REVERSIBLE CHANGES IN THE ULTRASTRUCTURE OF CHANG LIVER CELL MITOCHONDRIA FOLLOWING INCUBATION OF THE CELLS IN A GLUTAMINE-DEFICIENT MEDIUM

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Chang liver cells, from a suspension culture of a cell strain originally derived from liver (2), show a decrease in the rate of incorporation of labeled precursors into protein and nucleic acids when maintained in a glutamine-deficient medium (3). The rate of incorporation of labeled amino acids decreases to 20–30% of the rate in exponentially growing cells. 2–4 hr after the addition of glutamine, protein synthesis is restored to a rate comparable to that of normally growing cells (3).

It is of interest, then, to examine normally growing and glutamine-deprived cells to see what, if any, ultrastructural changes are associated with the decreased rate of protein synthesis in glutamine deprived cells.

MATERIALS AND METHODS

Cells from normally growing cultures in complete nutrient medium (3) and from cultures in glutamine-deficient medium were fixed for 25 min in 2% glutaraldehyde in balanced salt solution (6), postfixed for 1 hr in 1% osmium tetroxide in balanced salt solution, dehydrated in acetone, and then embedded in Vestopal W (8). All solution changes up to the Vestopal embedding involved centrifugation of the cells at 1000 *g*, removal of the old medium, and resuspension in the new medium

The embedded cells were sectioned with glass knives on an LKB Ultrotome. The sections were picked up on Formvar-covered copper grids and stained for 1–4 hr at room temperature with 5% uranyl acetate and for 1 min at room temperature with lead citrate. The grids were examined in a Siemens Elmiskop I.

RESULTS AND COMMENTS

Electron micrographs of control and glutamine-deprived cells show no difference in the grouping of ribosomes. There appears to be an equally large proportion of polyribosomal groupings under both conditions. This observation is puzzling since gradient centrifugation studies show a marked reduction in the proportion of rapidly sedimenting particles upon glutamine deprivation (3). It may be that the ribosomes remain grouped as polysomes in glutamine-deprived cells but the chemical bonds holding them in that configuration are either broken or altered, allowing the bonds to be more easily disrupted during isolation of the ribosomes.

The only striking change observed upon glutamine deprivation is the change in mitochondrial morphology. In normal exponentially growing cells in a complete nutrient medium, the mitochondria exhibit the classical structure (Figs. 1 and 2). The mitochondrial membrane appears as a five layered membrane, and the intracristal space is very narrow. The matrix appears indistinguishable in electron density and in composition from

the surrounding cytoplasm. All of the mitochondria in all of the cells examined have this appearance. On the other hand, in glutamine-deprived cells all of the mitochondria have an extremely electron-opaque (condensed) matrix (Figs. 3 and 4). The electron-lucid intracristal space is considerably expanded. The mitochondria appear to be smaller than control mitochondria, which is an indication of probable mitochondrial shrinkage.

When cells were fixed 2 and 4 hr after readdition of glutamine to the growth medium, the mitochondrial appearance in the majority of cells returned to that of the controls. The few cells which retained condensed mitochondria showed signs of swelling and of disrupting of the plasma membrane.

The fact that the changes in mitochondrial morphology upon glutamine deprivation of Chang liver cells is so striking and ubiquitious suggests that this system may be an excellent one for the study of associated ultrastructural and metabolic changes in mitochondria in the intracellular environment.

In the case of the observations described here, the possibility has not been ruled out that the changes in mitochondrial morphology are due to lack of mitochondrial proteins with a fairly rapid turnover rate which must constantly be replaced by the cells' protein-synthesizing machinery in order to maintain a normal mitochondrial structure.

Another possibility is that the changes in the structure of the mitochondria are associated with changes in their metabolism. A growing amount of literature relates mitochondrial metabolism to ultrastructure (1, 4, 5, 7, 9) and makes it tempting to think along such lines. A characteristic of cell strains grown in vitro is rapid growth. Consequently, the synthesis of macromolecules must be responsible for a large part of the cells' energy consumption. Removal of an essential amino acid effectively limits the rates of cell growth and synthetic activity, probably resulting in a decreased energy demand, while readdition of the missing component would reverse the situation. One can then speculate that the changes in the electron microscopic structure of the mitochondria upon glutamine deprivation and readdition are connected with shifts of the mitochondrion's energy production, shifts that occur in response to the altered energy demand.

It is too early to speculate which of the two possibilities is primarily involved in the structural changes in the mitochondria. Further interpretation of these observations must wait until more information is available concerning the effect of glutamine deprivation on the respiratory rate and on the metabolism of the cells.

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FIGURE 1. Survey micrograph showing a portion of a cell taken from an exponentially growing culture in complete nutrient medium. Note that the mitochondria do not appear very electron opaque. × 25,000.

