was the direct precursor of linoleic acid. The results indicated that desaturation could occur either (a) on the lipid, or (b) with the lipid-bound fatty acid in direct equilibrium with the enzyme, as conditions were such that added [^{14}C]oleate could not be desaturated. The implications of these findings will be discussed.

Harris, R. V., James, A. T. & Harris, P. (1965). In *The Biochemistry of Chloroplasts*, vol. 2, p. 241. Ed. by Goodwin, T. W. London and New York: Academic Press Inc.

Nichols, B. W. (1968). *Lipids*, 3, 354.

The Localization of the Neutral Glycosphingolipids in Rat Liver Cells

By BARBARA J. DOD and G. M. GRAY. (*The Lister Institute of Preventive Medicine, London, S.W. 1*)

Neutral glycosphingolipids occur as minor lipid components of most, if not all, mammalian tissues. Their localization in the mammalian cell has not been established, but they are able to function as haptens (Rapport, Graf & Schneider, 1964) and it is therefore possible that they may contribute to the immunological properties of the cell surface. Our previous work (Dod & Gray, 1968) showed that the concentration of neutral glycosphingolipids in the plasma membranes of rat liver cells was much higher than their concentration in whole liver, and this suggested that these lipids might be exclusive to the plasma membrane of the cell. Recent studies on the glycosphingolipid content of subcellular fractions of rat liver have supported this supposition.

Plasma membranes were prepared essentially by the method of Neville (1960), mitochondria by the method of Hogeboom (1955) and an endoplasmic-reticulum fraction by a modification of the method of Wallach & Kamat (1966). The purity of each membrane fraction was assessed by measuring the activity of specific marker enzymes: 5'-nucleotidase (EC 3.1.3.5) and leucine aminopeptidase (EC 3.4.1.1) for plasma membranes; succinate dehydrogenase (EC 1.3.99.1) for mitochondria; glucose 6-phosphatase (EC 3.1.3.9) for endoplasmic reticulum. The total lipids were extracted from each of the membrane fractions and the major portions of the phospholipids and neutral lipids were removed by treatment with mild alkali. The glycosphingolipids were separated from the other alkali-stable lipids (Gray, 1967) and individual components were isolated by two-dimensional thin-layer chromatography (Gray, 1967).

The glycosphingolipid composition in the subcellular fractions was the same as that in whole liver. The two major components were ceramide monohexoside and sulphatide (molar ratio 4:1); a

small amount of ceramide dihexoside and a trace of aminoglycolipid were also present. The total glycosphingolipids in the different fractions were quantitatively determined (as sphingosine) by the method of Lauter & Trams (1962), and the results expressed as μmoles of glycosphingolipid/ μmole of phospholipid phosphorus, were: plasma membrane, 7.1; endoplasmic-reticulum, 0.79; mitochondria, 0; total rat liver, 1.3. The glycosphingolipid found in the endoplasmic-reticulum fraction can be accounted for by the presence of some plasma-membrane fragments (enzyme assay indicated a contamination of approximately 10%).

If it is accepted that the glycosphingolipids are exclusive to the plasma membranes it is possible, for the first time, to calculate that proportion of the total phospholipid of the whole cell that is contained in the plasma membrane.

The support of this work by the Medical Research Council is gratefully acknowledged.

Dod, B. J. & Gray, G. M. (1968). *Biochim. biophys. Acta*, 150, 397.

Gray, G. M. (1967). *Biochim. biophys. Acta*, 144, 511.

Hogeboom, G. H. (1955). In *Methods of Enzymology*, vol. 1, p. 16. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Lauter, C. J. & Trams, E. G. (1962). *J. Lipid Res.* 3, 136.

Neville, O. M. (1960). *Biophys. biochem. Cytol.* 8, 413.

Rapport, M. M., Graf, L. & Schneider, H. (1964). *Arch. Biochem. Biophys.* 105, 431.

Wallach, D. F. H. & Kamat, V. B. (1966). In *Methods of Enzymology*, vol. 8, p. 164. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Properties of Glycerol Phosphate Acyl Transferase from Pigeon Liver Particles

By D. R. HUSBANDS* and W. E. M. LANDS. (*Department of Biological Chemistry, University of Michigan, Ann Arbor, Mich. 48104, U.S.A.*)

Glycerol phosphate acyltransferase (Acyl-CoA-L-glycerol 3-phosphate acyltransferase, EC 2.3.1.15) has been found in guinea-pig liver particles (Kornberg & Pricer, 1953), leaf tissue of plants (Cheniae, 1965), yeast (Kuhn & Lynen, 1965) and *Escherichia coli* (Ailhaud & Vagelos, 1966). It is a particulate enzyme and its activity can be inhibited by thiol-binding agents (Lands & Hart, 1965; Kuhn & Lynen, 1965).

The enzyme from pigeon liver is associated with the cell particles. It was assayed in a system containing [^{14}C]glycerol 3-phosphate and palmitoyl- or stearoyl-CoA. The product formed by this system was predominantly diacyl phosphatide.

* Present address: Unilever Research Laboratory, Sharnbrook, Bedford, England.