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The Effects of Manganese Deficiency During Prenatal and Postnatal Development on Mitochondrial Structure and Function in the Rat

SHERI ZIDENBERG-CHERR, CARL L. KEEN, AND LUCILLE S. HURLEY*

Department of Nutrition, University of California, Davis, California 95616

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ABSTRACT

The influence of manganese deficiency on liver trace element concentration, MnSOD activity, and mitochondrial structure and function during postnatal development was determined in rats. In both normal and manganese-deficient animals, liver manganese concentration increased with time, but in deficient rats liver manganese was lower than in controls at all ages measured. At 9 mo of age, liver manganese concentration in the deficient rats was only 20% that of controls. The developmental pattern observed for MnSOD paralleled that of liver manganese concentration in normal and deficient rats; it was lower than in controls on days 20 and 60. However, at 9 mo of age, MnSOD levels were similar in the two groups. Although there were no differences at 9 mo of age in MnSOD activity between the groups, manganese-deficient rats showed mitochondrial abnormalities in liver. Despite mitochondrial abnormalities, however, oxygen uptake and P/O ratios were normal. We suggest that the mitochondrial damage apparent at 9 mo of age is, at least in part, the result of lower than normal MnSOD activity occurring earlier. The functional significance of the abnormalities remains to be established.

*Author to whom all correspondence and reprint requests should be addressed.

Index Entries: Manganese, and mitochondrial functions; MnSOD, and mitochondrial functions; lipid peroxidation, and Mn deficiency; mitochondrial function, in Mn deficient rats; development, and trace elements, in rats.

INTRODUCTION

Manganese is essential for all species investigated so far, including rats, mice, rabbits, guinea pigs, pigs, poultry, sheep, goats, and cattle, and most recently, humans (*1,2*). Manifestations of its deficiency can include high neonatal death, impaired growth, skeletal abnormalities, depressed reproductive function, congenital ataxia, and defects in carbohydrate and lipid metabolism. Although it is evident that Mn is needed for several biological functions, its precise biochemical roles have not been delineated.

Manganese is involved in numerous biochemical reactions both as an integral part of metalloenzymes and as an enzyme activator. One of the manganese metalloenzymes in Mn-superoxide dismutase (MnSOD) (E.C. 1.15.1.1). Superoxide dismutases (SODs) function to protect the cell from free radical damage by catalyzing the following reaction: $O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2$ (*3*). SOD containing copper and zinc is found in the cytosol (CuZnSOD, MW 32,000), while SOD containing Mn is localized in the mitochondria. MnSOD isolated from chicken liver has a molecular weight of 80,000 and contains four subunits of equal size, each containing one atom of manganese (*3*).

The activity of MnSOD can be affected by dietary manganese status. Dietary deficiency of manganese resulted in a reduction in liver MnSOD activity in rats (*4,5*), mice (*6*), and chickens (*6*). In addition to diet, environment has also been shown to influence MnSOD; conditions that cause an increased production of superoxide radicals, such as exposure to hyperbaric oxygen (*7*), ozone, or ethanol, result in an increased level of MnSOD in rats and primates (*8–10*). The correlation of increased production of superoxide radicals and higher tissue MnSOD activity suggests a compensatory reaction by the animal. Taken together, these findings support the hypothesis that MnSOD provides protection to biological systems against oxygen and oxygen radical mediated toxicity.

As the mitochondrial fraction of the cell contains a major portion of the cellular Mn (*11*), it is reasonable to suggest that mitochondrial structure and/or function may be influenced by manganese status. Mitochondrial ultrastructural abnormalities have been observed in aged manganese-deficient mice. In liver, kidney, heart, and pancreas, mitochondria were elongated and stacked upon each other with crystae parallel to the outer membrane, in contrast to the normal perpendicular arrangement. Polymorphism, gigantism, and membrane damage were phenomena commonly observed in tissue from these animals (*12*).

Alterations of mitochondrial integrity can result in a reduction of coupled phosphorylation and an increase or decrease in oxygen consumption (*13*). Hurley and colleagues (*14*) reported that oxygen uptake of mitochondria from aged manganese-deficient mice was lower than normal; however, there were no differences in the ratios of ATP formed to oxygen consumed (P/O). There have been several reports that manganous ions together with ATP, ADP, or AMP can reverse or prevent swelling of mitochondria (*15–17*). Since swelling is associated with the functional capacity of mitochondria, it is possible that in Mn-deficient animals, mitochondria may be functionally compromised. Hunter et al. (*18*) have correlated mitochondrial swelling with the formation of lipid peroxides; one molecule of phospholipid peroxidized in 600 can cause detectable swelling (*19*). A reduction in the activity of the mitochondrially localized enzyme MnSOD therefore can interfere with the maintenance of normal structure and function in the manganese deficient animal.

Previous work from our laboratory has shown that in the rat, dietary Mn deficiency resulted in lower than normal activity of MnSOD and higher than normal levels of mitochondrial lipid peroxidation by 60 d of age (*5*), supporting the hypothesis that mitochondrial damage occurs in manganese-deficient animals because of depressed MnSOD activity leading to increased lipid peroxidation in the membranes (*20*). If this hypothesis is correct, a correlation between the age of onset of such mitochondrial lesions and reduced activity of MnSOD should be seen. We have therefore studied liver manganese concentration, MnSOD activity, and mitochondrial structure and function during postnatal development in manganese deficient and normal rats.

