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The Effects of Reserpine upon the Cardiac Enlargement of Copper Deficiency (42794)

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Abstract. Copper (Cu) deficiency, induced in rats by suckling from Cu-deficient dams and by offering a semisynthetic low-Cu diet from weaning, resulted in cardiac enlargement. This enlargement was not due to accumulation of excess fluid in the heart but was characterized by mitochondrial hypertrophy as demonstrated by electron microscopy and biochemical studies. Administration of reserpine limited the extent of cardiac enlargement; however, heart total noradrenaline (NA), unchanged by Cu deficiency, was significantly reduced by reserpine. It was concluded that cardiac enlargement in Cu deficiency was not directly related to NA concentration. An alteration in cardiac energy status, however, was suggested by reduction in activity of the nonheme iron-dependent enzyme, succinic dehydrogenase. © 1988 Society for Experimental Biology and Medicine.

Pathological lesions occur in many tissues of animals suffering from the effects of copper (Cu) deficiency, for review see (1). These defects, believed to result from failure of Cu-dependent enzymatic processes, range from overt signs such as hair depigmentation to those of a more serious but covert nature. Within the latter group, in addition to connective tissue defects, particularly in elastin, must be included "cardiac hypertrophy," a frequently reported manifestation of Cu deficiency (2–5). Such abnormal cardiac growth frequently leads to rupture of the heart and death in male rats reared on low-Cu regimes, particularly where diets contain a high proportion of fructose or sucrose (6).

Cardiac hypertrophy with concurrent anemia has also been shown to occur in iron (Fe)-deficient rats but it has been possible to reduce this hypertrophy by administering the alkaloid reserpine (7). The latter is an antihypertensive agent which depletes noradrenaline (NA) stores and prevents NA reuptake into storage granules in nerve terminals (8).

This study reports an attempt, by similar treatment with reserpine, to reduce the cardiac enlargement attributable to Cu deficiency and to obtain further insight into the events leading to its induction.

Materials and Methods. *Chemicals.* Double glass distilled water was used in the preparation of all solutions. Chemicals, of best purity, were obtained either from Sigma (Poole, Dorset, UK) or from BDH (Poole, Dorset, UK). Aluminum oxide, Brockman activity II, was obtained from the latter.

Animals, diets, and treatments. Three separate experiments were performed to investigate the effects in rat pups of low Cu intake from birth. To achieve this, pregnant female rats of the Rowett Hooded Lister strain were offered a diet of laboratory chow (CRM irradiated, Labshure, Ltd., Manea, Cambs, UK) until 5 days prepartum. These animals were then distributed into two groups, being fed *ad libitum* either a semisynthetic diet as previously described (9) with a Cu content of 5.0 mg Cu/kg DM (+Cu) or a similar diet with no added Cu containing 0.4–0.6 mg Cu/kg DM (−Cu). Distilled drinking water was supplied *ad libitum*. The pups used for the experiments were males selected from the +Cu dams and fostered to either control (+Cu) or low-Cu dams. Each dam, together with its eight foster pups, was separately housed in a stainless-steel/polypropylene cage until weaning at 19 days, from which time all pups were offered *ad libitum* the diets previously fed to their foster dams. All groups were injected with reserpine (2.5 mg/kg body wt) (+R) or carrier solution (−R) initially 12 hr after birth and then 3 days later. The carrier solution was an alcohol/citric acid medium (10) and the injections were made at the nape of the neck. Reserpine dosage and frequency of administration were based upon the work of Bartolomé and Slotkin (11) and of Rossi and Carillo (7)

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and were designed to deplete rat tissue catecholamine stores. The basic groups in each experiment were +Cu−R, +Cu+R, −Cu−R, and −Cu+R. Also, some rats of the −Cu−R group in Experiment 1 were treated with reserpine, but only from near weaning (RW) or from an "intermediate" stage 5 days later (RI) (Fig. 1). The more frequent regime of reserpine injections in Experiment 2 was discontinued at the third injection postweaning, due to adverse effects upon the rats. Some deaths occurred during the experiments, particularly in the −Cu reserpine-treated groups, and the numbers surviving are indicated in the tables. Rats were killed 3 weeks after weaning as preliminary experiments indicated that deaths from heart rupture were likely to occur from this time onward.

Tissue sampling. After ether anesthesia, all rats were weighed prior to killing. In Experiment 1 sets of rats from each basic group were killed near weaning and 5 days later as well as 3 weeks after weaning and the hearts were removed blotted, weighed, and dried for estimation of dry matter content. At 3 weeks on the diet other rats from each of the four groups were perfused retrogradely through the thoracic aorta with 250 m*M* sucrose to free heart tissue of blood. The hearts were then removed, weighed, and stored at −40°C for subsequent cytochrome c_1 and succinic dehydrogenase (SDH) (EC 1.3.99.1) analysis.

