subcellular components without recourse to the customary high shear forces.

Root tips (1–2 cm) were excised from 4-day-old seedlings of maize (*Zea mays*; Crow Hybrid WF9 × M14) and incubated for 12–18 h in a slightly hyperosmotic medium containing cellulase (5%, w/v, Onozuka SS) and Pectinase (0.5%, w/v, Macerozyme) obtained from R. W. Unwin and Co. Ltd., Welwyn, Herts., U.K. Protoplasts were produced in media based on sucrose, mannitol, a complete tissue-culture medium (Straus, 1960) or in a mixture of potassium chloride and calcium chloride of suitable osmolality.

A surface adenosine triphosphatase activity was found in protoplasts by biochemical assay and histochemical techniques, confirming the observations made by Hall (1970) on whole roots.

Digested roots retain cohesion, and the digestive enzymes can be washed away easily. The washed roots are then susceptible to homogenization by ten manual strokes in a Jencons Teflon-in-glass tissue grinder (150–200 μ m clearance).

The homogenate has been fractionated by differential and density-gradient centrifugation on sucrose and Ficoll (Pharmacia Ltd., London W.13, U.K.) gradients in 25 mM-tris-HCl buffer, pH 7.2, into eight discrete bands.

Electron-microscopical examination with differential phosphotungstic acid staining techniques (Glauert, 1965; Rambourg, 1967) localized a plasma-membrane-rich band (cf. Lembi & Morre, 1970). A K^+ -stimulated adenosine triphosphatase activity was found to be associated with this fraction. In addition, the distribution of univalent ion- and bivalent ion-stimulated adenosine triphosphatase activities in all the fractions was determined. Separate Mg^{2+} -stimulated, Mg^{2+} -inhibited and further K^+ -stimulated activities have been recognized. The highest specific activity of adenosine triphosphatase was associated with a very light vesicular fraction (cf. Matile, 1968).

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L-Tyrosine Decarboxylase from Barley Roots

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Hordenine (*NN*-dimethyltyramine) is synthesized in barley roots over a 5–8-day period after germination (Raoul, 1937). Isotope experiments have shown that tyrosine is decarboxylated to tyramine, which is methylated successively to *N*-methyltyramine and hordenine (Leete, Kirkwood & Marion, 1952; Leete & Marion, 1953). Only one enzyme in the sequence, *S*-adenosylmethionine-tyramine *N*-methyltransferase, which catalyses the methylation of tyramine to *N*-methyltyramine, has hitherto been isolated (Mann & Mudd, 1963).

L-Tyrosine decarboxylase has now been extracted from roots of 5-day barley seedlings and purified up to 80-fold by successive ammonium sulphate precipitation, passage through Sephadex G-50 and elution from DEAE-cellulose columns. Alternatively, a rapid 20-fold purification has been achieved by adsorption from the crude extract on DEAE-cellulose and subsequent elution. The enzyme was specific for the L-isomer and the reaction shown to produce equivalent quantities of tyramine and CO_2 . Although the preparations also showed L-glutamate decarboxylase activity (Beever, 1951), tyrosine decarboxylase was distinguished by its different pH optimum, stability and sensitivity to *p*-hydroxyphenylpyruvate and 2-oxoglytarate.

Inhibition by *p*-hydroxyphenylpyruvate was shown to be competitive, giving K_i 0.07 mM. Similar inhibitions by *p*-coumarate (K_i 0.07 mM), ferulate (K_i 0.06 mM) and chlorogenic acid (K_i 0.06 mM) were found, but caffeate and sinapate were relatively ineffective.

The enzyme was stimulated by *S*-adenosylmethionine over a concentration range from 0.1 μ M to 1 mM with an optimum at 0.1 mM. The products were then found to include *N*-methyltyramine and hordenine, which accumulated as the reaction progressed.

