The Pattern and Control of Isoprenoid Quinone and Tocopherol Metabolism in the Germinating Grain of Wheat (*Triticum vulgare*)

By G. S. HALL* AND D. L. LAIDMAN

Department of Biochemistry and Soil Science, University College of North Wales, Bangor

(Received 21 November 1967)

1. The syntheses of ubiquinone-9 and plastoquinone-9 were used as parameters respectively of mitochondrial and proplastid development in the germinating wheat grain. 2. The changes in the amounts of the tocopherols were also studied and the possible biological significance of these changes is discussed. During germination, the dimethyl tocopherols of the resting grain are probably not utilized for the synthesis of α -tocopherol. 3. It was demonstrated that ubiquinone synthesis, and hence probably mitochondrial development, in the aleurone cells during germination, is independent of control by gibberellic acid from the embryo. 4. The influence of light on the syntheses of the isoprenoid quinones in the etiolated wheat shoot was investigated. In particular, illumination did not stimulate the synthesis of either α -tocopherol or α -tocopherolquinone.

Despite the fact that the seeds of different plants differ anatomically and chemically, their basic processes of germination appear to be similar. The initial stages of germination transform a quiescent seed into an actively metabolizing organism within a matter of hours (Toole, Hendricks, Borthwick & Toole, 1956). Though the major biochemical events concerned with the subsequent breakdown of storage material in germinating seeds have been well documented (Wareing, 1963; Mayer & Poljakoff-Mayber, 1963), it is only in recent years that interest has turned to the biochemical mechanisms that control the development of metabolic activity and the initiation of cell growth and division.

Though the appearance of many enzymes during germination represents synthesis (Ingle & Hageman, 1965), others can be accounted for by the activation of pre-existing enzymes (Rowell & Goad, 1964). In the cereals it has been demonstrated that the formation of some hydrolytic enzymes in the endosperm is controlled by the hormone gibberellic acid. Gibberellic acid is secreted by the embryo early in germination (Yomo, 1960; Paleg, 1960, 1965) and response to the hormone is localized entirely in the aleurone cells (Briggs, 1964; Varner, 1964; Paleg, 1964). The hormone also stimulates the incorporation of labelled precursors into RNA (Ram Chandra & Varner, 1965) and of labelled amino acids into protein (Varner, 1964). These results suggest that gibberellic acid may function as a gene de-repressor

(Varner & Ram Chandra, 1964). Evidence for the hormonal control of the production of other enzymes during germination is less conclusive, and the formation of some endosperm enzymes appears to be independent of hormone control from the embryo (Marcus & Feeley, 1964; Tanner & Beevers, 1965). Paleg & Hyde (1964) have described extensive gibberellic acid-dependent changes in the sub-cellular morphology of aleurone cells in germinating barley.

In the studies described in this paper, ubiquinone and plastoquinone were used as parameters of mitochondrial and proplastid development respectively. Crane (1965) has pointed out that there has been no extensive study of the changes in amount of ubiquinone during growth and differentiation of cells or tissues. Seed germination provides such a system in the plant kingdom. During germination of the cereal grain, the cells of the embryonic axis exhibit both growth and division, followed by differentiation. In contrast, the scutellum and the aleurone layer consist of actively metabolizing cells that enlarge but do not undergo cell division or further differentiation.

Ubiquinone synthesis in isolated endosperm halves was studied in an attempt to illuminate mechanisms involved in the initiation and regulation of mitochondrial development in aleurone tissue of the germinating wheat grain. Amounts of the structurally related tocopherols, present in considerable quantities in the wheat grain and seedling, were also investigated. [The IUPAC-IUB nomenclature of quinones with isoprenoid side

* Present address: School of Biological Sciences, University of East Anglia, Norwich, NOR 77H.

chains [*Biochem. J.* (1967) **102**, 15] has been used throughout this paper. In addition the following trivial names have been used: trimethyl tocopherols, α -tocopherol + α -tocotrienol; dimethyl tocopherols, any mixture of two or more from β -tocopherol, β -tocotrienol, γ -tocopherol, γ -tocotrienol.]

A brief summary of this work has already appeared (Hall & Laidman, 1966).

