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, Warmsley & 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 2hr., 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 24mM-tris–HCl buffer, pH 7·4, containing 10mM-EDTA. After the unbroken cells had been centrifuged off, the homogenates were loaded on to a sucrose density gradient (buffered with 10mM-tris–HCl at pH 7·4) in a B XIV rotor and centrifuged at 35000rev./min. for 2·75hr. (100000*g* at the sample zone).

Under these conditions mitochondria (marker enzyme, cytochrome *c* oxidase) sedimented to their isopycnic density (1·19–1·21g./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.

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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 2hr. with a [^{14}C]palmitic acid–albumin complex in the presence of ATP,

CTP, CoA, phosphorylcholine chloride, α-glycerophosphate and $MgCl_2$, the isotope was rapidly incorporated into glycerides. At all times phospholipids contained the bulk of the esterified [^{14}C]-palmitic acid with only a small proportion in the neutral lipid. With incubations of up to 5min. phosphatidylcholine (lecithin) was the major labelled phospholipid. Thereafter phosphatidic acid was predominant although incorporation into both phosphatidylcholine and phosphatidic acid increased with time. After 2hr. phosphatidic acid contained 48·6%, phosphatidylcholine + phosphatidylethanolamine 24·4%, mono- and di-glycerides 19·2% and triglycerides 6·9% of the esterified [^{14}C]palmitic acid.

The acetic acid method of Warren, Glick & Nass (1966) for isolation of plasma membranes was applied to the isolated liver cells and a preparation obtained that under phase contrast appeared to consist of empty cell membranes and was enriched five- to six-fold in L-leucyl-β-naphthylamidase and alkaline phosphatase. In the early stages of [^{14}C]-palmitic acid uptake (less than 5min.) virtually all of the radioactive phospholipid formed was found in this cell fraction. As uptake continued the isotope accumulated in the remainder of the cell.

In the absence of added cofactors the cells (unlike those used in previous studies) still incorporated [^{14}C]palmitic acid into glycerides. The rate of incorporation was about 1.7μmole/g. dry wt./hr., which compares with a rate of about 5μmoles/g. dry wt./hr. for liver slices under the same conditions. The alterations in the pattern of fatty acid incorporation induced by starvation that were demonstrated by Vavrečka, Mitchell & Hübscher (1968) in liver slices can also be seen in isolated cells.

Incorporation into total glycerides by the cells was the same whether cofactors were added or not, but in the latter case the bulk of the [^{14}C]palmitic acid appeared in triglycerides (64·9% after 2hr.), not in phosphatidic acid. This suggests that in the former case phosphatidate phosphohydrolase was inhibited.

Addition of 1-acylglycerophosphorylcholine (lysolecithin) up to 0·1mM increased the incorporation of palmitic acid into cell lipids, but higher concentrations were inhibitory.

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The Transport of Palmitic Acid across Intestinal Brush-Border Membranes

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It is generally assumed that long-chain fatty acids are transported from the intestinal lumen into mucosal epithelial cells by a passive diffusion process (Johnston & Borgström, 1964). However, a study of palmitic acid uptake by everted rings of rat jejenum revealed that fatty acid uptake (expressed as μmoles/100mg. wet wt. of intestine/2hr.) was decreased from a control value of $0·73 \pm 0·02$ to $0·52 \pm 0·04$ ($P < 0·01$) in the absence of Ca^{2+} ions from the incubation medium. These findings led to a study of the effects of Ca^{2+} ions on the binding and release of palmitic acid by isolated brush-border membranes.

Isolated brush-border membranes were prepared by the method of Miller & Crane (1961) as modified by Harrison & Webster (1964). The brush borders were incubated for 1hr. at 37° in Krebs phosphate buffer, pH 6·3 (Krebs & Henseleit, 1932), containing albumin–[1-^{14}C]palmitic acid complex (Greenberger, N. J., Franks, J. J. & Isselbacher, K. J., 1965). Again, the uptake of palmitic acid, expressed as μmoles, was decreased from a control value of $1·15 \pm 0·05$ to $0·57 \pm 0·05$ ($P < 0·001$) in the absence of Ca^{2+} ions.

The release of previously incorporated [1-^{14}C]-palmitic acid from brush borders into the incubation medium was also studied. In a control medium $0·77 \pm 0·01$ μmole of palmitic acid was released and this increased to $0·99 \pm 0·03$ μmole ($P < 0·005$) in the absence of Ca^{2+} ions.

These results indicate that Ca^{2+} ions increase fatty acid transport into everted rings of rat intestine. Similarly the Ca^{2+} ion content of the incubation medium has also been shown to influence palmitic acid uptake and release by isolated brush-border membranes. These findings lead to the conclusion that the transport of long-chain fatty acids across brush-border membranes may involve two processes: (i) passive diffusion, shown in these experiments as uptake and release of palmitic acid in the absence of Ca^{2+} ions; (ii) a Ca^{2+}-dependent process that, with an imbalance of Ca^{2+} ions across the membrane, provides a mechanism directed to the movement of fatty acid into the cell.

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