Cardiac Ultrastructural and Electrophysiological Abnormalities in Postweanling Copper-Restricted and Copper-Repleted Rats in the Absence of Hypertrophy

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ABSTRACT Cardiac ultrastructural and functional characteristics were determined in copper-depleted and copper-repleted rats. Male weanling rats were randomized into five groups that were fed either copper-adequate or copper-deficient diets. After 5 wk, one group fed each diet was studied to obtain baseline values. At this time, one copper-adequate postweanling group continued to receive the adequate diet as control, one deficient postweanling group was fed the adequate diet to evaluate the effect of copper repletion and one postweanling adequate group was fed the deficient diet to evaluate copper depletion in relatively older rats. These dietary treatments were continued for six additional weeks. Copper-depleted rats of both ages exhibited significant cardiac ultrastructural pathology and electrocardiogram abnormalities and the postweanling copper-depleted rats exhibited these abnormalities in the absence of hypertrophy and anemia. Increased mitochondrial volume density, disarranged cristae, and nonaligned myofibrils with disturbances at Z-bands were displayed. Additionally, all copper-depleted rats demonstrated fragmented basal laminae at capillary-myocyte interface. Increased QRS amplitude and notching and greater QT intervals were displayed. Copper-repleted rats exhibited some, but not total, reversal of these abnormalities. These results suggest that capillary-myocyte interface changes may play an important role in the developing pathology of copper depletion. J. Nutr. 122: 1566–1575, 1992.

INDEXING KEY WORDS:
- copper deficiency
- electrocardiogram
- mitochondria
- ultrastructure
- rats


Similarly, several studies have reported greater cardiac mitochondrial volume densities in Cu-deficient rats [Borg et al. 1985, Dallman and Goodman 1970, Goodman et al. 1970, Kopp et al. 1983, McCormick et al. 1989, Medeiros et al. 1991a and 1991b]. Our laboratory recently reported that, additionally, cardiac myofibril volume density seems to be increased as the heart mass increases [Medeiros et al. 1991a]. Furthermore, disruption of the mitochondrial fine structure and the presence of increased glycogen granules and lipid droplets have been reported by Kopp et al. (1983) and Medeiros et al. (1991a and 1991b), with distorted myofibrils and poorly organized and nonaligned Z-bands [Borg et al. 1985, McCormick et al. 1989, Medeiros et al. 1991a and 1991b].

Reports on rats fed a Cu-adequate diet from weaning, followed by the feeding of a Cu-deficient diet during the postweanling period, have been limited. Additionally, abnormalities in cardiac ultrastructure and function in the absence of cardiac hypertrophy have not been reported. There seems to be a paucity of information on the reversibility of the

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cardiopathology in Cu-deficient rats, when these rats are repleted with a Cu-adequate diet. Viestenz and Klevay [1982] did report a decrease in the electrocardiogram abnormalities present in rats after being repleted for several weeks, following the feeding of a Cu-deficient diet from weaning.

Coronary vessels and the aorta have deleterious alterations in the Cu-deficient state. However, studies on the histopathology in Cu deficiency at the level of the capillary-myocyte interface have been limited. Farquaharson and Robins [1991] reported abnormal distribution of Type IV collagen in the endothelium and perimysium of hearts of Cu-deficient rats, and fragmentation and disorganization of myocyte basal laminae seemingly independent of myocardial fibrosis.

The objectives of the present study were to determine whether weanling rats fed a Cu-adequate diet for 5 wk, and then crossed to a Cu-deficient diet for another 6 wk, would develop abnormalities in cardiac electrophysiology and ultrastructure. Similarly, we investigated whether weanling rats fed a Cu-deficient diet for 5 wk, and then repleted with a Cu-adequate diet for another 6 wk, would demonstrate a decrease in the occurrence of abnormal cardiac pathophysiology.