METHODS

Sterilization and germination of the wheat grains. Wheat grains (var. Atle; 1963 harvest) were sterilized with NaOCl solution under reduced pressure (B. B. Stowe, personal communication). Each 5g. sample of grain (150 grains) was placed, along with about 20ml. of dilute NaOCl solution (0.05% available chlorine), in a 100ml. Buchner flask fitted with a Quickfit stopper. The flask contents were placed under reduced pressure (water pump) for 15min., the vacuum being released at 3min. intervals and immediately reapplied. The grain was then washed free of hypochlorite with ten 30ml. volumes of sterile water.

The sterilized grains were allowed to germinate in moist sterile vermiculite at 24° in the dark. The samples were harvested at periods up to 8 days and dissected into their component parts for analysis.

Preparation and incubation of endosperm halves. Initially, some difficulty was experienced in attempting to maintain incubated endosperm halves in a sterile state. Because of the important results that these early experiments yielded, however, they are reported in detail.

In the first experiments, endosperm halves were dissected from 5g. samples of grain that had been surface-sterilized by simply soaking for 10min. in dilute NaOCl solution. The grains were cut in half transversely and the embryo-containing halves were discarded. This ensures the complete removal of scutellar and embryonic tissue, which might otherwise act as a hormone source (Paleg, 1961). The endosperm halves dissected from different 5g. samples of grain were the same weight within the limits of our overall experimental error. This obviated the need to weigh the preparations after dissection. The halves were incubated on moist cotton wool in a Petri dish for periods up to 5 days at 24°. Similar samples were incubated at 4°.

In a second series of experiments endosperm halves, which had been cut from surface-sterilized grains, were soaked in an antibiotic mixture (streptomycin, 100 μ g./ml.; penicillin, 80 μ g./ml.) for 2hr. before being incubated as above on cotton wool moistened with the antibiotic mixture. Other endosperm halves were soaked for 15hr. in 1 μ M-gibberellic acid solution after the 2hr. antibiotic treatment, and were then incubated on cotton wool moistened with a solution of both the gibberellic acid and the antibiotics. Because these endosperm halves also became infected with moulds and bacteria, the latter series of experiments were repeated with strict aseptic conditions for the dissection and incubation. The incubations were carried out in Petri dishes covered by inverted filter funnels, the stems of which were plugged with cotton wool. Before use, the complete incubation units were sterilized by autoclaving, and, after cooling, the cotton wool in the Petri dishes was soaked either in the antibiotic solution or in the solution of gibberellic acid and antibiotic.

In the final series of experiments 5g. samples of grain were sterilized by vacuum infiltration with dilute NaOCl solution (see above). This procedure ensures that the sterilizing solution penetrates the intercellular spaces of the seed pericarp. Subsequent dissections and incubations were again carried out under aseptic conditions. In this series of experiments the effects of various inhibitors were also studied. Endosperm halves from 5g. of grain were soaked for 24hr. under aseptic conditions in a solution of the appropriate inhibitor before they were incubated on cotton wool soaked in the same solution. In other experiments the halves were immersed in the inhibitor solution for 24hr. at 4° before incubation at 24°. In addition other endosperm halves were dissected, under sterile conditions, into bran (aleurone layer and pericarp) and starchy-endosperm fractions, after soaking for 12hr. in sterile water. The bran was then incubated with the inhibitor in the same way as the endosperm halves. All antibiotic solutions were sterilized by ultrafiltration and samples were incubated for 3 days before analysis.

Addition of exogenous β -tocotrienol to the endosperm. Grain (4g.; 120 grains) was allowed to germinate in the dark for 6.5 days. By this time the endosperm was virtually a fluid-filled sac. In dim light, with a micro-syringe, 20.0 μ l. of a β -tocotrienol emulsion (200 μ g./ml.) was injected into the endosperm of each seedling. The emulsion was prepared immediately before use by dissolving 2mg. of β -tocotrienol in 0.2ml. of ethanol and syringing this rapidly into 9.8ml. of water. The suspension was completely dispersed by treating it ultrasonically for 1min. The injected seedlings were placed in moist vermiculite and incubated for a further 2 days in the dark at 24°. On harvesting, the seedlings were dissected into endosperm, root, shoot and scutellum. In this experiment the basal portions of the root and shoot were left attached to the scutellum. The lipid extracts from the dissected parts were fractionated and assayed for their contents of plastoquinone, ubiquinone and tocopherols.

