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 5 min. phosphatidylcholine (lecithin) was the major labelled phospholipid. Thereafter phosphatidic acid was predominant although incorporation into both phosphatidylcholine and phosphatidic acid increased with time. After 2 hr. 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 5 min.) 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 $1.7 \mu\text{mole/g. dry wt./hr.}$, which compares with a rate of about $5 \mu\text{moles/g. dry wt./hr.}$ 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 2 hr.), not in phosphatidic acid. This suggests that in the former case phosphatidate phosphohydrolase was inhibited.

Addition of 1-acylglycerophosphorylcholine (lysolecithin) up to 0.1 mM increased the incorporation of palmitic acid into cell lipids, but higher concentrations were inhibitory.

- Higgins, J. A. & Green, C. (1967). *Biochem. J.* **104**, 26P.
 Howard, R. B., Christensen, A. K., Gibbs, F. A. & Pesch, L. A. (1967). *J. Cell Biol.* **35**, 675.
 Vavrečka, M., Mitchell, M. P. & Hübscher, G. (1968). *Biochem. J.* **110**, 52P.
 Warren, L., Glick, M. C. & Nass, M. K. (1966). *J. Cell Physiol.* **68**, 269.

The Transport of Palmitic Acid across Intestinal Brush-Border Membranes

By K. A. MUNDAY, B. J. PARSONS and D. A. YORK.
 (Department of Physiology and Biochemistry,
 University of Southampton)

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 jejunum revealed that fatty acid uptake (expressed as $\mu\text{moles/100mg. wet wt. of intestine/2hr.}$) was decreased from a control value of 0.73 ± 0.02 to 0.52 ± 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 1 hr. at 37° in Krebs phosphate buffer, pH 6.3 (Krebs & Henseleit, 1932), containing albumin-[$1\text{-}^{14}C$]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 ± 0.05 to 0.57 ± 0.05 ($P < 0.001$) in the absence of Ca^{2+} ions.

The release of previously incorporated [$1\text{-}^{14}C$]palmitic acid from brush borders into the incubation medium was also studied. In a control medium $0.77 \pm 0.01 \mu\text{mole}$ of palmitic acid was released and this increased to $0.99 \pm 0.03 \mu\text{mole}$ ($P < 0.005$) 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.

D.A.Y. is in receipt of a Medical Research Council Training Scholarship.

- Greenberger, N. J., Franks, J. J., & Isselbacher, K. J. (1965). *Proc. Soc. exp. Biol. Med.* **120**, 468.
 Harrison, D. D. & Webster, H. L. (1964). *Biochim. biophys. Acta*, **93**, 662.
 Johnston, J. M. & Borgström, B. (1964). *Biochim. biophys. Acta*, **84**, 421.

Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
 Miller, D. & Crane, R. K. (1961). *Analyt. Biochem.* **2**, 284.

Inhibition of Palmitoyl-L-Carnitine Oxidation in Rat Liver Mitochondria by Pent-4-enoyl-L-carnitine

By P. C. HOLLAND and H. S. A. SHERRATT. (*Department of Pharmacology, University of Newcastle upon Tyne*)

Some fatty acids with a methylene group separated by two carbon atoms from a carboxyl group possess hypoglycaemic activity (Anderson *et al.* 1958). Of these, pent-4-enoic acid (Senior, Robson & Sherratt, 1968) and methylenecyclopropylacetic acid (Holt, Holt & Böhm, 1966) are known to inhibit strongly the formation of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ -palmitate in rat liver mitochondria. By contrast, the non-hypoglycaemic compounds *n*-pentanoic acid, cyclobutanecarboxylic acid and cyclopropanecarboxylic acid do not.