The results reported here revealed that weanling rats, fed a Cu-adequate diet, followed postweaning by a Cu-deficient diet, develop abnormalities in electrocardiograms (ECG) and cardiac ultrastructure, even in the absence of cardiac hypertrophy and anemia. Conversely, feeding a Cu-adequate diet postweaning to rats fed a Cu-deficient diet from weaning, did reverse the cardiac hypertrophy and some ECG abnormalities, but did not reverse the ultrastructural damage. Finally, these results suggest that, in all conditions, one of the pertinent cardiac abnormalities in Cu deficiency resides in the histopathological abnormalities at the capillary-myocyte interface, as demonstrated by increased distances between the capillary lumen and myocyte mitochondria and distortion of the basal laminae underlying the sarcolemma.

**MATERIALS AND METHODS**

**Animals and diets.** The protocol for the study was approved by the Institutional Laboratory Animal Care and Use Committee at The Ohio State University. Thirty-one male weanling Long-Evans rats [Harlan Sprague Dawley, Indianapolis, IN] were weighed upon arrival, and randomized into five groups with similar mean group weights. The groups were randomly assigned to either Cu-adequate [Cu+] [three groups, n = 6/group] or Cu-deficient [Cu−] [two groups, n = 6 and n = 7] diets. At the end of 5 wk, one group [n = 6] from each of the dietary treatments [Cu+ and Cu−] were randomly selected and studied to obtain baseline values and to confirm cardiac pathophysiology with Cu deficiency. At this time, one group of rats [n = 6] fed the Cu+ diet from weaning remained on the Cu+ diet to serve as a postweaning control [referred to as the Cu+ control group]. The remaining group of rats [n = 6] fed the Cu+ diet from weaning was crossed to the Cu− diet [referred to as the Cu+,Cu− group] to evaluate the effect of Cu depletion postweaning on cardiac pathophysiology in relatively older rats. The remaining group of rats fed the Cu− diet from weaning [n = 7] was crossed to the Cu+ diet [referred to as the Cu−,Cu+ group] to establish the effect of postweaning dietary Cu repletion in previously Cu-deficient rats. The latter three groups were maintained for six additional weeks on their respective diets.

All rats were fed a basal diet [U.S. Biochemical, Cleveland, OH], following the recommendations of the American Institute of Nutrition [1977], consisting of 500 g sucrose, 150 g cornstarch, 200 g casein and 50 g corn oil/kg diet, with the vitamin mix commonly used in AIN-76 diets. No Cu was added to the Cu− diet, while Cu was added as cupric carbonate [6 mg/kg food] to the Cu+ diet. Dietary Cu levels of 6.2 μmol Cu/kg diet [Cu− diet] and 92.4 μmol Cu/kg diet [Cu+ diet] were determined by flame atomic absorption spectrophotometry. This diet has been used consistently by our laboratory and has produced Cu deficiency among rats consuming the Cu-deficient diet.

Rats were housed singly in stainless steel cages, in a room with a 12-h light:dark cycle and mean temperature of 21.7°C, with free access to deionized-distilled water and food. Rats were weighed weekly.

**Experimental protocol.** Electrocardiograms were performed on the Cu+ and Cu− groups at the end of 5 wk, and ECG and tail cuff blood pressures on the remaining groups after a further 6 wk. The following day, rats were anesthetized by CO2 inhalation and the thoracic cavities opened for the necessary measurements as described below.

**Electrocardiograms.** Rats were lightly anesthetized with ketamine [85 mg/kg] and xylazine [15 mg/kg] intraperitoneally. The ECG were recorded, using leads I, aVF and V3, with subcutaneous needle electrodes as described by Medeiros et al. [1991a]. The ECG parameters statistically analyzed from the V3 lead were heart rate [beats per minute], duration of P, PQ, QRS and QT waves [msec], amplitudes of QRS, R and S waves [mV], and the ratio of R to S waves. Subjective interpretation of ECG patterns was done on all

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4Abbreviations used: Cu+, copper adequate; Cu−, copper deficient; Cu+,Cu−, copper adequate followed by copper deficient diet; Cu−,Cu+, copper deficient followed by copper adequate diet; Cu+ control, copper adequate control; ECG, electrocardiogram; SOD, cytoplasmic Cu-Zn superoxide dismutase.
three leads, with respect to gross indications of disturbances in intraventricular electrical conduction and left ventricular hypertrophy (increased QRS amplitude, increased QRS wave and QT interval duration and notching in the QRS complex).

