Q_{O_2} values when grown to the late exponential and stationary phases in Oxoid nutrient broth.

Strain UV-3 was unable to grow on a minimal medium containing the supplements necessary for the growth of the parent strain. The mutant grew to the small colony size when this medium was supplemented with lysine, methionine and proline. The proline deficiency was shown to be the result of an independent mutation. The blockage in methionine synthesis was shown to be at the level of cystathionine synthetase. It was found that the requirement for lysine + methionine could be overcome by the addition of succinate. However, concentrations of succinate higher than 5 mM inhibited the growth of the mutant on minimal medium. Although this inhibition could be overcome by casein hydrolysate, the nature of the inhibition has not yet been completely demonstrated.

With synthetic mixtures of amino acids, growth of the mutant in the presence of high succinate concentration did not occur unless isoleucine was present; however, isoleucine alone was insufficient to overcome the inhibition. α -Oxoglutarate or high concentrations of glutamate could, to a certain extent, replace the requirement for succinate. Acetate or other tricarboxylic acid-cycle intermediates were ineffective. Acetate did not improve growth on low concentrations of succinate (cf. Vise & Lascelles, 1967).

Yeast extract or lipic acid at the concentrations used to promote growth of the mutant studied by Vise & Lascelles (1967) promoted the growth of strain UV-3 to the small colony size; higher concentrations overcame the small colony phenotype and the cytochrome deficiency. Other vitamins, including *p*-hydroxybenzoic acid, were ineffective (cf. Jones & Lascelles, 1967).

Preliminary genetic mapping by interrupted mating suggests that the UV-3 mutation maps close to the mutation studied by Vise & Lascelles (1967) (Taylor & Trotter, 1967).

This mutant shares many features of other mutants responding to succinate or lysine + methionine, but does not appear to be identical with any previously isolated. The respiratory deficiency is probably a secondary consequence of this mutation.

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Cell-Wall Mucopolysaccharide of *Corynebacterium insidiosum* and *Corynebacterium sepe-donicum*

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The cell walls of *Corynebacterium tritici* contain as diamino acid L-2,4-diaminobutyric acid (Perkins & Cummins, 1964), about half of which has a free γ -amino group (Perkins, 1965). The cell walls of the related plant pathogens *Corynebacterium insidiosum* (N.C.P.P. 1110) and *Corynebacterium sepe-donicum* (N.C.P.P. 378) were prepared, and acid hydrolysates applied to an automatic amino acid analyser, glutamic acid, alanine, glycine and 2,4-diaminobutyric acid being found in the following respective molar proportions: *C. insidiosum*, 1.0, 1.0, 1.1, 1.7; *C. sepe-donicum*, 1.0, 1.0, 1.1, 2.0. The mucopolysaccharides therefore appeared to contain an excess of diamino acid.

A culture of *C. insidiosum* was inhibited with vancomycin and the accumulated mucopolysaccharide precursor nucleotide was purified chromatographically. It contained (molar proportions): UDP, 1.0; *N*-acetylhexosamine, 1.0; glutamic acid, 1.0; alanine, 2.0; glycine, 1.0; diaminobutyric acid, 1.0. A sample of this nucleotide was degraded (Perkins, 1967) and peptides of the following compositions were isolated: I, lactic acid, glycine, glutamic acid (? : 1 : 1); II, glutamic acid, diaminobutyric acid (1 : 1); III, diaminobutyric acid, alanine (1 : 2); IV, glutamic acid, diaminobutyric acid, alanine (1 : 1 : 2); V, alanylalanine. The results are consistent with the sequence muramyl-Gly-Glu-diaminobutyric acid-Ala-Ala. Contrary to expectations, the peptides II and IV were acidic at pH 7, and the peptide III was neutral. Dinitrophenylation and hydrolysis of peptides II and IV produced DNP-glutamic acid as the only DNP derivative, and free diaminobutyric acid was recovered.

The parent nucleotide was unchanged by treatment with fluorodinitrobenzene, so that both amino groups of diaminobutyric acid must have been blocked. Hydrolysis of the nucleotide and determination of volatile acids on gas-liquid chromatography showed that each molecule contained two residues of acetic acid. Presumably one was from *N*-acetylmuramic acid and the other blocked the γ -amino group of diaminobutyric acid.

Cell walls and precursor nucleotide were hydrolysed and the diaminobutyric acid was converted into its 2,4-di-DNP derivative for measurement of optical rotatory dispersion. The product from the nucleotide gave a curve like that of authentic L-compound, whereas the products from the cell walls of both species were almost optically inactive.

It seems probable that in the precursor nucleotide

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;