protein, 16.7% (1.17); cytochrome *c* oxidase, 79.8% (1.18, with maximum activity at 1.17 and a shoulder at 1.21); catalase, 4.94% (1.95); acid phosphatase (substrate *p*-nitrophenyl phosphate, pH4.2), 42.1% (heterogeneous density distribution mainly from 1.12 to 1.14). NADH- and NADPH-cytochrome *c* oxidoreductases were 57% and 50% sedimentable respectively and NADH- and NADPH-ferricyanide oxidoreductases 55% and 32% sedimentable respectively; all equilibrated at ρ 1.18.

For rate separations samples were homogenized in 0.25M-sucrose–1.0mM-EDTA–0.05M-phosphate buffer, pH7.4. Centrifugation to remove whole cells was followed by loading on to an unbuffered sucrose density gradient in a B XIV rotor. A linear stabilizing (ρ 1.04–1.09) sucrose density gradient was used followed by a cushion of 60% (w/v) sucrose.

The most rapidly sedimenting particles contained the following enzymes (percentage of total enzyme units in parentheses): protein (15%); cytochrome *c* oxidase (50%); succinate-, NADH- and NADPH-cytochrome *c* oxidoreductases (70%, 48% and 26% respectively); NADH- and NADPH-ferricyanide oxidoreductases (47% and 55% respectively).

A population of less rapidly sedimenting particles also contained cytochrome *c* oxidase, succinate-cytochrome *c* oxidoreductase, both NADH- and NADPH-cytochrome *c* oxidoreductase and also NADH-ferricyanide oxidoreductase.

Another population of particles was observed that were well separated from the oxidoreductases and contained 95% of the total catalase and 12% of total protein.

Acid phosphatase and esterase were 94% and 91% present in a broad band that had hardly migrated from the sample zone; 60% of the NADPH-cytochrome *c* oxidoreductase was also present in this region.

Alkaline phosphatase corresponded to the peak of non-sedimentable protein.

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The Effect of Growth with Chloramphenicol on the Mitochondria of *Tetrahymena pyriformis* Strain ST

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Mager (1960) has shown that chloramphenicol inhibits the incorporation of amino acids into a mitochondrial fraction prepared from *Tetrahymena pyriformis* strain W.

Growth of the organism in the presence of chloramphenicol (75μg/ml) leads to a prolonged lag phase, a longer mean generation time and a diminished cell yield (after 40h). When the antibiotic is added to an exponentially growing culture (200–500μg/ml) the cells continue to grow for two or three generations, and then growth virtually ceases. At the end of this period the cells are still normal in size (mean 50μm × 30μm) and the mitochondrial morphology appears normal. Between 24 and 48h after chloramphenicol addition cell size becomes progressively smaller, few cell divisions occur, cells become rounded (25μm × 25μm) and motility decreases. Mitochondria appear to be very greatly decreased in size and more numerous after 48–72h; the outer membranes are intact, but cristae structure has degenerated. Normal mitochondrial morphology is still in evidence after 72h growth in the absence of chloramphenicol.

Both cytochrome content per cell and per mg of mitochondrial protein were not significantly affected by growth with chloramphenicol after 72h. This confirms that growth with chloramphenicol results in the formation of a greater number of smaller mitochondria per cell; thus growth and division of mitochondria have become unlinked from cell growth and division.

Mitochondria isolated from 24h chloramphenicol-grown cells show normal succinoxidase and α-oxoglutarate oxidase activities, but these oxidations are not tightly coupled to phosphorylation. After 48–72h the mitochondria retain only one-fifth of their oxidative capacity and respiratory control is not detectable. Cells from a 72h chloramphenicol culture inoculated into fresh chloramphenicol-free growth medium show a lag of 24h followed by normal growth.

Mitochondria isolated from cells starved for 24h (no net growth) in the presence of 500μg of chloramphenicol/ml exhibit high respiratory control ratios. Cells starved in the absence of the antibiotic showed a progressive decline in mitochondrial protein and isolated mitochondria a loss of respiratory control. These results suggest that chloramphenicol leads to abnormal mitochondrial synthesis only in growing cells and that the synthesis of new mitochondrial membrane by the turnover of other cell proteins is negligible in non-growing cell suspensions.

G.T. held a Medical Research Council Studentship during the course of this work.

Mager, J. (1960). *Biochim. biophys. Acta*, **38**, 150.