu rats had a lower NE content and higher DA content in adrenal gland (P < 0.05).

Epinephrine content was not affected by dietary Cu deficiency.

Studies were extended in another model of Cu deficiency known to affect the CNS catecholamine milieu. Sprague-Dawley dams fed the two dietary treatments for the last two thirds of pregnancy delivered pups at the same time and in equal numbers, 9 ± 1.7 (mean ± se, n = 4) and 10 ± 1.7, for −Cu and +Cu dams, respectively. After lactation and weaning, a sample taken of one male and one female pup from each litter showed clear differences that depended on the diet history of their respective dams (Table 2). Both copper-deficient females (−CuF) and copper-deficient males (−CuM) were smaller and had enlarged hearts (data not shown) compared with their sex-matched controls, although body weight differences in the males were not significant (P < 0.06).

Weights of adrenal glands and brains were not affected by perinatal Cu deficiency (Table 2). Anemia was observed in −CuF and −CuM rats compared with their respective control groups (Table 2). Liver Cu concentration of −CuF and −CuM rats was 3.4 and 5.7%, respectively, that of +Cu rats. Liver Fe concentration was higher in −CuM than copper-adequate males (+CuM). The concentration of Cu in cerebral −CuF and −CuM rats was 18 and 20%, respectively, of +Cu values. Together, these results support the hypothesis that the −CuF and −CuM rats exhibited signs characteristic of severe Cu deficiency.

Male offspring adrenal glands were extracted and total RNA was isolated and subjected to Northern hybridization analysis (Fig. 4). A robust enhancement of the DBM mRNA steady-state level can be seen in all three lanes from the −CuM rats compared with the +CuM samples. On average, the ratio of DBM/18S for −CuM samples was 175% higher than that of the +Cu ratio, an enhancement similar to that observed in the adrenal samples of the −Cu rats in the postnatal model (Fig. 2). The 18S signal was not different between −Cu and +Cu lanes. The mean ratio of −Cu/+Cu was 0.91.

Female offspring adrenal glands were extracted and catecholamine content was determined by HPLC with electrochemical detection (Fig. 5). In contrast to the −Cu males in the postnatal model (Fig. 3), there was no difference in adrenal NE content in the 22-d-old females (Fig. 5). However, in confirmation of the postnatal model data, there was a signifi-

