of these *Corynebacteria* the L-diaminobutyric acid is masked by acetylation and that cross-linking occurs by introduction of D-diaminobutyric acid at a later stage, as proposed for D-ornithine in *Corynebacterium poinsettiae* (Perkins, 1967).

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A Polynucleotide Ligase from Polyoma Virus-Infected Mouse Cells

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A number of groups have demonstrated polynucleotide ligase activity in *Escherichia coli* and in *E. coli* infected with T4 phage (Gefter, Becker & Hurwitz, 1967; Gellert, 1967; Olivera & Lehman, 1967a; Weiss & Richardson, 1967). The enzymes have been partially purified and their cofactor requirements determined. Intermediates involved in the reactions have been investigated and reaction mechanisms proposed (Little, Zimmerman, Oshinsky & Gellert, 1967; Becker, Lyn, Gefter & Hurwitz, 1967; Olivera & Lehman, 1967b; Fareed & Richardson, 1967).

We now report that polynucleotide ligase activity has been detected in mouse cells infected with polyoma virus.

DNA molecules formed during the replication of covalently closed circular duplex DNA, such as polyoma virus DNA, must pass through a stage in which the molecule has at least one single strand scission. However, the DNA extracted from mature virus particles is mainly in the covalently closed, circular duplex form. Polyoma-infected cells, are therefore, likely to contain a DNA ligase.

Extracts of polyoma virus-infected mouse embryo cells were prepared by the method of Fried & Pitts (1968). Tertiary cultures of mouse embryo cells, maintained in Eagle's medium supplemented with 0.5% calf serum for four days, have a very low activity of DNA polymerase and probably also of other enzymes involved in DNA replication. Such cultures were infected with polyoma virus at a multiplicity of 20 plaque-forming units/cell. After 40 hr. incubation at 37°, the cells were washed, scraped into a small volume of buffer and homogenized with a Potter-Elvehjem homogenizer.

DNA ligase was assayed by following the conversion of circular polyoma DNA that had artificially induced single-strand nicks into the covalently closed, circular duplex form. Nicks were introduced

by the action of pancreatic deoxyribonuclease I at very low concentration. The reaction was stopped when there was an average of one nick per molecule. Such nicks have a phosphate group on the free 5'-end. About 1 µg. of this nicked polyoma DNA was incubated with cell extract in the presence of cofactors, and the product covalently closed, circular DNA was separated from substrate DNA by sedimentation through alkaline sucrose gradients.

Under these conditions, about 20% of the substrate DNA was converted into the covalently closed, circular form. The activity was not present in low-serum-cells that had been mock-infected, and in extracts from which the cell membranes had been removed by centrifugation at 10000g for 10 min. This ligase activity, therefore, may be required for the replication of polyoma virus in mouse embryo cells.

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Immunological Evidence for the Absence of a Polyoma Virus-Specific Deoxyribonucleic Acid Polymerase

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Polyoma virus replicates in mouse cells and transforms hamster cells. The virus DNA can code for between five and ten proteins of average size, three to six of which are accounted for by the viral coat proteins and specific antigens that appear in infected and transformed cells (Habel, 1961, 1965; Sjögren, Hellström & Klein, 1961). After the infection of mouse cells, the activities of at least nine enzymes, including DNA polymerase, are stimulated (Dulbecco, Hartwell & Vogt, 1965;

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