Electrocardiographic Activity and Cardiac Function in Copper-Restricted Rats (43396)

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Abstract. **The temporal sequence of events leading to cardiac dysfunction during copper restriction in the Long-Evans rat was studied over a 6-week period. Weanling rats were fed either copper-adequate (6 mg Cu/kg diet, $n = 25$) or copper-restricted (<1 mg Cu/kg diet, $n = 25$) diets for varying periods of time for up to 6 weeks. Beginning at 2 weeks after weaning and weekly thereafter, five rats from each diet were evaluated for cardiac function, and sacrificed, and indicators of copper deficiency were determined on several tissues. Electrocardiograms began showing indications of cardiac disease at Week 3 in the copper-restricted rats, at which time cardiac hypertrophy and other signs of copper deficiency were apparent. Greater QT intervals and QRS amplitudes were observed in copper-restricted rats at various weeks. Peak + and − dP/dt maxs did not differ by diet copper treatment for any of the time intervals studied, nor was any notable difference in developed left ventricular pressure apparent. Hematocrit and liver copper levels were decreased in copper-restricted rat hearts at all weeks. These results suggest that the onset of cardiac dysfunction in copper deficiency is rapid, with both dysfunction and hypertrophy apparent within 3 weeks after copper restriction and when liver copper levels have declined.** [P.S.E.B.M. 1992, Vol 200]

Cardiovascular defects in copper-restricted rats are well known. Some of the defects include abnormal blood vessel connective tissues, ventricular aneurysms, abnormal electrocardiograms (ECG; bundle branch block, supraventricular beats, wandering pacemaker), hemothorax, pleural effusion, and hypertrophy (1).

Studies reporting such findings have been limited to the late effects of copper restriction and have not examined cardiac changes over the time course in which these deficiency signs develop (1–6). Often, many of the copper-restricted rats used for these studies are but a few days away from death. Some of the alterations noted could be secondary to other conditions caused by the deficiency.

Morphological alterations may not always be functionally significant. Examination as to when cardiac functions and electrical properties change and the time point at which hypertrophy occurs would allow a better evaluation of whether the effects upon function occur before or after the cardiac enlargement.

The objectives of this study were to (i) monitor cardiac electrical and functional aspects of rats fed a copper-restricted diet as the effects of copper deficiency develop from Week 2 to Week 6 after weaning, and to (ii) better characterize the type of cardiac hypertrophy in the copper-restricted rat in contrast to previous studies reporting cardiac enlargements in a general sense.

Materials and Methods

The temporal sequence of physiological events was assessed in rats fed either copper-restricted or -adequate diets for various time periods following weaning. Weanling male Long-Evans rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN). The initial body weights of the rats were determined upon arrival, and the rats were randomly divided into two treatment groups at weaning or at 3 weeks of age such that the initial mean weights of the treatment groups were similar.

Rats were fed a basal diet that followed the recommendations of the American Institute of Nutrition (7), consisting of (by weight) 50% sucrose, 15% corn-

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starch, 20% casein, and 5% corn oil as energy sources. One group had copper in the form of cupric carbonate at a level of 6 mg/kg of feed (copper-adequate) and the other group had copper omitted from the diet (copper-restricted). These diets were prepared and purchased from U.S. Biochemical Corp. (Cleveland, OH). Mean analyzed copper values by atomic absorption spectrophotometry were 5.88 μg/kg of diet for the copper-adequate diet and 0.4 μg/kg of diet for the copper-restricted diet. Animals were given free access to deionized, distilled water.

Rats were housed singly in stainless steel cages in a room with a 12:12-hr, light:dark cycle and temperature control. The protocol used was approved by the Ohio State University Institutional Animal Welfare Committee.