MATERIALS AND METHODS

At 21 d of age, weanling female Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) were randomly assigned to one of two dietary treatment groups and fed a purified diet containing manganese at either 45 (control) or 1 μg/g (deficient). The diet contained 30% casein, 54.5% cerelose, 8% corn oil, 6% salt mix, and 1.5% vitamin mix. The detailed compositions of the vitamin and mineral mixes have been published previously (*5*). Diet and distilled water were provided *ad libitum*. The rats were individually housed in suspended stainless-steel cages in a temperature and light controlled room (22–23°C, 12 h light/dark cycle).

When the animals reached sexual maturity (approximately 60 d of age), they were mated overnight with stock-fed (Purina Rat Chow, Ralston Purina Co., St. Louis, MO) males. Pregnancy was confirmed by the presence of a copulatory plug, which was defined as day 0 of pregnancy. On day 0 of pregnancy and throughout the remaining experimental period, the rats previously fed the diet containing manganese at 1

μg/g were fed a diet of the same composition except that the manganese concentration was 3 μg/g [it was necessary to change the dietary Mn from 1 to 3 μg/g because in dams fed 1 μg/g, 90% of the offspring died during the first 7 d postpartum (*21*)]. The rats in the control group continued to receive dietary Mn at 45 μg/g. Daily food intake and weekly weight gain were measured in both groups during pregnancy. Body weight and survival of young from both groups were recorded from birth to weaning (25 d of age). At weaning, the young were housed individually in suspended stainless-steel cages. Rats continued to receive the same diets fed to their mothers. Offspring were killed by decapitation on 3, 20, and 60 d postpartum and at 9 mo of age; liver samples were taken for measurement of SOD activity, manganese concentration, oxygen uptake, and oxidative phosphorylation, and for transmission electron microscopy.

SOD Activity

Liver homogenates (10%) were prepared in cold 0.25*M* sucrose and sonicated for 2 min (30 s with 30 s cooling) with an Insonator Model 500 (Savant Instruments Inc., Hicksville, NY). Following sonication, the homogenates were centrifuged at 10,000*g* for 30 min at 4°C. The pellet was discarded, and the assay was conducted on the supernatant. Total SOD activity was determined by its ability to inhibit the autooxidation of pyrogallol (*22*). Total SOD activity was determined in 50 m*M* triscacodylic acid, 1 m*M* diethylenetriamine pentaacetic acid, pH 8.2 at 25°C. MnSOD activity was measured under the same conditions with the addition to the assay buffer of 1 m*M* potassium cyanide, which inhibits CuZnSOD. CuZnSOD activity was calculated by subtracting MnSOD activity from total activity. One unit of SOD was defined as the amount of enzyme needed to obtain 50% inhibition of pyrogallol autooxidation. The unit activity was computed by plotting the reciprocal of the slope of pyrogallol autooxidation versus the reciprocal of the volume of sample used for the assay.

Liver Trace Mineral Analysis

Tissue samples were wet ashed with 16*N* nitric acid, concentrated by evaporation, and diluted with distilled deionized water (*23*). Liver iron, copper, and zinc were determined by flame atomic absorption spectrophometry (IL551, Instrumentation Laboratories, Wilminton, MA). Manganese concentration was determined by flameless atomic absorption spectrophotometry (IL 551 in conjunction with the IL 55B flameless atomizer). Using these methods, recovery of added metal is 98 ± 2% (*24*).

Respiratory Control

Isolated mitochondria were prepared immediately according to the method of Hogeboom (*25*). Livers were homogenized in 5 m*M* MOPs buffer containing 250 m*M* mannitol and 0.5 m*M* EGTA at low speed

using a loose-fitting Elvehjem tissue homogenizer. The crude homogenate was centrifuged twice at 700*g* for 10 min at 0°C and the resulting supernatant was centrifuged at 12,000*g* for 10 min. The 12,000*g* pellet was suspended in the reaction medium containing 75 m*M* MOPS, 5 m*M* K_2HPO_4, 1 m*M* EGTA, 15 m*M* KCl, 16.6 m*M* $KHCO_3$, and 20 m*M* succinate. Oxygen uptake was determined by the polarographic assay of oxygen after the method of Chance and Williams (*26*) using succinate as the substrate in the reaction medium. A Gilson oxygraph was used to determine oxygen uptake and P/O ratios. The P/O ratio was calculated by determining the amount of oxygen consumed per unit ADP added to the reaction mixture. The concentration of protein was determined by a dye binding assay (Bio Rad, Richmond, CA).

Mitochondrial Ultrastructure

A 2 mm section of median lobular hepatic tissue was excised and minced in cold 3% glutaraldehyde buffered in 0.1*M* sodium cacodylate, pH 7.4, and fixed for 1 h. Fixation in glutaraldehyde was followed by fixation in 1% osmium tetroxide for 1 h. Fixed samples were dehydrated in graded acetone and embedded in Spurr's plastic (*27*). Thin sections were cut with a diamond knife on a 2128 LKB ultramicrotome and floated in a water bath. Sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 109 transmission electron microscope. Tissues from manganese deficient and control animals were always taken together and processed simultaneously.

