The Microsomal Fraction from *Tetrahymena pyriformis* Strain ST: Characterization and Subfractionation

By R. K. POOLE, W. G. NICHOLL, LYND A HOWELLS
AND D. LLOYD

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

(Accepted for publication 23 July 1971)

SUMMARY

Post-mitochondrial supernatants isolated from *Tetrahymena pyriformis* contained high specific activities of antimycin A-insensitive NADH- and NADPH-cytochrome *c* oxidoreductases, acid and alkaline *p*-nitrophenylphosphatases, DNase and ATPase; many of the enzymes associated with mammalian microsomes were not present at detectable levels. The only haem detected was protohaem; cytochromes *b*₅, *P*₄₅₀ and *P*₄₂₂ were present in microsomal fractions, and ethyl morphine and aniline HCl produced distinct changes in difference spectra. Post-mitochondrial supernatants were subfractionated by density gradient centrifugation. The distributions of NADH- and NADPH-cytochrome *c* oxidoreductases were different, and several distinct particles containing the latter enzyme system were distinguished. The distributions of acid *p*-nitrophenylphosphatase, acid DNase and ATPase were all different and indicative of several functionally distinct membrane systems. Alkaline *p*-nitrophenylphosphatase was non-sedimentable.

INTRODUCTION

The NADH- and NADPH-cytochrome *c* oxidoreductases of homogenates prepared from suspensions of *Tetrahymena pyriformis* show complex distributions after high speed zonal centrifugation on sucrose density gradients (Lloyd, Brightwell, Venables, Roach & Turner, 1971). While both these activities are in part associated with mitochondria (Turner, Lloyd & Chance, 1971), which equilibrate at $\rho = 1.21$, the regions of the gradient at $\rho < 1.20$ also contain both oxidoreductases. The distributions of these two enzyme systems are different, and they vary in homogenates of organisms harvested at different phases of the growth cycle of a batch culture (Lloyd *et al.* 1971).

In this paper we characterize the haemoproteins of the microsomal fraction of *Tetrahymena pyriformis*, strain ST and separate some of the component membrane systems by zonal centrifugation.

METHODS

Maintenance, growth and harvesting of the organism. *Tetrahymena pyriformis*, strain ST, was maintained, grown (in volumes of culture up to 15 l.), and harvested as previously described (Lloyd *et al.* 1971).

Disruption of organisms and preparation of post-mitochondrial supernatants and microsomal fractions. The organisms were washed once in preparation buffer consisting of 0.24 M-sucrose, 10 mM-tris HCl, and 0.5 mM-MgCl₂ (pH 7.4), and resuspended in 5 vol. of this buffer. The suspension was homogenized in a 50 ml. Kontes (Vineland, New Jersey, U.S.A.)

tight fitting (type B plunger) glass hand-homogenizer in ice, until almost 60% of the organisms were broken. Gentle strokes were used and care was taken to avoid cavitation. All centrifugation steps were carried out at 4° (in the 8 × 50 ml. rotor of an MSE 17 centrifuge except where stated). The whole homogenate was centrifuged for 5 min. at 300g ($r_{av} = 7.62$ cm.) to remove whole organisms, nuclei and pellicles. The supernatant was recentrifuged for 10 min. at 10,000g ($r_{av} = 7.62$ cm.) to remove mitochondria, peroxisomes and most of the lysosomes. The supernatant from this step is termed the 'post-mitochondrial supernatant' (PMS). The 'microsomal fraction' was prepared by centrifuging PMS for 1 h. at 106,000g ($r_{av} = 5.98$ cm.) in the 8 × 10 ml. rotor of an MSE 40 centrifuge. The sedimented material was resuspended in preparation buffer to a protein concentration of about 1 mg./ml.