A second experiment was designed to indicate whether or not exogenous β -tocotrienol was degraded by endosperm fluid. Duplicate samples of grain (2.5g.) were germinated for 6 days and the endosperms carefully removed from the shoot and root. The endosperm fluid could then be collected by gently crushing the endosperms with a mortar and pestle and pressing the resultant slurry through several layers of cheesecloth. The fluid from each sample was made up to 14ml. with water and placed in a 50ml. conical flask, plugged with cotton wool. One sample was boiled and acted as control. A portion (1ml.) of β -tocotrienol emulsion (200 μ g./ml.) was added to each flask and to a third flask containing 14ml. of water (water control). All three flasks were incubated at 24° in a metabolic shaker. At 0, 20 and 40hr., 3ml. samples were withdrawn from each flask and placed into 15ml. volumes of hot propan-2-ol. The mixture was boiled under reflux for 20min. and cooled. After addition of water, the lipids were extracted with three 10ml. lots of light petroleum and evaporated to dryness under N₂. The extracts were dissolved in ethanol and their tocopherol contents determined directly by the modified Dilley & Crane (1963) method (Hall & Laidman, 1968).

Effect of light on tocopherol synthesis in the wheat shoot. Samples (4g.) of wheat grain were allowed to germinate in the dark for 6 days. Growth was then allowed to continue for periods of 24hr. or 72hr. in the light of two

40 w bulbs at a distance of 50 cm. The shoots were cut off just above the scutellum and were analysed for their content of ubiquinone, plastoquinone, phyloquinone, α -tocopherol and β -carotene. Their chlorophyll contents were measured by the method of Arnon (1949) on a portion of the lipid extract. Control samples of 6-day seedlings were left in the dark for 24 and 72 hr. and analysed similarly.

Determination of ubiquinone, plastoquinone and tocopherols. The ubiquinone, plastoquinone and tocopherol contents of the dissected parts of the harvested seedlings were determined by our routine method (Hall & Laidman, 1968). The tocopherol values obtained by gas-liquid chromatography were checked (on duplicate samples) by the modified Dilley & Crane (1963) spectrophotometric method. The ubiquinone contents of the endosperm halves were determined by the same method, but, on this occasion, the tocopherols were determined as α -tocopherol + α -tocotrienol and β -tocopherol + β -tocotrienol, by the modified Dilley & Crane (1963) procedure.

RESULTS

Changes in amounts of quinone and tocopherol during germination. Preliminary experiments with the whole grain and seedling demonstrated that, during germination in the dark, there was an early and rapid synthesis of ubiquinone-9, followed by a synthesis of plastoquinone-9. Since photosynthesis was prevented in these studies, the dry-matter content of the germinating grains progressively decreased. Therefore, to relate these syntheses to growth and development, it was necessary to relate the values to seed numbers rather than to dry matter.

The changes in the amounts of quinones are shown in Fig. 1. At no stage were any tocopherol-

quinones or phyloquinone detected. Ubiquinone was synthesized in both the embryo and the endosperm and increases could be detected after 12 hr. of imbibition in both parts of the grain. The synthesis of ubiquinone in the endosperm was confined entirely to the bran fraction and presumably to the aleurone tissue. Within the embryo, ubiquinone was continuously synthesized in the growing shoot and root. A synthesis was also observed in the scutellum, this being similar to that in the aleurone layer in that the amount of ubiquinone doubles before reaching a maximum. The tissues differed, however, in the time at which these changes took place. Whereas ubiquinone synthesis in the aleurone cells and the embryonic axis had commenced 12 hr. after imbibition, the increase in the scutellum did not commence until about 2 days after imbibition. The synthesis of plastoquinone in the embryonic axis commenced some 36 hr. later than the start of ubiquinone synthesis in the axis. Plastoquinone occurred almost entirely in the growing shoot and to a much smaller extent in the root (Table 1). It was not found in the scutellum or endosperm. As would be expected, ubiquinone occurred in both the shoot and root.

The changes in the amounts of the tocopherols are shown in Figs. 2 and 3. There was no apparent synthesis of tocopherols in the endosperm. The amount of β -tocotrienol in this part decreased by about 40%, whereas the amounts of the other

Fig. 1. Isoprenoid quinones in the germinating wheat grain. ●, Ubiquinone-9 in the root and shoot; ▲, plastoquinone-9 in the root and shoot, ○, ubiquinone-9 in the endosperm; △, ubiquinone-9 in the scutellum.