At Weeks 2, 3, 4, 5, and 6 following weaning, five rats from each copper treatment group were anesthetized with ketamine (85 mg/kg) and xylazine (15 mg/kg) given intraperitoneally. Electrocardiogram Leads I, aVF, and V3 were recorded at 100 mm/sec paper speed on a photographic oscillograph, with frequency response flat to over 1000 Hz. Subcutaneous needle electrodes were used. Leads I, aVF, and V3 were utilized because they permit representation of projections of cardiac vectors on the two axes (I = X, aVF = Y) constituting the frontal plane, and because Lead V3 is a precordial lead with great proximity to the left ventricular free-wall and is, therefore, sensitive to alterations in ventricular depolarization and repolarization. The following ECG parameters were analyzed from lead aVF: heart rate, durations of P, PQ, QRS, and QT; amplitudes of P, R, and S waves; and the peak to peak value of R to S. The P wave and QRS complex represent electrical stimulation in the atria and ventricles, respectively. The T wave represents repolarization of the ventricles. PQ represents the interval between onset of atrial stimulation and onset of ventricular stimulation; it is known that the greatest portion of this interval is generated by the delay of conduction through the AV node itself. The QT interval, electrical systole, is the duration required for ventricular depolarization (stimulation) and repolarization. Durations were measured directly from the traces inscribed on oscillographic paper. The speed of 100 mm/sec permitted the resolving of intervals to at least 10 msec. In addition, we noted subjectively: changes in contours of QRS and/or ST-T in all leads, occurrence of high frequency notching or slurring to QRS, and alterations in polarity of component deflections. The R:S ratio in V3 was used because in dogs and cats, its ratio changes in different directions with right or left ventricular hypertrophy. Additionally, orientation of the mean QRS in the frontal plane (mean electrical axis) was estimated from analysis of the QRS in Leads I and aVF.

Hemodynamic studies were done on the same anesthetized rats. Short-beveled, 21-gauge hypodermic needles attached directly to a pressure transducer were thrust through the left thoracic wall into the left ventricle. Pulsatile pressures and their derivatives were recorded using a modification of the method of Saragoca and Tarazi (8). The frequency response of the overall recording system is flat to over 150 Hz. Measurements of maximal rates of pressure rise and fall and total developed left ventricular pressure were made. In some instances, the needle did not enter the left ventricle initially. If after two attempts a tracing was not obtained, no further attempt was made, since this could compromise the heart integrity and influence the tracing. Therefore, data on rats for each week may represent less than five per treatment group.

After these cardiac tests were completed and while rats were fully anesthetized, a small amount of blood was removed by cardiac puncture. The blood was transferred to heparinized tubes for hematocrit determination. Rats were sacrificed by severing the aorta.

Following sacrifice, hearts from copper-restricted and copper-adequate rats were removed, blotted relatively free of blood, weighed, and frozen for future studies. Rat hearts from Week 6 were fixed in 10% buffered formalin. Median sagittal sections were cut, and hearts were photographed for measurements of thickness of right and left ventricular free-walls and ventricular septa and for total length (apicobasilar) and width (at level of the apex of the posterior papillary muscle). The histological cross-sections were stained with hematoxylin and eosin and Masson's trichrome and analyzed by a board-certified veterinary pathologist in a blind manner.

The liver was removed, rinsed in deionized, distilled water, blotted, weighed, and frozen until copper analysis, as described below.

Liver and Diet Copper Determination. Liver and diet samples were weighed, dried in a vacuum oven, reweighed, and wet-ashed as described previously (4). Liver and diet copper were determined with a Varian flame atomic absorption spectrophotometer. Reference bovine liver purchased from the National Institute of Standards and Technology (Gaithersburg, MD) was used to verify accuracy of results.

Statistical Analysis. Parameters of cardiac function and hematocrit, body weight, liver copper, heart weight, and heart weight to body weight data were analyzed by a two-way analysis of variance with weeks and copper treatment as main effects using the Statistical Analysis Software system (9). Where significant interactions or significant week effects occurred, means were separated by the least significant difference method (10). Two-tailed tests were used for the analysis of variance.

Results

At 6 weeks after copper restriction, rats assigned to the copper-restricted group had significantly depressed body weight and hematocrit, but elevated heart weight and heart to body weight (Table I). Furthermore, rats at earlier time points and assigned to the copper-restricted group also showed some of these same alterations. Heart weight was also increased at Week 5, but the heart to body weight ratio was elevated beginning at Week 3 and remained elevated at later points as well. Body weight was only significantly depressed at Week 4 in addition to Week 6 among the copper-restricted rats. Hematocrit was lower at all weeks in the rats assigned to the copper-restricted group. There were no mortalities in any of the treatment groups throughout the study. However, at week 5, one of the copper-adequate rats died after being anesthetized and, consequently, ECG, contractility, and hematocrit data were not obtained, although heart weight, final body weight, and liver copper were determined.

