limitations were markedly less toxic. In fact, the walls of Mg^{2+} -limited organisms grown at a low rate (dilution rate 0.1 h^{-1}) appeared to be non-toxic.

The 3-deoxy-2-octulonic acid contents of walls of fast-growing (dilution rate 0.8 h^{-1}) glycerol-limited organisms and walls of slow-growing (dilution rate 0.1 h^{-1}) Mg^{2+} -limited organisms were similar, but the toxicities of these walls were very different. This suggests that no correlation between the 3-deoxy-2-octulonic acid content of walls and their toxicity exists.

A characteristic component of the lipid A portion of the lipopolysaccharide of the Enterobacteriaceae is 3-hydroxymyristic acid. Thus again it would be expected that the 3-hydroxymyristic acid content of wall preparations would correlate with the toxicity result. The 3-hydroxymyristic acid content of these wall preparations was determined by methanolysis of the walls and g.l.c. of the methyl esters of the fatty acids formed. The 3-hydroxymyristic acid (characterized by retention times and mass spectra of its methyl ester) content of these walls again varied markedly, but there was no apparent correlation with the toxicity results.

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The Effect of Growth Conditions on the Muropeptide Composition of Walls of *Bacillus subtilis* W23

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This communication reports some preliminary findings on the influence of growth conditions on the muropeptide composition of *Bacillus subtilis* W23 when grown in a chemostat.

B. subtilis W23 was grown in a chemostat with different nutrient-limiting growth. Samples of organisms were removed from cultures and immediately heated at 100°C (for 20 min) to inactivate the autolytic enzymes. After the heating the organisms were isolated (after being washed three times with phosphate buffer) and incubated with lysozyme. Results showed that the rate of lysis of the organisms was different with respect to the growth limitation applied. Mg^{2+} -limited organisms lysed at a lower rate than did organisms from phosphate- or nitrogen-limited cultures. The difference in lysozyme sensitivity between Mg^{2+} - and phosphate-limited organ-

isms could be due to the different anionic polymers (teichoic acid and teichuronic acid) being present in the walls. This is unlikely, however, since ammonia-limited organisms, which also have teichoic acid in their wall (Ellwood, 1970) lysed at a faster rate than did phosphate-limited organisms. In fact, no correlation could be found between the wall anionic polymer content and the rate of lysis by lysozyme, suggesting that this difference might be due to the structure of the muropeptide.

Walls were prepared from organisms grown in a chemostat under a variety of conditions, and the anionic polymers present in the wall were removed by trichloroacetic acid extraction to leave a residue composed of muropeptide together with some protein. This residue was then analysed for muropeptide components and was found to be composed of glucosamine, muramic acid (presumably as the *N*-acetyl derivatives), alanine, glutamic acid and diaminopimelic acid. In all the wall preparations examined the qualitative composition was the same. However, quantitative analysis showed marked differences in the ratios of muramic acid and glucosamine to glutamic acid, whereas the ratio of the peptide components one to another was roughly constant. The molar ratios of muramic acid and glucosamine relative to glutamic acid in the muropeptide of ammonia-limited organisms were about unity, close to the theoretical value assuming that the sugar backbone of the muropeptides is unsubstituted. With Mg^{2+} - and sulphate-limited organisms the muropeptide contained a higher proportion of glucosamine than muramic acid, whereas the muropeptide of K^{+} -limited, glucose-limited and phosphate-limited organisms contained a higher proportion of muramic acid than glucosamine.

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The Respiratory-Chain Dehydrogenases of *Azotobacter vinelandii*

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Phosphorylating respiratory membranes from the nitrogen-fixing aerobe *Azotobacter vinelandii* catalyse the rapid oxidation of NADH, L-malate [NAD(P)^{+} -independent] and NADPH (Pandit-Hovenkamp, 1967).

NADH (K_m 0.040 mM), L-malate (K_m 0.167 mM) and NADPH (sigmoidal saturation kinetics, $s_{0.5}$ 0.440 mM) were oxidized at rates of 1.25, 1.59 and $1.49\text{ }\mu\text{g-atom of O/min per mg of protein}$ respectively with corresponding P/O ratios of 0.88, 0.54 and 0.58.

Oxidase activities with multiple substrates indicated the dehydrogenases were rate-limiting for the oxidation of single substrates.