RESULTS

Weight Gain and Food Intake During Gestation

Weight gain (Table 1) and caloric intake (data not shown) of the dams fed Mn deficient diets (3 μg/g) during pregnancy did not differ from those of the controls. Both groups consumed approximately 80 kcal/d during the first 2 wk and increased their caloric intake to approximately 110 kcal/d during the last week of gestation.

Physical Characteristics of the Offspring

Despite the lack of differences in food intake and weight gain during pregnancy, birth weight of offspring of Mn-deficient dams was 10% lower than that of offspring from control dams (Table 1). Manganese deficiency did not influence the number of pups born per litter. Survival to weaning was lower in offspring of Mn-deficient dams than in controls. Approximately 86% of the offspring born to control dams survived to weaning (day 25) whereas only 53% of those born to Mn-deficient dams survived to this age (Table 1).

TABLE 1
Effect of Manganese Deficiency on Maternal Weight Gain, and Birth Weight, Survival to Weaning, and Swimming Ability of the Offspring

	Control	Deficient
Pregnant females, No.	9	9
Pups/litter, No.[a]	10.0 ± 2	11.3 ± 0.7
Weight gain[b]	147.1 ± 15	140.1 ± 0.5
Birth weight[c]	6.6 ± 0.1	6.0[e] ± 0.1
Survival to weaning,[c] %	86	53
Able to swim[d]	100	6

[a]Mean ± SEM.
[b]Gross weight gain (grams) of dams from day 0 to day 21 of pregnancy (not excluding litters).
[c]$\frac{\text{Young alive at 25 d}}{\text{Live young born}} \times 100$.
[d]Percent of litter with swimming ability at 25 d of age.
[e]Significantly different from control animals at $p < 0.05$.

The surviving offspring exhibited signs typically observed in Mn-deficient rats, including retraction of the head and the inability to right themselves from a supine position (*1*). At 25 d of age they were examined for their body-righting ability by the swimming test (*1*). Only 6% of the Mn-deficient offspring were able to swim compared to 100% with swimming ability in the control group (Table 1).

The offspring of Mn-deficient females also showed less weight gain. Pup weight was significantly lower in the Mn-deficient group at all days measured than in controls. By day 21, pup weight in the deficient group was only 66% that of the pups in the control group (Fig. 1).

Liver Manganese Concentration and MnSOD Activity

Liver Mn concentration in deficient offspring was lower than that of controls at all times measured (Table 2). In control offspring, liver Mn concentration increased over threefold between 3 d and 9 mo of age, with values (means ± SEM) of 0.72 μg Mn/g tissue and 2.40 ± 0.1 μg Mn/g tissue, respectively. Liver Mn concentration in deficient offspring increased fivefold during this time period, but was only 0.1 ± 0.06 μg Mn/g at 30 d of age and 0.5 ± 0.3 μg Mn/g at 9 mo of age.

The activity of liver MnSOD is shown in Fig. 2. (Some of these values have been reported previously.) Through 60 d of age, the activity of MnSOD paralleled that of liver Mn; offspring from both Mn control and Mn-deficient dams had increasing values during this time period. By day 20, values for MnSOD activity tended to be lower in deficient rats than in controls; however this difference was not significant until day 60 (615 ± 15 U/g liver, 288 ± 25 U/g liver for control and deficient rats, re-

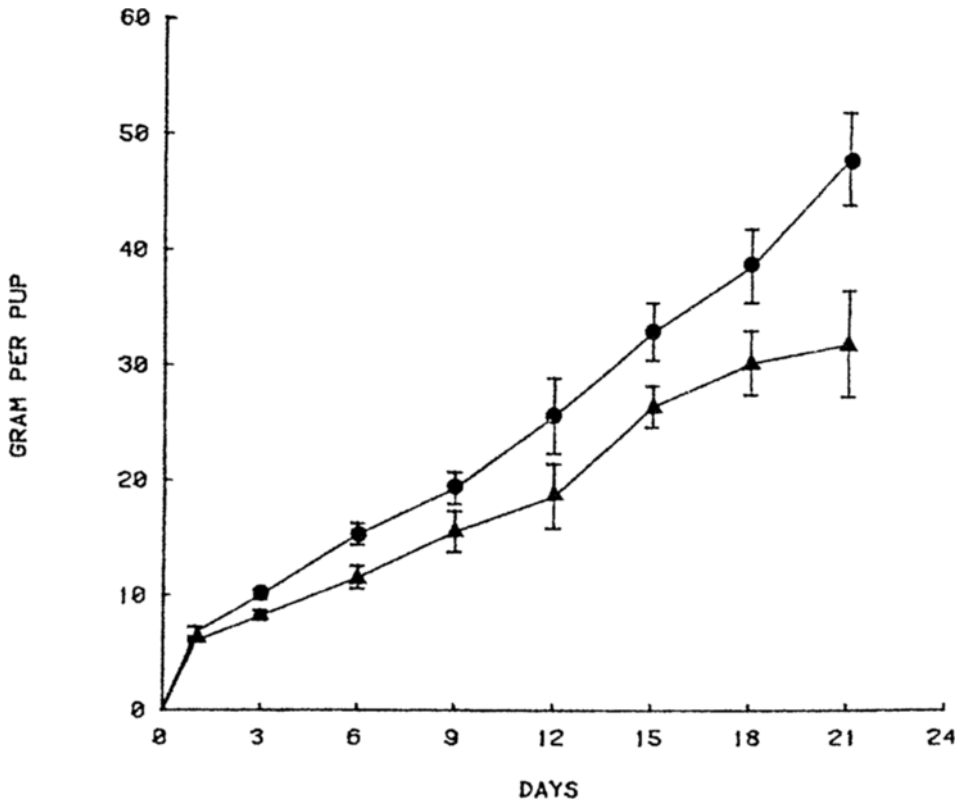

Fig. 1. Postnatal weight gain of offspring from control (●) and Mn-deficient (¡) rats from birth through 21 d of age. Each point represents the mean weight per pup in nine litters for controls and seven litters for deficient animals.

spectively). At 9 mo of age, MnSOD activity had decreased in both control and deficient animals (240 ± 26 U/g, 188 ± 20 U/g liver, respectively).