Preparation of samples for transmission electron microscopy. After the thoracic cavities were opened, <0.5 mL of blood was removed by cardiac puncture and placed in heparinized tubes for hematocrit analysis. The hearts were arrested in diastole by injection of 1 mol/L KCl into the right ventricle, removed, rinsed in Dulbecco’s PBS [Gibco Laboratories Life Technologies, Grand Island, NY] at 37°C and weighed. Samples from the left ventricular free wall were cut tangentially to the outer wall to obtain fibers in the longitudinal plane. Processing of tissue into blocks for transmission electron microscopy was by the method of Medeiros et al. (1991a), using glutaraldehyde and osmium tetroxide as fixatives and spurr as resin.

Morphometric analysis. Electron micrographic prints (x17,000) were analyzed morphometrically by overlay of a plastic grid with 1-cm square divisions. Mitochondrial, myofibrillar and other volume densities (µm³/µm³) were determined by a point system as described by Weibel (1979) and Steer (1981). By definition, “other” included volume density occupied by intracellular material other than clearly defined mitochondria and myofibrils.

A 1–4 point qualitative scale (increments of 0.5) was used to analyze the integrity of the capillary-myocyte interface, in a blind manner, by examination of electron micrographic prints (x25,000). The normal appearance includes membranes with minimal convolutions, intact basal laminae, narrow intermembrane areas, normally arrayed myofibrillar and mitochondrial structure and minimal debris. Samples with these characteristics were assigned a maximum value of 4. Samples exhibiting gross pathology, convoluted membranes, broad intermembrane spaces, obviously damaged basal laminae, disarrayed mitochondria and accumulated intermembrane debris, were assigned values approaching 1. Mean values were obtained by averaging two prints. A subsample of prints was scored several weeks later and scores were compared for reliability.

Superoxide dismutase activity. Liver cytoplasmic Cu-Zn superoxide dismutase (SOD, EC 1.15.1.1) activity was determined spectrophotometrically to assess relative Cu status of rats in the different treatment groups. This assay was based on the autoxidation of pyrogallol, as described by Marklund and Marklund (1974) and modified by Prohaska (1983). The amount of pyrogallol added to give a change in absorbance of 0.016/30 s was determined, followed by determination of the amount of liver sample to reduce the change in absorbance to 0.008/30 s. One unit of SOD activity was defined as the amount of activity to inhibit the auto-oxidation of pyrogallol by 50%, expressed as U/g.

Hematocrit. Blood obtained by cardiac puncture was drawn into heparinized tubes and hematocrit determined using a microhematocrit centrifuge.

Statistical analysis. The dependent variables of body weight, heart weight, heart to body weight ratio, hematocrit, SOD, ECG measurements, mitochondrial, myofibrillar and other volume densities, mitochondrial to myofibrillar ratio, capillary-myocyte interface scores for all groups and blood pressure for the latter three groups, were analyzed, using the Statistical Analysis System (SAS Institute, Cary, NC). General Linear Models procedure was used to determine significant differences by ANOVA. When significant F values were obtained, the least significant difference procedure was used to determine which of the five treatment group means differed from one another. A repeated measures ANOVA was performed to determine significant differences in means of body

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td><strong>Body and heart weights, liver superoxide dismutase (SOD) activity and hematocrit in rats fed Cu-adequate and Cu-deficient diets</strong></td>
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<tr>
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<tr>
<td>-----------</td>
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<tr>
<td>Body weight, g</td>
</tr>
<tr>
<td>Heart weight, g</td>
</tr>
<tr>
<td>Heart:body weight&lt;br&gt;(×10⁻³</td>
</tr>
<tr>
<td>Liver SOD, u/g</td>
</tr>
<tr>
<td>Hematocrit</td>
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</table>

¹Values are means ± SEM. Values with different letter superscripts differ significantly, P < 0.05.
TABLE 2
Histopathological cardiac indices in rats fed Cu-adequate and Cu-deficient diets

