Sedimentation Characteristics of Mitochondria, Peroxisomes and Lysosomes from the Ciliate Protozoon *Tetrahymena pyriformis* Strain ST after Chloramphenicol-inhibited Growth

By R. K. POOLE, W. G. NICHOLL,* G. TURNER, G. I. ROACH AND D. LLOYD

Department of Microbiology, University College of South Wales and Monmouthshire, Newport Road, Cardiff; CF1 3NR

(Received for publication 14 May 1971)

**SUMMARY**

Homogenates of *Tetrahymena pyriformis* suspensions were prepared after growth in the absence and presence of chloramphenicol (500 μg./ml.). Sedimentation of organelles through gradients of aqueous sucrose solutions was studied by means of a zonal centrifuge. Mitochondria (marker enzyme, malate dehydrogenase) from organisms grown with chloramphenicol sediment more slowly (median sedimentation coefficient in water at 20° = 10,600 S) than those from normal cells (median sedimentation coefficient in water at 20° = 90,000 S), which confirms that the former are of smaller volume than normal mitochondria. After high-speed zonal centrifugation both mitochondria (marker enzymes, NADH and NADPH-cytochrome c oxidoreductases) attain similar equilibrium densities (median density $\rho = 1.21$ to $1.22$). The buoyant density (median $\rho = 1.23$) of peroxisomes (marker enzyme, catalase) is not altered by the inclusion of chloramphenicol in the growth medium, but the proportion of non-sedimentable catalase is increased. Chloramphenicol-inhibited growth also leads to a change in the distribution of lysosomes (marker enzymes, acid $p$-nitrophenolphosphatase, and N-acetyl-$p$-glucosaminidase); a marked reduction in the population at $\rho = 1.15$ to $1.20$ is accompanied by the appearance at $\rho = 1.09$ to $1.15$ of a new class of organelles containing acid hydrolases.

**INTRODUCTION**

Growth of *Tetrahymena pyriformis* with chloramphenicol leads to the production of grossly abnormal mitochondria which have a pleiotropic respiratory deficiency (Turner & Lloyd, 1969, 1971). This system differs from all those previously studied, e.g. yeast (Clark-Walker & Linnane, 1966), *Pythium ultimum* (Marchant & Smith, 1968), regenerating rat liver (Firkin & Linnane, 1969) and *Polytornella caeca* (Lloyd, Evans & Venables, 1970), in that in addition to producing respiratory deficient mitochondria with distorted and reduced ultrastructure of the crista membrane the antibiotic appears to unlink mitochondrial division from cell division. This leads to the formation of large numbers of smaller mitochondria.

The present study was carried out to examine the sedimentation properties of the modified mitochondria and other organelles in chloramphenicol-inhibited organisms. The observed sedimentation behaviour confirms that although the mean radius of the isolated mitochondrion is only one-third that of the normal mitochondrion, the median densities are not significantly different. A marked alteration in the density distribution of lysosomes is evident.

* Present address: Department of Bacteriology, The Medical School, University Walk, Bristol, BS8 1TD.
METHODS

Maintenance, growth, harvesting of the organism and preparation of homogenates. Tetrahymena pyriformis ST (from Dr Y. Suyama) was maintained, grown, harvested and disrupted as described previously (Lloyd, Brightwell, Venables, Roach & Turner, 1971).

Subcellular fractionation by zonal centrifugation. High-speed zonal centrifugation was carried out in a BXIV zonal rotor (Anderson et al. 1967) as previously described (Lloyd et al. 1971). Rate separations were also performed in this rotor. All sucrose solutions were buffered at 4°C to pH 7.4 with 10 mM-tris base and HCl. The rotor was loaded at 2500 rev./min. through the 'edge line' with the following volumes of sucrose solutions: 50 ml. each of 10, 11.5, 13, 14.5, 16.0, 17.5, 19.0, 20.5 and 170 ml. of 60% (all w/w). The homogenate was then pumped into the centre of the rotor followed by 60 ml. of 6.5% (w/w) sucrose overlay. As the sample was loaded, a stop-watch was started and the times of loading, acceleration and deceleration were followed. The contents of the rotor were unloaded at 2500 rev./min. through the flow-cell of a Beckman DB recording spectrophotometer. Light-scattering was measured in this system at 520 nm. Fractions (10 ml.) were collected at 5°C. Sucrose concentrations, measured with a refractometer, were converted to densities by means of the data of de Duve, Berthet & Beaufay (1959), and viscosity values were also obtained from these tables.

