The reaction mixtures contained, in 0.48 ml, 1.25 μ g of native DNA, 40 nmol of each of the four deoxynucleoside triphosphates, of which either dTTP or dCTP was labelled with tritium, 2 μ mol of magnesium chloride, 0.3 μ mol of 2-mercaptoethanol, 0.3 mg of bovine serum albumin, 20 μ mol of glycine-NaOH buffer, pH 9.2, 35 μ mol of sodium chloride and 10 units of enzyme. These were incubated at 37°C. Samples were taken at intervals and acid-insoluble radioactivity was counted in a toluene-based scintillation fluid after precipitation on filter-paper discs (Bollum, 1959).

As in previous experiments there were two successive phases of thymidine incorporation into polymer. No incorporation of deoxycytidine took place during the second phase, supporting the conclusion that a polymer containing only deoxyadenosine and thymidine was formed. Incubation of the product of [³H]dTTP incorporation with formic acid-diphenylamine (Burton & Petersen, 1960) rendered the thymidine incorporated into polymer in the second phase entirely acid-soluble, indicating that it was the alternating co-polymer poly[d(A-T)].

When the products of a polymerase reaction, in which [³²P]DNA and [³H]dTTP were employed, were centrifuged in alkaline caesium chloride density gradients, the main bulk of tritium banded at the same density as poly[d(A-T)]. Neither the distribution of tritium between this band and the [³²P]DNA band, nor the distribution of ³²P in the gradient, suggested that there was any appreciable amount of DNA covalently linked to the poly-[d(A-T)], thus providing no evidence that the DNA served any primer role in the poly[d(A-T)] synthesis.

This work was supported by grants from the Medical Research Council of Ireland, the Irish Cancer Society and the National Science Council. D.S.B. received a maintenance grant from the Department of Education, Irish Republic.

Bollum, F. J. (1959). *J. biol. Chem.* **234**, 2733.

Burton, K. & Petersen, G. B. (1960). *Biochem. J.* **75**, 17.

McNulty, M. S. & Winder, F. G. (1972). *Biochim. biophys. Acta* (in the Press).

Absence of Single-Stranded Regions in Deoxyribonucleic Acid during the Action of an Adenosine Triphosphate Dependent Deoxyribonuclease

By F. G. WINDER and P. A. SASTRY. (*Department of Biochemistry, Trinity College, Dublin 2, Irish Republic*)

The ATP-dependent deoxyribonuclease from *Mycobacterium smegmatis* excises short oligonucleotides from the ends of strands of DNA, and evidence

has been obtained that it attacks both strands of the duplex more-or-less simultaneously (Winder & Lavin, 1971). It was important to confirm the latter point, since it is probable from work with other micro-organisms that this enzyme is involved in recombination (Oishi, 1969; Barbour & Clark, 1970; Vovis & Buttin, 1970) and since exonucleolytic attack on both strands of the duplex does not readily fit into most models of recombination (see Whitehouse, 1970). Adsorption on nitrocellulose is a sensitive test for single-stranded regions (Berger & Irvin, 1970), which should be formed if the enzyme preferentially digests one strand of the DNA.

The enzyme was purified about 180-fold from iron-limited *M. smegmatis* by a modification of the procedure of Winder & Lavin (1971). Incubation mixtures contained, in 1 ml, 1.68 nmol of [³²P]DNA from *Escherichia coli* (which had been passed through Millipore filters, type HAWP), 2 μ mol of ATP, 20 μ mol of magnesium chloride, 75 μ mol of tris-HCl buffer, pH 8.0, 50 μ mol of potassium chloride, 0.15 μ mol of dithiothreitol and 288 units of enzyme. After incubation at 37°C, mixtures were deproteinized with phenol-sodium dodecyl sulphate, pH 9.0, and dialysed against 0.15 M-sodium chloride-15 mM-sodium citrate, pH 7.0. Samples were removed, dried on Millipore filters and their radioactivities counted. The remainder was made 0.3 M with respect to sodium chloride and 30 mM with respect to sodium citrate, pH 7.0, and passed through Millipore filters (which had been pre-washed with the same buffer), followed by washing with buffer. The filters were dried and their radioactivities counted in a toluene-based scintillation fluid.

At zero time 6% of the [³²P]DNA recovered from the reaction mixtures was adsorbable on the filters. After 0.5, 1, 2, 3, 5 and 10 min of reaction the total amount of DNA recovered had fallen by 14, 25, 51, 73, 76 and 79% respectively, and the DNA adsorbable on the filters had fallen by 1, 15, 20, 36, 52 and 49% respectively. Thus the action of the enzyme led to a continuous decline in the amount of DNA adsorbable on the filters (even if this was slightly slower than the decline in total DNA), instead of the increase that would be expected if the enzyme produced single-stranded regions. This is further evidence that the enzyme attacks both strands more-or-less simultaneously.

This work was supported by grants from the Medical Research Council of Ireland, the Irish Cancer Society and the National Science Council.

Barbour, S. D. & Clark, A. J. (1970). *Proc. natn. Acad. Sci. U.S.A.* **65**, 955.