The hearts from the RW and RI groups were prepared for dry matter and cytochrome c_1 determinations at 3 weeks on diet as above. In Experiment 2 a small portion of left ventricle was taken for ultrastructural studies and in Experiments 2 and 3 hearts and livers were excised, weighed, frozen in liquid N_2, and stored at −40°C to await analysis.

Tissue preparation and analyses. Catecholamines were determined in left ventricular tissue using standard perchloric acid extraction and alumina purification techniques (12) followed by isocratic reversed-phase HPLC separation and electrochemical detection (13). Livers were wet ashed as described previously (9) and atomic absorption spectrometry was used to determine Cu and Fe concentrations. Packed red cell volumes (PCV) were measured in blood which had been centrifuged in microhematocrit tubes on a Beckman microfuge (Beckman RIIC Ltd., Glenrothes, UK). Hemoglobin was determined by the cyanmethemoglobin technique.

Cytochrome c_1 was estimated in supernatants prepared from perfused hearts which had been homogenized in phosphate buffer and then briefly sonicated prior to centrifugation. Difference spectra in the range 540 to 554 nm were obtained by comparing an oxidized preparation with one reduced by NADH and inhibited with sodium cyanide. Concentration of cytochrome c_1 was calculated from the difference in absorbance between these wavelengths using an extinction coefficient of 18.8 cm^{-1} mM^{-1} (14). Succinic

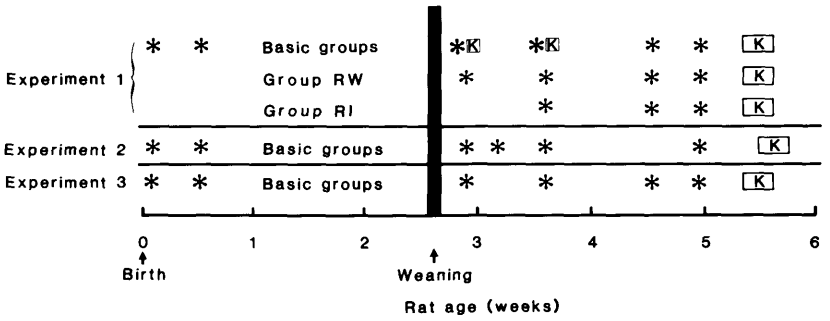

FIG. 1. Timing of reserpine (R) or carrier injections to copper (Cu)-sufficient or -deficient rats* and timing of killings [K]. The basic groups in each experiment were +Cu−R, +Cu+R, −Cu−R, and −Cu+R. Groups RW and RI rats, fed low-Cu diet, were reserpine-treated only from "near weaning" or from an "intermediate" stage 5 days later. Reserpine doses were, prior to weaning, 2.5 mg/kg body wt and after weaning 0.3 mg/kg body wt.

dehydrogenase was estimated on the same supernatant preparations using the method of Cooney *et al.* (15).

Methods of electron microscopy. Pieces of left ventricular tissue from the free wall of the heart close to the apex were taken from four animals from each of the four groups in Experiment 2. The tissue was fixed initially in 2.5% glutaraldehyde, buffered with 0.1 *M* sodium cacodylate for 4 hr, and postfixed for 1 hr in 1% osmium tetroxide in the same buffer. The specimens were stained *en bloc* in uranyl acetate and embedded in Araldite. Ultrathin sections were cut with a diamond knife, stained with lead citrate, and examined on an AEI 801 electron microscope.

Calculation of mitochondrial/myofibrillar ratios. The micrographs used were all taken at the same original magnification and printed to a final magnification of 14,970. In all cases fields were selected randomly from several blocks taken from the same animal. Care was taken not to include areas close to the nuclei, where mitochondria are greater in number than elsewhere in the cytoplasm. Twelve micrographs from each group covering an area of 1830 μm^2 were examined using a Hipad digitizing tablet (Bausch & Lomb, Austin, TX) linked to a Prime 550 computer (Prime Computer Inc., Framingham, MA) programmed to calculate the ratio of mitochondrial to myofibrillar areas.

Statistical analysis. Data from the four groups were analyzed by nonorthogonal analysis of variance to compensate for inequalities in group numbers. Where statistically significant differences were indicated by *F* tests, individual group comparisons were made by Student's *t* test using the residual mean square from the analysis of variance to estimate the SEDM. Where comparisons were made only between +Cu and −Cu groups, the Student *t* test was used.