Enzyme assays. Assay conditions for acid p-nitrophenolphosphatase, acid N-acetyl-β-(D)-glucosaminidase, catalase, NADH and NADPH-cytochrome c oxidoreductases have been described previously (Lloyd et al. 1971). Malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37) activity was measured by the rate of decrease in extinction at 340 nm. (Ochoa, 1955). The reaction mixture contained, in a total volume of 3 ml.: 3.7 mM-MgCl₂, 0.05 mg. NADH and enzyme in 37 mM-K phosphate buffer at pH 7.4. The blank cuvette contained reaction mixture lacking NADH only. The reaction was started by the addition of 0.04 mg. Na-oxaloacetate to both cuvettes.

Assays for acid hydrolases were performed at 29°C, while all the spectrophotometric assays were carried out at room temperature (18°C ± 2°C), using a Cary model 14 recording spectrophotometer fitted with a 0 to 0.1 extinction slide wire. Preliminary experiments were performed with whole homogenates to ensure that the rates of reactions measured were proportional to the amount of homogenate protein used, were not limited by substrate concentration, and were linear over the incubation times employed. When assaying fractions, a suitable volume of the most active fraction was used so that the measured reaction velocity was within the range determined for whole homogenate. In a typical experiment whole homogenate was diluted 1 in 40, such that 0.1 ml. containing 0.02 mg. protein was in each assay; 0.1 ml. amounts of the fractions were then found to be suitable volumes for determination of

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Fig. 1. Fractionation of a homogenate of Tetrahymena pyriformis grown in the absence of chloramphenicol. The cultures was harvested when the population reached 4 x 10⁶ organisms/ml.; the homogenate (1.8 ml.) contained 191 mg. total protein. Whole homogenate was diluted 1 in 40, the volumes of diluted homogenate and of fractions taken for assays were as follows: acid p-nitrophenolphosphatase, 0.2 ml.; malate dehydrogenase, 0.1 ml.; catalase, 0.1 ml.; and NADH-cytochrome c oxidoreductase, 0.1 ml. (a) Centrifugation was at 12,300 rev./min. for 2 min. (= 2.7 x 10⁶ g min. at the sample zone: ω²dr = 5.88 x 10⁶ rad. sec⁻²). (b) Sucrose density gradient (○), protein (●), and light-scattering at 520 nm. (continuous line). (c) Acid p-nitrophenolphosphatase (○) and malate dehydrogenase (●). (d) Catalase (○) and NADH-cytochrome c oxidoreductase (●). Recoveries were: protein, 118%; acid p-nitrophenolphosphatase, 108%; malate dehydrogenase, 127%; and NADH-cytochrome c oxidoreductase, 122%.
Fig. 1. For legend see opposite page.
Fig. 2. For legend see opposite page.
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Fig. 2. Fractionation of a homogenate of Tetrahymena pyriformis grown in the presence of chloramphenicol. The culture was harvested in late phase 2 (Turner & Lloyd, 1971) after 24 h. normal growth and a further 48 h. with chloramphenicol (500 μg./ml.). The population was 6·1 × 10⁴ organisms/ml., and the homogenate (6·2 ml.) contained 47·0 mg. total protein. Whole homogenate was diluted 1 in 40, and volumes of diluted homogenate and of fractions taken for assays were as follows: acid p-nitrophenolphosphatase, 0·2 ml.; malate dehydrogenase, 0·1 ml.; catalase, 0·1 ml.; and NADH-cytochrome c oxidoreductase, 0·1 ml. (a) Centrifugation was at 12,300 rev./min. for 2 min. (≡ 2·42 × 10⁴ g min. at the sample zone (Jk²dt = 4·8 × 10⁷ rad. s⁻¹). (b) Sucrose density gradient (○), protein (●) and light-scattering at 520 nm. (continuous line). Acid p-nitrophenolphosphatase (●) and malate dehydrogenase (■). (d) Catalase (○) and NADH-cytochrome c oxidoreductase (■). Recoveries were: protein, 125%; acid p-nitrophenolphosphatase, 110%; malate dehydrogenase, 106%; catalase, 72%; and NADH-cytochrome c oxidoreductase, 102%.

