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

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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.

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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

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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 phosphatidate.

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This was shown by chromatography of the triethylamine salts of phosphatidic acid on silicic acid-impregnated glass-fibre paper, with the solvent system chloroform-methanol-0.73M-ammonia soln. (200:45:6, by vol.). When the phosphate group was removed with L- α -phosphatidate phosphohydrolase (EC 3.1.3.4) (Weiss, Kennedy & Kiyasu, 1960), diglyceride accounted for 85% of the phosphatidate hydrolysed. The remainder was monoglyceride (Hill, Husbands & Lands, 1968). A twofold purification of the enzyme was achieved by ultrasonic treatment in 0.01% deoxycholate. Further purification was not successful. The apparent K_m for L-glycerol 3-phosphate was $50\mu\text{M}$. This is similar to the value calculated from the results of Tzur, Tal & Shapiro (1964) for rat liver, but is lower than the value for either the yeast enzyme (Kuhn & Lynen, 1965) or the *E. coli* enzyme (Ailhaud & Vagelos, 1966).

The enzyme can be protected from inhibition by the thiol-binding agent *N*-ethylmaleimide (NEM) by prior incubation with acyl-CoA. The amount of thiol group so protected was determined by using [^{14}C]NEM. When 42% protection of the enzyme activity was achieved by incubation of the enzyme with palmitoyl-CoA before the addition of NEM, the amount of thiol group so protected was determined to be 0.1 $\mu\text{mole/mg}$. of enzyme protein. This was calculated from the difference in binding of [^{14}C]NEM to the protected enzyme in the presence and absence of L-glycerol 3-phosphate. These thiol groups are essential for the activity of the enzyme. There relationship to the active site of the enzyme is very close; we were unable to separate the thiol groups from the enzyme activity.

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- Ailhaud, G. P. & Vagelos, P. R. (1966). *J. biol. Chem.* **241**, 3866.
Cheniae, G. M. (1965). *Plant Physiol.* **40**, 235.
Hill, E. E., Husbands, D. R. & Lands, W. E. M. (1968). *J. biol. Chem.* **243**, 4440.
Kornberg, A. & Pricer, W. E., jun. (1953). *J. biol. Chem.* **204**, 345.
Kuhn, N. J. & Lynen, F. (1965). *Biochem. J.* **94**, 240.
Lands, W. E. M. & Hart, P. (1965). *J. biol. Chem.* **240**, 1905.
Tzur, R., Tal, E. & Shapiro, B. (1964). *Biochim. biophys. Acta*, **84**, 18.
Weiss, S. B., Kennedy, E. P. & Kiyasu, J. Y. (1960). *J. biol. Chem.* **235**, 40.

Gas-Liquid Chromatography of Mono-glycerides and Diglycerides

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Qualitative parameters defining the gas-liquid-chromatographic behaviour of trimethylsilyl (TMS) ethers of mono- and di-glycerides are largely lacking in the literature (see Kuksis, 1967). We therefore extended our studies of factors affecting the separation of triglycerides on SE-30 and QF-1 (Watts & Dils, 1968) to the TMS ethers of a wide range of mono- ($\text{C}_2\text{--C}_{18}$) and di-glycerides ($\text{C}_4\text{--C}_{36}$).

Both gas-liquid and thin-layer chromatography were used to determine completeness of silylation, and conditions that have been used (Tallent & Kleiman, 1968) were shown to yield partially silylated products.

Isothermal analysis showed that on SE-30 or QF-1, log(retention volume) was directly proportional to carbon-atom number and inversely proportional to absolute temperature. However, the TMS ethers of lower monoglycerides ($\text{C}_2\text{--C}_6$) and diglycerides ($\text{C}_4\text{--C}_{12}$) deviated from these relationships. Since no single temperature could be employed to analyse the full range of either mono- or diglyceride TMS ethers, temperature-programmed analysis was used. The temperature-programmed conditions that enabled triglycerides to be separated (Watts & Dils, 1968) gave good separation of both mono- and di-glyceride TMS ethers. Relative elution temperatures (T_{RE}) were therefore used to compare the chromatographic properties of different glyceride classes. We defined the carbon equivalent of a TMS group (ΔC_{TMS}) as the difference in carbon-atom number (per TMS group) between a mono- or di-glyceride TMS ether and the triglyceride with the same T_{RE} under the same conditions of temperature programming. Though the TMS group is equivalent to about 5.3 carbon atoms, the apparent increase in molecular weight, as indicated by ΔC_{TMS} , was found to be only 1.7–2.5 for monoglycerides, and varied from –2.0 to 4.8 for diglycerides. This indicates specific interactions between these solute molecules and column components.

To compare the behaviour of free diglycerides, we defined the carbon equivalent of hydroxyl group (ΔC_{OH}) in an analogous manner to the definition of ΔC_{TMS} . Values of ΔC_{OH} (3.1–4.4) showed that free and silylated diglycerides are eluted at the same T_{RE} . Thus the usefulness of silylation for diglycerides appears to be solely in minimizing adsorption on to column walls or supports (or both).

By using weight and molar correction factors, the composition of the TMS ethers of diglycerides