Zonal centrifugation. Post-mitochondrial supernatants were fractionated using a B XIV rotor (Anderson *et al.* 1967). The rotor was loaded with the following volumes of sucrose solutions (all buffered to pH 7.4 at 4° with tris HCl and containing 0.5 mM-MgCl₂): 40 ml. each of 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5 and 37.5%; 140 ml. 60%; from 10 to 15 ml. PMS, 30 ml. each of 7 and 9% (all w/w). Loading was at 2500 rev./min.; the rotor was then accelerated to 35,000 rev./min. (36,500g at sample zone) and held at this speed. The rotor was unloaded at 2500 rev./min. through the flow cell of a Beckman recording spectrophotometer; extinction was monitored at 260 nm. Fractions (10 ml.) were collected at 5°. Sucrose concentrations were measured with a refractometer and density calculated from the data of de Duve, Berthet & Beaufay (1959). *S*-values were calculated by the method of Halsall & Schumaker (1969) as detailed by Poole *et al.* (1971).

Enzyme assays. Assays of acid *p*-nitrophenylphosphatase (EC 3.1.3.2), NADH- and NADPH-cytochrome *c* oxidoreductases (EC 1.6.99.3) were exactly as described by Lloyd *et al.* (1971). Methods for assay of ATPase (at pH 6.8, in the presence of 4 mM-MgCl₂), alkaline phosphatase and all the enzymes looked for but not detected have been previously published by Cartledge & Lloyd (1972). Precautions taken to ensure linearity of enzyme assays and proportionality of reaction rates to volumes used in assays of fractions from zonal centrifugation experiments have also been described previously in detail (Lloyd *et al.* 1971).

Analytical methods. Difference spectra were traced with a Cary model 14 split-beam spectrophotometer fitted with a 0 to 0.1 extinction slide wire. Sometimes an attachment for scanning at the temperature of liquid N₂ was also used.

Pyridine haemochrome derivatives were prepared according to the method of Falk (1964). Protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine plasma albumin as a standard. P_i was measured by the method of Leloir & Cardini (1957).

Materials. Antimycin A, ATP, NADH, NADPH, cytochrome *c* (horse-heart type III) and acid phosphatase substrate tablets were from Sigma Chemical Co., London, S.W. 6. Bovine Albumin Powder (fraction V from bovine plasma) was obtained from Armour Pharmaceutical Co. Ltd, Eastbourne, Sussex.

Presentation of results. Enzyme units are expressed as nmoles substrate transformed/min. or measured product formed/min. In Fig. 5 to 7 vertical lines divide the diagrams at density intervals of 0.05 g./ml.

RESULTS

Enzymes present in post-mitochondrial supernatants. Post-mitochondrial supernatants contained the following enzymes: NADH- and NADPH-cytochrome *c* oxidoreductases, acid and alkaline *p*-nitrophenylphosphatases, acid DNase and ATPase. Specific activities and percentage of the total units of the whole homogenate present in this fraction for cells harvested from 1-day (exponential phase) and 6-day-old (late-stationary phase) cultures are presented in Table 1. Enzymes looked for but not present at detectable levels included PPase, TPPase, AMPase, UDPase, GDPase, ADPase, glucose-6-Pase, monoamine oxidase, aryl sulphatase, β -glucuronidase, *N*-ethyl morphine demethylase, UDP-glucosyl transferase, UDP-glucuronyl transferase, aniline hydroxylase, and aromatic nitroreductase.

Difference spectra of microsomal fractions. Extinction maxima at 555 to 558 nm. and 428 nm. in difference spectra (dithionite reduced minus oxidized) at room temperature (Fig. 1, B), or at 557, 526 to 530 and 425 nm. at low temperature (Fig. 1, C) were similar to

Fig. 1. Difference spectra of *Tetrahymena* microsomal fraction. The suspension of microsomes contained 0.66 mg. protein/ml. Curve A represents the baseline (oxidized minus oxidized). Curve B was obtained after the addition of excess sodium dithionite to the sample cuvette. Curve C is the difference spectrum (dithionite reduced minus oxidized) obtained at the temperature of liquid N_2 . Path lengths were 1 cm. in A and B, and 2 mm. in C.

those observed for microsomes of mammalian origin (Estabrook & Cohen, 1969), and may be indicative of the presence of both cytochromes b_5 and P_{450} . The former component was specifically reduced by NADH or NADPH (Fig. 2, A), while the latter was best seen in dithionite reduced-CO minus dithionite reduced spectra (Fig. 2, B). Another CO-binding pigment is also present which gives a maximum at 422 nm.