### TABLE 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Copper-adequate</th>
<th>Copper-deficient</th>
<th>Copper-adequate</th>
<th>Copper-deficient</th>
</tr>
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<tbody>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>66.7 ± 3.0</td>
<td>49.2 ± 3.4*</td>
<td>64.3 ± 1.8</td>
<td>51.8 ± 5.0</td>
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<tr>
<td>Adrenal weight, mg</td>
<td>22.0 ± 1.2</td>
<td>20.8 ± 1.9</td>
<td>27.5 ± 1.8</td>
<td>21.5 ± 2.3</td>
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<tr>
<td>Brain weight, g</td>
<td>1.62 ± 0.02</td>
<td>1.56 ± 0.02</td>
<td>1.64 ± 0.02</td>
<td>1.65 ± 0.02</td>
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<td>Hemoglobin, g/L</td>
<td>94.1 ± 4.6</td>
<td>63.5 ± 2.9*</td>
<td>80.3 ± 4.3</td>
<td>62.0 ± 1.9*</td>
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<td>Liver copper, nmol/g</td>
<td>294 ± 30.4</td>
<td>9.91 ± 0.05*</td>
<td>176 ± 7.1</td>
<td>10.1 ± 0.94*</td>
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<tr>
<td>Liver iron, μmol/g</td>
<td>1.20 ± 0.25</td>
<td>0.79 ± 0.09</td>
<td>0.40 ± 0.01</td>
<td>0.68 ± 0.52*</td>
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<td>Cerebrum copper, nmol/g</td>
<td>33.1 ± 1.9</td>
<td>5.98 ± 0.25*</td>
<td>31.3 ± 0.72</td>
<td>6.17 ± 0.30*</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM (n = 4); Student’s t test between groups of each gender, P < 0.05.
2 Brain cerebral cortex.
two samples from male offspring and found only a weak signal in
4). We attempted to detect DBM mRNA in the "midbrain"
and more robust response observed in adrenal gland (Figs. 2,
DBM mRNA after Cu deficiency in contrast to the consistent
2
2
gata/pons for
Panel B
FIGURE 4 Northern blot hybridization analysis of rat adrenal total
RNA (10 µg/lane) subjected to denaturing electrophoresis, capillary
transfer and binding to 32P-labeled DNA probes specific for rat DBM
and murine 18S ribosomal RNA. Arrows indicate migration position of
28S and 18S ribosomal RNA visualized with acridine orange. Lanes were
loaded with RNA isolated individual adrenal glands from three
copper-deficient male [−CuM; (−)] and three copper-adequate male
+CuM; (+)] 22-d-old rats after perinatal Cu deficiency. The DBM/18S
density ratio was determined and the mean ± sem for −Cu rats (4.93
± 0.55) was higher than the ratio for +Cu rats (1.79 ± 0.20) (P < 0.05).
dicant 3.8-fold elevation in DA content, suggesting DBM impair-
ment. Adrenal epinephrine content was not altered in the perinatal model of Cu deficiency, in agreement with the data on older rats (Fig. 3). The absolute levels of the adrenal catecholamines in the 48-d-old Holtzman males and 22-d-old Sprague-Dawley females (Figs. 3, 5) seem roughly proportional to adrenal gland size (Tables 1 and 2).
RNA from the medulla oblongata/pons, a region rich in noradrenergic cell bodies, was evaluated for DBM mRNA
content by Northern hybridization analysis (Fig. 6). Panel A shows 8 lanes loaded with 20 µg of total RNA alternating with four −CuM and four +CuM samples. In only the first pair of loaded samples did there appear to be an enhancement of the DBM mRNA signal. The mean DBM/18S area ratio for −CuM samples was not different than the value for +CuM. However, in panel B, the mean DBM/18S ratio for −CuF was 52% higher than the corresponding ratio for copper-adequate females (+CuF), P < 0.05. The 18S signal for medulla oblongata/pons for −Cu rats was not different from that of +Cu rats. The mean ratio for −CuM+/CuM was 1.02 and for −CuF+/CuF was 0.95. Overall, in only about half of the brain −Cu samples did there appear to be a clear cut elevation in DBM mRNA after Cu deficiency in contrast to the consistent and more robust response observed in adrenal gland (Figs. 2, 4). We attempted to detect DBM mRNA in the "midbrain" samples from male offspring and found only a weak signal in two +CuM samples and none in the four −CuM samples (data not shown). We were also unable to detect a DBM mRNA signal in hypothalamus using RNA from rats of another study (data not shown) despite the highest DBM enzyme activity in this region (Prohaska and Bailey 1995).
Some of the 22-d-old offspring were placed in stainless steel cages and followed a Cu-adequate dietary protocol for 6 wk. Analyses of samples of these repleted and control male rats indicated that Cu status was no longer different (body weight, liver Cu, liver Fe, hemoglobin) between groups with the exception of cerebrum Cu, which demonstrated that the Cu-repleted mean was still lower at 33% of the +Cu mean (Table 3). Furthermore, adrenal DBM mRNA levels were no longer significantly elevated, suggesting that the observations made in the 22-d-old −CuM rats were reversible.

**DISCUSSION**

Both models of dietary Cu deficiency produced a phenotype consistent with severe Cu deficiency including growth retardation, depletion of liver Cu concentration and anemia. In both models, there was a consistent and robust (twofold) elevation in DBM mRNA in adrenal glands of −Cu rats. Sabban et al. (1991) communicated a similar observation in 7-wk-old −Cu rats in a perinatal Cu deficiency model. In the current postnatal studies, the mRNA increase and enzyme activity increase were approximately equal. This suggests that the mRNA level is reflecting protein levels. In the perinatal model, the increase in DBM mRNA was much greater than the 44% increase in adrenal enzyme activity that was found in another group of rats treated similarly (Prohaska and Bailey 1994). Hesketh (1981) also reported higher adrenal DBM activity in a postnatal Cu-deficient rat model. This rise in adrenal DBM mRNA was eliminated by restoring Cu to the diet of −CuM rats, suggesting the transitory nature of the induction.