Results. Liver Cu concentration (Table I) decreased significantly with low-Cu diet ($P < 0.001$) in all experiments, ranging from 1.12 to 1.26 μg Cu/g wet liver compared with control values which were 4.85 μg Cu/g or greater. Thus the rats were considerably Cu depleted. Liver Fe concentration, however, was not significantly changed by Cu deficiency. Although body weight (Table I) was not significantly affected by Cu deficiency or reserpine in Experiment 1, it was signifi-

TABLE I. THE EFFECT UPON RAT LIVER COPPER (Cu) CONCENTRATION, LIVER IRON (Fe) CONCENTRATION, BODY WEIGHT, HEART WET WEIGHT, AND HEART TO BODY WEIGHT RATIO OF OFFERING RATS COPPER-SUFFICIENT OR -DEFICIENT DIETS WITH OR WITHOUT CONCURRENT RESERPINE (R) TREATMENT

		Treatment							
		+Cu		−Cu				Significance of treatment effects	
Parameter	Experiment	−R	+R	−R	+R	Residual SD	Cu	Reserpine	Interaction
Liver Cu concentration	1	5.77 (6)	6.58 (5)	1.12 (6)	1.98 (6)	0.91	***	*	NS
(μg/g wet tissue)	2	4.85 (8)	5.29 (8)	1.26 (8)	1.89 (5)	0.80	***	NS	NS
	3	5.71 (8)	5.76 (6)	1.12 (6)	1.91 (5)	0.57	***	NS	NS
Liver Fe concentration	2	76.4 (8)	101.2 (8)	75.6 (8)	134.6 (5)	18.2	*	***	*
(μg/g wet tissue)	3	89.0 (8)	98.6 (6)	106.5 (6)	99.1 (5)	25.6	NS	NS	NS
Body wt (g)	1	131 (6)	124 (6)	122 (6)	109 (5)	14.2	NS	NS	NS
	2	151 (8)	115 (8)	132 (8)	102 (5)	15.8	*	***	NS
	3	150 (8)	110 (7)	113 (6)	91 (5)	9.6	***	***	***
Heart wet wt (mg)	1	474 (6)	491 (5)	689 (6)	514 (6)	64.7	***	**	**
	2	637 (8)	531 (8)	934 (8)	485 (5)	86.6	***	***	***
	3	667 (8)	509 (7)	884 (6)	482 (5)	63.6	***	***	***
Heart to body wt ratio	1	0.382 (6)	0.395 (5)	0.565 (6)	0.472 (6)	0.050	***	NS	*
(%)	2	0.423 (8)	0.461 (8)	0.722 (8)	0.482 (5)	0.083	***	***	***
	3	0.445 (8)	0.465 (7)	0.782 (6)	0.531 (6)	0.055	***	***	***

Note. Results are means of observations. Number of animals in parentheses.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

cantly reduced by both factors in Experiments 2 and 3; however, the overall effect of reserpine was greater than that of Cu. There was a significant interaction between the Cu and the reserpine effects in Experiment 3.

Despite the tendency for decreased body weight in rats offered a low-Cu diet, their heart weights were increased significantly in each of the three experiments (Table I), the increases ranging from 32 to 47% above that of control. Reserpine, however, significantly decreased heart weight when administered to low-Cu rats and the reductions were such that the heart weights of the reserpine-treated groups did not differ significantly with the Cu status of the diet. When the ratios of heart weight to body weight (BW) were examined (Table I), there were no significant differences in the +Cu rats with or without reserpine. In the Cu-deficient groups of Experiments 2 and 3, reserpine caused a highly significant reduction in heart to BW ratio to the extent that the ratios for the −Cu+R groups were not significantly different from those of the +Cu+R groups. In Experiment 1, although the heart to BW ratio was significantly reduced ($P < 0.05$) by reserpine treatment of −Cu rats by the final killing, it did not decrease to the values of +Cu rats (Fig. 2). In this experiment the heart to BW ratio of the untreated −Cu rats remained consistently high during the postweaning period. Reserpine given to the −Cu rats from birth significantly reduced this ratio but only during the last 16-day period of the experiment. Delaying treatment with reserpine until weaning decreased its effectiveness in limiting heart to body weight ratios and a further delay of 5 days ("intermediate" stage) abolished the effect.

At each of the three ages at which rats were killed in Experiment 1 dry matter determinations on the hearts revealed no differences between groups but there were slight differences with age. Mean values (±SEM) were 20.89 ± 0.10% at weaning, 22.21 ± 0.05% at the intermediate stage, and 22.37 ± 0.08% at 3 weeks postweaning, for 22, 23, and 35 rats respectively.