Q-T interval and the QRS and R heights differed significantly either by copper treatment or by an interaction of copper treatment and time (Table II). The Q-T interval was greater for copper-restricted rats at Weeks 4 ($P \leq 0.05$) and 6 ($P \leq 0.01$), with similar trends observed at other weeks. The QRS amplitude was significantly higher ($P \leq 0.01$) over all time periods for copper-restricted rats compared with copper-adequate rats, except for Week 3. The R amplitude was elevated ($P \leq 0.01$) in copper-restricted rats at all weeks except Week 2.

ECG patterns of rats in the copper-restricted group showed aberrations beginning at Week 3 (Fig. 1). The P wave in one rat was inverted. At Week 4, one rat had an inverted T wave (No. NC406) and one rat had large R and peak to peak QRS amplitude (No. NC442). At Week 5, notching was apparent in the QRS of one copper-restricted rat (No. NC452) and another rat had an inverted T wave. At Week 6, all copper-restricted rats showed some abnormal ECG patterns, including notching (No. NC412) and inverted T waves (Nos. NC440, NC460, NC422, and NC412). Voltages were increased in copper-restricted rats as well. There was no significant difference ($P > 0.05$) of orientation of mean QRS in the frontal plane.

Values for + and − dP/dt max as well as total left developed ventricular pressure did not differ significantly ($P \geq 0.05$) between copper-restricted and copper-adequate rats, nor was there any noticeable trend (Table III).

Cross-sectional views of the hearts from rats sacrificed at Week 6 showed that copper-restricted rats had thicker ventricular walls compared with copper-adequate rats (Fig. 2). Cardiac length and width, as well as thickness of right and left ventricular free-wall and septum thickness, were significantly ($P \leq 0.001$) increased in the copper-restricted rat heart. The mean measurements in millimeters (± SE) for copper-adequate and -restricted rats, respectively, were as follows: length, 16.3 ± 0.38 vs 19.2 ± 0.83; width, 10.7 ± 0.20 vs 12.2 ± 0.31; right ventricular free-wall thickness, 1.3 ± 0.09 vs 1.7 ± 0.20; septum thickness, 3.4 ± 0.36 vs 3.9 ± 0.32; left ventricular free wall thickness, 4.3 ± 0.15 vs 4.7 ± 0.26; epicardial perimeter, 45 ± 1.5 vs 53 ± 1.2. Differences between groups were significant ($P \leq 0.01$). The type of hypertrophy appeared to be mixed with predominantly concentric characteristics since the free-walls were grossly thickened. Since lumen size in fixed hearts is difficult to assess, we base this suggestion on a comparison with other rat models of hypertrophy. As a basis for comparison, we used the spontaneously hypertensive heart failure corpulent rat mode (SHHF/Mcc-cp). This model has three genotypes, but only two phenotypes: (i) corpulent (cp/cp), exhibiting obesity, diabetes, hypertension and cardiac hypertrophy; (ii) heterozygous lean (cp/±), exhibiting hypertension and hypertrophy without obesity and diabetes; and (iii)

Table I. Indices of Copper Deficiency (Mean ± SE)

Parameter	Week 2		Week 3		Week 4		Week 5		Week 6	
	Cu− ($n = 5$)	Cu+ ($n = 5$)	Cu− ($n = 5$)	Cu+ ($n = 5$)	Cu− ($n = 5$)	Cu+ ($n = 5$)	Cu− ($n = 5$)	Cu+ ($n = 5$)	Cu− ($n = 5$)	Cu+ ($n = 5$)
Heart wt[a] (g)	0.58 ± 0.02	0.57 ± 0.02	0.95 ± 0.08	0.77 ± 0.02	0.95 ± 0.06	0.88 ± 0.04	1.26 ± 0.05	0.85 ± 0.02	1.52 ± 0.10	1.08 ± 0.04
Body wt[b] (g)	123 ± 5.6	118 ± 8.5	144 ± 2.2	147 ± 0.9	156 ± 11.2	200 ± 9.0	197 ± 13.4	206 ± 8.6	228 ± 24.4	286 ± 15.8
Heart:body wt[c] ($\times 10^{-3}$)	4.7 ± 0.2	4.9 ± 0.3	6.6 ± 0.6	5.2 ± 0.2	6.2 ± 0.4	4.4 ± 0.1	6.5 ± 0.7	4.1 ± 0.1	6.7 ± 0.3	3.8 ± 0.1
Hematocrit[d] (%)	28.0 ± 3.0	39.5 ± 1.4	27.5 ± 1.0	37.0 ± 0.9	34.5 ± 1.6	39.5 ± 1.4	28.5 ± 4.1	38.0 ± 1.4	29.5 ± 3.3	41.5 ± 0.9
Liver copper[e] (μgCu/g wet wt)	0.95 ± 0.32	4.24 ± 0.35	0.28 ± 0.17	4.67 ± 0.10	0.33 ± 0.19	3.94 ± 0.77	0.32 ± 0.14	3.69 ± 0.92	0.33 ± 0.10	4.21 ± 0.33
Liver copper[e] (μgCu/g dry wt)	3.38 ± 1.02	14.31 ± 1.05	0.96 ± 0.57	16.29 ± 0.33	1.09 ± 0.62	11.95 ± 2.08	1.08 ± 0.49	12.47 ± 3.00	1.10 ± 0.34	14.23 ± 1.04