The inhibition of enzyme activity by *p*-hydroxyphenylpyruvate suggests that tyrosine decarboxylation may take place only while the roots are dependent on tyrosine from the reserve protein and is inhibited when this has been exhausted and tyrosine is synthesized *de novo*. It may also be inhibited in cells producing lignin precursors or other phenolic substances by the hydroxylated cinnamic acids. Stimulation by *S*-adenosylmethionine may be held to support the view that alkaloids can act as pools for methyl groups (Mothes, 1955), but there is no evidence that these are subsequently

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used (Frank & Marion, 1956). The limitation of hordenine synthesis to the early stages of development after germination may thus rest on the availability of tyrosine from the endosperm (Folkes & Yemm, 1956) and its inhibition by intermediates in the synthesis of tyrosine and its conversion into other phenolics.

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The Isolation and some Properties of a Factor causing Gelling in the Phloem Sieve-Tube Exudate of the Squash Plant (*Cucurbita pepo*)

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In a communication to a Meeting of the Society for Experimental Biology held at University College, Dublin, Irish Republic, in July 1970 T. S. Walker & R. Thaine established the presence in the phloem sieve-tube exudate from *Cucurbita pepo* stems of some glycolytic and respiratory dehydrogenases. In that communication and in a subsequent paper (Walker & Thaine, 1971) they reported the presence in the exudate of a protein factor that caused gelling and showed that the gelling reaction could be inhibited by the presence of 2-mercaptoethanol and other thiol compounds. This communication describes some properties of a homogeneous preparation of the gelling factor.

Exudate from the stems of mature *C. pepo* plants was collected into a buffered medium of 0.1M-Tricine-KOH, pH 7.5, containing 0.05M-2-mercaptoethanol, 0.2M-EDTA, 0.4M-mannitol and 0.1% of polyvinylpyrrolidone. The exudate was clarified by centrifugation, batch treated with DEAE-cellulose, and the gelling factor was isolated from the filtrate after Sephadex G-100 chromatography and CM-cellulose chromatography. The final peak eluted from the CM-cellulose column ran as one component on electrophoresis at different pH values and formed a gel when 2-mercaptoethanol was removed by dialysis.

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The gelling factor was found to be a basic protein with the following amino acid composition: lysine, 40; histidine, 10; arginine, 17; aspartic acid, 35; threonine, 7; serine, 20; glutamic acid, 46; proline, 14; glycine, 25; alanine, 14; valine, 33; methionine, 3; isoleucine, 38; leucine, 38; tyrosine, 14; phenyl-alanine, 22; cysteine, 6; tryptophan, 1; the minimum molecular weight was 43371.

The protein sedimented as a single boundary in the ultracentrifuge with a sedimentation coefficient, $s_{20,w}^0$, of 4.32S. A molecular weight of 127000 was derived from the sedimentation and diffusion coefficients.

The gelling phenomenon was found to be dependent on protein concentration, thiol concentration and dissolved O_2 in the collecting medium. The relative effectiveness of several thiols decreased in the order: 2-mercaptoethanol < dithiothreitol < cysteine < GSH. Cleland's reagents were only partially effective in preventing gelling in the crude exudate, since the reduced gelling protein was continually reoxidized by aerial oxidation.

The evidence indicates that, although the gelling factor is not the sole constituent of the phloem sieve-tube slime plugs in *C. pepo*, it alone may be responsible for the formation of the plugs.

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The Effects of Depletion of Unsaturated Fatty Acids on the Energy-Dependent Reactions of Yeast Mitochondria

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Studies with a mutant of *Saccharomyces cerevisiae* that cannot synthesize unsaturated fatty acids have demonstrated that depletion of unsaturated fatty acids leads to a specific loss of mitochondrial oxidative phosphorylation both *in vivo* and *in vitro* (Proudlock, Haslam & Linnane, 1969, 1971; Haslam, Proudlock & Linnane, 1971). Mitochondria depleted of unsaturated fatty acids possess a full complement of cytochromes, oxidize both NAD-linked and flavoprotein-linked substrates at normal rates and have succinate dehydrogenase and malate dehydrogenase activities similar to those of mitochondria supplemented with unsaturated fatty acids. However, oxidative phosphorylation by the mitochondria is markedly affected by the fatty acid composition. Phosphorylative ability decreases progressively with the mitochondrial content of unsaturated fatty acids, and is completely lost in mitochondria in which less than 20% of the fatty acids are unsaturated. The incorporation *in vivo* of unsaturated fatty acids into the lipids of