Heart Cu concentrations in Experiments 2 and 3 (Table II) were severely reduced ($P < 0.001$) by Cu deficiency, but the decrease was less in the hearts of low-Cu rats treated with reserpine than in low-Cu controls ($P < 0.05$). The total Cu contents of hearts, although lowered by Cu deficiency ($P < 0.001$), were not significantly different in low-Cu rats with or without reserpine treatment.

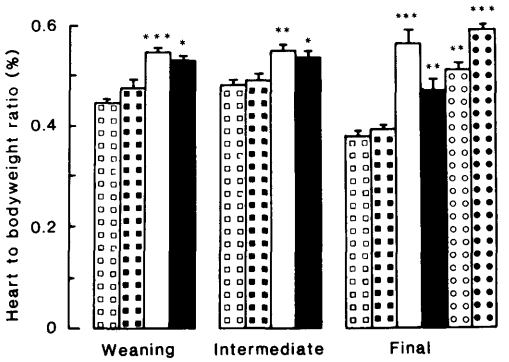

FIG. 2. Heart to body weight ratios (%) in Experiment 1. The rat groups are +Cu−R▦, +Cu+R▦, −Cu−R□, and −Cu+R■. The low-Cu group first reserpine-treated "near weaning" (W) is designated ▩ and that from 5 days later ("intermediate" treatment (I))▦. Time points are those of killing. The significances of the differences between "starred" groups and their appropriate +Cu controls are designated as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. Results are means ± SEM with $n = 6$ rats/group except in killings W, groups +Cu−R, and −Cu+R; I, −Cu−R, and F, +Cu+R where $n = 5$. At the final killing there was a significant difference ($P < 0.01$) between the −Cu−R group and the −Cu+R group which had been reserpine-treated from birth.

Rat heart NA concentrations were significantly depleted ($P < 0.001$) by Cu deficiency per se in both Experiments 2 and 3 (Table II), but treatment with reserpine also significantly reduced heart NA concentrations below control values in both Cu-adequate and Cu-deficient groups. Reserpine treatment resulted in notably lower NA concentrations in Experiment 3 probably because of the shorter time between the last injection of reserpine and the sacrifice of the rats (Fig. 1).

The normal ultrastructural appearance of the myocardium (+Cu−R, Experiment 2) is shown in Fig. 3a. A similar appearance was noted in the animals which were fed the control diet but subjected to reserpine administration (not shown). There was little difference seen between the two Cu-deficient groups (Figs. 3b and 3c). Thus, reserpine ap-

TABLE II. THE EFFECTS UPON HEART COPPER (Cu) CONCENTRATION, HEART TOTAL Cu, HEART NORADRENALINE CONCENTRATION, HEART TOTAL NORADRENALINE, AND HEART TOTAL NORADRENALINE TO BODY WEIGHT RATIO OF OFFERING RATS COPPER-SUFFICIENT OR -DEFICIENT DIETS WITH OR WITHOUT CONCURRENT RESERPINE (R) TREATMENT

Parameter	Experiment	Treatment +Cu −R	Treatment +Cu +R	Treatment −Cu −R	Treatment −Cu +R	Residual SD	Significance of treatment effects: Cu	Reserpine	Interaction
Heart copper (Cu) concentration (μg Cu/g wet tissue)	2	4.13 (7)	4.73 (7)	1.14 (8)	3.36 (5)	0.87	***	**	*
	3	5.00 (7)	6.03 (7)	1.40 (6)	3.52 (5)	1.42	***	*	NS
Heart total Cu (μg)	2	2.67 (7)	2.50 (7)	1.04 (8)	1.64 (5)	0.55	***	NS	NS
	3	3.31 (7)	2.90 (7)	1.24 (6)	1.68 (5)	0.87	***	NS	NS
Heart noradrenaline (NA) concentration (ng NA/g wet tissue)	2	694 (8)	535 (8)	431 (8)	352 (5)	149.6	***	*	NS
	3	659 (7)	151 (7)	432 (6)	163 (5)	87.2	***	***	***
Heart total NA (ng)	2	441 (8)	277 (8)	395 (8)	172 (5)	93.7	*	***	NS
	3	440 (7)	78 (7)	304 (6)	80 (5)	70.7	*	***	*
Heart total NA:ng/135 g body wt[a]	2	398 (8)	329 (8)	427 (8)	230 (5)	127	NS	*	NS
	3	394 (7)	96 (7)	358 (6)	117 (5)	72	NS	***	NS

Note. Results are means of observations. Number of animals in parentheses.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.
[a] For further details, see Discussion.

peared to have no further effect on the heart ultrastructure of Cu-deficient rats. There were, however, considerable differences in ultrastructure when Cu-deficient hearts were compared with control hearts. The most obvious change was the large increase in area occupied by the mitochondria which led to displacement and distortion of the myofibrils. The individual mitochondria of the two −Cu groups also appeared larger than normal and there was some evidence of disruption of the mitochondrial fine structure. In the +Cu hearts mitochondria occupied 44% of the combined area of mitochondria and myofibrils, which together accounted for approximately 95% of the total area of the tissue, but in the Cu-deficient hearts the area of the mitochondria increased to 58% (Table III). There was thus a 32% increase in the area occupied by mitochondria in the Cu-deficient hearts, relative to the control hearts. The results of the assays for cytochrome c_1 as an index of mitochondria in perfused hearts, from Experiment 1, are shown in Table III.