FIGURE 2 Detail of two mitochondria in a cell taken from an exponentially growing culture in a complete nutrient medium. The mitochondrial membrane is a five layered structure, and the intracristal space is very narrow. Note that the mitochondrial matrix is about as electron dense as the surrounding cytoplasm. × 100,000.

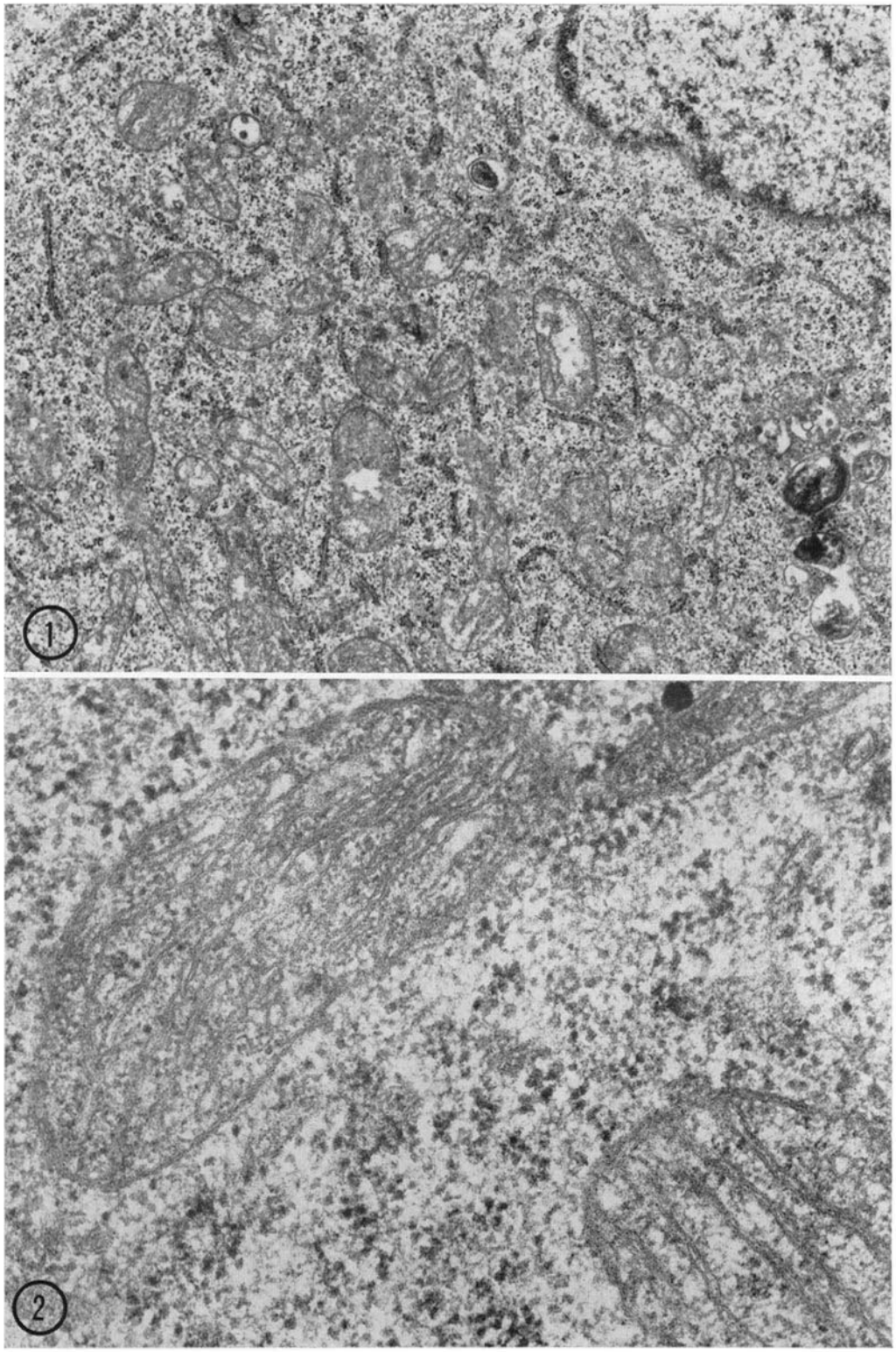
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FIGURE 3 Survey micrograph showing a portion of a cell taken from a culture which had been in a glutamine-deficient medium for about 20 hr. The mitochondrial matrix is extremely electron opaque. × 25,000.

FIGURE 4 Detail of three mitochondria in a cell taken from a culture which had been in a glutamine-deficient medium for about 20 hr. The intracristal spaces (electron-lucid spaces) are wider than those of control mitochondria. Compare the size of these mitochondria with the size of those in Fig. 2. × 100,000.