Fig. 2. Difference spectra of *Tetrahymena* microsomal fraction. The suspensions contained 3.7 mg. protein/ml. Curve A was obtained after the addition of 1 mg. of NADH to the sample cuvette (NADH-reduced minus oxidized) and has been corrected for the baseline (oxidized minus oxidized). An identical spectrum was obtained when NADPH replaced NADH in this experiment. Further increases in extinction occurred at 555 and 424 nm. on adding excess Na-dithionite. Curve B represents the CO spectrum (dithionite reduced—CO minus dithionite reduced) corrected for the appropriate baseline (dithionite reduced minus dithionite reduced).

After treatment of the microsomal fraction by the method of Falk (1964), the presence of the pyridine haemochromogen derivative of protohaem (extinction maxima at 557 and 527 nm., Fig. 3, B) was demonstrated. This confirmed the presence of *b*-type cytochrome; no haems *a* or *c* were detected in this fraction.

Fig. 3. Difference spectra for pyridine haemochrome derivatives from *Tetrahymena* microsomal fraction. The extract containing protohaem (and haem *a* if present; Falk, 1964) was resuspended in distilled water, as was the pellet obtained after extraction (containing haem *c*, if present). The difference spectrum was obtained after the addition of pyridine (1 ml.) and M-NaOH (0.5 ml.) to the preparations (4.5 ml.). To one cuvette 1 mg. of K-ferricyanide was added, and to the other 1 mg. of Na-dithionite. Curve A indicates that haem *c* was absent while curve B shows the presence of protohaem only.

Fig. 4. Difference spectra of the pigments of *Tetrahymena* microsomal fraction resulting from additions of aniline HCl and ethyl morphine. Curve A, oxidized minus oxidized (baseline). Curve B was obtained after adding 3 mM-aniline HCl to one cuvette (oxidized + aniline HCl minus oxidized) and curve C was traced after adding 3 mM-ethyl morphine (oxidized + ethyl morphine minus oxidized). Protein concentration was 0.68 mg./ml.

The addition of 3 mM-aniline hydrochloride to an oxidized microsomal preparation gave a spectrum with a trough at 390 nm. and a peak at 410 nm. (Fig. 4, B). Ethyl morphine (3 mM) produced an extinction minimum at 415 nm. and a maximum at 435 nm. (Fig. 4, C).

Fig. 5. Post-mitochondrial supernatant from organisms harvested after growth for 1 day (population 36,000 organisms/ml.) fractionated in a BXIV zonal rotor. The post-mitochondrial supernatant (7.8 ml.) contained 42 mg. protein. This was diluted 1 in 40 and 0.2 ml. was found to be a suitable volume for enzyme, phosphate and protein assays. Centrifugation was at 35,000 rev./min. for 165 min. ($\equiv 6 \times 10^6 \text{ g min.}$ at the sample zone; $\int_0^t \omega^2 dt = 1.3 \times 10^{11} \text{ rad.}^2 \text{s.}^{-1}$). (a) Sucrose density gradient ($\circ$), protein ($\bullet$) and $E_{260\text{nm}}$. (continuous line). (b) NADH- ($\blacksquare$) and NADPH- ($\blacktriangle$) cytochrome *c* oxidoreductases. (c) Acid *p*-nitrophenylphosphatase ($\bullet$) and P_i ($\circ$).

The supernatant obtained after sedimentation of the microsomal fraction contained no spectrally-detectable haemoproteins.

Subfractionation of post-mitochondrial supernatants by zonal centrifugation. In order to separate the component membrane systems of the PMS, they were centrifuged through sucrose gradients in the BXIV zonal rotor. The distributions of enzymes through the gradient after centrifugation of PMS (prepared from organisms from a 1-day-old culture) at 35,000 rev./min. for 165 min. is shown in Fig. 5. The profile of extinction at 260 nm. is complex

Fig. 6. Post-mitochondrial supernatant from organisms harvested after growth for 1 day (population 54,000 organisms/ml.) fractionated in a BXIV zonal rotor. The post-mitochondrial supernatant (17.5 ml.) contained 195 mg. protein. This was diluted 1 in 40 and 0.2 ml. was found to be a suitable volume for all the assays. Centrifugation was at 35,000 rev./min. for 6 h. ($\equiv > 12 \times 10^6 g$ min. at the sample zone: $\int_0^t \omega^2 dt = 3.0 \times 10^{11} \text{ rad.}^2 \text{ s.}^{-1}$). (a) Sucrose density gradient (○), protein (●), P_i (△) and $E_{260\text{ nm}}$ (continuous line). (b) NADH- (■) and NADPH- (▲) cytochrome *c* oxidoreductases. (c) Acid *p*-nitrophenylphosphatase (●), DNase (○) and ATPase (▲).