Copper deficiency significantly reduced blood hemoglobin (Hb) and heart mitochondrial SDH activity (Table IV). Heart Fe concentration and blood PCV (Table IV) were also significantly reduced in Experiment 2, with a similar trend in Experiment 3. However, total Fe contents of hearts were not altered by the deficiency—the decline in concentration was largely balanced by the increase in size of the hearts.

Discussion. The present experiments were designed to ascertain if the cardiac enlargement associated with Cu deficiency could be overcome by reserpine treatment and whether such an effect could be related to altered heart NA concentrations.

Copper deficiency increased not only the wet weight of the heart but also produced a corresponding increase in dry weight such that the percentage dry matter was unaltered by the deficiency. Cardiac enlargement was thus not caused by excessive water uptake by the heart.

In the Cu-depleted heart, the cytochrome c_1 concentration increased (Table III). This cytochrome forms part of the mitochondrial membrane and therefore may be used as a marker for mitochondria. The increase from 9.9 to 13.3 nmole/g tissue represented a 34% increase in the mitochondrial compartment

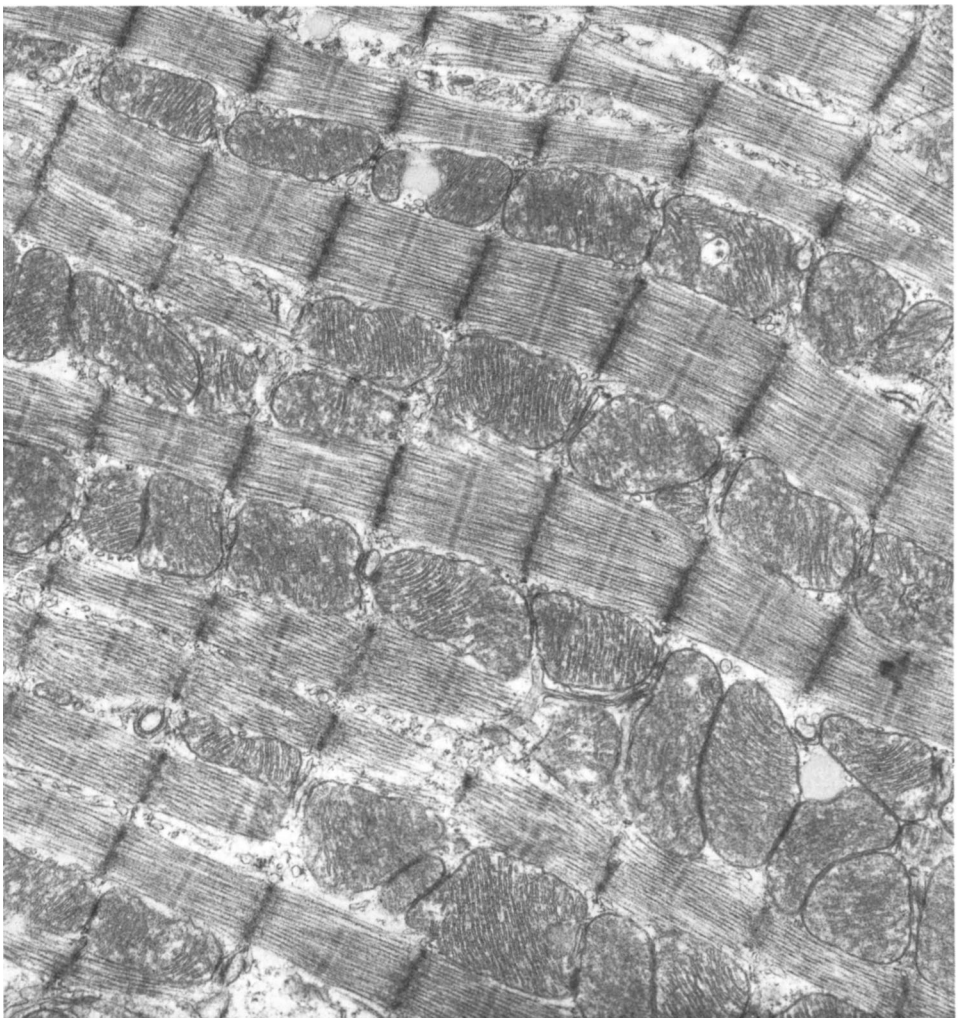

FIG. 3a. Myocardium of normal rat (+Cu−R) showing orderly arrangement of rows of mitochondria and myofibrils. The area occupied by the myofibrils exceeds that of the mitochondria. ×14970.

