coefficient (Atkinson, Hathaway & Smith, 1965) increases from 1 in the absence of UMP to approx. 3 at UMP concentrations of $50\text{ }\mu\text{M}$ or higher. Changing the UMP concentration does not appreciably alter V_{max} . At fixed concentrations of substrates plots of initial rate versus UMP concentration are also sigmoidal, with Hill coefficients approx. 3.

These results suggest that at pH 10 the enzyme has allosteric properties. The kinetic data are consistent with (but do not prove) the concerted-transition mechanism proposed by Monod, Wyman & Changeux (1965), as applied to a trimeric enzyme in which ligand binding and dissociation are rapid compared with the product-forming process. UMP is seen to be a sensitive regulator of the enzyme *in vitro*, especially when the concentration of carbamoyl phosphate is low. However, the significance *in vivo*, if any, of the unusual pH-dependence of this regulatory behaviour is not understood.

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Acid Phosphatase and Ribonuclease in the Cotyledons of Germinating Peas

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The activities of acid phosphatase and ribonuclease in pea cotyledons increase early in germination. Experiments described below indicate that acid phosphatase is synthesized *de novo* but that ribonuclease is not.

Peas were germinated for 4 days in presence of ^{14}C -labelled *Chlorella* protein hydrolysate. The cotyledons were removed, homogenized and centrifuged at $20000g$. Acid phosphatase in the supernatant was purified by precipitation with ammonium sulphate, chromatography on Whatman DE-32 cellulose and electrophoresis on polyacrylamide gel. Radioactivity and enzymic activity in the gel were found to coincide precisely, suggesting that the enzyme had been synthesized *de novo*. This was confirmed by submitting the partially purified enzyme from peas germinated in 80% (v/v) D_2O to equilibrium centrifugation in caesium chloride (Filner & Varner, 1967; Longo, 1968). Enzymic activity was located in the part of the gradient of density $1.381\text{ g}\cdot\text{cm}^{-3}$. For seed germination in H_2O the enzyme was located in the region of density $1.360\text{ g}\cdot\text{cm}^{-3}$. This difference represents a shift of

1.62% in the buoyant density of acid phosphatase after germination in 80% (v/v) D_2O . It is concluded that synthesis of the enzyme occurs during this process.

Ribonuclease was isolated from cotyledons of peas germinated for 4 days in the presence of ^{14}C -labelled *Chlorella* protein hydrolysate, when activity of the enzyme is maximal (Barker & Hollinshead, 1966). After purification by precipitation with ammonium sulphate, chromatography successively on hydroxyapatite and DE-32 cellulose, and electrophoresis on polyacrylamide gel, enzymic activity was confined to three closely associated bands. It did not coincide exactly with a peak of radioactivity, but association of radioactivity with the enzyme could not be excluded. The enzyme was partially purified from peas germinated in 80% (v/v) D_2O and centrifuged as described above. Ribonuclease activity was found in the region of density $1.341\text{ g}\cdot\text{cm}^{-3}$. For peas germinated in H_2O the enzyme was present in the fraction of density $1.339\text{ g}\cdot\text{cm}^{-3}$. It is concluded that ribonuclease is not synthesized *de novo* under these conditions.

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The Sites of Synthesis of Chloroplast Proteins

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Previous attempts to determine the sites of synthesis of chloroplast proteins in higher plants have involved the treatment of greening cells with chloroamphenicol and cycloheximide, but it is now clear that neither of these compounds is sufficiently specific in its action *in vivo* to allow definite conclusions to be made (Ellis & MacDonald, 1970). Protein synthesis by isolated chloroplast ribosomes, unlike that by cytoplasmic ribosomes, is inhibited by spectinomycin, lincomycin and erythromycin (Ellis, 1970). We now report that lincomycin is a highly effective and specific inhibitor of chloroplast development in pea apices.