<table>
<thead>
<tr>
<th>Index</th>
<th>Weaning Cu+ (n = 6)</th>
<th>Cu- (n = 6)</th>
<th>Postweaning Cu+ control (n = 6)</th>
<th>Cu+ depleted (n = 6)</th>
<th>Cu+-Cu+ depleted (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial volume density, μm²/μm³</td>
<td>0.318 ± 0.001c*</td>
<td>0.482 ± 0.013b*</td>
<td>0.322 ± 0.020c*</td>
<td>0.462 ± 0.009b**</td>
<td>0.508 ± 0.022a</td>
</tr>
<tr>
<td>Myofibril volume density, μm²/μm³</td>
<td>0.590 ± 0.009a</td>
<td>0.375 ± 0.018b**</td>
<td>0.650 ± 0.021a</td>
<td>0.396 ± 0.007b**</td>
<td>0.528 ± 0.132b</td>
</tr>
<tr>
<td>Other volume density, μm²/μm³</td>
<td>0.045 ± 0.003c*</td>
<td>0.112 ± 0.008b**</td>
<td>0.042 ± 0.001c*</td>
<td>0.142 ± 0.007a</td>
<td>0.100 ± 0.010b**</td>
</tr>
<tr>
<td>Mitochondria:myofibrils</td>
<td>0.541 ± 0.025b*</td>
<td>1.375 ± 0.107a</td>
<td>0.502 ± 0.044b*</td>
<td>1.170 ± 0.038a</td>
<td>1.164 ± 0.195a</td>
</tr>
<tr>
<td>Capillary:myocyte interface</td>
<td>3.33 ± 0.20a</td>
<td>1.67 ± 0.25b**</td>
<td>2.88 ± 0.17a</td>
<td>1.71 ± 0.16b**</td>
<td>1.79 ± 0.16b**</td>
</tr>
</tbody>
</table>

1Values are means ± SEM. Values with different letter superscripts differ significantly. Levels of significance are *P < 0.0001 and **P < 0.05.

weight by treatment and weeks of treatment. The alpha level was set a priori at 0.05.

RESULTS

Weanling rats after 5 wk of dietary treatment. We had planned to continue this phase of the study for 6 wk. However, early morbidity of the Cu- rats necessitated completion after only 5 wk. The Cu- weanling rats had significantly lower body weight, hematocrit values and liver SOD activity than Cu+ rats (Table 1). Cardiac hypertrophy was clearly established by significantly greater heart weight and heart:body weight ratio in the Cu- rats (Table 1). Cardiac ultrastructural abnormalities included significantly greater mitochondrial and other volume densities, significantly lower myofibrillar volume density, with a significantly greater mitochondrial:myofibrillar ratio in Cu-compared with Cu+ rats (Table 2). Degenerative changes in cardiac myocytes were characterized by lower mitochondrial cristae, sparse disarranged myofibrils and Z-band disturbances (Fig. 1). Evaluation of histopathology at the capillary-myocyte interface on the four-point scale, revealed significantly greater intermembrane areas, subsarcolemmal accumulation of debris and pericapillary collagen deposition in Cu-compared with Cu+ rats (Fig. 2). Copper-deficient rats displayed significantly greater QRS wave amplitude and duration (Table 3), QRS notching (Fig. 3) and QT duration compared with Cu+ rats.

Postweanling rats after six additional weeks of dietary treatment. All rats fed Cu- diets from weaning and then crossed to the Cu+ diet postweaning were alive after six additional weeks, despite early morbidity displayed after the first 5 wk of dietary treatment. There were no visual differences in appearance among any of the remaining rats. Body weight (Table 1) was significantly lower in the postweanling depleted [Cu+,Cu-] compared with the Cu+ control rats, with no significant difference between repleted [Cu-,Cu+] and either depleted [Cu+,Cu-] or Cu+ control rats (Table 1). Liver SOD activity of all three groups differed significantly. Depleted [Cu+,Cu-] rats had very low SOD activity (Table 1) compared with repleted [Cu-,Cu+] and Cu+ control rats, although these levels were still significantly higher than that of the weanling Cu- rats. There was no significant age difference in liver SOD activity between rats fed Cu+ diets. In contrast to the weanling rats, there was no significant difference in hematocrit between the postweanling rats (Table 1).