Table 1. Percentage distribution of tocopherols and quinones between the shoot and root of 8-day seedlings

	Percentage distribution of tocopherols and quinones			
	Total lipid	Ubiquinone-9	Plastoquinone-9	α-Tocopherol
Shoot	81.5	78	97	88
Root	18.5	22	3	12

Fig. 2. Tocopherols in endosperm of the germinating wheat grain. ●, α -Tocopherol; ▲, β -tocopherol; ○, α -tocotrienol; △, β -tocotrienol.

Fig. 3. Tocopherols in the embryo of the germinating wheat grain. ●, α -Tocopherol; ▲, β -tocopherol; △, β -tocotrienol.

tocopherols remained virtually constant. The greater part of this loss of β -tocotrienol was associated with the digestion of the starchy endosperm. The amounts of tocopherols in the bran thus showed only very slight decreases during germination. In the embryo, on the other hand, β -tocopherol and β -tocotrienol had disappeared after 4 days and a 2.5-fold dark synthesis of α -tocopherol was observed within 8 days of imbibition. Separate examination of the root and shoot of the 8-day seedlings (Table 1) revealed that α -tocopherol was present in both parts, but that its distribution between shoot and root was in a ratio unlike that of either ubiquinone or plastoquinone. Dissection of the 1- and 2-day embryos into scutellum and embryonic axis showed that the sudden fall in amounts of α -tocopherol between these two times (see Fig. 3) occurred in both parts of the embryo.

Ubiquinone synthesis in the isolated endosperm. The results of the first experiments are shown in Fig. 4. When endosperm halves were incubated at 24°, ubiquinone synthesis commenced within 24 hr., but continued beyond the control values obtained from halves dissected from 3- and 5-day seedlings. Samples incubated at 4° showed no increase in ubiquinone though they imbibed water, which indicated that a metabolic process was involved. The observed increase, over and above the control, was thought to be due to microbial contamination, since the incubated halves became heavily infected with moulds and bacteria and the amounts of ubiquinone may have been modified by the presence of these organisms. Accordingly, the antibiotic mixture was used in the second series of experiments in an effort to decrease this infection. These results are shown in Fig. 5. Ubiquinone synthesis in the endosperm halves was delayed and partially inhibited by the antibiotic mixture, though the samples were still heavily infected with moulds and

Fig. 4. Ubiquinone-9 synthesis in incubated endosperm halves. ■, Control endosperm halves from germinated whole grains; ●, surface-sterilized endosperm halves incubated at 24°; ○, surface-sterilized endosperm halves incubated at 4°.

Fig. 5. Ubiquinone-9 synthesis in incubated endosperm halves. ▲, Surface-sterilized endosperm halves incubated with antibiotic mixture; △, surface-sterilized endosperm halves incubated with antibiotic mixture + gibberellic acid; ●, surface-sterilized endosperm halves incubated under sterile conditions with antibiotic mixture; ○, surface-sterilized endosperm halves incubated under sterile conditions with antibiotic mixture and gibberellic acid; ■, control endosperm halves and vacuum-sterilized endosperm halves incubated under sterile conditions.

bacteria. By streaking samples of fluid from the Petri dishes on to nutrient-agar plates containing the antibiotic mixture it was demonstrated that the bacteria were resistant to this combination of antibiotics, rather than that the antibiotics were inactivated during the incubation period. It was found that gibberellic acid counteracted the effect

of the antibiotics since a large increase in ubiquinone was detected when both solutions were used together. When the experiment was repeated with aseptic dissection techniques the increase of ubiquinone in the endosperm halves was still significantly delayed in the presence of antibiotics, but in the presence of antibiotics and gibberellic acid a pattern more typical of the changes in the intact seed was observed (Fig. 5). However, the samples were still heavily contaminated, suggesting that the micro-organisms were not entirely responsible for the high level of synthesis of ubiquinone in the first experiment.