Liver Iron, Copper, and Zinc Concentrations

Iron levels observed in liver from 9-mo-old Mn-deficient rats were 45% higher than in 9-mo-old control animals (Table 2). The postnatal changes in liver iron concentration were similar in Mn deficient and control rats and seemed to occur in three stages. Liver iron was highest in the very young rat (323.4 ± 31.3 and 330.9 ± 28 μg/g for control and deficient day 3 rats, respectively, decreasing to much lower values measured at day 20 (90.48 ± 8.4 and 95.5 ± 28 μg/g for control and deficient rats, respectively). At day 60, liver iron had reaccumulated and continued to increase as evidenced by the values observed in 9-mo-old animals.

Liver copper concentration decreased from 3 d of age (24.6 ± 3.4 and 29.2 ± 0.6 μg Cu/g for control and deficient rats, respectively), to day 60 (4.49 ± 0.3 and 4.14 ± 0.4 μg Cu/g for control and deficient rats, respec-

TABLE 2
Liver Trace Element Concentration[a,b]

Diet group	Age	Mn, μ/g	Fe, μg/g	Cu, μg/g	Zn, μ/g
Control	3 d	0.72 ± (0.2)	323.4 ± (32)	24.57 ± (3.4)	67.18 + (2.8)
Deficient	3 d	0.10* ± (0.06)	330.9 ± (28)	29.17 ± (0.6)	69.04 ± (10.0)
Control	20 d	1.77 ± (0.3)	90.5 ± (8.4)	14.97 ± (1.5)	35.33 ± (1.5)
Deficient	20 d	0.78* ± (0.8)	95.5 ± (29)	16.56 ± (1.9)	36.63 ± (2.6)
Control	60 d	2.49 ± (0.51)	148.6 ± (15)	4.49 ± (0.3)	34.94 ± (1.8)
Deficient	60 d	0.73* ± (0.10)	195.0 ± (13)	4.18 ± (0.3)	31.91 ± (2.4)
Control	9 mo	2.40 ± (0.1)	287.1 ± (22)	4.0 ± (0.2)	17.1 ± (0.6)
Deficient	9 mo	0.51* ± (0.3)	418.1* ± (21)	5.9* ± (0.8)	16.2 ± (1.0)

[a]Wet weight of liver; mean ± (SEM) of at least five rats.
[b]Significantly different from the corresponding age group fed control diet at $p < 0.05$ indicated by an asterisk.

tively). Control rats maintained similar copper concentration at 9 mo as observed at day 60; in contrast, copper concentration in livers from deficient rats was 30% higher at 9 mo of age than at day 60, and were significantly higher than levels observed in control rats.

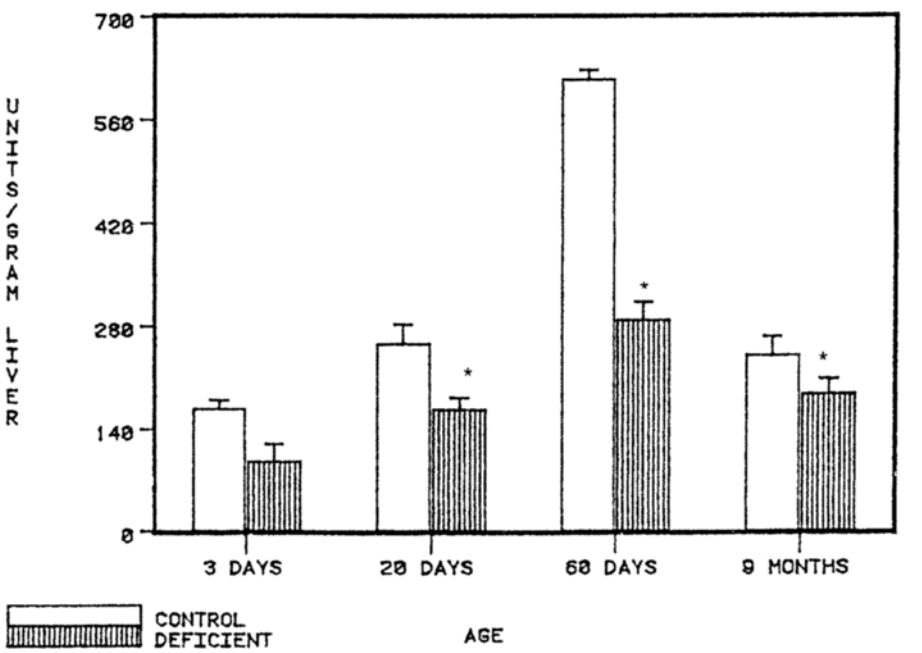

Fig. 2. Liver MnSOD activity (U/g liver) in control (□) and Mn-deficient (▨) offspring at 3, 20, and 60 d and 9 mo of age. Each point represents the Mean + SEM of at least four rats. Significant differences from the controls of the corresponding age group at $P < 0.05$ is indicated by an asterisk.