Berger, H., jun. & Irvin, J. L. (1970). *Proc. natn. Acad. Sci. U.S.A.* **65**, 152.

Oishi, M. (1969). *Proc. natn. Acad. Sci. U.S.A.* **64**, 1292.

Vovis, G. F. & Buttin, G. (1970). *Biochim. biophys. Acta*, **224**, 42.

Whitehouse, H. L. K. (1970). *Biol. Rev.* **45**, 265.

Winder, F. G. & Lavin, M. F. (1971). *Biochim. biophys. Acta* (in the Press).

Dye-Buoyant-Density-Gradient-Analysis of *Rhizobium* Deoxyribonucleic Acid

By F. C. CANNON, L. K. DUNICAN and F. O'GARA.
(Department of Microbiology, University College, Galway, Irish Republic)

It has been suggested (Dunican & Cannon, 1971) that the genetic elements controlling effectiveness (*Eff*⁺) and infectivity (*Inf*⁺) in *Rhizobium trifolii* can exist extrachromosomally. This hypothesis was based mainly on the fact that effectiveness was lost when cultures were grown in acridine dyes and ethidium bromide. In the present study dye-buoyant-density-gradient centrifugation has been used to separate these elements from chromosomal DNA.

Cultures of *Rhizobium trifolii* strains T1 and 205 were labelled with [³H]thymidine and their respective viomycin-resistant variants, strains T1V^r and 205V^r, were labelled with [¹⁴C]thymidine. Cell lysates were prepared by the method of Bazaral & Helinski (1968) with minor modifications. The sheared lysate (1.5 ml) was added to a tube containing caesium chloride (3.8 or 4.2 g), 0.5 ml of ethidium bromide (1 mg/ml) and 2.0 ml of water. The mixture was centrifuged for 44 h at 130 000g at 24°C. Two-drop fractions were collected on glass-fibre discs (Whatman GF/A) and assayed in a liquid-scintillation spectrometer.

Dye-buoyant-density-gradient centrifugation of cell lysates resulted in a satellite-DNA peak in addition to the main chromosomal peak for both the parent strains T1 and 205 and their derivatives, strains T1V^r and 205V^r. The effect of sublethal doses of u.v. radiation on the dye-buoyant-density-gradient profiles of DNA before sarkosyl lysis was also examined. No significant effect on the satellite-DNA peaks was observed with this treatment. These and related findings support the suggestion that the *Rhizobium* strains being examined have extrachromosomal DNA. The relationship between this DNA and the symbiotic characters in *Rhizobium* will be discussed.

F.C.C. holds a fellowship awarded by An Foras Taluntais.

Bazaral, M. & Helinski, D. R. (1968). *J. molec. Biol.* **36**, 185.

Dunican, L. K. & Cannon, F. C. (1971). *Pl. Soil* (in the Press).

Comparative Study of a Novel Dual-Fibred Muscle

By J. J. A. HEFFRON and J. V. McLOUGHLIN. (*The Agricultural Institute, Castleknock, Co. Dublin, Irish Republic*)

The semitendinosus muscle of the pig has anterior red-fibre and posterior white-fibre areas. Both areas have the same origin and insertion. The consistent occurrence of parallel longitudinal bands of red fibres and white fibres in this muscle was first reported by Briskey, Sayre & Cassens (1962). In view of the considerable biochemical and physiological differences between red muscle and white muscle (Mommaerts, 1970; Dubowitz, 1970), several parameters of the red-fibre and white-fibre areas of pig semitendinosus muscle were examined.

The myoglobin content and succinate dehydrogenase activity of the red-fibre area were four times those of the white-fibre area, whereas the lactate dehydrogenase activity in the white fibres was twice that in the red fibres. All five isoenzymes of lactate dehydrogenase were present in the red fibres, whereas isoenzymes 2 and 3 were absent from the white fibres. Isoenzyme 1 was barely detectable in the white fibres, but the bands of isoenzymes 4 and 5 were very intense. The white fibres had a larger diameter and shorter sarcomere length than the red fibres. Since these measurements were made on post-rigor muscle, the differences in diameter and sarcomere length could be due to a greater degree of contraction of the white fibres during the onset of rigor. In resting muscle obtained under total anaesthesia the initial concentrations of phosphocreatine and ATP were higher in the white fibres than in the red fibres. Resting concentrations of glycogen were greatest in the white fibres and more lactate was formed *post mortem*. Stimulation via the sciatic nerve depleted the phosphocreatine and ATP of both the red fibres and the white fibres, and accelerated the subsequent disappearance of ATP and the onset of rigor. Anaerobic glycolysis was activated to a greater extent in the white fibres than in the red fibres after the stimulus. The glycolytic response of the red fibres to stimulation was increased when the blood supply was discontinued.

Myosin and natural actomyosin adenosine triphosphatase activity of the red fibres was 50–70% of that of the white fibres when assayed at optimum substrate concentrations. The Mg²⁺-stimulated adenosine triphosphatase activities of natural actomyosin from red fibres and from white fibres showed similar sensitivity towards ethanedioxybis-(ethylamine)tetra-acetate, and potassium chloride had a marked inhibitory effect in both cases (70% inhibition occurring with 40 mM-potassium