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Non-Specific Uptake and Specific Binding of Sulphate by *Aspergillus nidulans*

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Yamamoto & Segel (1966) noted that the sulphate transport curves (plots of sulphate uptake versus time) for *Penicillium chrysogenum* did not go through the origin. They suggested that the intercept on the ordinate axis (sulphate uptake) resulted either from adsorption of sulphate on the mycelial surface or from penetration, by diffusion, into a free space (cf. 'apparent free space'; Briggs *et al.*, 1961). Under conditions of no net transport of sulphate, the uptake was non-saturatable, energy-independent, temperature-independent and unaffected by pH in the range 4–10.

Aspergillus nidulans wild type and its sulphite-requiring mutants (Arst, 1968) showed a similar sulphate uptake. In the transport-negative mutant sB-3 uptake of sulphate into the free space was non-saturatable and non-specific, and was independent of the energy and sulphur status of the mycelium and the time of exposure to external sulphate. Equilibrium with the external medium was achieved rapidly (15 s–1 min). Under conditions of no net transport, non-specific initial uptake also occurs in transport-positive mutants. The uptake accounts for 55–80% of the total volume of the pressed-dry mycelium, of which less than 20% is interstitial. The 'apparent free space' volume varies with osmotic changes (cf. Briggs, 1970). For example, the initial overshoot and delay in the establishment of steady sulphate uptake, reported by Scott & Spencer (1965) in transport studies, were observed only when harvested mycelia had been equilibrated with deionized water before being transferred to the buffered medium containing $\text{Na}_2^{35}\text{SO}_4$.

At an external sulphate concentration of $50\text{ }\mu\text{M}$ the 'apparent free space' of *Asp. nidulans* sB-3 accumulated $0.1\text{ }\mu\text{mol}$ of $\text{SO}_4^{2-}/\text{g}$ dry wt., but the intercept on the ordinate axis could increase to $0.7\text{ }\mu\text{mol}/\text{g}$ dry wt. in transport-positive mycelia after a period of sulphur starvation. In general, higher values for the intercept on the ordinate axis were associated with increased sulphate transport rates, but inhibition of protein synthesis during sulphur starvation prevented these increases. The elevation of the intercept on the ordinate axis was not affected by prior incubation of

the mycelium with 2,4-dinitrophenol, but pre-pulsing with $5\text{ mM-K}_2\text{CrO}_4$ restricted the value to that of the transport mutant. These and other results indicate that the increase in the intercept on the ordinate axis, above the 'apparent free space' value, measures specific sulphate-binding to a protein, the synthesis of which is repressed during sulphur sufficiency. A specific sulphate-binding protein is known to form part of the transport system in bacteria (Pardee, 1966).

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The Effect of Growth Conditions on the Chemical Composition and Endotoxicity of Walls of *Aerobacter aerogenes* N.C.T.C. 418

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The core structure of the lipopolysaccharide of walls of Gram-negative bacteria contains two characteristic components, i.e. 3-deoxy-2-octulonic acid and heptose. Walls of *Aerobacter aerogenes* grown in a chemostat under different growth conditions were examined for these two components. It was found that the 3-deoxy-2-octulonic acid and heptose contents and their ratio to one another varied quite markedly, suggesting that the composition of the lipopolysaccharide was phenotypically variable (Ellwood & Tempest, 1967; Tempest & Ellwood, 1969).

3-Deoxy-2-octulonic acid is the sugar that links the lipid A (the lipid A moiety gives the lipopolysaccharide its endotoxic properties; Lüderitz *et al.*, 1966) to the core polysaccharide, and some correlation between the 3-deoxy-2-octulonic acid content of walls and their toxicity could be expected.

Wall preparations of *A. aerogenes* used in the previous chemical study have now been examined further with respect to their toxicity in mice. The LD_{50} was determined by standard procedures, and it was found that organisms grown glycerol-limited at a dilution rate of 0.8 h^{-1} had walls that gave an LD_{50} in mice of $3\text{ mg}/20\text{ g}$ body wt. (walls of batch-grown *Escherichia coli* give LD_{50} in mice of $2\text{--}3\text{ mg}/20\text{ g}$). All other wall preparations prepared from organisms grown at different growth rates or different growth