Fig. 3. Plots of $\eta_T/1 - \bar{v}_p \rho_p$ against ln r for mitochondria from cells grown in the absence (a) and in the presence (b) of chloramphenicol. See methods section for explanation of symbols.
the total amount of enzyme per fraction. The validity of this procedure was confirmed by the satisfactory enzyme recovery figures finally obtained.

Protein was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951); the standard used was bovine serum albumin.

**Electron microscopy.** Isolated mitochondria were fixed as suspensions in fractions from the aqueous sucrose gradients by the addition of 1% (w/v) osmium tetroxide in 50 mM-K phosphate buffer (pH 7.4), fixation was continued for 30 min. and the mitochondria centrifuged down. After dehydration in ethanol the mitochondria were embedded in a mixture of n-butyl and methyl methacrylates (4:1, v/v). Sections were cut at a nominal 5 nm. thickness (grey/silver colour) with a Reichert ultramicrotome and stained on the grids with lead citrate. The specimens were examined and photographed in an AEI EM6 electron microscope operating at 60 kV.

**Treatment and presentation of results.** Enzyme units are expressed as nmoles of substrate transformed/min., or measured product formed/min. In the graphical presentations (Fig. 1, 2, 4) vertical lines divide the diagrams at density intervals of 0.05 g./ml. Percentage sedimentability is arbitrarily expressed in the high-speed centrifugation experiments as that proportion of the total enzyme units recovered which sedimented beyond \( \rho = 1.10 \) after centrifugation at \( 6 \times 10^5 \text{g} \text{ min.} \).

In rate–zonal separations centrifugal speeds were converted into values for squared angular velocities (rad. \( 2 \text{s.}^{-2} \)). These were plotted against time (Fig. 1, 2, 4) in order to obtain a value for the time integral of the squared angular velocity

\[
\left( \int_{0}^{t} \omega^2 dt \right) \text{ rad. } 2 \text{s.}^{-1}.
\]

Values for integrated field-times were calculated from the centre of the sample zones. Sedimentation coefficients \( (S_{20,w}) \) were calculated by the method of Halsall & Schumaker (1969).

The sedimentation coefficient of a particle in water at 20° is given by

\[
S_{20,w} = \frac{1 - \bar{\nu}_m \rho_{20,w}}{\eta_{20,w}} \int_{\ln r}^{\ln r_0} \frac{\eta_s}{1 - \bar{\nu}_m \rho_s} \, d \ln r,
\]

where

- \( \eta_s \) = viscosity of gradient solution at radius \( r \) (cm.), in poise,
- \( \eta_{20,w} \) = viscosity of water at 20°, in poise,
- \( \bar{\nu}_m \) = partial specific volume of the particle at radius \( r \), in ml./g.,
- \( \rho_s \) = density of gradient solution at radius \( r \), in g./ml.,
- \( \rho_{20,w} \) = density of water at 20°, in g./ml.,
- \( r \) = distance of particle from the axis of rotation, in cm.,
- \( S_{20,w} \) = sedimentation coefficient of the particle in water at 20°,

and

\( \omega = \) angular velocity, in rad./s.

Values of \( r \) were obtained from the volume data by use of data calculator (M.S.E. Ltd, Crawley, Sussex). Calculations of \( a \) (particle radius in cm.) were by use of the equation:

\[
S = \frac{2a^2(\rho_m - \rho_{20,w})}{9\eta_{20,w}}
\]

where \( \rho_m \) = density of the particle in g./ml.
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RESULTS

Rate-zonal centrifugations

Distributions of marker enzymes after low-speed centrifugation of homogenate of organisms grown in the absence of chloramphenicol. After centrifugation for 2 min. at 12,300 rev./min. (= 2.7 \times 10^4 \text{g min. at the sample zone;})

\[ \int_0^t \omega^2 dt = 5.88 \times 10^8 \text{ rad.} \text{s}^{-2}, \text{Fig. 1a}, \]

the light-scattering profile of the gradient indicated that a peak of rapidly sedimenting particles had arrived at the cushion and was thus well separated from the sample zone (Fig. 1b). Protein assays confirmed that 34 % of the total protein of the homogenate had sedimented beyond fraction 15. The presence of a non-protein component in the rapidly sedimenting

Table I. Percentage sedimentabilities of enzymes in homogenates of Tetrahymena pyriformis after growth in the absence or presence of chloramphenicol