(Fig. 5a); the major zone (4) contained (74S) ribosome monomers while the other zones contained (in order of increasing *S*-values) soluble nucleotides (1), 30S and 56S ribosome subunits (2, 3), 102S ribosome dimers (5) and 125S trimers (6). The gradient was not designed to demonstrate the presence of larger polyribosomes (Cameron *et al.* 1966; Hartman & Dowben, 1970*a, b*); an exponential gradient is best for this purpose (Marcus *et al.* 1967). Five distinct sedimentable populations of particles containing NADPH-cytochrome *c* oxidoreductase were revealed under these conditions (Fig. 5*b*). The distribution of this

Fig. 7. Post-mitochondrial supernatant from organisms harvested after growth for 6 days (population 2.5×10^8 organisms/ml.) fractionated in a BXIV zonal rotor. The post-mitochondrial supernatant (7.5 ml.) contained 120 mg. protein. This was diluted 1 in 40 and 0.2 ml. was found to be a suitable volume for all the assays. Centrifugation was at 35,000 rev./min. for 165 min. ($\cong 6 \times 10^6 g$ min. at the sample zone: $\int_0^\omega \omega dt = 1.45 \times 10^{11} \text{ rad. } ^2\text{s.}^{-1}$) (a) Sucrose density gradient (○), protein (●) and $E_{260\text{nm}}$. (b) Acid (●) and alkaline (○) *p*-nitrophenylphosphatases. (c) NADH- (■) and NADPH- (▲) cytochrome *c* oxidoreductases.

enzyme system was clearly different from that of the NADH-linked system which gave only one major zone at $\rho = 1.24$. Both these activities were completely insensitive to antimycin A, which inhibits mitochondrial nicotinamide nucleotide-linked cytochrome *c* oxidoreductases (Turner, Lloyd & Chance, 1971). The distribution of P_i (Fig. 5c) followed that of protein (Fig. 5a) closely at $\rho < 1.10$, but was quite different throughout the remainder of the gradient. Acid *p*-nitrophenylphosphatase gave a single major zone at $\rho = 1.165$.

In order to find whether these membrane components had attained their equilibrium densities, a similar experiment was performed, but the time of centrifugation was increased to 6 h. at 35,000 rev./min. The 260 nm. profile (Fig. 6a) indicates that ribosome subunits and monomer had moved further down the gradient. The sedimentable NADPH-cytochrome *c* oxidoreductase then showed a single zone at $\rho = 1.22$ and this corresponded also to the position of the NADH-driven enzyme system. The disappearance of the multiple zones of NADPH-cytochrome *c* oxidoreductase activity demonstrated that in the previous experiment (Fig. 5), membrane fragments with which this activity was associated have not attained their isopycnic density. In this experiment a large proportion of the total NADPH-cytochrome *c* oxidoreductase was non-sedimentable; this can only be accounted for by assuming that the conditions of cell disruption were more severe than in the last experiment. Further confirmation of this conclusion came from the presence of a trace of antimycin A sensitivity in fraction 53. Acid *p*-nitrophenylphosphatase again gave a single major zone at $\rho = 1.165$. ATPase showed an extremely heterogeneous distribution. A major zone at $\rho < 1.10$ did not correspond with any of the other enzymes assayed while the activity found above $\rho = 1.15$ overlapped the zones of acid *p*-nitrophenylphosphatase and those of the two reductases.

Zonal centrifugation of a post-mitochondrial supernatant from late stationary phase organisms confirmed the previous observations of Lloyd *et al.* (1971) that the density-distributions of both oxidoreductases is markedly altered in homogenates from 'aged' organisms (compare Fig. 5 and 7). The major sedimentable zone (Fig. 7c) appeared at $\rho = 1.20$ and the distribution of both NADH- and NADPH-cytochrome *c* oxidoreductases was multimodal. Distributions of the two oxidoreductases were still different from one another. Alkaline *p*-nitrophenylphosphatase (Fig. 7b) was almost entirely non-sedimentable, while the distribution of acid *p*-nitrophenylphosphatase was similar to that in the corresponding experiment with 1-day-old organisms.