Oxidations in mitochondria were measured polarographically at 30° and pH 7.0 in the presence of 20mM-malonate. Pent-4-enoic acid (0.1mM) inhibited the oxidation of palmitoyl-L-carnitine by 80% and this inhibition was abolished under uncoupling conditions (addition of 0.02mM-dinitrophenol and 10mM-arsenate), indicating that an activated derivative is responsible. Preincubation of uncoupled mitochondria with pent-4-enoyl-L-carnitine for 3min. inhibited palmitoyl-L-carnitine oxidation (80% at 0.1mM; 50% at 0.01mM), when the maximum remaining rate of oxidation was only reached 2–3min. after the addition of palmitoyl-L-carnitine, in contrast with the linear rate immediately obtained in the absence of inhibitor. *n*-Pentanoyl-L-carnitine, cyclobutanecarbonyl-L-carnitine and cyclopropanecarbonyl-L-carnitine only inhibited slightly under these conditions.

Intramitochondrial pent-4-enoyl-CoA is probably formed from exogenous pent-4-enoyl-L-carnitine and this might inhibit long-chain fatty acid oxidation, either directly or by binding CoA. Alternatively, possible products of β -oxidation of pent-4-enoyl-L-carnitine may be inhibitory; although any acryloyl-CoA formed may not inhibit strongly as suggested by Williamson, Scholz & Fukami (1969), since the oxidation of palmitoyl-L-carnitine is only inhibited weakly by acrylic acid in coupled mitochondria (50% at 2mM, 10% at 0.1mM). There was a pulse of oxygen uptake of 30ng.atoms/mg. of protein when 0.1mM-pent-4-enoyl-L-carnitine was added to uncoupled mitochondria, which may be compared with the amount of pent-4-enoyl-CoA that could be formed assuming sequestration of the total mitochondrial CoA content of about 2nmoles/

mg. of protein (Garland, Shepherd & Yates, 1965). It is therefore not yet possible to explain how pent-4-enoic acid or methylenecyclopropylacetic acid inhibits long-chain fatty acid oxidation (see Senior *et al.* 1968).

We thank Dr G. A. Swan for help with the synthesis and characterization of pent-4-enoyl-L-carnitine and the British Diabetic Association for financial support.

Anderson, H. V., Johnson, J. L., Nelson, J. W., Olson, E. C., Speeter, M. E. & Vavra, J. J. (1958). *Chem & Ind.* p. 330.
 Garland, P. B., Shepherd, D. & Yates, D. W. (1965). *Biochem. J.* **97**, 587.

Holt, L. von, Holt C. von & Böhm, H. (1966). *Biochim. biophys. Acta*, **125**, 11.

Senior, A. E., Robson, B. & Sherratt, H. S. A. (1968). *Biochem. J.* **110**, 511.

Williamson, J. R., Scholz, R. & Fukami, M. (1969). *Abstr. 6th Meet. Fed. Europ. biochem. Soc., Madrid*, p. 131.

The Chain Length of Fatty Acids Synthesized by Fatty Acid Synthetase

E. M. CAREY and R. DILS (*Department of Biochemistry, University of Birmingham*) and H. HANSEN (*Atomic Energy Commission, Roskilde, Denmark*)

The triglycerides of many tissues contain predominantly long-chain fatty acids, and the synthesis of these acids by fatty acid synthetase has been extensively studied in a number of tissues. In pigeon and rat liver, the product of fatty acid synthetase at optimum substrate concentrations is predominantly free palmitic acid (Wakil, 1961; Hsu, Wasson & Porter, 1965; Burton, Haavik & Porter, 1968).

The triglycerides of milk from a number of species contain significant amounts of short-chain and medium-chain fatty acids (Garton, 1963; Glass, Troolin & Jenness, 1967). In rabbit milk over 70% of the esterified fatty acids are octanoic acid and decanoic acid, and these are not of dietary origin (Smith, Watts & Dils, 1968). Particle-free extracts of lactating rabbit mammary gland synthesize a wide range ($\text{C}_{4:0}$ – $\text{C}_{18:0}$) of even-numbered saturated fatty acids from acetate by the malonyl-CoA pathway (Smith & Dils, 1964, 1966). Increasing the activity of acetyl-CoA– CO_2 ligase (ADP) in these extracts increased the rate of synthesis and the proportion of long-chain fatty acids synthesized. A high proportion of butyric acid was always synthesized, though none occurs in rabbit milk (Smith *et al.* 1968).

Fatty acid synthetase has now been isolated from rabbit mammary gland and its properties have been compared with rat liver fatty acid synthetase. At