These results suggested that the infections must be seed-borne and that the surface-sterilization procedure was unsatisfactory. The vacuum-infiltration technique was then introduced. Dilute sodium hypochlorite solution was found to have no effect on the germination of the intact grains, as compared with untreated controls, with respect to oxygen uptake, amounts of ubiquinone and visible growth. By this procedure, endosperm halves could be incubated for periods of up to 8 days in the sterile Petri dishes without any sign of microbial infection. Analyses then demonstrated that the pattern of ubiquinone synthesis in the endosperm halves dissected from germinated grains, and the increase in isolated endosperm halves incubated under germination conditions, either with or without gibberellic acid, were identical (Fig. 5). By incubating dissected whole endosperms as well as the endosperm halves it was found that the pattern of development of ubiquinone was the same in both cases. That is, there was no differential increase of ubiquinone between the proximal and distal halves of the endosperm, owing to progressive diffusion of the hormone from the embryo, as has been observed in α -amylase formation (Briggs, 1964).

When endosperm halves were incubated in the absence of gibberellic acid their amounts of tocopherol remained constant. The partial loss during germination of β -tocotrienol from the endosperm of the intact grain may therefore be indirectly dependent on the hormonal action of gibberellic acid on the aleurone cell. Direct proof of this was not possible because endosperm halves that are incubated in the presence of gibberellic acid undergo extensive degradation of their starchy endosperm as a result of hormonally induced α -amylase activity. The soluble products flow out from the halves and therefore there would, in any case, be a loss of the tocopherols of the starchy endosperm.

Table 2 shows the effect of various metabolic inhibitors on the synthesis of ubiquinone in incubated endosperm halves and starch-free bran. Neither actinomycin D nor puromycin, at either relatively high or low concentrations, prevented ubiquinone synthesis in either endosperm halves or

Table 2. *Ubiquinone content of grain parts after incubation with inhibitors for 3 days*

Values are given as $\mu\text{g./150}$ grain parts. Experimental details are given in the text.

	Pericarp+ aleurone layer	Endosperm halves
Actinomycin D		
10 $\mu\text{g./ml.}$ (8 μM)	18	20
100 $\mu\text{g./ml.}$	—	21.7
100 $\mu\text{g./ml.}$ (initial period 4°)	—	19
100 $\mu\text{g./ml.}$ + KBrO_3 (1mm)	—	21.5
Puromycin hydrochloride		
50 $\mu\text{g./ml.}$ (0.1mm)	20	20
500 $\mu\text{g./ml.}$	—	20
Chloramphenicol		
1mg./ml.	—	14.4
2,4-Dinitrophenol		
1.0mm (pH 5.0)	—	16
0.05mm	—	16.5
0.1mm	15	—
0.1mm + gibberellic acid (0.05mm)	11.8	—
Halves steeped for 3 days	—	12.9
Controls		
0 day (ungerminated)	6	8
3 days	19.6	20

isolated bran layers. Chloramphenicol partially inhibited the synthesis. When the halves were soaked in the inhibitor solution for 24hr. at 4° before incubation at 24° the grain still absorbed water, but ubiquinone synthesis during incubation was not inhibited. It was hoped that, by using this method, the antibiotic would have time to penetrate the aleurone cells before active metabolism commenced. At 4° RNA and protein synthesis is appreciably decreased (Marcus & Feeley, 1965). The addition of potassium bromate to inhibit possible breakdown of actinomycin D by proteolytic enzymes (Varner & Ram Chandra, 1964) had no effect on the results.

When endosperm halves were steeped in water for 3 days there was only a partial synthesis of ubiquinone, indicating an oxygen requirement. Similarly 2,4-dinitrophenol, either alone or in combination with gibberellic acid, caused a partial but smaller inhibition of ubiquinone synthesis in both endosperm halves and isolated aleurone layers. The importance of the oxidative metabolism as a source of energy for the aleurone cell and for ubiquinone synthesis is thus established.

The observation that the amounts of ubiquinone in the control samples of the endosperm halves and isolated aleurone layers were identical (Table 2) confirmed that ubiquinone synthesis outside the

embryo is confined to the aleurone layer, and suggests that the synthesis is independent of carbohydrate or other energy reserves from the starchy endosperm. It was, however, impossible to remove all traces of starch from the dissected aleurone layer, and it is conceivable that this was utilized by the aleurone cells.

Addition of exogenous β -tocotrienol to the endosperm. Seedlings injected with β -tocotrienol continued to grow normally, compared with untreated controls. Though 400 μg . of β -tocotrienol was

injected into 120 seedlings, after a further 2 days of growth no trace of this compound could be detected in the root, shoot or scutellum, though a small amount (36 μg .) was found in the endosperm. This, however, could be accounted for by the endogenous β -tocotrienol present in the bran. The amounts of α -tocopherol, plastoquinone and ubiquinone in the injected seedlings were not different from those of the controls (Table 3).