<table>
<thead>
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<th></th>
<th>Absence of chloramphenicol</th>
<th>Presence of chloramphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td>Acid p-nitrophenolphosphatase</td>
<td>19</td>
<td>37</td>
</tr>
<tr>
<td>Catalase</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>83</td>
<td>9</td>
</tr>
<tr>
<td>NADH-cytochrome c reductase</td>
<td>35</td>
<td>12</td>
</tr>
</tbody>
</table>

populations of particles is evident, as the major light-scattering zone was slightly behind that of protein. Of the total malate dehydrogenase, 83 % was present in the particles which migrated beyond fraction 15 (Fig. 1c), and almost all of this sedimentable enzyme was present in the population of particles distributed around \( \rho = 1.19 \). The remaining 17 % was left at the starting zone. The sedimentable portion (19 %) of \( \rho \)-nitrophenolphosphatase (Fig. 1c) was sedimenting as a heterogeneous population. Only 1 % of the catalase had moved beyond fraction 15, and of the total NADH-cytochrome c oxidoreductase 35 % had sedimented (Fig. 1d). The distributions of the non-sedimentable portions of \( \rho \)-nitrophenolphosphatase and catalase were much broader than that of the starting zone, suggesting that if a higher field-time had been used further populations of sedimentable particles would have been separated.

Distributions of marker enzymes after low-speed centrifugation of homogenate of organisms grown in the presence of chloramphenicol. After centrifugation for 2 min. at 12,300 rev./min. (= 2.42 \times 10^4 \text{g min. at the sample zone;})

\[ \int_0^t \omega^2 dt = 4.8 \times 10^8 \text{ rad.} \text{s}^{-2}, \text{Fig. 2a} \]

the light-scattering profile and distributions of marker enzymes obtained was quite different for the homogenate from organisms grown with chloramphenicol (Fig. 2b to d). A
Fig. 4. For legend see opposite page.
Fig. 4. Fractionation of a homogenate of *Tetrahymena pyriformis* after growth in the presence of chloramphenicol. The culture was harvested in late phase 2 (Turner & Lloyd, 1971) after 24 h. normal growth and a further 48 h. with chloramphenicol (500 μg./ml.). The population was 6 × 10^4 organisms/ml., and the homogenate (11 ml.) contained 112.2 mg. total protein. Whole homogenate was diluted 1 in 30, and volumes of diluted homogenate and of fractions then taken for assay were as follows: acid p-nitrophenolphosphatase, 0.1 ml.; acid N-acetyl-3-(D)-glucosaminidase, 0.3 ml.; catalase, NADH- and NADPH-cytochrome c reductases, all 0.1 ml. (a) Centrifugation was at 35,000 rev./min. for 165 min. (= 6 × 10^6 g min. at the sample zone; \[\int_0^\infty \rho_0^2 \, dt = 1.45 \times 10^{14} \text{ rad. s}^{-2}\). (b) Sucrose density gradient (○), protein (●) and light-scattering at 520 nm. (continuous line). (c) Acid p-nitrophenolphosphatase (●) and acid N-acetyl-β-(D)-glucosaminidase (□). (d) Catalase (○), NADH- (□) and NADPH-cytochrome c oxidoreductase (▲). Figures for recoveries were as follows: protein, 102.7%; acid p-nitrophenolphosphatase, 89.9%; acid N-acetyl-β-(D)-glucosaminidase, 99.3%; catalase, 61.25%; NADH-cytochrome c oxidoreductase, 115.3%; and NADPH-cytochrome c oxidoreductase 119.3%.

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major peak of light-scattering at around fraction 12 corresponded to a shoulder of protein which was just separating from the starting zone. This zone accounted for about 80% of the total malate dehydrogenase and also for some of the catalase; only 9% of the dehydrogenase enzyme was found beyond fraction 15, while about 10% had not moved from the starting zone (Fig. 2c). In this experiment 37% of the p-nitrophenolphosphatase had sedimented, and a large proportion of this had reached the sucrose cushion. The distribution of catalase was similar to that in the experiment with the homogenate of normal organisms, while the sedimentability of NADH-cytochrome c oxidoreductase was decreased to 12% (Fig. 2d). The percentage sedimentabilities of marker enzymes in normal and chloramphenicol-grown organisms are compared in Table 1.