The postweanling depleted [Cu+,Cu-] rats did not display cardiac hypertrophy compared with Cu+ control rats as measured by differences in absolute heart weight (Table 1). Absolute heart weight was significantly greater only in the repleted [Cu-,Cu+] compared with the depleted [Cu+,Cu-] rats. There was greater heart weight:body weight in the Cu-,Cu+ repleted rats compared with the Cu+,Cu- depleted rats and Cu+ control rats (Table 1).

Morphometric analysis disclosed significant cardiac ultrastructural pathology in the postweanling depleted [Cu+,Cu-] and repleted [Cu-,Cu+] rats (similar to that of the weanling Cu- rats), compared with Cu+ rats (Table 2 and Fig. 2). The Cu-depleted [Cu+,Cu-] and repleted [Cu-,Cu+] rats demonstrated significantly higher mitochondrial and other volume densities and mitochondrial:myofibrillar volume density ratios than Cu+ control rats. There were no significant differences in volume densities between postweanling and weanling rats fed copper-adequate diets, suggesting that there was no age effect. However, there were significant differences in volume densities between depleted [Cu+,Cu-] and repleted [Cu-,Cu+] rats. Mitochondrial volume density of repleted [Cu-,Cu+] rats was significantly lower than...
FIGURE 1 Transmission electron micrographs of cardiac myocytes from weanling (a) Cu+ and (b) Cu− rats, and postweanling (c) Cu+ control, (d) depleted (Cu−,Cu+) and (e) depleted (Cu+,Cu−) rats. Weanling Cu− and postweanling depleted (Cu+,Cu−) and repleted (Cu−,Cu+) rats displayed greater volume densities of mitochondria, sparse and disorganized mitochondrial cristae and reduced and disorganized myofibrils, with Z-band splitting. M = mitochondria; my = myofibrils; O = other; L = lipid droplet; G = glycogen granule. Bar = 1 μm.
FIGURE 2 Transmission electron micrographs of the capillary-myocyte interface of left ventricles from weanling (a) Cu+ and (b) Cu− rats, and postweanling (c) Cu+ control, (d) repleted (Cu−,Cu+) and (e) depleted (Cu+,Cu−) rats. Histopathology is evident in all Cu-depleted and repleted rats, regardless of age. The sarcolemma is convoluted and asymmetrical, with broad intermembrane areas and subsarcolemmal debris, and disorganized myofibrils and mitochondrial disarray. Basal laminae appear focally fragmented and thickened, and pericapillary collagen seem increased. S = sarcolemma; BL = basal laminae; C = capillary; A = intermembrane area. Bar = 1 μm.
TABLE 3

Electrocardiogram variables and blood pressure in rats fed Cu–adequate and Cu–deficient diets

<table>
<thead>
<tr>
<th></th>
<th>Weanling</th>
<th>Postweanling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cu+</td>
<td>Cu–</td>
</tr>
<tr>
<td></td>
<td>[n = 6]</td>
<td>[n = 6]</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>351 ± 16.2</td>
<td>320 ± 8.3**</td>
</tr>
<tr>
<td>P wave duration, ms</td>
<td>15 ± 1.8b*</td>
<td>15 ± 0.2b*</td>
</tr>
<tr>
<td>P-R interval, ms</td>
<td>48 ± 1.1</td>
<td>48 ± 1.7</td>
</tr>
<tr>
<td>QRS wave duration, ms</td>
<td>12.5 ± 1.12**</td>
<td>17.7 ± 2.0a</td>
</tr>
<tr>
<td>QRS amplitude, mV</td>
<td>1.9 ± 0.09bc**</td>
<td>2.7 ± 0.31a</td>
</tr>
<tr>
<td>R amplitude, mV</td>
<td>1.6 ± 0.10b*</td>
<td>1.9 ± 0.19a</td>
</tr>
<tr>
<td>S amplitude, mV</td>
<td>0.3 ± 0.07</td>
<td>0.7 ± 0.16</td>
</tr>
<tr>
<td>QT interval, ms</td>
<td>56 ± 1.6b**</td>
<td>40 ± 1.1b**</td>
</tr>
<tr>
<td>Control</td>
<td>67 ± 3.3**</td>
<td>100 ± 4.5*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Values with different letter superscripts differ significantly. Levels of significance are *P < 0.01 and **P < 0.05.