In the experiments in which β -tocotrienol was incubated with samples of endosperm fluid it was found that there was no rapid breakdown of β -tocotrienol by enzymic or other agents (Table 4). All the samples, including the controls, showed only a relatively slow breakdown over the 40 hr. incubation period, though there was an initial sharp decrease in the flasks that contained endosperm fluid. In view of the natural instability of the tocopherol emulsion and the similarity of the values obtained from both the control and experimental incubations, there was no appreciable destruction of β -tocotrienol *in vitro* due to enzymic activity of the endosperm fluid. No β -tocotrienolquinone could be detected in any of the incubations or in the seedlings that had been injected with β -tocotrienol.

Effect of light on the synthesis of isoprenoid quinones in the shoot. Though the syntheses of plastoquinone, phyloquinone, β -carotene and chlorophyll were stimulated after the illumination of etiolated shoots, the syntheses of ubiquinone and α -tocopherol were not (Table 5). In contrast with the results of Threlfall & Griffiths (1967) in barley and maize only insignificant amounts of α -tocopherolquinone could be detected in the wheat shoots that had been illuminated for 24 or 72 hr., and we were unable to detect phyloquinone in the etiolated shoot. The light-independent nature of α -tocopherol synthesis in the wheat seedling is in agreement with that demonstrated in the pea by Gaunt & Stowe (1967) and in maize by Threlfall & Griffiths (1967).

DISCUSSION

The increases in amounts of ubiquinone during the early stages of germination support the con-

Table 3. *Analysis of wheat seedlings after injection of β -tocotrienol into the endosperm sac*

Values are given as μg ./120 seedlings. Experimental details are given in the text.

	Plasto- quinone-9	Ubiquinone-9	α -Tocopherol
Shoot			
Experimental	88	59	97
Control	83	64	94
Root			
Experimental	2	17	13
Control	3	24	16
Scutellum			
Experimental	5	24	23
Control	3	25	20
Total			
Experimental	95	100	133
Control	89	113	130

Table 4. *Incubation of β -tocotrienol with endosperm fluid*

Values are given in μg . Experimental details are given in the text.

	Time		
	0 hr.	20 hr.	40 hr.
Endosperm fluid + β -tocotrienol	150	134	109
Boiled endosperm fluid + β -tocotrienol	146	122	117
Water control + β -tocotrienol	202	135	132

Table 5. *Changes in isoprenoid compounds in etiolated 6-day wheat shoots after additional growth periods in the light and in the dark*

Values are given as μg ./120 shoots. Experimental details are given in the text.

Additional growth period	Phyloquinone	Ubiquinone-9	Plastoquinone-9	α -Tocopherol	β -Carotene	Total chlorophyll
Dark (24 hr.)	—	57	81	60	16	—
Light (24 hr.)	Trace	53	176	67	104	3440
Dark (72 hr.)	—	68	87	96	20	—
Light (72 hr.)	25	74	293	92	336	5210

clusions of Lund, Vatter & Hanson (1958) that a significant growth of mitochondria occurs during cell growth. Available histological evidence indicates that mitochondria in scutellar tissues do not increase in number, but that they do increase in size and degree of development of their cristae after about 3 days of germination (Hanson, Vatter, Fisher & Bils, 1959; Nieuwdorp & Buys, 1964). The precise timing of ubiquinone synthesis and mitochondrial development in the various tissues is presumably synchronized with demands for energy.

Treharne, Mercer & Goodwin (1966) have shown that, during the 'greening-up' of etiolated shoots, isoprenoid synthesis in the chloroplast is regulated by a combination of enzyme segregation and specific membrane permeability. We wish to take the discussion one stage further. The ratio of the rate of synthesis in the light to the rate of synthesis in the dark in the shoots of 6-day dark-grown seedlings is different for each isoprenoid compound. In the wheat seedlings used in our 'greening-up' experiments the ratios range from unity, for α -tocopherol, to infinity, for phyloquinone. In other words, the syntheses of different isoprenoids in the developing chloroplast are stimulated to different degrees by light. It is clear therefore that some other factor, besides that of simple compartmentation, is involved in the control of isoprenoid synthesis in this organelle.