Seeds of *Pisum sativum* were germinated in the dark for 7 days. Etiolated shoots were excised, the cut ends placed in lincomycin, and the shoots illuminated with white light (14000lx) for 48h. The

apical leaves were ground with glass beads in a hypo-osmotic medium and centrifuged at 10000g for 10 min. The supernatant solution was assayed for NADP-specific triose phosphate dehydrogenase and ribulose diphosphate carboxylase. RNA polymerase was assayed in suspensions of washed pellets prepared by centrifuging extracts made in iso-osmotic medium at 1500g for 10 min. The synthesis of chlorophyll by the apices is inhibited 50% by 1 μ M-lincomycin. The specific activities of the dehydrogenase, carboxylase and polymerase increased about sixfold, tenfold and twofold respectively during illumination. The increases in dehydrogenase and polymerase activities are unaffected by 5 μ M-lincomycin, but the increase in carboxylase activity is almost totally prevented; 50% inhibition of the increase in carboxylase activity is produced by about 1 μ M-lincomycin. The activities of all three enzymes are unaffected by lincomycin *in vitro*. The increase in dehydrogenase activity is inhibited by D-threo-chloramphenicol, but a similar inhibition is produced by the L-threo isomer, indicating that the antibiotic is not acting solely at the ribosomal level (Ellis, 1969).

Analysis of RNA by polyacrylamide-gel electrophoresis showed that the chloroplast ribosomal RNA components increased in amount on illumination of the pea apices. This increase was prevented by 2.5 μ M-lincomycin. Incorporation of [32 P]phosphate into cytoplasmic ribosomal RNA was unaffected by 5 μ M-lincomycin.

Assuming that the increases in activity are due to net synthesis, these results suggest that the synthesis of the carboxylase and probably of chloroplast-ribosomal proteins requires the activity of plastid ribosomes, whereas that of the dehydrogenase and polymerase does not.

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Turnover of Deoxyribonucleic Acid in Seedlings of *Pisum sativum*

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Pelc (1968) has suggested that dividing and non-dividing cells of eukaryotic organisms contain a species of DNA ('metabolic DNA') that turns over much more rapidly than the chromosomal

DNA, and that consists of extra copies of active genes. In support of this suggestion Pelc (1968) cited observations made by several authors on a wide range of animal and plant tissues. Recently, however, the reliability of results obtained from experiments on plant tissue (Sampson & Davies, 1966) has been questioned (Bryant & Wildon, 1971). A series of experiments has therefore been undertaken to establish whether or not 'metabolic DNA' may be detected in plant tissue.

Pea (*Pisum sativum*) seedlings (five days old) were supplied with [6- 3 H]thymidine for 4 h; half the seedlings were then incubated in unlabelled thymidine (7000-fold excess) for a further 20 h. The roots were harvested and sectioned and the cells in the post-meristematic maturation zone were examined by radioautography. The results indicated that after a 4 h incubation in radioactive thymidine there was a high level of incorporation into the nuclei of the cells. Further, there was a marked decrease in the incorporation after 20 h in unlabelled thymidine. Treatment of the tissue sections with deoxyribonuclease I (EC 3.1.4.5) caused a loss of most of the radioactivity from the nuclei; treatment with ribonuclease (EC 2.7.7.16) or Pronase did not lead to a loss of radioactivity. These results led to the conclusion that radioactivity from [6- 3 H]thymidine may be incorporated into the nuclear DNA of cells in the maturation zone of the roots, and that part of the incorporated radioactivity may be 'chased' out by unlabelled thymidine. On their own these results cannot be taken to show that 'metabolic DNA' is present in plants. It is emphasized, however, that the results cannot be explained in terms of the kinetics of cell division reported for the maturation zone of the root (Clowes & Juniper, 1968).

Before any conclusions can be reached about the existence of 'metabolic DNA', it is important that these radioautographic experiments should be followed up by experiments involving more direct biochemical methods, including extraction of DNA and assay of radioactivity by scintillation counting. Such experiments are at present in progress.

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