[a] There were significantly higher heart weights in Cu− rats than in Cu+ rats at Weeks 5 and 6, $P \leq 0.01$.
[b] There were significantly lower body weights in Cu− rats than in Cu+ rats at Weeks 4 and 6, $P \leq 0.01$.
[c] There were significantly higher heart:body weight ratios in Cu− rats than in Cu+ rats at Weeks 3, 4, 5, and 6, $P \leq 0.01$.
[d] There were significantly lower values in Cu− rats than in Cu+ rats (over all time periods) ($n = 4$ for Cu+ rats at Week 5).
[e] There were significantly lower liver copper levels in Cu− rats than in Cu+ rats over all time periods, $P \leq 0.001$.

Table II. ECG by Treatment (Mean ± SE)

ECG	Week 2		Week 3		Week 4		Week 5		Week 6	
	Cu− (n = 5)	Cu+ (n = 5)	Cu− (n = 5)	Cu+ (n = 5)	Cu− (n = 5)	Cu+ (n = 5)	Cu− (n = 5)	Cu+ (n = 4)	Cu− (n = 5)	Cu+ (n = 5)
P (ms)	21 ± 1.2	19 ± 1.0	29 ± 4.3	20 ± 4.2	17 ± 3.4	18 ± 2.6	17 ± 1.2	21 ± 1.5	20 ± 2.7	17 ± 2.6
P-R (ms)	46 ± 2.5	46 ± 2.5	50 ± 0.0	52 ± 2.0	44 ± 2.5	54 ± 2.5	46 ± 2.5	45 ± 5.0	46 ± 2.5	51 ± 2.5
QRS (ms)	20.2 ± 1.3	18.6 ± 1.9	18.8 ± 3.2	20.0 ± 1.6	20.0 ± 0.0	20.0 ± 0.0	18.6 ± 1.0	19.0 ± 0.6	18.6 ± 1.0	19.0 ± 1.0
QT (ms)[a]	76 ± 7.5	80 ± 4.1	63 ± 2.5	66 ± 6.8	82 ± 9.7	54 ± 4.0	72 ± 12.4	55 ± 5.0	112 ± 14.3	60 ± 5.8
Heart rate (beats/min)	315 ± 13.8	336 ± 20.9	322 ± 27.4	324 ± 10.4	308 ± 11.5	322 ± 14.6	313 ± 6.2	357 ± 23.8	315 ± 21.3	299 ± 19.7
QRS ht[b] (mm)	12.4 ± 1.2	12.4 ± 2.2	11.3 ± 1.9	9.2 ± 1.0	13.8 ± 3.6	8.4 ± 1.0	14.6 ± 0.5	9.3 ± 1.8	12.8 ± 1.7	8.6 ± 1.4
R/S	2.1 ± 0.80	2.2 ± 0.78	2.9 ± 1.23	1.1 ± 0.10	2.0 ± 0.79	2.4 ± 0.95	1.9 ± 0.50	0.8 ± 0.21	1.7 ± 1.08	1.4 ± 0.79
R ht[c] (mm)	74 ± 16.0	78 ± 21.0	93 ± 14.9	40 ± 7.1	89 ± 36.6	52 ± 10.7	86 ± 15.0	35 ± 13.2	68 ± 10.7	43 ± 6.3

[a] QT was significantly longer in Cu− rats than in Cu+ rats at Weeks 4 ($P \leq 0.05$) and 6 ($P \leq 0.01$).
[b] There were significantly higher values in the Cu− group rats than in the Cu+ group rats over all weeks, $P \leq 0.01$. Cu+ (Mean ± SE), 9.58 ± 0.70; Cu−, 13.01 ± 0.84.
[c] R waves were significantly higher in Cu− rats than in Cu+ rats at Weeks 3, 4, 5, and 6, $P \leq 0.01$.

