Teichoic Acid or Teichuronic Acid in the Walls of *Bacillus subtilis* var. *niger*, Grown in a Chemostat

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The phosphorus contents of Mg^{2+}- or K^+-limited *Bacillus subtilis* var. *niger* are considerably greater than those of similarly limited *Aerobacter aerogenes*. This suggests the presence of large amounts of phosphorus-containing polymers (other than RNA and DNA) in the bacilli (see previous communication).

The walls of *B. subtilis* are known to contain teichoic acids (Baddiley, 1964) which, since they are phosphorus-containing polymers, could account for the high phosphorus levels in *B. subtilis*. Therefore, walls were prepared from Mg^{2+}-limited *B. subtilis*, grown at a dilution rate (D) of 0·2 hr.$^{-1}$ (35°, pH 7·0), and analysed. These walls, which amounted to 24% of the bacterial dry wt. contained 6% phosphorus—sufficient to account for the difference in phosphorus content between *B. subtilis* and *A. aerogenes* (i.e. 3·0 and 1·7% of the bacterial dry wt. respectively, at D = 0·2 hr.$^{-1}$). Extraction of the wall preparation with 10% (w/v) trichloroacetic acid, followed by precipitation with 5 vol. of acetone, gave a polymer which accounted for 40% of the wall dry wt. Hydrolysis of this polymer (2N-HCl, 100°, 3 hr.) and chromatographic analysis revealed the presence of glycerol, glycerol phosphates and glucose only, indicating a teichoic acid-type compound.

B. subtilis contained little non-nucleic acid phosphorus when grown in a PO_4^{3-}-limited environment (Tempest, Dicks & Ellwood, 1967) and analysis of walls from these organisms (D = 0·2 hr.$^{-1}$, 35°, pH 7·0), which amounted to 20% of the bacterial dry wt. showed them to contain only 0·2% phosphorus. However, treatment of these walls with trichloroacetic acid (as above) again extracted polymer amounting to 40% of the wall wt. Acid hydrolysis and chromatographic analysis showed the absence of glycerol, glycerol phosphates and glucose but the presence of uronic acid and an amino sugar, indicating a teichuronic acid-type compound (Janczura, Perkins & Rogers, 1961).

Thus, the absence of teichoic acid from the walls of PO_4^{3-}-limited *B. subtilis* accounts for their lowered phosphorus content, and the replacement of teichoic acid by teichuronic acid (another anionic polymer) may explain the continued large requirement of potassium for growth.

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Effect of Aflatoxin B_1 on a Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerase *in vitro*

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Several compounds which bind to DNA have been shown to inhibit DNA-dependent RNA synthesis. Aflatoxin B_1, a toxic and carcinogenic product of *Aspergillus flavus*, has been reported to bind to DNA (Sporn, Dingman, Phelps & Wogan, 1966) and to inhibit protein synthesis in an *in vitro* rat-liver ribosomal system (Smith, 1965).

The effect of aflatoxin B_1 on the DNA-dependent RNA polymerase from *Escherichia coli* has been investigated. The enzyme was isolated from frozen cells by the method of Chamberlin & Berg (1962) and was assayed by measuring the incorporation of [8-^{14}C]ATP into the acid-insoluble product (Nicholson & Peacocke, 1966). Activity was dependent on exogenous DNA and 2–30 μg. of calf-thymus DNA were present routinely in the 0·25 ml incubation mixture. Aflatoxin B_1 was isolated from a mixture of aflatoxins B_1, B_2, G_1, G_2 by thin-layer chromatography on silica gel in chloroform–ether–acetic acid (2 : 2 : 1). The addition of a solution of aflatoxin B_1 (25 to 42 mμmoles in 0·02 ml. of propylene glycol or of a crude mixture of aflatoxin B_1, B_2, G_1, G_2 (approx. 49 mμmoles) in 0·01 ml. propylene glycol to the incubation mixture (pH 8·0) was without effect on RNA synthesis when compared with controls containing propylene glycol. This held for maximal or limiting concentrations of DNA primer, although actinomycin D (10 μg./ml.) inhibited the activity by over 90%.

Aflaxtoxin appeared to bind to DNA under the conditions of the polymerase assay. By maintaining a sufficiently high molar ratio of DNA to aflatoxin, it was possible to study the spectrophotometric shift induced in the 365 mμ absorption maximum of aflatoxin by DNA. It was concluded that aflatoxin interacted with DNA at pH 7·4 as previously reported (Sporn *et al.* 1966) and also at pH 8·0, where a very similar shift was induced. The interaction appears to be reversed by increasing ionic strength, since the maxima in the difference spectrum was reduced by half on increasing the sodium chloride concentration from 0·015M to 0·15M. The binding which has been inferred from this appears to be rapid, reversible and probably weak.

Smith's (1965) finding that the inhibition of protein synthesis by aflatoxin in an *in vitro* ribosomal system could be reversed by glutathione might indicate involvement of SH- enzymes. In the polymerase system rigorous exclusion of thiol groups from the last stages of the enzyme purification and