Though the α -tocopherol content of the wheat embryo axis had increased by 103 $\mu\text{g.}/150$ embryos after 8 days of germination in the dark, only 47 $\mu\text{g.}$ of other tocopherols had disappeared in that time from the whole grain. Thus the methylation of the dimethyl tocopherols of the resting grain could account for only a part of the increases in α -tocopherol. The fact that injected β -tocotrienol did not influence the rate of α -tocopherol synthesis tends to uphold the view that the dimethyl tocopherols are not utilized in this way during germination. Tocopherols have been associated with the flowering process in plants (Michniewicz & Kamienska, 1965) and the presence of the dimethyl tocopherols in the resting grain may simply reflect their distribution at the end of the grain maturation period; they may have no function during germination. Further, the breakdown of exogenous β -tocotrienol by the starchy endosperm may be nothing more than one aspect of the general hydrolysis and breakdown of this tissue during germination. However, enzymic breakdown of exogenous tocopherol by tissues that do not appear to be catabolizing their own endogenous tocopherol has been noted (Gaunt & Stowe, 1967; Laidman, 1964).

With respect to the tocopherols in the aleurone cells, however, a more significant proposition may be advanced. This is that the α -tocopherol in these cells is associated with the lipid-rich spherosomes

(Paleg & Hyde, 1964), and that this α -tocopherol and some of the other lipids of the spherosome are involved in the gibberellic acid-triggered development of the endoplasmic reticulum during germination. Such a system would be similar to that described by Lichtenthaler & Sprey (1966) and Sprey & Lichtenthaler (1966) as being associated with lamellar formation during chloroplast development.

The experiments on endosperm halves have demonstrated that the synthesis of ubiquinone, and therefore the complete development of the mitochondria, in the aleurone tissue are independent of control by gibberellic acid. This is in contrast with the gibberellic acid-initiated development of the endoplasmic reticulum in these cells during the early stages of germination (Paleg & Hyde, 1964). Some other factor must be responsible for initiating ubiquinone synthesis in this tissue during germination. There is clearly yet another controlling mechanism that terminates ubiquinone synthesis in the aleurone cell after 3 days of germination. Similar factors probably initiate and terminate ubiquinone synthesis in the scutellum during germination.

The inhibitory effect of the penicillin-streptomycin mixture on ubiquinone synthesis in the aleurone tissue is important. It is assumed that this effect is due to the streptomycin rather than the penicillin since the former is more toxic to plants (Brian, 1957). The use of streptomycin to inhibit bacterial growth in isolated endosperm halves cannot therefore be recommended. The finding that gibberellic acid reverses the inhibitory effect of streptomycin but not the inhibitory effect of 2,4-dinitrophenol suggests that the gibberellic acid-streptomycin interaction is specifically associated with RNA and protein synthesis. The apparent anomalies between the results of the inhibitor studies in our aleurone system and those in some other plant systems (e.g. Marcus & Feeley, 1965; Morton & Raison, 1964; Parisi & Ciferri, 1966) can probably be explained to some degree by the demonstration in yeast that two distinct protein-synthesizing systems exist (Clark-Walker & Linnane, 1966). One, the cytoplasmic ribosomal system, is sensitive to cycloheximide but largely insensitive to the inhibitors of bacterial protein synthesis; the other, the mitochondrial system, is inhibited by chloramphenicol and other inhibitors of bacterial protein synthesis. Thus it might be construed that ubiquinone synthesis is controlled by a mitochondrial DNA-RNA system. A further aspect of antibiotic action that must be considered is that streptomycin and chloramphenicol also inhibit energy-linked processes in plant mitochondria (Stoner, Hodges & Hanson, 1964). Such a mode of action in aleurone mitochondria might appear to be supported by the inhibitory effect of

2,4-dinitrophenol on ubiquinone synthesis, but would offer no explanation for the reversal of the inhibitory effect of streptomycin by gibberellic acid. Obviously a clearer understanding of the modes of action of antibiotics on plant systems is required before the present results can be fully interpreted.

We are indebted to the Agricultural Research Council for financial support, to the Science Research Council for the award of a research studentship to G.S.H., to Dr A. L. Morrison (Roche Products Ltd., Welwyn Garden City, Herts.) for the gift of authentic tocopherol samples, to Dr F. W. Hemming (University of Liverpool) for the gift of authentic quinone samples and to Dr J. S. Tzoumertic (Merck, Sharp and Dohme Research Laboratories, Rahway, N.J., U.S.A.) for the gift of actinomycin D.