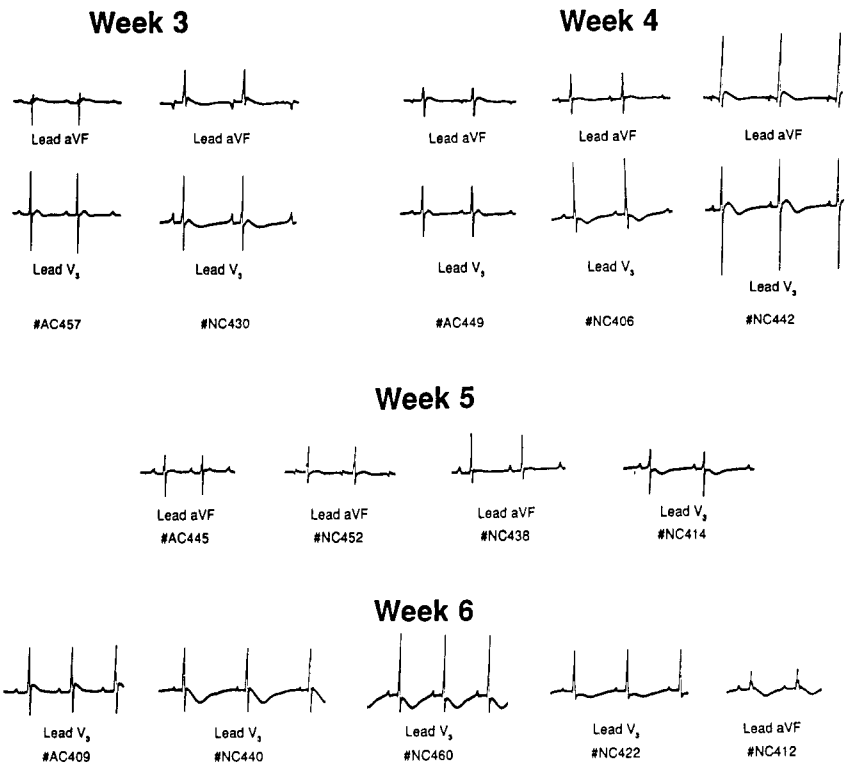

Figure 1. ECG patterns of copper-adequate and -deficient rats at different weeks. Changes in ECG become apparent at Week 3 with an inverted P wave in Lead aVF and an altered T wave in Lead V_3. At Week 4 inverted T waves are more apparent and increased QRS and R heights are evident. Similar findings are also present at Peak 5 for more copper-restricted rats. In Week 6, inverted T waves are apparent and notching can be observed in one copper-restricted rat (No. NC412). AC refers to copper-adequate rats and NC to copper-restricted rats.

Table III. Heart Contractility and Left Total Developed Pressure (Mean ± SE)[a]

Contractility	Week 2		Week 3		Week 4		Week 5		Week 6	
	Cu− (n = 3)	Cu+ (n = 4)	Cu− (n = 3)	Cu+ (n = 3)	Cu− (n = 3)	Cu+ (n = 3)	Cu− (n = 5)	Cu+ (n = 5)	Cu− (n = 5)	Cu+ (n = 4)
+dP/dt (mm Hg/sec)	6767 ± 1233	7200 ± 850	5500 ± 2255	6867 ± 1968	7367 ± 984	4600 ± 379	6800 ± 566	5275 ± 795	5640 ± 1479	5700 ± 1116
−dP/dt (mm Hg/sec)	4833 ± 664	5600 ± 1163	4433 ± 1337	4600 ± 917	4733 ± 384	3100 ± 379	5900 ± 1249	4350 ± 850	4020 ± 996	3925 ± 993
Ps (mm Hg)	133 ± 4	135 ± 7	103 ± 30	155 ± 8	133 ± 7	122 ± 6	143 ± 5	141 ± 10	110 ± 27	106 ± 6

[a] There was no significant difference for +dP/dt, −dP/dt, and left total developed pressure (Ps) between Cu− and Cu+ groups.

