0·82. Cyclic nitrosamines as, for example, nitrosomorpholine and nitroso-piperidine and others can be resolved in the system hexane/ether/dichloromethane = 5 : 7 : 10. We succeeded in separating up to 20 different nitrosamines by two-dimensional thin-layer chromatography.

This work will be continued, especially in relation to the mechanism of the colour reaction of nitrosamines with diphenylamine/palladium chloride.

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Distribution of Dehydroascorbic Acid Reductase in the Wheat Grain

Hopkins and Morgan[1] showed that the juices of cauliflower and cabbage contain an enzyme which catalyses the reduction of dehydroascorbic acid (DHA) by glutathione (GSH). Crook[2] determined the properties of this reductase and separated it from the associated ascorbic acid oxidase. In further work Crook and Morgan[3] examined thirty species of plants for the presence of the enzyme. This survey included extracts of barley grain and sprouted wheat grain and the enzyme was found in both. Later, Maltha[4] showed that an extract of wheaten flour catalysed the reaction.

In experiments concerned primarily with the influence of sulphydryl groups on the properties of dough from wheaten flour (cf. Frater and Hird[5], McDermott and Pace[6]) we observed that suspensions of flour in water containing GSH rapidly reduced DHA. The activity could be measured with as little as 10 mg flour and this suggested it would be practicable to investigate the distribution of the enzyme in the resting wheat grain, using the small quantities of material which are obtained by dissecting out the anatomical parts by hand. Dissected fractions of the grain were prepared as described by Hinton[7]. Enzyme activity was measured essentially as described by Hopkins and Morgan, using suspensions of material in McIlvaine buffer of *p*H 6·2 at 25° C. The suspensions were dispersed in the buffer for 30 min before adding the reactants GSH and DHA. DHA was prepared either by oxidizing ascorbic acid with iodine immediately before use or by the method of Kenyon and Munro[8]. No activity was found in samples of wheat which had been heated with steam at 100° C for 4 min. Results obtained are given in Table 1.

The concentration of enzyme is much higher in the germ than in the rest of the grain, but is appreciable in the endosperm. Because of the relative proportions of germ and endosperm in the grain (2·4 and 82·5 per cent respectively[7]) most of the total activity is found in the endosperm. Appreciable activity in the endosperm of resting grain is not usually found with other enzymes which have been investigated.

Table 1. Activity of DHA Reductase in Dissected Fractions of the Wheat Grain (var. Cappelle Desprez)

(μg ascorbic acid formed per min, reaction time 10 min at 25° C)

	From 1962 harvest	From 1963 harvest
Pericarp and testa	1·0	0·6
Aleurone	9·4	11·2
Endosperm	15·1	14·3
Embryo	70·7	70·7
Scutellum	68·5	71·0

Reaction mixture: 10 mg material in 4 ml. buffer, *p*H 6·2, containing $1·25 \times 10^{-3}$ M DHA and $2·54 \times 10^{-3}$ M GSH. Reaction stopped with 1 ml. metaphosphoric acid (25 per cent), centrifuged, 4 ml. supernatant titrated with 2,6-dichlorophenol indophenol (80 mg/l.).

The distribution of the enzyme in a dicotyledonous plant, *Pisum sativum*, has been determined by Yamaguchi and Joslyn[9]. They found that in the germinating seed the embryo had the highest activity.

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Activation of the Latent Tyrosinase of Broad Bean

Extracts of leaf tissue of broad bean (*Vicia faba*, L.) contain an enzyme which shows little tyrosinase (E.C. 1.10.3.1)[1] activity unless treated with a denaturing agent[2–5] when the activity toward both orthodiphenols and monophenols normally increases 50-fold. Conditions under which the denaturing agents are used for activation (Table 1) are normally considered to be insufficient to break covalent bonds associated with the protein molecules but are known to alter the tertiary structure and to cause some proteins to dissociate into sub-units[6]. Like other tyrosinases[7] the isolated latent enzyme contains copper and exists in several forms separable by column chromatography or by electrophoresis on starch gel. Inhibition investigations have shown that the latent enzyme is less sensitive toward copper complexing agents, such as cyanide and diethyldithiocarbamate, than the active form, indicating that activation is probably accompanied by an unmasking of the prosthetic group.

We believe that the observed increase in tyrosinase activity brought about by denaturing agents is most probably due to a re-arrangement of tertiary structure and not to liberation of a masking sub-unit. First, when the denaturing agent, including H^+ or OH^-, is removed the enzyme reverts to a latent form (an observation over-

Table 1. Conditions for Activation

Reagent	Optimum *p*H	Time of incubation (min)	Concentration
H^+	—	3–5	10^{-3} M*
OH'	—	3–5	$10^{-3} - 10^{-4}$ M*
Sodium dodecylsulphate	5·1	1	$10^{-3} - 10^{-4}$ M†
Sodium dioctylsulphosuccinate	5·1	1	$10^{-3} - 10^{-4}$ M
Carboxymethylcellulose	4·8	15	10 mg/ml.
Urea	4·8	5	7 – 10 M
Guanidine salts	4·8	5	2 M

* Higher concentrations cause irreversible denaturation.
† Prolonged incubation causes denaturation apparently due to dissociation of the enzyme into sub-units as judged by examination of the sedimentation coefficient.