Calculations of values of \(S_{20,w}\) for mitochondria from organisms grown in the absence and in the presence of chloramphenicol. Calculations of values for \(S_{20,w}\) for mitochondria employed the method of Halsall & Schumaker (1969). From the density gradient profiles (Fig. 1b, 2b) \((\eta_p / 1 - \bar{v}_p \rho_0)\) was plotted as a function of \(\ln r\) for the experiments with normal and chloramphenicol-inhibited organisms (Fig. 3a, b). The normalized areas enclosed by the curves thus constructed

\[
\left(\text{expressing } \int_{\ln r_1}^{\ln r_2} \frac{\eta_p}{1 - \bar{v}_p \rho_0} \, d \ln r\right)
\]

were substituted into equation (1), and \(S_{20,w}\) values so obtained were converted into Svedberg units. The \(S\) values obtained for normal and chloramphenicol-grown cells were \(9 \times 10^4 S\) and \(10.6 \times 10^4 S\) respectively. Corresponding values for the radii of the isolated mitochondria were calculated (from equation (2)) as 0.45 μm. and 0.15 μm. respectively.

Isopycnic–zonal centrifugation

Distribution of marker enzymes after high-speed centrifugation of homogenate of organisms grown with chloramphenicol. After centrifugation of a homogenate from organisms grown in the presence of chloramphenicol for 165 min. at 35,000 rev./min. (≡ 6 × 10^6 g min. at the sample zone:

\[
\int_0^\infty \rho_0^2 \, dt = 1.45 \times 10^{14} \text{ rad. s}^{-1}\)

the major peak of light-scattering (Fig. 4b) corresponded to the position of mitochondrial activities of NADH and NADPH-cytochrome c oxidoreductases which had attained their equilibrium positions in the gradient (Fig. 4d). A shoulder on the light-scattering profile at \(\rho = 1.23\) corresponded to the position of catalase (Fig. 4d). Thus the median buoyant den-
sities of mitochondria and of peroxisomes were no different when organisms were grown in the absence of chloramphenicol (Lloyd et al. 1971) or in its presence (Table 2), although there was a pronounced reduction in the percentage sedimentabilities of the two oxidoreductases and also of catalase after growth with the antibiotic. The acid hydrolases assayed were markedly altered in distribution by the inclusion of chloramphenicol in the growth medium. Although the percentage of the total units of these two enzymes sedimenting beyond \( \rho = 1.10 \) was not significantly altered, there was a shift of lysosomal activities normally found in the \( \rho = 1.15 \) to 1.20 region of the gradient (Lloyd et al. 1971) to the 1.10 to 1.15 zone (Fig. 4c).

Table 2. Percentage sedimentabilities and median buoyant densities of the sedimentable portions of enzymes in homogenates of Tetrahymena pyriformis after growth in the absence or in the presence of chloramphenicol

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Absence of chloramphenicol</th>
<th>Presence of chloramphenicol</th>
</tr>
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<tbody>
<tr>
<td>Protein</td>
<td>52 (1.20)</td>
<td>38 (1.21)</td>
</tr>
<tr>
<td>Acid p-nitrophenolphosphatase</td>
<td>92 (1.18)</td>
<td>89 (1.19)</td>
</tr>
<tr>
<td>Acid N-acetyl-(\beta)-(D)-glucosaminidase</td>
<td>67 (1.19)</td>
<td>68 (1.20)</td>
</tr>
<tr>
<td>Catalase</td>
<td>89 (1.23)</td>
<td>66 (1.23)</td>
</tr>
<tr>
<td>NADH-cytochrome c oxidoreductase</td>
<td>92 (1.16)</td>
<td>84 (1.21)</td>
</tr>
<tr>
<td>NADPH-cytochrome c oxidoreductase</td>
<td>81 (1.17)</td>
<td>60 (1.21)</td>
</tr>
</tbody>
</table>

Electron microscopy of isolated mitochondria after high-speed centrifugation. Mitochondria were fixed while they were suspended in isopycnic aqueous sucrose solutions (\( \rho = 1.21 \) to 1.22) (Fig. 5a, b). The normal mitochondria showed well-preserved ultrastructure. Although the inner membranes had shrunk slightly, the tubules of the inner membranes were clearly defined as randomly orientated structures, cut in many different planes. The mitochondria from chloramphenicol inhibited organisms (Fig. 5c, d) were preserved as intact spheres with outer membranes of normal appearance, but the cristae and matrix spaces filled with a structureless osmiophilic material. The mean particle diameter of the normal mitochondria was 0.53 \( \mu \)m; while the mitochondria from chloramphenicol-inhibited organisms measured 0.125 \( \mu \)m. on average.