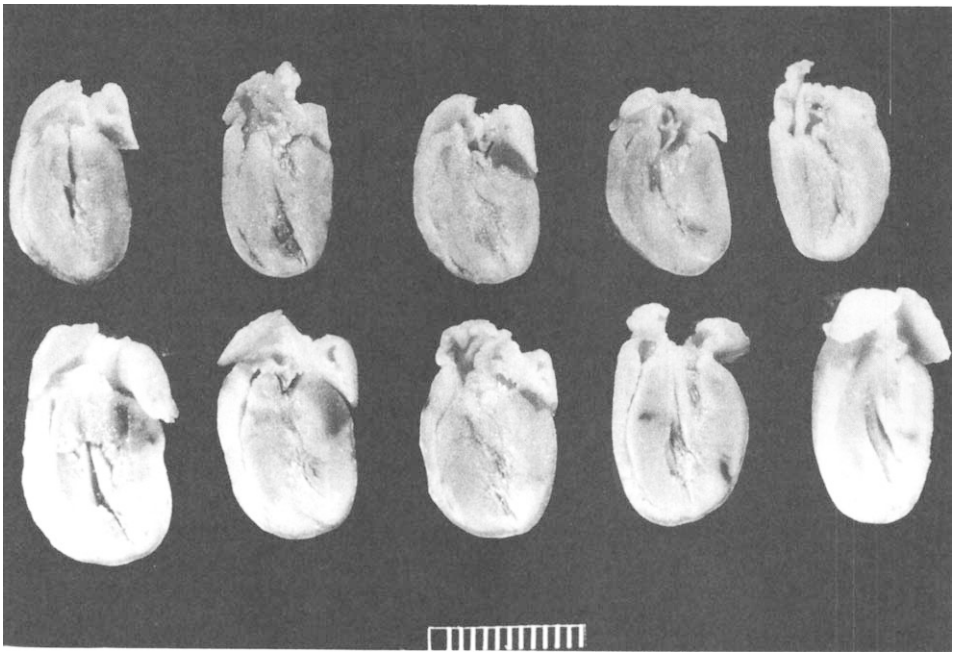

Figure 2. Sagittal views of copper-adequate (top row) and copper-restricted (bottom row) rat hearts at Week 6 of the study. Note the increased thickness of the free ventricular walls as well as the septum in the copper-restricted rats. Rules at bottom is in 1-mm increments.

homozygous lean, (±/±) normal. In Figure 3, a 9-month-old normal rat heart (±/±) is compared with the eccentric hypertrophy pattern from a 9-month-old corpulent rat. The eccentric pattern shows a greater lumen size and greater width and length in comparison with the normal. Conversely, the concentric pattern shows grossly thickened walls and septum with an apparent reduction in lumen size and little increase in epicardial perimeter compared with the normal. This latter pattern was produced by treating a 9-month-old heterozygous lean rat with clonidine (Boehringer Mannheim, Indianapolis, IN).

Light microscopy results suggested that copper-restricted rat hearts had minimal to mild fibrosis.

Discussion

Rats began showing signs of copper deficiency at Week 2 with a lower hematocrit. The heart to body weight ratio, an index of cardiac hypertrophy, was increased by Week 3 and the absolute heart weight was greater by Week 5 in the copper-restricted rats. With respect to the latter, the copper-restricted rat hearts were 1.26–1.52 g for Weeks 5 and 6, respectively, compared with control values of 0.85 and 1.08 g. Body weight depression appeared to be the least consistent sign of copper deficiency.

Viestenz and Klevay (1), studied ECG patterns of copper-deficient rats and copper-adequate rats. They described changes in ST-T, PQ interval, and QRS duration, as well as supraventricular and ventricular arrhythmias. Increased height of QRS was observed. Kopp *et al.* (11) analyzed ECG and His-bundle electrocardiograms from copper-adequate and -restricted rats. No differences in heart rates or orientation of QRS vectors in the frontal plane were observed, but prolongation of PR interval and prolongation of H-V intervals, indicating reduced conductivity of the His-Purkinje system, were reported. They also observed increased amplitudes of R waves and alterations in ST-T in 75% of the copper-deficient rats. Often the ST-T changes were produced by inversion of T waves in leads in which they were normally positive. With the exception of the PR interval, our study produced similar results.