of the heart in Experiment 1. In the absence of estimates of the cytochrome c_1 content of the mitochondria, these figures for c_1 per gram tissue could not be used to estimate the weight of mitochondria per gram tissue. However, the relative increase in cytochrome c_1 agreed well with the 32% increase in mitochondrial area induced by Cu deficiency in the hearts from Experiment 2. It therefore seemed appropriate to use the values for mitochondrial areas to estimate the volume or weight fractions of the heart occupied by the mitochondria. The mean heart weight of the four +Cu controls used for electron microscopy studies was 704 mg, which on the basis of areas comprised 310 mg mitochondria and 394 mg of myofibrils per heart. The corresponding figures for −Cu hearts were 895 mg consisting of 519 mg mitochondria and 376 mg of myofibrils. However, the rats of the two groups had different body weights, their mean weights being 153 g for +Cu rats and 116 g for deficient rats. This difference in size would be reflected in their heart

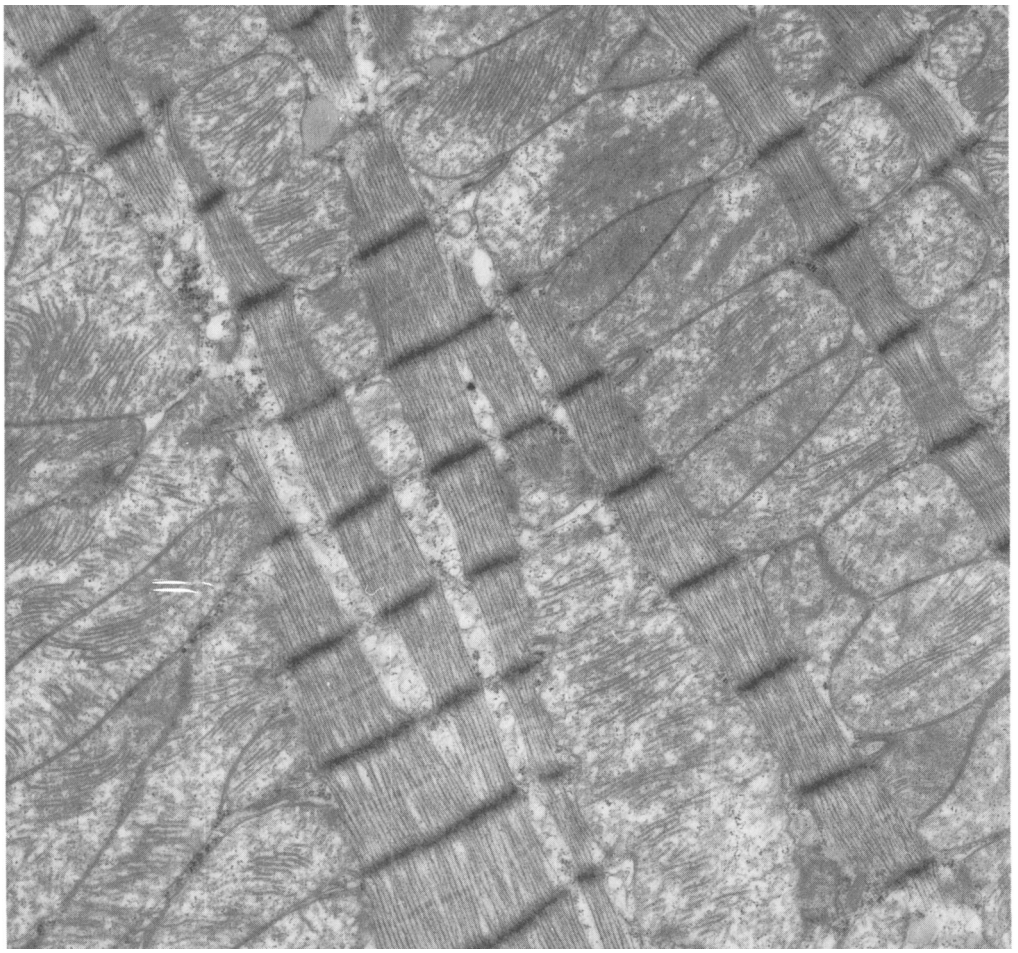

FIG. 3b. Myocardium of copper-deficient rat (−Cu−R). The area occupied by the mitochondria is increased and exceeds the area occupied by the myofibrils. The myofibrils are displaced by the enlarged mitochondria. ×14970.