Fig. 5 (a) Mitochondria from homogenate of Tetrahymena pyriformis grown in the absence of chloramphenicol. Fraction of density 1.21 after isopycnic–zonal centrifugation. Average mitochondrial radius = 0.53 \( \mu \)m. (15 measurements).

(b) As in (a); at high magnification to show well-preserved membrane ultrastructure. Outer membranes \( (om) \) and inner membranes \( (im) \) forming randomly orientated cristae are still clearly defined.

(e) Mitochondria from homogenate of Tetrahymena pyriformis grown in the presence of chloramphenicol. Fraction of density 1.21 (fraction 35 of the experiment described) after isopycnic–zonal centrifugation. Average mitochondrial radius = 0.125 \( \mu \)m. (15 measurements).

(d) As in (c); at high magnification. Although the outer membrane \( (om) \) is still intact and the inner membrane \( (im) \) is apparent, no organized cristae can be distinguished.
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(a) 12 µm

(b) 0.5 µm

(c) 2 µm

(d) 0.5 µm
DISCUSSION

The behaviour of subcellular organelles, present in homogenates of normally grown Tetrahymena pyriformis, subjected to high-speed centrifugation on sucrose density gradients has previously been studied by Müller, Baudhuin & de Duve (1966), Müller, Hogg & de Duve (1968) and by Lloyd et al. (1971). Thus by isopycnic banding it is possible to distinguish between mitochondria, peroxisomes and lysosomes by their equilibrium densities, although the density distributions of the organelles containing acid hydrolases is always extremely heterogeneous and there appear to be several distinct populations of these particles (Lloyd et al. 1971). The present work shows that growth in the presence of chloramphenicol did not lead to detectable changes in the median buoyant densities of mitochondria or peroxisomes, but did lead to a profound alteration in the density distribution of lysosomes. A similar change has previously been noted after starvation of normally-grown cells (Lloyd et al. 1971) and warrants further investigation.

As chloramphenicol affects protein synthesis and hence the gross membrane morphology of mitochondria, and particularly as the effect is entirely on the inner membrane (as judged by electron microscopic evidence: Turner & Lloyd, 1971) which has a much higher buoyant density than the outer membrane (D. Lloyd & G. Turner, unpublished results), it might be expected that the mitochondria from chloramphenicol-grown organisms would be less dense than normal mitochondria. However, this was not the case; an explanation for this surprising result might be sought either by measurement of protein/lipid ratios of the membranes or studies on permeability to sucrose.

The conditions for rate-zonal centrifugations were designed with a view to detecting an alteration in mitochondria size to confirm the reduced dimensions of mitochondria as observed in sections of chloramphenicol-grown organisms (Turner & Lloyd, 1971). The distribution of malate dehydrogenase (which is 85% mitochondrial in Tetrahymena pyriformis (Müller et al. 1968) in two similar experiments with organisms grown in the absence and presence of the antibiotic, indicated that while the proportions of enzyme intra- and extramitochondrially were not altered, the velocity of sedimentation of mitochondria was greatly reduced in the homogenates of organisms which had been grown with chloramphenicol. This is also confirmed by the reduced sedimentability of NADH-cytochrome c oxidoreductases under these conditions. Such conditions were chosen in order to separate particles of mitochondrial size from all smaller particles (e.g. ‘microsomes’ which also contain part of the total NADH-cytochrome c oxidoreductase: Lloyd et al. 1971). The median sedimentation coefficients (90,000 and 10,600 respectively) ideally correspond to median radii of 0.45 and 0.15 μm, which correspond to average values of 0.53 and 0.125 μm, measured in electron micrographs after fixation of mitochondria while they were suspended in isopycnic concentrations of sucrose.

The acid ρ-nitrophenolphosphatase of homogenates of organisms grown with chloramphenicol has a more rapidly sedimenting component than those of normal organisms and thus a detailed study of changes of lysosomes after growth with the antibiotic might also prove rewarding.

We would like to express our thanks to Professor D. E. Hughes for his continued interest and encouragement, and to Mr W. J. Henderson and Miss S. Davies for expert technical assistance with electron microscope techniques. G. T. was the holder of an M.R.C. Postgraduate Studentship.
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REFERENCES