that of depleted [Cu+, Cu–] rats, while not significantly different from that of the younger Cu– rats. Additionally, other volume density of depleted [Cu–, Cu+] rats was significantly higher than that of both the depleted [Cu+, Cu–] and the weanling Cu– rats, indicating degenerative changes. Myofibrillar volume density of depleted [Cu+, Cu+] rats was significantly lower than that of both weanling Cu– and Cu+ control rats, whereas that of depleted [Cu+, Cu–] rats was not significantly different from other groups, illustrating differences between repleted and depleted rats.

Similar degenerative abnormalities were exhibited in Cu–depleted [Cu+, Cu–] rats and repleted [Cu–, Cu+] rats compared with Cu+ control rats, as in the weanling Cu– rats (Fig. 2). Depleted [Cu+, Cu–] and repleted [Cu–, Cu+] rats compared with Cu+ control rats displayed fragmented mitochondrial cristae, with disturbed inner and outer membranes, and translucent matrices. Myofibrils were sparse with considerable loss of normal organization, highly convoluted intercalated discs, accumulation of Z-band material and some splitting of Z-bands. Glycogen granules were increased and nuclei were often contorted in rats fed Cu– compared with Cu+ diets.

Evaluation of the cardiac capillary-myocyte interface (Table 2 and Fig. 3) evidenced a significantly greater pathology in depleted [Cu+, Cu–] and repleted [Cu–, Cu+] rats compared with Cu+ control rats, with no significant differences between depleted [Cu+, Cu–] and repleted [Cu–, Cu+] rats. Furthermore, there were no differences between weanling Cu– and postweanling Cu+ control rats. The sarcolemmal membranes in depleted [Cu+, Cu–] and repleted [Cu–, Cu+] rats were convoluted, with focally fragmented and thickened basal laminae, compared with Cu+ control rats. Subsarcolemmal debris was evident, with increased intermembrane areas and pericapillary collagen deposition in all Cu– compared with Cu+ rats. Capillary membranes seemed less affected than myocyte membranes.

Electrocardiograms. Blood pressure and heart rate did not differ significantly between depleted [Cu+, Cu–], repleted [Cu–, Cu+] and Cu+ control rats (Table 3).

There was no significant difference in P-R interval, S amplitude and R:S between any of the postweanling groups (Table 3). P wave duration of repleted [Cu–, Cu+] rats was significantly longer, but there was no difference in P wave duration between depleted
(Cu+,Cu-) and Cu+ control rats (Table 3). There were no differences in QRS wave duration and R amplitude between the depleted (Cu+,Cu-) and weanling Cu− rats, whereas both group means were significantly greater than of the repleted (Cu−,Cu+) and Cu+ control rats. QRS wave duration and R amplitude were similar between repleted (Cu−,Cu+) and Cu+ controls. Depleted (Cu+,Cu−) rats had significantly greater QRS amplitude than repleted (Cu−,Cu+) and Cu+ control rats, whereas those of repleted (Cu−,Cu+) and Cu+ control rats were similar. However, the QT intervals of depleted (Cu+,Cu−) and repleted (Cu−,Cu+) rats were not significantly different, whereas that of depleted (Cu+,Cu−) rats was greater than that of Cu+ control rats. There were no significant differences between the weanling Cu+ and postweanling Cu+ control rats in QRS wave duration or amplitude, R amplitude and QT intervals, suggesting that the differences observed between groups were not related to age.