The quinones juglone (5-hydroxy-1,4-naphthoquinone), duroquinone (2,3,5,6-tetramethyl-1,4-benzoquinone; see Ruzicka & Crane, 1970) and ubiquinone-1 (Schatz & Racker, 1966; Eilermann *et al.*, 1970) were used as electron acceptors to investigate the nature and energy-conservation properties of these dehydrogenases. Anaerobic electron transfer from NADH to ubiquinone-1 yielded a P/2e ratio (Site 1) of 0.18 [when calculated from P/O ratio (NADH) minus P/O ratio (malate) this ratio was 0.34], and was stimulated by ADP in the presence of P_i, which indicated a mitochondrial type of respiratory control. Juglone and duroquinone failed to support either ATP synthesis or respiratory control with NADH, although they readily accepted reducing equivalents from NADH dehydrogenase (3.54 and 0.90 μ mol of NADH oxidized/min per mg of protein respectively). NADPH dehydrogenase was only weakly coupled to ATP synthesis [P/2e with ubiquinone-1, 0.05; P/O ratio (NADPH) minus P/O ratio (malate), 0.04], but was stimulated by the uncoupler carbonyl cyanide *m*-chlorophenylhydrazine, was highly active with juglone (2.59 μ mol of NADPH oxidized/min per mg of protein) and was virtually inactive with duroquinone. In contrast, malate dehydrogenase was completely non-phosphorylating with ubiquinone-1, showed no stimulation by ADP + P_i or carbonyl cyanide *m*-chlorophenylhydrazine, and was virtually inactive with both juglone and duroquinone.

These results indicate that ubiquinone-1 accepts electrons from a carrier located between Sites I and II that is accessible to all three dehydrogenases (probably the endogenous ubiquinone-8; see Klingenberg & Kroger, 1966), of which only the NADH dehydrogenase is strongly coupled to ATP synthesis or reacts significantly with duroquinone. The ability of juglone to accept electrons from the NADH (or NADPH) dehydrogenase, both very rapidly and without concomitant energy conservation, suggests that it reacts with the carrier located on the substrate side of the energy-conservation site.

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The Involvement of Iron in the Respiratory System of *Azotobacter vinelandii*

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Respiratory membranes from *Azotobacter vinelandii* rapidly oxidize L-malate [independent of NAD(P)⁺], NADH (Eilermann *et al.*, 1970; Ackrell & Jones, 1971) and NADPH with the concomitant synthesis of ATP. Similar preparations, on reduction with NADH, yield an electron-paramagnetic-resonance signal characteristic of a non-haem iron protein (Beinert *et al.*, 1962) and an absorption spectrum typical of an iron-sulphur flavoprotein (Dervartanian & Bramlett, 1970).

Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) sulphonate, a water-soluble metal chelator with a high affinity for Fe²⁺ ions (Palmer, 1970), completely inhibits malate oxidase ([I]₅₀ 0.21 mM), NADPH oxidase ([I]₅₀ 0.88 mM) and electron transfer from NADH to O₂, juglone or duroquinone ([I]₅₀ 3.60 mM), but did not affect the oxidation of ascorbate + 2,6-dichlorophenol-indophenol or ascorbate + *NNN'*-tetramethyl-*p*-phenylenediamine. Hill plots of the inhibition curves yielded slopes of approx. 2 with NADH or NADPH as substrate and of approx. 1 with malate. Kinetically, the chelator acted as a pure competitive (dead-end) inhibitor with respect to NADH, malate or NADPH and also, with NADH as substrate, with respect to juglone or duroquinone.

Low concentrations (<0.25 mM) of bathophenanthroline sulphonate completely uncoupled site I phosphorylation and abolished respiratory control with NADH by stimulating the State 4 rate without affecting State 3. Slightly higher concentrations (<0.62 mM) increased the efficiency of ATP synthesis at Site 2 but failed to induce respiratory control with the Site 2 substrate malate. Bathophenanthroline at 0.15 mM has been shown to inhibit substantially the trypsin-induced adenosine triphosphatase of similar membrane preparations (Eilermann *et al.*, 1971).

Incubation of respiratory membranes with bathophenanthroline sulphonate yielded a weak absorption spectrum that was greatly intensified after the addition of NADH, malate or NADPH ($\lambda_{\max}$ 539 nm, shoulder at 495 nm). An identical spectrum was also obtained with dithionite-reduced spinach ferredoxin and with Fe²⁺ ions; Fe³⁺ ions were less effective, and all other metal ions tested were completely ineffective.

These results suggest that, in *A. vinelandii* respiratory membranes, iron may function both as an electron-transfer component (at the dehydrogenase level) and also in an unknown capacity in energy conservation at Sites 1 and 2.