Norman, A. & Shorb, M. (1962). *Proc. Soc. exp. Biol. Med.* **110**, 552.
Norman, A. & Widstrom, O. A. (1964). *Proc. Soc. exp. Biol. Med.* **117**, 442.

The Effect of Pent-4-enoic Acid and Some Simple Related Compounds on the Oxidation of Fatty Acids by Rat-Liver Mitochondria

By A. E. SENIOR and H. S. A. SHERRATT. (*Department of Physiology, University of Newcastle-upon-Tyne*)

Pent-4-enoic acid, methylenecyclopropaneacetic acid and 3-methylenecyclobutanecarboxylic acid are hypoglycaemic compounds structurally related to hypoglycin (Anderson *et al.* 1958). Pent-4-enoic acid partially uncouples oxidative phosphorylation and strongly inhibits pyruvate oxidation in rat-liver mitochondria (Sherratt, 1963). However, these effects are also shown to varying extents by pentanoic acid, pent-2-enoic acid, cyclopropanecarboxylic acid and cyclobutanecarboxylic acid which are not hypoglycaemic (Senior & Sherratt, 1966). von Holt, von Holt & Böhm (1966) attributed the hypoglycaemic activity of hypoglycin to the inhibition of mitochondrial long-chain fatty acid oxidation by methylenecyclopropaneacetic acid, a metabolite of hypoglycin. Pent-4-enoic acid inhibits palmitate oxidation in skin (Yardley, 1964).

Here we report the effects on fatty acid oxidation by pent-4-enoic acid and the four related simple fatty acids used in our previous work (Senior & Sherratt, 1966). [1-^{14}C]-Labelled butyrate (1mM), octanoate (1mM), laurate (0·16mM, apparent concentration) or palmitate (1mM, apparent concentration) and rat-liver mitochondria were incubated in the Warburg apparatus at pH 7·0 and 30°. The incubation medium included fumarate (0·4mM), ATP (2mM), hexokinase (EC 2.7.1.1.) (15 units) and glucose (20mM). The $^{14}CO_2$ produced was trapped in KOH and counted in a liquid scintillation spectrometer (Herberg, 1960). Laurate and palmitate suspensions were prepared by adjusting their solutions at pH 12 to pH 7·0. Control oxygen uptakes of 60–80 and 40–50mμ atoms O/min./mg. of protein were obtained using laurate and palmitate respectively.

Pent-4-enoic acid (0·01mM) inhibited $^{14}CO_2$ production from palmitate by 82%, from butyrate by 6%, from octanoate by 2% and from laurate by 10%. This strong inhibition of palmitate oxidation was not found with the four related acids and none gave a marked inhibition of butyrate, octanoate or laurate oxidation. DL-Carnitine (1mM) increased the oxidation of laurate and palmitate by up to 100% but did not affect the percentage inhibition of palmitate oxidation by pent-4-enoic acid. Only cyclopropanecarboxylic acid significantly reduced acetoacetate formation from fatty acids.

The strong inhibition of palmitate oxidation by pent-4-enoic acid and methylenecyclopropaneacetic acid may explain their hypoglycaemic activity. This conclusion is supported by the demonstration that chemically similar yet nonhypoglycaemic fatty acids do not inhibit palmitate oxidation at low concentrations.

We thank the Medical and Science Research Councils for financial support.

Anderson, H. V., Johnson, J. L., Nelson, J. W., Olsen, E. C., Specter, M. E. & Ugura, J. J. (1958). *Chem. & Ind.* (Rev.), p. 330.
Herberg, R. J. (1960). *Analyt. Chem.* **32**, 42.
Holt, von C., Holt, von M. & Böhm, H. (1966). *Biochem. biophys. Acta*, **125**, 11.
Sherratt, H. S. A. (1963). *Biochem. J.* **88**, 46P.
Senior, A. E. & Sherratt, H. S. A. (1966). *Biochem. J.* **100**, 71P.
Yardley, H. J. (1964). 6th Internat. Congr. Biochem., New York. I.U.B. **32**, p. 602.

Unesterified Fatty Acid in Brain and its Release in Subcellular Fractions

By G. G. LUNT and C. E. ROWE. (*Department of Physiological Chemistry, University of Birmingham, Birmingham* 15)

Unesterified fatty acids from brain were isolated by thin-layer chromatography (Skipski, Smolowe, Sullivan & Barclay, 1965). Acids containing 12 to 20 carbon atoms were estimated by gas chromatography using methyl heptadecanoate as an internal standard. From fresh mouse brain yields of 3μmoles/g. of protein were obtained. From mouse brain slices incubated at 0° and at 37° the yields were 9 and 8μmoles/g. of protein respectively. There was considerable incorporation of [1-^{14}C] acetate into unesterified fatty acids of mouse brain slices at 37° but little or no incorporation at 0°.

Homogenates of whole rat brain were fractionated by centrifugation in sucrose density gradients (Eichberg, Whittaker & Dawson, 1964) into fractions containing predominantly nuclei and cell fragments, synaptosomes and myelin, mitochondria, microsomal fragments, and supernatant fluid. Unesterified fatty acids were obtained from all fractions. The total yield from fractionated homogenate (2·8mg. methyl ester/g. of protein) was greater than that obtained from fresh tissue (1·5mg. of methyl ester/g. of protein), indicating that fatty acids had been released during the preparation of the fractions. The distributions of fatty acids from