microscopy. Nuclear, chloroplast and mitochondrial fractions were almost completely resolved, as indicated by DNA, chlorophyll and ubiquinone distribution.

α -Tocopherol and α -tocopherolquinone were largely present in the chloroplasts (92% and 88% respectively). γ -Tocopherol occurred in significant quantities in all fractions, but was concentrated in chloroplasts (55%) and the microsomal fraction (24%). δ -Tocopherol was fairly evenly distributed among fractions: nuclear, 16%; chloroplast, 28%; mitochondrial, 17%, microsomal, 13%; supernatant, 26%.

Barlow, S. M. & Gaunt, J. K. (1968). *Biochem. J.* **109**, 8p.
Bucke, C. (1969). In *Progress in Photosynthesis Research*, vol. 1, p. 325. Ed. by Metzner, H. Berlin: Lange & Springer.

Dilley, R. A. & Crane, F. L. (1963). *Pl. Physiol., Lancaster*, **38**, 452.

Lichtenthaler, H. K. (1966). *Ber. dt. bot. Ges.* **79**, 111.

Peake, I. R. (1970). Ph.D. Thesis: University of Liverpool.

A Study of Mitochondrial Complementation in Wheat

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It has been reported that mixtures of mitochondria from various inbred lines of wheat occasionally exhibit oxidative and phosphorylative activities that are higher than those of mitochondria from either pure line, as also are the activities of mitochondria from hybrids of these lines (Sarkissian & Srivastava, 1969a). This phenomenon has been called 'mitochondrial complementation' (McDaniel & Sarkissian, 1966).

Attempts were made to confirm the published results of Sarkissian & Srivastava (1969b,c) by using their method for the very rapid isolation of mitochondria from varieties of wheat that, when crossed, showed heterosis. No success was achieved until a number of modifications were made to the procedure and very strict attention was paid to details of the method. A standard oxygen-electrode system was used to measure the oxygen consumption during the phosphorylation of known amounts of ADP by mitochondria isolated from each of the pure lines under consideration, and also from 1:1 mixtures of mitochondria from these lines selected in pairs and from the relevant hybrids, if available.

Measurements of mitochondrial activity in two varieties of wheat provided by the Plant Breeding Institute showed evidence of complementation, and the hybrid demonstrated positive heterosis with respect to yield of grain. One of these varieties also gave mitochondria that when mixed with particles

from a third variety demonstrated negative complementation, and the hybrid showed negative heterosis. These results are judged to be sufficiently encouraging to warrant further investigations into the phenomenon of mitochondrial complementation and its relation with hybrid vigour.

This work forms part of a joint project between the Glasshouse Crops Research Institute and the Plant Breeding Institute, Cambridge. Specialized laboratory facilities at the University of Sussex were generously provided by Professor J. F. Sutcliffe.

McDaniel, R. G. & Sarkissian, I. V. (1966). *Science, N.Y.*, **152**, 1640.

Sarkissian, I. V. & Srivastava, H. K. (1969a). *Proc. natn. Acad. Sci. U.S.A.* **63**, 302.

Sarkissian, I. V. & Srivastava, H. K. (1969b). *Pl. Physiol., Lancaster*, **43**, 1406.

Sarkissian, I. V. & Srivastava, H. K. (1969c). *Life Sci.* **8**, 1201.

Allosteric Properties of Wheat-Germ Aspartate Transcarbamoylase

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Aspartate transcarbamoylase (EC 2.1.3.2), purified approx. 20-fold from commercial wheat germ, is inhibited by UMP; the enzyme is thus potentially a site for feedback regulation of the pyrimidine-biosynthetic pathway in the germinating wheat embryo. This and other features of the enzyme have been reported in a previous communication (Yon, 1971).

The same partially purified preparation has now been further characterized. Catalytic activity and sensitivity to inhibition by UMP are both pH-dependent. The most prominent feature of the pH-activity profile is a single sharp peak between pH 9.5 and 11.0, with a maximum at pH 10.5 of 0.11 $\mu\text{mol/min per mg}$ of protein at 25°C. Elsewhere in the range of activity (which extends from pH 6.5 to 11.2) the activity is less than 0.04 $\mu\text{mol/min per mg}$ of protein. In the range pH 6.5–7.5 the enzyme is insensitive to UMP. Above pH 7.5 sensitivity to UMP gradually increases to a maximum at pH 10.0.

Kinetic behaviour has been studied in detail at pH 10.0 and 25°C. In the absence of UMP initial-rate plots are hyperbolic for both substrates (L-aspartate and carbamoyl phosphate). In the presence of UMP kinetic plots for aspartate remain hyperbolic; the inhibition is non-competitive with respect to aspartate. However, in the presence of UMP plots for carbamoyl phosphate become sigmoidal. The degree of sigmoidicity is a function of the UMP concentration; the interaction (Hill)

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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Atkinson, D. E., Hathaway, J. A. & Smith, E. C. (1965). *J. biol. Chem.* **240**, 2682.

Monod, J., Wyman, J. & Changeux, J. P. (1965). *J. molec. Biol.* **12**, 88.

Yon, R. J. (1971). *Biochem. J.* **121**, 18P.

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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Barker, G. R. & Hollinshead, J. A. (1966). *Nature, Lond.*, **212**, 926.

Filner, P. & Varner, J. E. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 1520.

Longo, C. P. (1968). *Pl. Physiol., Lancaster*, **43**, 660.

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