Medulla oblongata/pons was chosen to represent CNS tissue enriched in cell bodies that contain DBM. The activity of DBM in this region is ~1% that of adrenal gland (Prohaska and Bailey 1994 and 1995); thus, it was more challenging to detect DBM mRNA levels. However, results in these studies indicate a modest effect (significant in females) that supports adrenal data indicating an up-regulation of DBM mRNA after perinatal Cu deficiency. In −CuF, the average increase in medulla oblongata/pons DBM mRNA was 52%, similar to the elevation in DBM enzyme activity measured previously for −CuF (25%) and −CuM (43%) (Prohaska and Bailey 1995). The degree of Cu deficiency in brain in the current model, as assessed by cerebrum Cu concentration, is similar to that observed previously (Prohaska and Bailey 1994), suggesting that the RNA data are representative of this model. Previous results in the postnatal model indicated that although brain Cu was lower in −Cu rats by 36%, there was no change in

**FIGURE 5** Adrenal catecholamine content following perinatal Cu deficiency (Experiment 2). Catecholamines were extracted from pairs of adrenal glands and determined by HPLC with electrochemical detection. Bars represent means and error bars SEM for n = 4 pairs of adrenal glands from 22-d-old female rats receiving either Cu-adequate or Cu-deficient treatment. Means were compared; *P < 0.05 compared with Cu-adequate group.
DBM activity (Prohaska et al. 1995); thus, RNA analyses were not performed on brains of the older rats.

What might be the cellular signal responsible for increasing DBM mRNA? A likely candidate is depletion of NE. Treatment with reserpine, a drug that depletes catecholamines, results in induction of rat adrenal DBM mRNA (McMahon et al. 1990). Reserpine treatment of rats also elevates DBM activity and protein level in the brain in a time course that parallels depletion of monoamines (Reis et al. 1975). In the CNS, there is reproducibly lower NE in the medulla oblongata/pons of −Cu rodents, supporting the hypothesis that low NE induces DBM transcription.

However, in the adrenal gland, the data are less clear. In the current studies, for example, there was evidence of lower NE in the postnatal model but not the perinatal model, yet both yielded similar mRNA enhancements. Others have reported varying outcomes of postnatal Cu deficiency on adrenal NE content. Hesketh (1981) reported lower NE in −CuM rats. Fields et al. (1991) reported higher NE in −CuM rats. Prohaska et al. (1990) reported no changes in NE content in −CuM rats. In those cases in which DA was measured, a robust increase was detected even though NE data were not consistent. Others have shown that DBM is transcriptionally regulated by many factors such as cAMP, glucocorticoids, bradykinin, nicotine and immobilization stress (McMahon and Sabban 1992). Perhaps the rise in DBM mRNA is due to one or more of these factors. A candidate for further research is glucocorticoids because it is known that −Cu rats have elevated levels in the adrenal gland and plasma (Fields et al. 1991).

Negative results of mixing experiments in the current studies support the proposal that there is more DBM protein present after Cu deficiency rather than abnormal levels of endogenous factors. It is possible that the extra apo-DBM is not active in vivo because of limiting free Cu ion. Thus far, no specific Cu-chaperon protein for DBM has been identified; thus, the specific details of how Cu is inserted into apo-DBM are not known. However, because of the facile and rapid exchange of free Cu with apo-DBM (Skotland and Flatmark 1983), it is feasible that extra apo-DBM can be activated with traces of free Cu when assayed in the laboratory. This would explain the higher DBM activity detected in the laboratory assay and is consistent with the higher mRNA levels. Measurement of DBM protein will be required to confirm this supposition.

Catecholamine data presented in these two experiments and those discussed previously suggest that DBM activity is impaired in vivo. Catecholamine data in humans with Cu deficiency due to Menkes' syndrome also support defective DBM function (Hoeldtke et al. 1988). It will be more challenging to determine whether any of the phenotypic and developmental abnormalities associated with Cu deficiency are due to catecholamine imbalance.

### Acknowledgment

We appreciate the advice and generous gift of the DBM plasmid from Ester Sabban, New York Medical College, Valhalla, NY.

### Literature Cited


### Table 3

**Characteristics of 64-d-old male Sprague-Dawley rats after 6 wk of copper repletion**

<table>
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<th>Characteristic</th>
<th>Copper-adequate</th>
<th>Copper-repleted</th>
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<tr>
<td>Body weight, g</td>
<td>426 ± 16</td>
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<tr>
<td>Adrenal weight, mg</td>
<td>73 ± 7</td>
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<tr>
<td>Hemoglobin, g/L</td>
<td>135 ± 14.2</td>
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<td>Liver copper, nmol/g</td>
<td>70.5 ± 1.10</td>
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<td>1.84 ± 0.22</td>
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<td>Cerebrum copper, nmol/g</td>
<td>38.1 ± 0.79</td>
<td>20.3 ± 0.16*</td>
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<tr>
<td>Adrenal DBM/18S mRNA, density</td>
<td>8.72 ± 0.72</td>
<td>10.2 ± 0.6</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM (n = 4 copper-adequate; n = 3 copper-repleted); Student’s t test, P < 0.05.

2 Brain cerebral cortex.


