sperm extracts consistently gives two distinct peaks of enzyme activity. One peak is eluted at a position corresponding to a molecular weight of 100 000, whereas the other peak is eluted at the void volume from Sephadex G-150 (mol.wt. > 500 000). No significant change from one peak to the other was observed when either form was re-run separately on Sephadex, even when run in the presence of high salt concentrations (0.75M). No difference in the K_m of fructose or glucose could be detected between the two forms of hexokinase.

Horizontal starch-gel electrophoresis separates hexokinase into three bands. One of these migrates very slowly from the origin and is characteristic of sperm hexokinases. Katzen (1967) discovered this slowly migrating form of hexokinase in testis and epididymal extracts and deduced that it resulted from the sperm in these tissues. Separate electrophoresis of each of the two peaks from gel filtration strongly suggests that this 'sperm-type' hexokinase band on electrophoresis is identical with the higher-molecular-weight enzyme peak from gel filtration. The low mobility of the 'sperm-type' band may well be due to its being a relatively large molecule, retarded by the sieving action of starch gel, rather than to its charge or chemical properties.

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Characteristics of Growth of a *Pseudomonas* Species in Culture Media containing Chlorhexidine

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Pseudomonads are able to grow in media containing many types of bactericide. We have used a strain isolated by Mr R. Hall that will grow aerobically in media containing up to 200 µg. of chlorhexidine/ml. In the absence of bactericide, cultures consisted mainly of motile bacteria that gave smooth colonies on agar; on transfer to media containing chlorhexidine the organisms became non-motile and had rough colonial morphology. When cultures grown in chlorhexidine were transferred to media containing less, or no, chlorhexidine, growth occurred either immediately or after a lag during which viability was roughly

constant, but on transfer to a medium containing more chlorhexidine viability first decreased, then remained constant, then increased. After this growth phase viability again decreased, usually fairly rapidly, and the greatest absorption of bactericide occurred. The ratio of viable cells to the total cell mass was always lower in the presence than in the absence of bactericide. Electron micrographs of sections revealed, amongst apparently normal bacteria, many greatly damaged bacteria; large numbers of empty vesicles of varying size were present; some had the complete bacterial wall structure, some only part of it. The vesicles probably came from deformed cells, some having no cytoplasm and some in which the cytoplasm was replaced by electron-dense granules.

Suspensions of washed bacteria grown in medium containing radioactively labelled nutrients without chlorhexidine release labelled components from their metabolic pool on boiling, on treatment with acids or on addition of chlorhexidine. Similar suspensions prepared from cultures grown in presence of chlorhexidine have a metabolic pool extractable by boiling or treatment with acids but only partly extractable or not extractable by chlorhexidine depending on the concentration during growth.

At pH 7 cultures in media containing chlorhexidine moved with an electrophoretic mobility of either 1.0×10^{-4} or 1.0×10^{-5} cm.²sec.⁻¹V⁻¹. It is suggested that in a normal population a small proportion of the pseudomonads can grow in chlorhexidine, and these are selected when chlorhexidine is added; only the most highly adapted of their progeny survive; on transfer to drug-free medium, the culture reverts to the original composition; resistance to chlorhexidine is due to non-absorption by the cells. This is in accordance with the hypothesis of Hamilton (1968).

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A Mutant Strain of *Escherichia coli* Requiring Lipic Acid for Normal Growth

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A mutant of *Escherichia coli* K-12, W-945 (original strain from Professor W. Hayes) was obtained by u.v. irradiation. This mutant, named UV-3, has an exceptionally small colony form when grown on Oxoid nutrient agar and low cytochrome concentrations (particularly cytochrome a_2), and low concentrations of respiratory quinones, and low

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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