Letter to the Editor: Copper Metabolism in Menkes Disease

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We would like to thank Drs. Rennert and Chan for their comments on our paper (1). In their letter they describe their findings in Menkes disease and point out certain apparent differences, mainly quantitative, from our data. They quote a number of abstracts and papers in press, which do not provide us with the necessary information needed to interpret these differences. In these studies they have used skin fibroblasts cultured in the presence of various concentrations of nonradioactive copper or fibroblasts cultured in the presence of ^{64}Cu . Undoubtedly, the culturing conditions employed in these studies, e.g., type of culture medium, presence or absence of fetal calf serum or type and batch of fetal calf serum used, phase of fibroblasts (growth vs. stationary) etc., could affect the time course of the labeling experiments or the degree of the copper toxicity. We know that phosphate present in the medium and the fetal calf serum used in our studies precipitated some of the copper added to the culture medium. In addition, we did not indicate that cell toxicity occurred in Menkes fibroblasts only at medium copper concentration of 60 $\mu\text{g}/\text{ml}$. We reported that at 60 μg CuCl_2/ml , distinct signs of copper toxicity and cell death were apparent only in the Menkes fibroblasts. Measuring the rate of DNA synthesis in the presence of various concentrations of copper, we have demonstrated that DNA synthesis was reduced in the mutant cells even when they were cultured at 2 $\mu\text{g}/\text{ml}$. In contrast to the Menkes fibroblasts, normal fibroblasts cultured in CuCl_2 concentrations of 2–30 $\mu\text{g}/\text{ml}$ synthesized DNA at a greater rate than did untreated normal cells.

We do not know why Rennert and Chan have failed to identify the large molecular weight species eluted in the void volume of the Sephadex G-75 column. Methodologic differences could account for this discrepancy.

In order to study directly the binding of copper to the metallothionein(s), we labeled in the test tube skin fibroblast lysates derived from both normal subjects and patients with Menkes. These experiments demonstrated clearly that the amount of ^{64}Cu bound to the 10,000 molecular weight species in the Menkes fibroblast lysates was approximately 2.5 times greater than that in the normal lysates. Also, a significant amount of radioactivity was bound to the large molecular weight species (molecular weight greater than 100,000) in both normal and Menkes fibroblast lysates (3). Because there was similar competition between the small and the large molecular weight species for binding copper in controls and patients with Menkes, and because the copper bound to the 10,000-molecular weight component, both under saturating and unsaturating conditions, was approximately 2.5 times greater in Menkes cells than in normal cells, we think that the affinity of the metallothionein(s) for copper is the same in

normal and mutant fibroblasts. Our findings indicate that the increased copper binding by the 10,000-molecular weight species in the Menkes cells results from an increased binding capacity of this molecule or from the presence of an increased amount of this molecule in the cells. Also, the labeling experiments of the lysed cells indicate that the defect in the Menkes fibroblasts is not caused by a primary error in the transport of copper across the cell membranes, but rather is related to the increased binding of copper by the metallothionein(s). This agrees with our reported data demonstrating that several transport inhibitors did not affect to a different degree the uptake and efflux of ^{64}Cu by normal and Menkes-cultured fibroblasts (1).

Finally, we did not suggest that the copper concentration in the brain is normal, but we quoted Reske-Neilsen *et al.* (4) who found normal copper concentrations in the brain of a patient with Menkes disease. We think that the copper concentrations in the brain and other organs of patients with Menkes disease depend to a great extent on whether or not the patients were previously treated with parenterally administered copper, which circumvents the gut barrier. The finding of reduced concentrations of copper in the whole brain of a patient with Menkes disease (2) does not answer the basic question as to whether or not the neuronal cells of patients with Menkes disease have an increased, normal, or reduced affinity to copper. This patient died at 31 months old and the brain volume was less than newborn size. Severe neuronal loss and replacement by glial cells in the cortex, virtual absence of the Purkinje cells and decreased numbers of granular cells with gliosis in the cerebellum, and marked demyelination of the white matter were found.

In summary, we think that Menkes fibroblasts accumulate more copper than normal fibroblasts because of increased copper binding by the metallothionein(s) of the mutant cells than by those of the normal cells. Subsequently this increased copper accumulation causes cell malfunction and death occurring at lower extracellular copper concentrations in Menkes fibroblasts than in normal fibroblasts.

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