weights regardless of Cu status. Assuming heart weight to be proportional to body weight the hearts of +Cu rats weighing 135 g would have contained 274 mg mitochondria and 345 mg myofibrils while for Cu-deficient rats the figures would have been 605 mg and 436 mg respectively (Table III). Therefore, although the heart enlargement of Cu-deficiency is generally referred to as hypertrophy, the above figures suggest that the effect of Cu deficiency on the heart is very largely one of mitochondrial hypertrophy. This confirms and extends the findings of Goodman *et al.* (2). These authors also reported a similar effect on the enlarged hearts of Fe-deficient rats (2).

Copper concentrations and total Cu were low in hearts from Cu-depleted rats. However, although reserpine treatment increased Cu concentration, it did not significantly increase the total Cu in the heart. Thus, reserpine influenced heart size rather than Cu supply.

Noradrenaline concentration was depleted in the enlarged hearts of Cu-deficient rats as has been previously recorded (16, 17). However reserpine treatment, which normalized heart weight in Cu-deficient rats, caused a

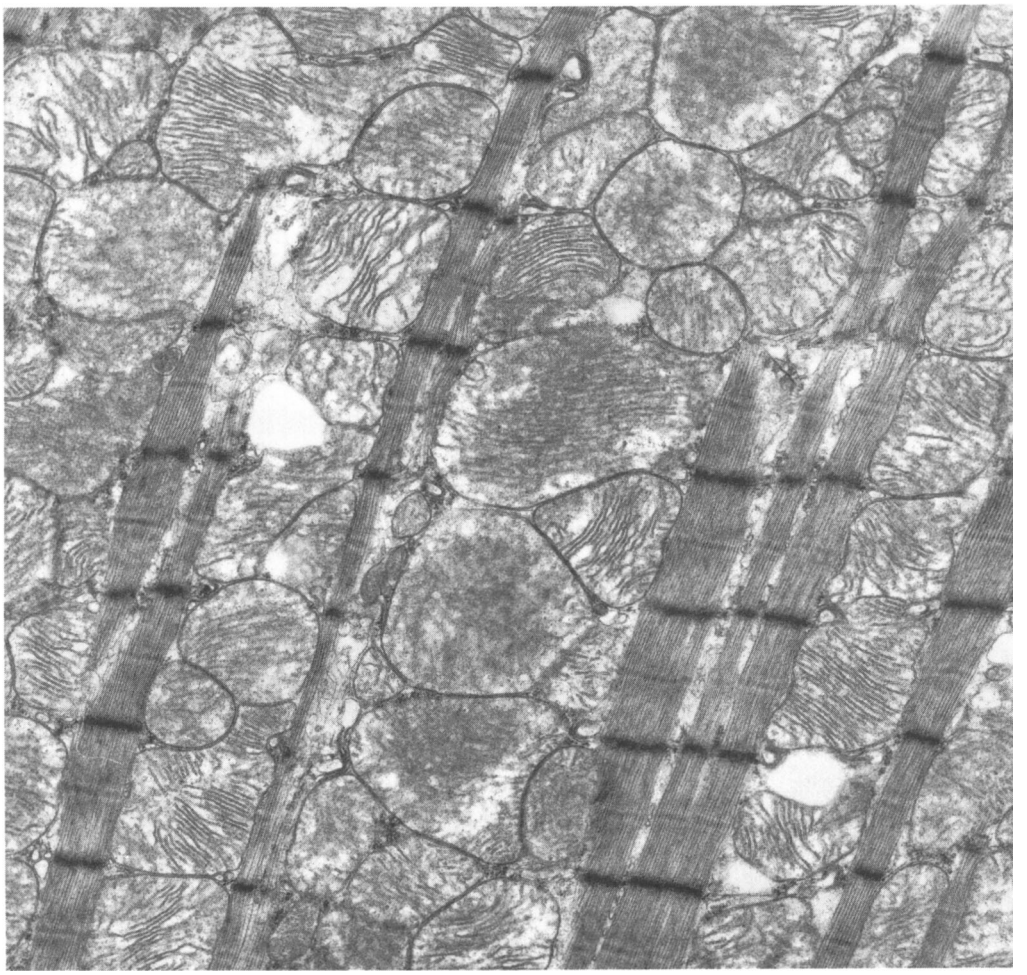

FIG. 3c. Myocardium of copper-deficient rat treated with reserpine (−Cu+R) showing the same appearance as that on Fig. 3b (−Cu−R). ×14970.

further reduction of NA concentration. After correction for differences in body size the total quantity of NA in the heart was actually unaltered by Cu deficiency (Table II). In contrast, reserpine administration significantly reduced total NA in the hearts of Cu-deficient rats. It therefore seems unlikely that NA was directly associated with the cardiac enlargement of Cu deficiency. Furthermore, reserpine did not reverse the change in mitochondrial/myofibrillar ratio (Table III). Therefore, the effect of reserpine on cardiac enlargement was due to a generalized reduction in heart size rather than the result of overcoming the defects caused by Cu deficiency.