A summary of recoveries of enzyme units and proportion of total units of the whole homogenates present in the post-mitochondrial supernatants is presented in Table 1.

DISCUSSION

There have been few studies on the components of post-mitochondrial supernatants of homogenates prepared from eukaryotic micro-organisms. Fatty-acid synthase and desaturase are present in yeast microsomal fractions (Klein, Volkmann & Chao, 1967), and anaerobically grown yeast contains five haemoproteins including cytochromes b_1 and P_{450} (Ishidate, Kawaguchi, Tagawa & Hagiwara, 1969). The subcellular distributions of NADH- and NADPH-cytochrome *c* oxidoreductases in aerobically grown yeast originally described by Schatz & Klima (1964) have been reappraised and examined in more detail by Cartledge & Lloyd (1972). Finally, polyribosome separations have been described for yeast (Marcus *et al.* 1967) and for *Tetrahymena pyriformis* (Cameron *et al.* 1966; Hartman & Dowben, 1970a, b).

The preparation from *Tetrahymena* of post-mitochondrial supernatants and microsomal fractions which do not contain fragments of membrane of mitochondrial origin necessitates

Table 1. *Specific activities of enzymes and their recoveries in whole homogenates and post-mitochondrial supernatants*

Data calculated for the experiments shown in Fig. 5 to 7. Absence of a figure indicates enzyme not assayed (WH = whole homogenate, PMS = post-mitochondrial supernatant and P₁ = pellet obtained on centrifuging WH at 10,000 g for 10 min.).

	Enzyme units				
	Specific activities of enzymes in		Recovery $\left(\frac{P_1 + PMS}{WH}\right)\%$	Recovery in 6 fractions from PMS (%)	Total units of WH in PMS (%)
	WH	PMS			
Homogenates from 1-day organisms (Fig. 5)					
Protein	—	—	93	105	—
NADH-cytochrome <i>c</i> oxidoreductase	71.2	26.0	102	74	22
NADPH-cytochrome <i>c</i> oxidoreductase	11.6	14.4	120	102	73
Acid <i>p</i> -nitrophenylphosphatase	24.4	7.0	65	53	17
Alkaline <i>p</i> -nitrophenylphosphatase	—	—	—	—	—
ATPase	—	—	—	—	—
DNase	—	—	—	—	—
Homogenates from 1-day organisms (Fig. 6)					
Protein	—	—	100	88	—
NADH-cytochrome <i>c</i> oxidoreductase	57.8	26.1	67	140	39
NADPH-cytochrome <i>c</i> oxidoreductase	13.5	9.7	99	113	61
Acid <i>p</i> -nitrophenylphosphatase	40.7	38.6	103	111	41
Alkaline <i>p</i> -nitrophenylphosphatase	—	—	—	—	—
ATPase	85.5	170.0	86	97	75
DNase	6.1	23.0	99	130	57
Homogenates from 6-day organisms (Fig. 7)					
Protein	—	—	96	114	—
NADH-cytochrome <i>c</i> oxidoreductase	23.5	19.4	96	85	75
NADPH-cytochrome <i>c</i> oxidoreductase	8.5	9.5	83	87	76
Acid <i>p</i> -nitrophenylphosphatase	20.3	10.0	112	—	—
Alkaline <i>p</i> -nitrophenylphosphatase	18.6	31.3	—	102	77
ATPase	—	—	—	—	—
DNase	—	—	—	—	—

gentle disruption; if more than 60% of the organisms are broken, mitochondrial contamination does occur and is detected spectrophotometrically by the presence of the typically mitochondrial haemoproteins (Turner *et al.* 1971) and by partial antimycin-A sensitivity of the NADH-cytochrome *c* oxidoreductase. These criteria do not exclude the possibility of contamination by outer mitochondrial membranes and there are no unequivocal markers for these in *Tetrahymena* (G. Turner & D. Lloyd, unpublished work).