In both control and deficient rats, zinc concentration was highest at day 3, and by 9 mo of age, zinc levels were 25% of values observed in the neonate. There were no differences between control and deficient rats.

Respiratory Control

The average respiratory control index (RCI; ratio of state 3 to state 4 respiration) for isolated mitochondria was 3.5, with a range of 3.0–4.0, indicating that the mitochondria were properly isolated. There were no differences between control and deficient animals for oxygen uptake or P/O ratios at any time tested (Table 3). In both control and deficient animals, O_2 uptake at 9 mo of age was approximately 65% lower than at 60 d of age.

Mitochondrial Ultrastructure

Livers from control and deficient rats from day 3 to day 60 exhibited normal ultrastructure (Fig. 3–6). At 9 mo of age, liver from three of the four manganese-deficient rats showed abnormal mitochondria, whereas those of control rats had normal ultrastructure. Representative micrographs are shown in Fig. 7–10. In the deficient animals, large vacuoles were present in the matrix of many mitochondria. The inner and outer mitochondrial membranes often separated from each other, creating open spaces.

DISCUSSION

Results from this study show that Mn deficiency during gestation results in lower than normal birth weight of the offspring. Additionally, survival to weaning was significantly reduced. Manganese has previously been shown to be necessary for optimal growth and survival in

TABLE 3
Oxidative Phosphorylation and Oxygen Uptake in Liver Mitochondria from Manganese-Deficient and Control Rats

Group	Age	O_2 uptake[a]	P/O ratio[b]
Control	60 d	87.5 ± 11.1	1.2 ± 0.1
Deficient	60 d	111.8 ± 20.9	1.0 ± 0.1
Control	9 mo	30.2 ± 1.64	1.5 ± 0.2
Deficient	9 mo	34.3 ± 2.8	1.3 ± 0.2

[a]Oxygen uptake is measured in nanomoles of oxygen consumed per minute per milligram of mitochondrial protein.
[b]μmoles of ADP added per μmoles of O_2 consumed.

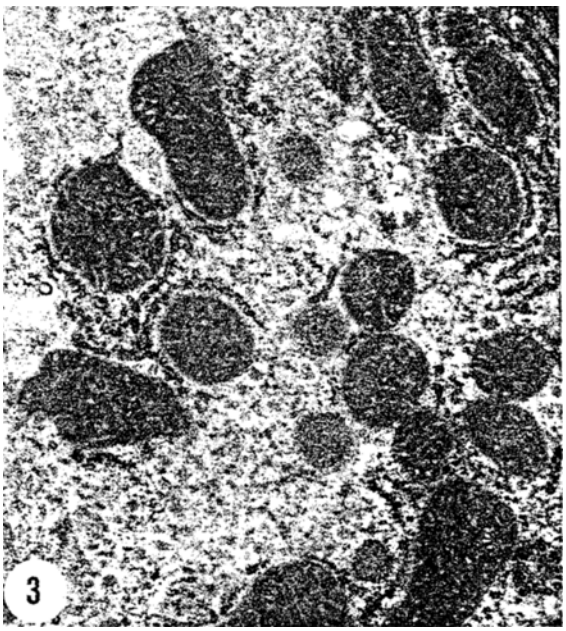

Fig. 3. Liver mitochondria from a control rat, 20 d of age, showing normal cristae parallel to the outer mitochondrial membrane. ×23,000.

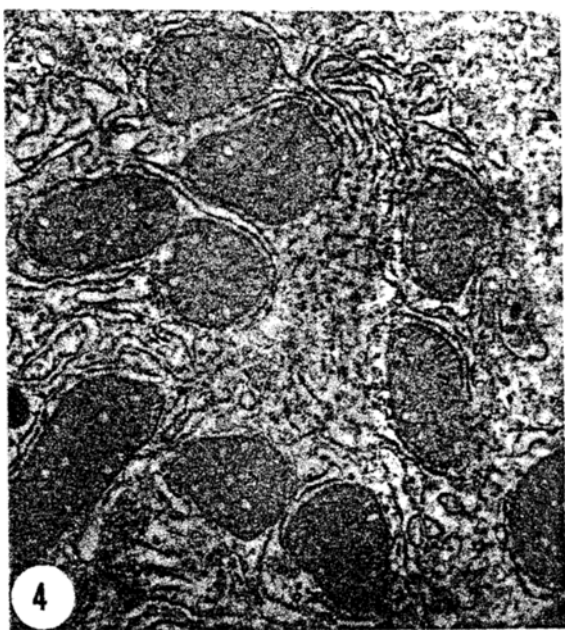

Fig. 4. Liver mitochondria from a deficient rat, 20 d of age, showing normal ultrastructure. ×24,000.

