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;