In agreement with Redman *et al.* (12), there was increased thickness of both left and right ventricular free-walls and increased thickness in the septum of copper-restricted rats as compared with the copper-adequate rats. Fibrosis was also evident, in agreement with Redman *et al.* (12).

The most notable finding from the present study is how soon after copper restriction cardiac hypertrophy and ECG abberations become apparent. Within 3 weeks after the copper restriction was initiated, ECG patterns appeared abnormal, hematocrit was depressed, and cardiac hypertrophy had developed. Other literature cited in this paper report results from rats fed copper-restricted diets for much longer periods. Six to eight weeks after copper restriction is initiated, the mortality rate in the copper-restricted groups increases rapidly. Biochemical, ultrastructural, and functional alterations at later points in time could be caused by secondary effects of copper deficiency, such as anemia and lack of oxygen delivery to organs.

The voltage generated at a body surface electrode

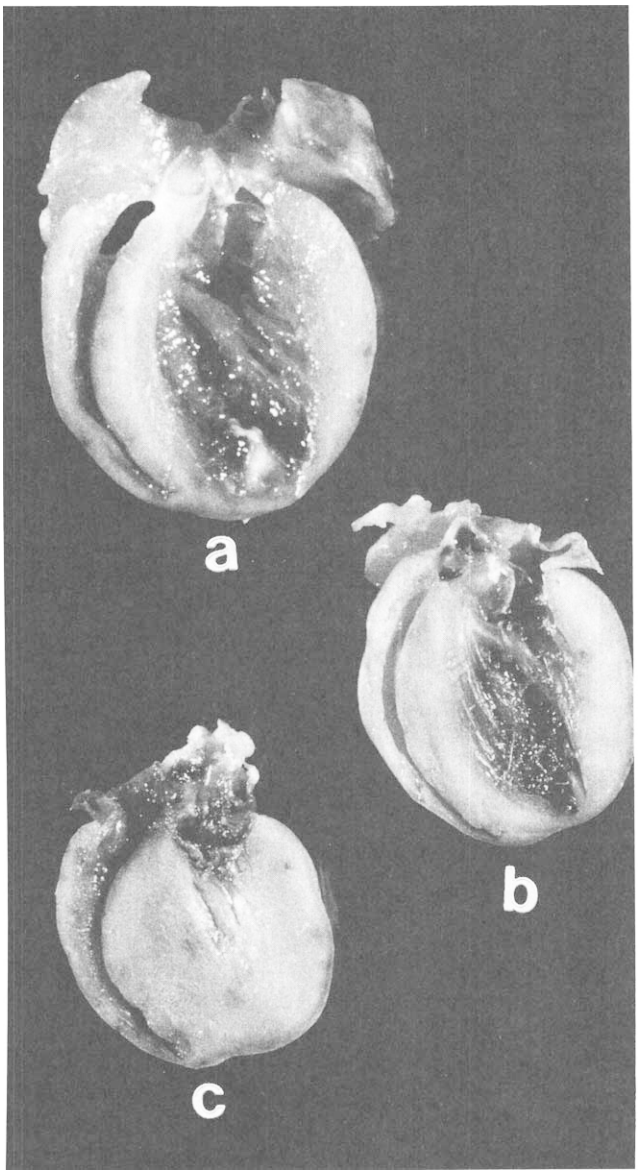

Figure 3. Sagittal views of different patterns of cardiac hypertrophy from 9-month-old SHHF/Mcc-cp rats. (a) Eccentric, (b) normal, and (c) concentric patterns are shown in this figure. Note the differences in muscle wall thickness and the apparent lumen size differences. The epicardial perimeter in the (a) eccentric type appears increased over both the (b) normal and (c) concentric hearts. (See text for details.)