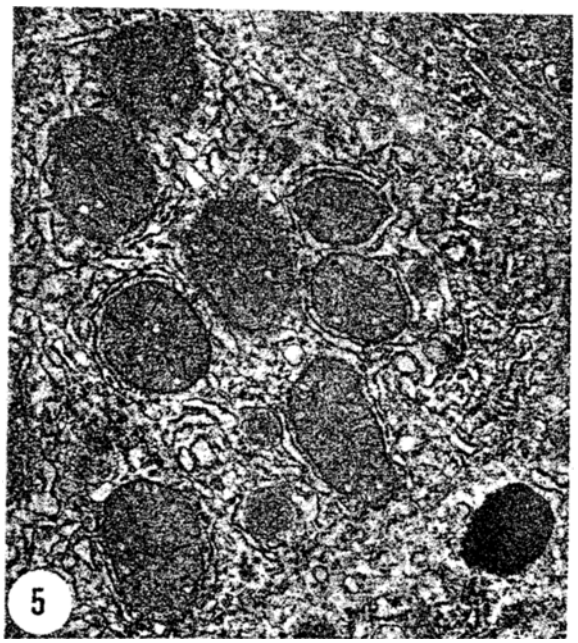

Fig. 5. Liver mitochondria from a control rat, 60 d of age, showing normal ultrastructure. ×24,000.

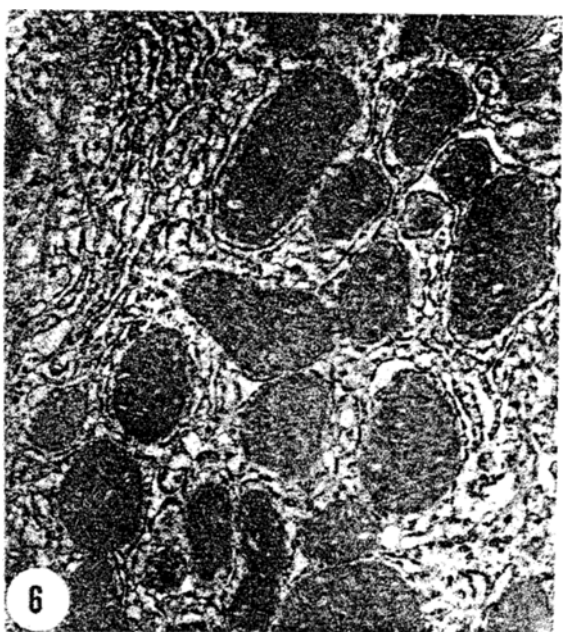

Fig. 6. Liver mitochondria from a deficient rat, 60 d of age, showing normal ultrastructure. ×29,000.

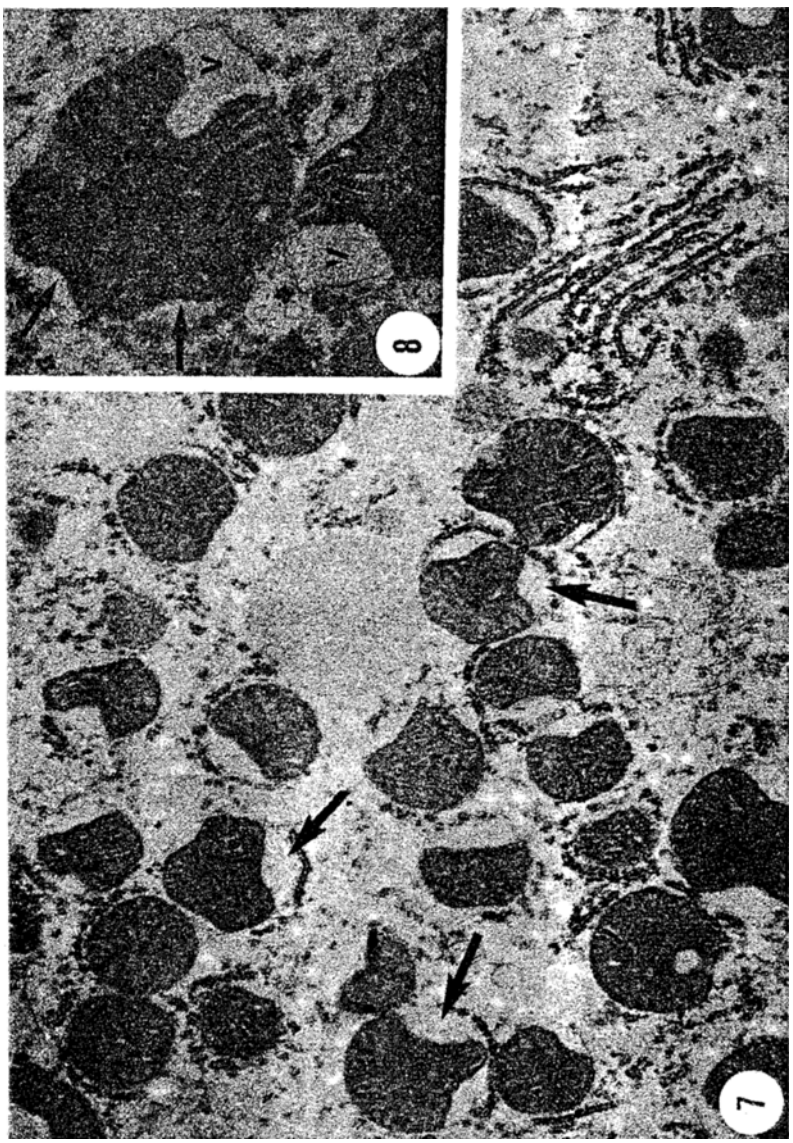

Fig. 7. Liver mitochondria from a deficient rat, 9 mo of age. Inner and outer mitochondrial membranes are separated creating open spaces (arrows). Lack of detail is observed in the cytoplasm. ×18,000.

