Frearson, Kit & Dubbs, 1965; Kit, Dubbs & Frearson, 1966; Sheinin, 1966; Kara & Weil, 1967). The remaining viral genes cannot code for all these enzymes, and it is of interest to determine which, if any, are virus specific.

New DNA polymerase activity appears after infection of cells by many viruses, and immunological methods have been used to distinguish some of the enzymes from those of the host cells, e.g. the herpes virus-induced polymerase (Keir, Subak-Sharpe, Shedden, Watson & Wildy, 1966).

Extracts were prepared of uninfected mouse embryo cells, grown in Eagle's medium containing 10% of calf serum, and of polyoma and herpes virus-infected mouse embryo cells, maintained in Eagle's medium containing 0.5% of calf serum. The DNA polymerase activities of the uninfected and infected cell extracts were similar. Most of the activity in the infected extracts was virus induced, because of the low activities in the low-serum-cells before infection (Smart, Fried & Pitts, 1967).

Rabbit antisera were prepared against uninfected and polyoma virus-infected cell extracts. Antiserum against similar extracts of herpes virus-infected rabbit kidney cells was a gift of Professor H. Subak-Sharpe.

After treatment of cell extracts with antisera for 19 hr. at 4°, the residual DNA polymerase activities were assayed. Antisera against uninfected and polyoma virus-infected mouse-cell extracts inhibited the enzyme activities of each extract by up to 60%, but did not inhibit the activity of herpes virus-infected mouse cell extract. The antiserum against extracts of herpes virus-infected rabbit cells, on the other hand, inhibited the activity of the herpes virus-infected mouse cell extract by 45%, but not the activities of uninfected or polyoma virus-infected extracts.

These results indicate that the DNA polymerase induced by polyoma virus is indistinguishable, by this technique, from the uninfected-cell enzyme.

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On the Tight Coupling of Phospholipid Synthesis and Fatty Acid Desaturation in *Chlorella vulgaris*

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Chlorella vulgaris chloroplasts can introduce double bonds into oleic acid to produce successively linoleic and linolenic acids by an oxidative mechanism requiring molecular oxygen and NADPH as cofactors (Harris, James & Harris, 1965). The fatty acid precursor must be in an 'activated' form; the CoA thiol ester is produced in the soluble fraction of the *Chlorella* cell and can be desaturated by the chloroplasts. It has not been proved, however, whether the CoA derivative is the immediate precursor of the polyunsaturated fatty acid, or whether the true substrate is in another, possibly protein-bound, form.

In numerous experiments in our Laboratory, we have noticed a striking association between certain polar lipids and the sequential introduction of double bonds into saturated fatty acids. Nichols (1968) concluded that certain fatty acids become associated with a particular lipid class and that transformations between members of a fatty acid series occur within that lipid class. [^{14}C]Oleic acid is incorporated by whole cells and isolated chloroplasts almost exclusively into the 2-position of phosphatidylcholine. Only two species of phosphatidylcholine molecules (the 0,1 and 1,1 species) contain appreciable amounts of the label. Desaturation to linoleate occurs only after a large proportion of the label has been incorporated into phosphatidylcholine. In the early stages, the product is only detected in the phosphatidylcholine fraction and not until later does the free fatty acid contain linoleate.

The conversion of [^{14}C]oleoyl-CoA into labelled linoleate by isolated chloroplasts could be completely inhibited by very small amounts of a phosphatidylcholine dispersion; dispersions of other lipids were inhibitory but only at much higher concentrations. Only part of the phosphatidylcholine inhibition is therefore likely to be due to a 'detergent' effect; the major part could be due to a preference for phosphatidylcholine as a substrate for the desaturation.

Direct experiments were performed to determine whether oleic acid esterified in phosphatidylcholine

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.

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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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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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