is determined by (1) the magnitude of the unit dipole, thousands of which constitute the wave of depolarization traversing the myocardium, (2) the net area of the wave of depolarization traversing the myocardium at each instant, and (3) a contribution made by an image dipole within the lumen of the ventricle. The contribution of the image dipole becomes greater the thinner the left ventricular free-wall becomes and the greater the difference in resistivity between the myocardium and the blood within the lumen. The increased height of QRS may be due to an increase in muscle mass and, therefore, an increase in the area of the boundaries of depolarization, or it may be due to an increase in differences of resistivity between blood (due to mild anemia) and myocardium (due to increased volume density of mitochondria). Anemic blood has decreased resistivity, while mitochondrial-laden myocardium has increased resistivity. We (13) have shown that the increased QRS height due to even severe anemia is much less than that observed in our copper-restricted rats. This increased difference in resistivity could augment the contribution of the image dipole. Between the two possible explanations for increased height of QRS, the former explanation (i.e., increased muscle mass) is more likely. Notching of the QRS may have occurred because of myocardial fibrosis and subsequent splittering of waves of depolarization, or the notching could be due to competition between the hypertrophied right and left ventricles for dominance over resultant vectors. Since we did not observe right ventricular enlargement out of proportion to the left ventricle and since we observed fibrosis in the copper-deficient rat myocardium, we feel that it is more likely that the QRS notching was due to fibrosis. Prolongation of the QT interval may have resulted from delayed repolarization, which in turn may have been due to decreased potassium conductance. T wave inversion may reflect an alteration in the time course of ventricular repolarization. Such changes can be due to ventricular hypertrophy, slowing of the rate of ventricular depolarization or to altered ionic fluxes.

It appears that the pathophysiology of this model may not include a substantial valvular component, i.e., mitral regurgitation, since with that lesion, one would expect a predominant volume overload—eccentric hypertrophy or dilatation as opposed to mixed hypertrophy with predominant concentric characteristics observed in our copper-restricted rats. In concentric hypertrophy, an increase in thickness of the cardiac muscle walls without an increase in the ventricular chamber size is observed, and normally results from increased pressure load as observed in aortic stenosis. In eccentric hypertrophy, both dilatation and increased muscle mass occur, but the ventricular lumen is large. *In vivo* measurements by echocardiography of the ventricular lumen in copper-deficient rats could confirm this contention.

Studies on the effect of copper restriction upon heart function have been limited. Prohaska and Heller (14) noted that copper-deficient rats had lower spontaneous heart rates *in vitro* and decreased coronary resistance, and consumed more oxygen per unit pressure developed. The heart rates were 161 and 215 beats/min between copper-deficient and copper-adequate rats respectively. This is in contrast to the present study, in which no differences were apparent, and is in contrast to a previous finding from our group using the SHHF strain (15).

Myocardial function in both systole and diastole

was estimated by analysis of peak + and − dP/dt. In the rats from this study, neither parameter altered significantly. That peak +dP/dt-max did not increase despite increased ventricular wall thickness may, in fact, indicate that there was a reduction in wall stress and rate of change of wall stress during systole. However, if ventricular preload was reduced due to concentric hypertrophy, one might have expected a decrease in peak +dP/dt. We do not know what the preload was during life, since our measurements were made only during postmortem examination of a formalin-fixed heart. That peak −dP/dt did not decrease indicates little change in at least the early phases of relaxation.

Many models of heart disease manifest ventricular hypertrophy which is predominantly eccentric (i.e., the mass is increased and the epicardial perimeter is increased). Hearts from the copper-restricted rats in this study were hypertrophied (i.e., increased mass), but, since the walls were very thick and the epicardial perimeter was increased, the hypertrophy was termed mixed. If the heart were hypertrophied only concentrically, then we would have anticipated increased mass and gross wall thickening, but little increase in epicardial perimeter. A previous study on copper deficiency in young SHHF rats indicated a mixed hypertrophy where both concentric and eccentric patterns were noted (15). Stemmer *et al.* (16) and Petering *et al.* (17) reported that deficient rats developed dilated cardiomyopathy. Dilatation in the heart is reflected by increased epicardial perimeter, width, and/or length of the organ. However, their results are difficult to interpret, since no data on free-wall or septal thickness, ventricular size, or cross-sectional photographs were presented. Also, their study lasted 90 days. Our results suggest that dilatation does occur in that the width, length, and mass of the hearts are increased in the copper-restricted rats.

Additional studies designed to quantitate *in vivo* ventricular lumen size as a function of copper restriction and also evaluate biochemical and histological aspects of the heart earlier in feeding studies would help determine the primary effects of copper upon cardiac physiology.

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