Fig. 8. Higher magnification of mitochondria from a deficient rat, 9 mo of age. Open spaces (arrows) and vacuoles (v) are seen in the matrix. ×56,000.

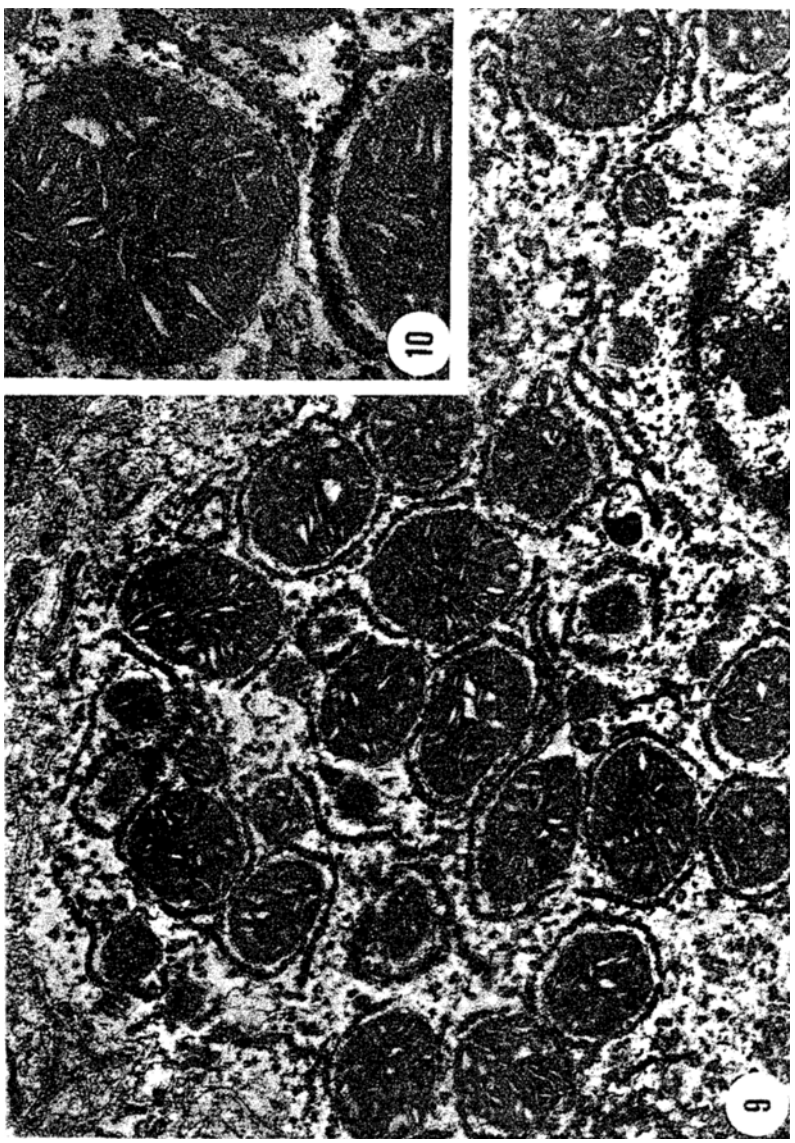

Fig. 9. Liver mitochondria from a control rat, 9 mo of age. Mitochondria appear normal. ×17,000.

Fig. 10. Higher magnification of mitochondria from a control rat, 9 mo of age. ×42,000.

mice and rats. Shils and McCollum (*28*), using rats, reported a 93% mortality of Mn-deficient offspring within 48 h of birth. Similarly, Hurley et al. (*29*) observed that the percentage of rat pups found dead at birth was significantly higher in litters from dams fed manganese-deficient diets than in offspring of dams fed Mn-adequate diets. Survival of the remaining pups to 28 d was also considerably lower than in controls. Similar survival data for mice have been reported by Bell and Hurley (*12*) and de Rosa et al. (*6*). In the current study, we observed a depression in the postnatal growth rate in the manganese deficient pups. In contrast, Bell and Hurley (*12*) using mice as the experimental model, observed no depression in body growth of the offspring from Mn-deficient mice. These differences may be caused by varying responses to the deficient diet among species.

Reduced growth rates in mice and rats fed Mn-deficient diets during postnatal development only (weaning through adulthood) have also been reported (*30–32*). As maternal food intake and weight gain were not low in dams fed Mn-deficient diets during pregnancy in the present study, the lower birth weight in this group may be caused by preferential use of Mn by the dam, which may have interfered with normal skeletal development and growth of the fetus. Skeletal abnormalities resulting from pre- and early postnatal manganese deficiency have been reported in avian species and mammals (*1,33*). These abnormalities are thought to result from a generalized defect in mucopolysaccharide synthesis (*1*). In manganese-deficient animals, otoliths are missing or are only partially calcified (*34*), resulting in ataxia of the offspring (*1,34*). In the present report, we observed ataxia as characterized by delayed righting reflexes in the Mn-deficient offspring.

