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

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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.

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Comparative Study of a Novel Dual-Fibred Muscle

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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

chloride). The rate and extent of superprecipitation of natural actomyosin from white fibres were 25–50% greater than those of natural actomyosin from red fibres. It remains to be established how the contraction times of the red fibres and the white fibres are related to their myosin adenosine triphosphatase activities.

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Post-Mortem Autolysis in Bovine Muscle

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Muscle becomes hard and inextensible during the onset of rigor (Bendall, 1960), but during subsequent storage at 2°C there is a progressive increase in tenderness. The ability to maintain isometric tension is lost (Jungk, Snyder, Goll & McConnell, 1967), but the muscle does not regain its extensibility. These changes during storage are believed to be caused by post-mortem autolysis. A study of the myofibrillar ultrastructural changes over the storage period essential to an understanding of the nature of this autolysis is reported. Proteolysis in the sarcoplasmic fraction and release of lysosomal cathepsin D were also investigated.

Bovine semitendinosus muscle was sampled immediately *post mortem* and after 24h, 4 days and 10 days storage at 2°C for light and electron microscopy. The supernatants obtained after centrifuging homogenates from 0h, 2-day and 15-day post-mortem samples for 30min at 1000g (r_{av} 7.62cm) at 4°C were applied to an exponential sucrose density gradient in the BXIV aluminium zonal rotor and centrifuged for 45min at 43000g (r_{av} 4.7cm) at 5°C. Collected fractions were analysed for protein (Schuel & Schuel, 1967) and cathepsin D activity (Anson, 1937).

The phase-contrast microscope showed a progressive increase in incidence of transverse fibre breaks during the storage period. These breaks always occurred in the I band.

Pre-rigor myofibrils in the electron microscope had the appearance of resting muscle (Huxley, 1960). The Z lines showed a well-defined fibrillar

pattern (Landon, 1970), which gradually turned amorphous during subsequent storage. The other components of the myofibril were not significantly affected by storage for 10 days. The ultrastructural locus of the fibre breaks seen in the light microscope was the Z line/I band junction (Reville, Joseph & Harrington, 1971).

The amount of sedimentable protein decreased during storage. In pre-rigor samples 52.5% of the total protein was sedimentable, whereas 2-day samples showed 27.5% and 15-day samples only 18% sedimentable protein.

The total activity of cathepsin D did not change appreciably over the storage period, but of this 84.5% was sedimentable in pre-rigor samples and 40.7% after 15 days.

The limited and specific ultrastructural disruption of the myofibrillar fraction occurring during storage would undoubtedly result in increased tenderness and loss of ability to maintain isometric tension. The sarcoplasmic fraction undergoes a more extensive autolysis, but all the cathepsin D activity does not seem to be released during storage. This agrees with Valin (1970).

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Light-Scattering Rayleigh Linewidth Measurements on G-Actin

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Light-scattering by solutions has been used as a method for finding macromolecular parameters for the past 25 years. During the past decade, however, it has become possible, largely owing to the advent of the laser, to investigate the spectral distribution of the scattered light (see Cummins, Knable & Yeh, 1964; Dubin, Lunacek & Benedek, 1967; French, Angus & Walton, 1969; Rimai, Hickmott, Cole & Carew, 1970; Pecora, 1970; Ford, Karasz & Owen, 1970; Sellen, 1970; Foord