Inability to detect many of the enzymes present in microsomal fractions from mammalian tissues led us to study nicotinamide nucleotide linked oxidoreductases which are often associated with rough and smooth endoplasmic reticulum (see review by Reid, 1967); on isolation these membranes give rise to the 'microsomal fraction'. The natural carriers in the mammalian microsomal electron-transport chains are cytochromes *b*₅ and *P*₄₅₀; similar respiratory pigments were detectable in *Tetrahymena*. All of the cytochrome *b*₅ was reducible by either NADH or NADPH, while dithionite also reduced *P*₄₅₀. In mammalian systems, *P*₄₅₀ acts as the oxygen-activating enzyme and site of substrate interaction for oxidative transformations of various lipophilic foreign compounds and steroids, while cytochrome

b_5 is involved in oxidative desaturation of fatty acids (Sato, Nishibayashi & Ito, 1969). Mammalian cytochrome *c* acts as an artificial acceptor in these electron-transport chains, and its reduction provides a convenient marker for microsomal membranes.

The addition of drugs to microsomal fractions of mammalian cells gives distinctive spectral changes because they bind with cytochrome P_{450} (Imai & Sato, 1966). With *Tetrahymena* microsomal fractions, aniline HCl gave the 'type II' trough at 390 nm. but the maximum was at 410 nm. rather than at 430 nm. Ethyl morphine also gave a spectrum which was quite different from that with mammalian microsomes. Thus although enzymes involved in the metabolism of these drugs could not be detected in *Tetrahymena* (and could not be induced by growth in the presence, for example, of phenobarbitone, L. Howells & D. Lloyd, unpublished results), these drugs can be bound to microsomal haemoprotein. Possibly the P_{420} plays a role in this binding, as the spectral changes observed did not follow the classical pattern. This pigment (which may be similar to that formed on denaturation of mammalian P_{450} , Omura & Sato, 1964) must be a *b*-type cytochrome, as only protohaem was detectable in *Tetrahymena* microsomes.

Heterogeneity of microsomal membranes was evident after rate separation on a size basis in the zonal centrifuge. The most rapidly moving component had reached its isopycnic density ($\rho = 1.23$), contained both NADH- and NADPH-cytochrome *c* oxidoreductases, and had an extinction at 260 nm.; this was followed by membrane fragments (sedimentation coefficients 125*S*, 102*S* and 74*S*) rich in the NADPH-linked activity. Extended centrifugation sedimented all three zones to $\rho = 1.20$ to 1.23. As previously observed (Lloyd *et al.* 1971) the pattern of sedimentability of these enzyme systems was altered in homogenates of stationary phase organisms.

The simplicity of distributions of acid *p*-nitrophenylphosphatase contrasted with the heterogeneity of organelles containing this enzyme seen when whole homogenates were centrifuged under similar conditions (Lloyd *et al.* 1971). Thus it seems that the smallest lysosomal vacuoles containing this enzyme were very homogeneous with respect to density, and that several of the populations of the whole homogenate (including that not sedimentable beyond $\rho = 1.10$) previously observed consisted of particles large and dense enough to have been sedimented with the mitochondria at $10^5 g$ min. in 0.24 M-sucrose. Acid DNase was present in two major zones; the sedimentable zone was distinct from that containing the *p*-nitrophenylphosphatase. ATPase also shows a density distribution different from the other enzymes assayed.

These results suggest that there are many different populations of membranes in the microsomal fraction of *Tetrahymena* and may reflect a structural and functional diversity of the cytomembranes of intact organisms.

We thank Professor Hughes for his interest and encouragement, and Dr M. G. Townsend for information on mammalian drug metabolism.

REFERENCES

- ANDERSON, N. G., WATERS, D. A., FISHER, W. D., CLINE, G. B., NUNLEY, C. E., ELROD, L. H. & RANKIN, C. T. (1967). Analytical techniques for cell fractionations. V. Characteristics of the BXIV and BXV zonal centrifuge rotors. *Analytical Biochemistry* **21**, 235-252.
- CAMERON, I. L., CLINE, G. B., PADILLA, G. M., MILLER, JUN., O. L. & VAN DREAL, P. A. (1966). Polysomes from *Tetrahymena* following pyrimidine deprivation and replacement. *National Cancer Monograph* **21**, 361-369.

