early in their life cycle (phase G1), and cells near the bottom at the end of the cycle (phase G2). The experiment with labelled thymidine shows that cells near the middle of the gradient are in the period of DNA synthesis (phase S).

These results, in confirmation of other work (Bergeron, Warmley & Pasternak, 1969), indicate that incorporation of proline, uridine and choline into protein, RNA and phospholipid respectively is not limited to a specific portion of the cell cycle (as is the case with thymidine), nor is the rate of incorporation constant throughout the cell cycle. Rather incorporation appears to increase from a low rate in phase G1 to a higher rate in phase G2. Such an increase has been observed by using other techniques in the case of RNA and protein synthesis (e.g. Robbins & Scharff, 1966; Pfeiffer & Tolmach, 1968; Martin, Tomkins & Granner, 1969).

A.M.H.W. is a Medical Research Council Scholar.
J.J.M.B. is a Rhodes Scholar.

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Subcellular Fractionation of *Polytomella caeca* by Zonal Centrifugation

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Lloyd, Evans & Venables (1968) have shown that resting cells of acetate-grown *Polytomella caeca* oxidize propionate only after a lag of 2 hr., and that this adaptation involves the formation of enzymes of β -oxidation. Two of these enzymes, β -hydroxypropionate dehydrogenase and malonic semialdehyde dehydrogenase, were shown by differential centrifugation to be located in a mitochondrial fraction. Present studies were undertaken to confirm this observation and to investigate the distribution of other enzymes in homogenates prepared from suspensions of this organism.

Cells were disrupted in an all-glass hand-operated homogenizer in a medium consisting of 0.32M-sucrose in 24 mM-tris-HCl buffer, pH 7.4, containing

10 mM-EDTA. After the unbroken cells had been centrifuged off, the homogenates were loaded on to a sucrose density gradient (buffered with 10 mM-tris-HCl at pH 7.4) in a B XIV rotor and centrifuged at 35 000 rev./min. for 2.75 hr. (100 000g at the sample zone).

Under these conditions mitochondria (marker enzyme, cytochrome c oxidase) sedimented to their isopycnic density (1.19–1.21 g./ml.). A major peak of catalase activity at density 1.24 (1.22 in an unbuffered gradient) suggests that this organism contains peroxisomes. Three peaks of acid phosphatase activity (substrate, *p*-nitrophenyl phosphate) were located: a 'non-sedimentable' activity and activities associated with particles sedimenting to densities 1.14 and 1.20–1.21 respectively. The distribution of esterase activity (substrate, *p*-nitrophenyl acetate) was similar to that of acid phosphatase. Most of the NADPH-cytochrome c oxidoreductase activity was 'non-sedimentable', whereas NADH-cytochrome c oxidoreductase activity was mainly located in the mitochondrial peak and also at a density of 1.11. A portion of the malate dehydrogenase (40–60%), citrate synthase (70–80%) and NADP-linked isocitrate dehydrogenase (8%) activities was mitochondrial; variation in the sedimentability of these activities may be used as an index of mitochondrial breakage. Isocitrate lyase was nearly all (> 99%) detected as a 'soluble' activity.

β -Hydroxypropionate dehydrogenase was located mainly (> 95%) in the mitochondrial fractions of both propionate-grown cells and acetate-grown cells that had been adapted to propionate.

R.A.C. holds a Medical Research Council Research Studentship.

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Palmitic Acid Uptake and Metabolism by Isolated Rat Liver Cells

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Previous studies on isolated liver cells (Higgins & Green, 1967) raised the possibility that incorporation of fatty acids into plasma-membrane phospholipids was involved in their uptake. The cells used in these earlier studies displayed metabolic deficiencies, but new techniques (Howard, Christensen, Gibbs & Pesch, 1967) give suspensions of cells in much better condition. When such cell suspensions were incubated for up to 2 hr. with a [14 C]palmitic acid-albumin complex in the presence of ATP,