differential centrifugation as described by Falcone & Nickerson (1956). Defatted cell wall gave a typical analysis of carbohydrate, 85%; N, 1.3%; P, 0.6%; mannose:glucose ratio, 1.05:1. Pronase digestion of this material solubilized 54% of the total carbohydrate and 38% of the total phosphorus. The mannose:glucose ratio of the residue was 1:2. The soluble fraction from the Pronase digest was fractionated on DEAE-cellulose to give phosphoglycopeptide material (250 mg. from 1g. defatted cell wall; carbohydrate, 94%; mannose:glucose ratio, 4.6:1; N, 0.8%; P, 0.9%).

The nature of the phosphodiester linkages in the phosphoglycopeptide was investigated by measuring the elimination of inorganic phosphate from its periodate-oxidation product. The native phosphoglycopeptide yielded no inorganic phosphate when treated with 1N-KOH at room temperature for 12 hr., but mild alkali treatment (pH 10.5) of the periodate-oxidation product resulted in the elimination of 59% of the phosphorus as inorganic phosphate. Almost complete conversion into inorganic phosphate was achieved only by adding the periodate-oxidation product to 1N-KOH. Our results suggest that the phosphodiester linkages in the glycopeptide are similar to those reported for the extracellular phosphomannan from *H. holstii* (Jeanes & Watson, 1962) in which the phosphodiester groups are linked between C-6 of one mannose unit and C-1 of another, which is in turn glycosidically linked at C-2 by another mannose residue.

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Falcone, G. & Nickerson, W. J. (1956). *Science*, **124**, 272.

Jeanes, A. & Watson, P. R. (1962). *Canad. J. Chem.* **40**, 1318.

Lampen, J. O. (1968). *Leeuwenhoek ned. Tijdschr.* **34**, 1.

Partial Purification of Deoxyribonucleic Acid Polymerase from *Mycobacterium smegmatis*

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A nucleoside triphosphate-dependent DNA-breakdown system has been found in *Mycobacterium smegmatis* (Winder & Coughlan, 1967a) and in bacteria from two other genera (Tsuda & Strauss, 1964; Anai, 1967). It has been suggested that this system might represent the reversed action of a DNA polymerase (deoxynucleoside triphosphate-DNA deoxynucleotidyltransferase, EC 2.7.7.7) able to use ATP in place of pyrophosphate, with a consequent shift of equilibrium towards depolymerization (Winder & Coughlan, 1967c). Hence,

the purification of DNA polymerase from *M. smegmatis* was undertaken as a preliminary to testing this hypothesis.

The bacteria were grown in iron-limited medium and harvested after 72 hr., and extracts were prepared as described by Winder & Coughlan (1967a,b). DNA polymerase was determined by using [³H]TTP in the assay mixture of Lehman (1963) but by employing the procedure of Patel, Howk & Wang (1967).

The crude extracts incorporated about 0.2 mμmoles of thymidine into DNA/30 min./mg. of protein. Treatment with 0.5% streptomycin gave a precipitate that showed a 50-fold increase in specific activity over the crude extract, with a recovery of 65%. The supernatant contained a similar total activity, the total recovery in this step being about 130%. Dialysis of this supernatant, addition of 5 mg. of DNA/ml. and addition of 2% streptomycin gave a second precipitate with a similar specific activity and recovery to the first. The dialysed solutions of the two precipitates were treated with deoxyribonuclease and then with C₆ alumina gel, and eluted as described by Lehman (1963). The total activity was substantially increased by the deoxyribonuclease treatment, but there was difficulty in eluting the activity from the gel. However, a good recovery was achieved with the material from the second precipitate, and a 430-fold increase in specific activity over the crude extract was obtained.

Determination of ATP-dependent DNA-breakdown activity was carried out during the purification. A marked variation in the ratio of this activity to DNA polymerase activity was observed. This suggests that the two activities are not due to one enzyme, but further investigations will be required to show this conclusively.

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Anai, M. (1967). *J. Jap. Biochem. Soc.*, **39**, 167.

Patel, G., Howk, R. & Wang, T. Y. (1967). *Nature, Lond.*, **215**, 1488.

Lehman, I. R. (1963). In *Methods in Enzymology*, vol. 6, p. 34. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Tsuda, Y. & Strauss, B. S. (1964). *Biochemistry*, **3**, 1678.

Winder, F. G. & Coughlan, M. P. (1967a). *Biochim. biophys. Acta*, **134**, 215.

Winder, F. G. & Coughlan, M. P. (1967b). *Biochem. J.* **103**, 59 p.

Winder, F. G. & Coughlan, M. P. (1967c). *Biochem. J.* **103**, 67 p.