The effects of reserpine on the hearts of Cu-deficient rats were therefore similar to those on Fe-deficient rats (7) in that there was a further reduction in NA concentration and a normalization of heart size.

In the rats which did not receive reserpine, Cu deficiency tended to reduce blood PCV and significantly reduced Hb levels (Table IV). These data suggest some impairment of Fe metabolism in this study although liver Fe concentration was still unaffected. The total Fe of hearts was not decreased but Fe con-

TABLE III. THE EFFECTS UPON HEART CYTOCHROME C_1 CONCENTRATIONS AND MITOCHONDRIAL AND MYOFIBRILLAR FRACTIONS OF OFFERING RATS COPPER-SUFFICIENT OR -DEFICIENT DIETS WITH OR WITHOUT CONCURRENT RESERPINE (R) TREATMENT

Parameter	Treatment				Residual SD	Significance of treatment effects		
	+Cu		−Cu					
	−R	+R	−R	+R		Cu	Reserpine	Interaction
Cytochrome c_1[a]	9.9 (5)	12.8 (6)	13.3 (6)	16.4 (6)	2.51	**	*	NS
Mitochondria per heart (mg)[b]	274	267	605	417	53.5	***	**	**
Myofibrils per heart (mg)[b]	345	382	436	282	52.8	NS	*	**

Note. Results are means of observations. For heart "fraction" data only the first four rats of each group in experiment 2 were sampled; four replicates from each rat heart. For cytochrome data, number of animals in parentheses. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, not significant.

[a] Calculated as nmole/g wet tissue.

[b] Calculated as mg per 135 g body wt using appropriate EM areas. For further details, see Discussion.

centration was. Copper deficiency also produced a significant decline in activity of the heart mitochondrial respiratory enzyme SDH for which nonheme Fe is required. One explanation for this reduction may be that delivery of Fe to mitochondria was impaired and that SDH activity was then compromised. A similar reduction in SDH activity also occurs in the enlarged hearts of Fe-deficient rats (18). Our finding of reduced SDH in Cu deficiency is at variance with that of Kelly *et al.* (3). These authors, however, estimated SDH by a histochemical method.

Both the decline in SDH and the previously observed reduction of cytochrome-*c*-oxidase activity (2, 3) in Cu-deficient hearts suggest a potential decline in energy production in the mitochondrion.

Our results therefore indicate that mitochondrial enlargement may be related to an energy deficit occurring in the Cu-deficient heart, as has been previously suggested (16,

TABLE IV. THE EFFECTS UPON HEART IRON (Fe) CONCENTRATION, HEART TOTAL Fe, BLOOD HEMOGLOBIN, PACKED RED CELL VOLUMES, AND HEART SUCCINIC DEHYDROGENASE OF OFFERING RATS COPPER-SUFFICIENT OR -DEFICIENT DIETS

Parameter	Experiment	Treatment		Significance[a]
		+Cu	−Cu	
Heart iron (Fe) concentration (μg Fe/g wet tissue)	2	74.6 ± 2.43 (7)	56.6 ± 3.93 (8)	**
	3	69.7 ± 4.27 (7)	59.6 ± 5.33 (6)	NS
Heart total Fe (μg)	2	48.1 ± 2.50 (7)	54.0 ± 6.80 (8)	NS
	3	47.0 ± 2.55 (7)	52.4 ± 4.46 (6)	NS
Hb (g/100 ml)	2	10.2 ± 0.27 (8)	8.0 ± 0.40 (8)	***
	3	12.6 ± 0.77 (6)	9.2 ± 0.14 (6)	**
PCV (%)	2	39.3 ± 0.80 (8)	29.1 ± 1.46 (8)	***
	3	39.0 ± 1.43 (8)	35.9 ± 1.17 (6)	NS
SDH (μmole/min/g wet wt)	1	9.36 ± 1.72 (5)	3.47 ± 0.26 (5)	**

Note. Results are means of observations ± SEM. Number of animals in parentheses. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; NS, not significant.

[a] Significance between groups tested using student's *t* test.

19), and that NA has no direct role in the establishment of the defect. Whether the energy deficit results directly from Cu deficiency or is secondary to an induced Fe deficiency remains to be investigated.

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