Subjective evaluation of ECG tracings confirmed quantitative ECG analysis (Fig. 3). The most notable differences between the experimental and control groups were greater QRS amplitudes and notching of the QRS complex in leads aVF and V3. Three of the repleted (Cu−,Cu+) rat tracings displayed notching in lead V3, compared with none in the Cu+ control group. Depleted (Cu+,Cu−) rats tended to exhibit more frequent notching in the QRS complex in lead V3 compared with Cu+ control rats.

**DISCUSSION**

Results of this study supported the results reported by others that weanling rats fed a Cu− diet for 5 wk displayed cardiac ultrastructural (Borg et al. 1985, Goodman et al. 1970, Kopp et al. 1983, McCormick et al. 1989, Medeiros et al. 1991a and 1991b) and electrophysiological abnormalities (Medeiros et al. 1991a and 1991b, Viestenz and Klevay 1982). Additionally, this study showed that Cu− rats developed histopathological abnormalities at the capillary-myocyte interface, involving the sarcolemma and basal laminae.

It was clear that postweanling rats fed Cu− diets developed similar pathophysiology, despite the absence of cardiac hypertrophy and anemia. It is noteworthy that the repleted (Cu−,Cu+) rats were still alive and of healthy appearance after consuming the Cu+ diet for 6 wk, whereas they were morbid before initiation of repletion. However, some ultrastructural and functional differences between repleted (Cu−,Cu+) and Cu+ control rats remained at the end of the study.

**Superoxide dismutase.** Paynter et al. (1979) reported decreased SOD values in various tissues of rats with Cu deficiency. In this study, liver SOD activity was found to vary with dietary Cu manipulation, but not with age. This suggests that SOD activity is sensitive to different stages of Cu depletion and repletion.

**Hematology.** Unlike among the weanling rats, no significant difference in hematocrit was found among any of the postweanling rats. It is therefore unlikely that anemia was primarily involved in the development of the cardiac pathology observed in these rats, in agreement with the suggestion by Medeiros et al. (1991a and 1991b).

**Gross pathology and myocardial hypertrophy.** In contrast to the weanling rats, the heart weight and heart weight:body weight ratio of postweanling rats recorded in this study indicated that there was no cardiac hypertrophy in the relatively older depleted (Cu+,Cu−) rats, compared with the Cu+ control rats. This indicates that the ultrastructural and ECG abnormalities observed developed in the absence of cardiac hypertrophy. The greater heart weight:body weight ratio of repleted (Cu−,Cu+) rats compared with Cu+ control and depleted (Cu+,Cu−) rats suggested that cardiac hypertrophy remained despite 6 wk of Cu repletion.

**Electrocardiograms.** Blood pressure response of rats fed Cu− diets is variable and may be species and age dependent. Decreased blood pressure has been reported by Medeiros et al. (1984), Wu et al. (1984) and Klevay et al. (1988), in rats fed Cu− diets from weaning, whereas Medeiros (1987) and Klevay (1987) reported increased blood pressure when rats were fed a Cu− diet after weaning. In this study, the lack of a significant difference in blood pressure between the postweanling rats suggested increased blood pressure was not a prerequisite for the developing myocardial pathology. In agreement with this study, Prohaska and Heller (1982) reported decreased heart rates in weanling rats fed Cu− diets.