- CARTLEDGE, T. G. & LLOYD, D. (1972). Distributions of marker enzymes in aerobically-grown, glucose derepressed *Saccharomyces carlsbergensis* after zonal centrifugation: effects of EDTA and magnesium ions. *Biochemical Journal* (in the Press).
- DE DUVE, C., BERTHET, J. & BEAUFAY, H. (1959). Gradient centrifugation of cell particles. Theory and applications. *Progress in Biophysical Chemistry* **9**, 326-369.
- ESTABROOK, R. W. & COHEN, B. (1969). Organization of the microsomal electron transport system. In *Microsomes and Drug Oxidations*, p. 95. Edited by J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts & G. J. Mannering. New York and London: Academic Press.
- FALK, J. E. (1964). *Porphyryns and Metalloporphyrins*. New York: Elsevier Publishing Co.
- HALSALL, H. B. & SCHUMAKER, V. N. (1969). Sedimentation coefficients in the zonal centrifuge. *Analytical Biochemistry* **30**, 368-376.
- HARTMAN, H. & DOWBEN, R. M. (1970a). Polyribosomes from *Tetrahymena*. *Journal of Cell Biology* **44**, 676-678.
- HARTMAN, H. & DOWBEN, R. M. (1970b). Polyribosomes profiles from heat synchronized *Tetrahymena*. *Biochemical and Biophysical Research Communications* **40**, 964.
- IMAI, Y. & SATO, R. (1966). Substrate interaction with hydroxylase system in liver microsomes. *Biochemical and Biophysical Research Communications* **22**, 620-626.
- ISHIDATE, K., KAWAGUCHI, K., TAGAWA, K. & HAGIHARA, B. (1969). Haemoproteins in anaerobically-grown yeast cells. *Journal of Biochemistry, Tokyo* **65**, 375-383.
- KLEIN, H. P., VOLKMANN, C. M. & CHAO, F. C. (1967). Fatty acid synthetase of *Saccharomyces cerevisiae*. *Journal of Bacteriology* **93**, 1966-1971.
- LELOIR, L. F. & CARDINI, C. E. (1957). Characterization of phosphorus compounds by acid lability. *Methods in Enzymology* **3**, 840.
- LLOYD, D., BRIGHTWELL, R., VENABLES, S. E., ROACH, G. I. & TURNER, G. (1971). Subcellular fractionation of *Tetrahymena pyriformis* ST by zonal centrifugation: changes in activities and distribution of enzymes during the growth cycle and on starvation. *Journal of General Microbiology* **65**, 209-223.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275.
- MARCUS, L., RIS, H., HALVORSON, H. O., BRETTHAUER, R. K. & BOCK, R. M. (1967). Occurrence, isolation and characterization of polyribosomes in yeast. *Journal of Cell Biology* **34**, 505-512.
- OMURA, T. & SATO, R. (1964). The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its haemoprotein nature. *Journal of Biological Chemistry* **239**, 2370-2378.
- POOLE, R. K., NICHOLL, W. G., TURNER, G., ROACH, G. I. & LLOYD, D. (1971). Sedimentation characteristics of mitochondria, peroxisomes and lysosomes from the ciliate protozoon, *Tetrahymena pyriformis*, strain ST after chloramphenicol-inhibited growth. *Journal of General Microbiology* **67**, 161-173.
- REID, E. (1967). Membrane systems. In *Enzyme Cytology* p. 321. Edited by D. B. Roodyn. London and New York: Academic Press.
- SATO, R., NISHIBAYASHI, H. & ITO, A. (1969). Characterization of two haemoproteins of liver microsomes. In *Microsomes and Drug Oxidations*, p. 111. Edited by J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, G. J. Mannering. New York and London: Academic Press.
- SCHATZ, G. & KLIMA, J. (1964). Triphosphopyridine nucleotide cytochrome *c* reductase of *Saccharomyces cerevisiae*: a microsomal enzyme. *Biochimica et biophysica acta* **81**, 448-461.
- TURNER, G., LLOYD, D. & CHANCE, B. (1971). Electron transport in phosphorylating mitochondria from *Tetrahymena pyriformis* ST. *Journal of General Microbiology* **65**, 359-374.