The developmental pattern observed for liver Mn concentration paralleled that for MnSOD activity. Both control and deficient animals exhibited a rise in Mn concentration and MnSOD activity from birth through 60 d of age. These results differ from those of Widdowson et al. (*35*) and Bruckman and Zondek (*36*), who reported that in human liver there was a relatively constant concentration of manganese throughout life. However, these early studies employed colorimetric assays and flame atomic absorption analysis, whereas we have used flameless atomic absorption, increasing sensitivity approximately 100–1000-fold (*24*). Therefore the differences in results may be a consequence of the different methods used.

By 9 mo of age, in both control and deficient animals, liver Mn concentration was approximately the same as that observed at 60 d of age. In contrast, in control animals, the activity of MnSOD was 50% of values observed at 60 d of age. In the 9-mo-old animals, there was no difference in MnSOD activity between control and deficient animals. However, since the liver Mn concentration in the 9-mo-old dificient rat was 80% lower than in controls, questions arise concerning other functions of liver Mn. As a component of two other metalloenzymes, pyruvate carboxylase

and arginase, Mn has a significant role in carbohydrate metabolism and urea formation. If most of the available Mn is present as MnSOD, the activity of pyruvate carboxylase may be compromised, resulting in decreased gluconeogenic capacity. Baly and coworkers (*37*) have shown that pyruvate carboxylase activity is lower in adult fasted Mn-deficient rats than in fasted control rats. Reductions may be even more dramatic under conditions of oxygen stress following the ingestion of ethanol, which results in production of superoxide radicals during its metabolism. Roe et al. (*38*) demonstrated that prenatal ethanol exposure caused a lag in gluconeogenic enzyme development. Thus, stress on the gluconeogenic capacity, such as that which may result from manganese deficiency may lead to impaired gluconeogenesis in the newborn, making it more susceptible to neonatal hypoglycemia. Similarly, a deficiency of Mn may result in reduced arginase activity (*39*), resulting in high ammonia levels.

Our findings of lower levels of MnSOD in the 9-mo-old control rat than in the 60-d-old rat are intriguing when one considers the biochemical changes that occur with aging. One theory is that free radical-induced membrane damage contributes either directly or indirectly to the aging process. Our findings suggesting that reductions in MnSOD activity may occur after maturity in the rat support this approach.

The developmental pattern of liver iron was similar to that reported for mice by Keen and Hurley (*40*). We found that the rat is born with high levels of iron that subsequently decrease after 3 d of age. Leslie and Kaldor (*41*) reported a similar time lag in the mobilization of iron in the postnatal rat. It is generally accepted that the high concentration of this element in the liver of the newborn represents fetal storage, which compensates for the low concentration of iron in milk (*11*). By 60 d of age, liver iron had increased, possibly because of the higher concentration of iron in solid food than in milk. Liver iron was 50% higher in the 9-mo-old deficient rat than in the similarly aged control. Since iron has been implicated as a catalyst for lipid peroxidation either in the heme protein or nonheme forms (*42,43*), such high concentrations may contribute to excessive lipid peroxidation and subsequent membrane damage.

The developmental patterns of liver copper were similar in control and deficient rats until 9 mo of age. At this time, livers from deficient rats had higher levels of copper; we have suggested that this may be the result of a compensatory relationship between MnSOD and CuZnSOD, as CuZnSOD activity has been reported to be higher in Mn-deficient animals than in controls (*5*).

Liver zinc concentration, like liver copper, decreased with age. This developmental pattern differs from that observed in mice (*40*), but is similar to that of humans in which the newborn usually shows liver zinc concentration higher than the adult.

Although there were no differences at 9 mo of age in MnSOD activity, ultrastructural examination revealed mitochondrial abnormalities in

9-mo-old manganese-deficient rats. There were large vacuoles within the mitochondria. The inner and outer mitochondrial membranes often separated from each other, creating open spaces. Similar abnormalities have been observed in liver from patients with Wilson's disease (*44*), diseases of mitochondrial myopathy (*45*), and Adriamycin treatment (*46*). The underlying mechanisms of these changes are unknown; however, excessive lipid peroxidation has been suggested as a contributing factor. We suggest that the mitochondrial abnormalities observed in this study are at least in part the result of the lower MnSOD activity occurring at 60 d of age, accompanied by excessive mitochondrial lipid peroxidation. Since no structural abnormalities were apparent earlier, the resulting mitochondrial damage observed in this study may result from numerous factors contributing to structural damage over a period of time. Lipid composition of the membrane may be altered because of elevated lipid peroxidation. Additionally, since Mn is a cofactor for several lipogenic enzymes (*11*), a consequence of its deficiency may be abnormal lipid metabolism. Manganese is also a cofactor for enzymes functioning in cholesterol synthesis and fatty acid synthesis; thus alterations in the synthesis of these compounds could contribute to abnormal membranes.

Although the functional capacity of the mitochondria measured in this study was normal, biochemical abnormalities other than those of respiratory control may be present. For example, biochemical abnormalities such as lesions in the transport and utilization of substrates (*47–49*) and in the oxidation of NADH, cytochrome b, and cytochrome oxidase have been reported.

In conclusion, the results presented confirm that mitochondrial abnormalities occur in mature manganese-deficient rats. These findings suggest that damage to mitochondrial membranes produced by manganese deficiency may be the result of an earlier depression of MnSOD activity with concomitant elevated peroxidation observed at 60 d of age.

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