ECG abnormalities in weanling rats fed Cu− diets have been reported in a number of studies. Viestenz and Klevay (1982) reported abnormal ST segments, increased PR intervals, R wave duration and amplitude and some ventricular and supraventricular beats. Kopp et al. (1983) reported His Bundle electrogram abnormalities, and Medeiros et al. (1991a) indicated increased QT intervals and QRS amplitudes. Some regression of ST segment abnormalities and survival after several weeks of Cu repletion of Cu− rats has been reported by Viestenz and Klevay (1982). In this study, the predominant ECG abnormalities of the postweanling depleted (Cu+,Cu−) rats were centered in the QRS complex and QT interval. These ECG abnormalities, in the absence of cardiac hypertrophy, seem to indicate disturbances in electrical conductance and ion flux. The ECG tracings of repleted (Cu−,Cu+) rats in many instances showed responses similar to those of Cu+ control rats, indicating regression toward normal from the weanling Cu− state. However, after 6 wk of repletion, some damage was still evidenced by increased P wave duration.
Cardiac ultrastructural changes. The ultrastructural abnormalities revealed by morphometric analysis in this study in postweanling rats resemble those of the weanling rats in this study and reported by others (Goodman et al. 1970, Kopp et al. 1983, Medeiros et al. 1991a and 1991b), even in the absence of cardiac hypertrophy. The most notable abnormalities in Cu− rats appeared in the changed volume densities and degeneration of the subcellular organelles. The reduced mitochondrial volume density of the postweanling repleted (Cu−, Cu+) rats compared with the depleted (Cu+, Cu−) rats was still significantly greater than that of the Cu+ control rats. This suggests that residual damage remained even after 6 wk of Cu repletion.

Histopathology of capillary-myocyte interface. In addition to the ultrastructure of cardiac cell organelles, the integrity of the capillary endothelium, myocardial sarcolemma and pericellular area could be of great importance in cellular respiration and function. The basal laminae underlying all cell membranes, consists primarily of type IV collagen and the adhesive proteoaminoglycan laminin, and fibronectin. Functions of the basal laminae include structural membrane support and cellular regeneration (Alberts et al. 1989).

Leigh (1975) first described abnormalities in the boundaries between capillaries and adjacent myocytes in Cu− steers. Increases in amounts (Borg et al. 1985, Farquaharson et al. 1989) and changes in the type (Dawson et al. 1982) of interstitial and pericellular collagen deposition have been reported. Bird et al. (1966) in chicks and Farquaharson et al. (1989) in rats suggested that diminished collagen strength could be due to insufficient 3-hydroxy pyridinium crosslinking by reduced activity of the cuproenzyme, lysyl oxidase, in the Cu− deficient state. Recently, Farquaharson and Robins (1991), using immunohistochemical methods in hearts of Cu− rats, confirmed the presence of abnormal Types III and I collagen in focal areas and distortion and disorganization of basal laminae underlying the myocytes. In the present study, electron micrographs revealed significantly greater pathology at the capillary-myocyte interface in all Cu− compared with Cu+ rats, with no significant difference of age or stage of repletion or depletion apparent (Table 2). In agreement with Farquaharson and Robins (1991) it seemed that the capillary wall was less affected than that of the sarcolemma. This consistent pathology in Cu deficiency suggests involvement in the development of abnormalities in more than one way. Increased pericellular collagen and distortion of subsarcolemmal mitochondria could contribute to increased oxygen diffusion distances and relative ischemia. The swelling of the mitochondria may be an attempt to increase surface area to maximize uptake of both oxygen and Cu. Damage to the basal laminae could reduce cellular regeneration and contribute to sarcolemmal malfunction. Early response to Cu deficiency by decreased deaminative crosslinking of type IV collagen through decreased activity of lysyl oxidase may be one of the mechanisms in the etiology of functional and ultrastructural changes in Cu deficiency.

In summary, this study demonstrated that typical electrophysiological and cardiac ultrastructural pathology developed when postweanling rats were fed a Cu− diet, despite absence of myocardial hypertrophy, high blood pressure and anemia. It further demonstrated that postweanling Cu− rats that were Cu repleted by feeding a Cu+ diet demonstrated significant signs of pathological regression. However, after 6 wk of repletion some ECG and cardiac ultrastructural abnormalities remained. The role of the basal laminae in the complex pathophysiology of dietary Cu deficiency warrants further investigation, because our data suggest that a defect in this substance could be an initiating factor in the onset of the pathology. We have obtained similar results to those reported here in another study conducted to determine the influence of aerobic exercise upon Cu deficiency.

These results suggest that a temporary reduction in dietary copper during different times of the life cycle may result in cardiac damage without apparent cardiac hypertrophy and that the cardiac damage due to copper restriction may not be completely reversible with copper repletion.

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LITERATURE CITED