REFERENCES

- Arnon, D. I. (1949). *Plant Physiol.* **24**, 1.
 Brian, P. W. (1957). *Annu. Rev. Plant Physiol.* **8**, 413.
 Briggs, D. E. (1964). *J. Inst. Brew.* **70**, 14.
 Clark-Walker, G. D. & Linnane, A. W. (1966). *Biochem. biophys. Res. Commun.* **25**, 8.
 Crane, F. L. (1965). In *Biochemistry of Quinones*, p. 183. Ed. by Morton, R. A. London and New York: Academic Press Inc.
 Dille, R. A. & Crane, F. L. (1963). *Analyt. Biochem.* **5**, 531.
 Gaunt, J. K. & Stowe, B. B. (1967). *Plant Physiol.* **42**, 859.
 Hall, G. S. & Laidman, D. L. (1966). *Biochem. J.* **101**, 5P.
 Hall, G. S. & Laidman, D. L. (1968). *Biochem. J.* **108**, 465.
 Hanson, J. B., Vatter, A. E., Fisher, M. E. & Bils, R. F. (1959). *Agron. J.* **51**, 295.
 Ingle, J. & Hageman, R. H. (1965). *Plant Physiol.* **40**, 672.
 Laidman, D. L. (1964). *Biochem. J.* **91**, 31C.
 Lichtenthaler, H. K. & Sprey, B. (1966). *Z. Naturf.* **21b**, 690.
 Lund, H. A., Vatter, A. E. & Hanson, J. B. (1958). *J. biophys. biochem. Cytol.* **4**, 87.
 Marcus, A. & Feeley, J. (1964). *Biochim. biophys. Acta*, **89**, 170.
 Marcus, A. & Feeley, J. (1965). *J. biol. Chem.* **240**, 1675.
 Mayer, A. M. & Poljakoff-Mayber, A. (1963). *The Germination of Seeds*, pp. 101-143. Oxford: Pergamon Press Ltd.
 Michniewicz, M. & Kamienska, A. (1965). *Naturwissenschaften*, **52**, 623.
 Morton, R. K. & Raison, J. K. (1964). *Biochem. J.* **91**, 528.
 Nieuwdorp, P. J. & Buys, M. C. (1964). *Acta bot. neerl.* **13**, 559.
 Paleg, L. (1960). *Plant Physiol.* **35**, 293.
 Paleg, L. (1961). *Plant Physiol.* **36**, 829.
 Paleg, L. (1964). In *Regulateurs Naturels de la Croissance Végétale*, p. 303. Ed. by Nitsch, J. P. Paris: Centre Nationale du Recherche Scientifique.
 Paleg, L. (1965). *Annu. Rev. Plant Physiol.* **16**, 291.
 Paleg, L. & Hyde, B. (1964). *Plant Physiol.* **39**, 673.
 Parisi, B. & Ciferri, O. (1966). *Biochemistry*, **5**, 1638.
 Ram Chandra, G. & Varner, J. E. (1965). *Biochim. biophys. Acta*, **108**, 583.
 Rowsell, E. V. & Goad, L. J. (1964). *Biochem. J.* **90**, 12P.
 Sprey, B. & Lichtenthaler, H. K. (1966). *Z. Naturf.* **21b**, 697.
 Stoner, C. D., Hodges, T. K. & Hanson, J. B. (1964). *Nature, Lond.* **203**, 258.
 Tanner, W. & Beevers, H. (1965). *Z. Pflanzenphysiol.* **53**, 72.
 Threlfall, D. R. & Griffiths, W. T. (1967). In *Biochemistry of Chloroplasts*, vol. 2, p. 255. Ed. by Goodwin, T. W. London and New York: Academic Press Inc.
 Toole, E. H., Hendricks, S. B., Borthwick, H. A. & Toole, V. K. (1956). *Annu. Rev. Plant Physiol.* **7**, 299.
 Treharne, K. J., Mercer, E. I. & Goodwin, T. W. (1966). *Biochem. J.* **99**, 239.
 Varner, J. E. (1964). *Plant Physiol.* **39**, 413.
 Varner, J. E. & Ram Chandra, G. (1964). *Proc. nat. Acad. Sci., Wash.* **52**, 100.
 Wareing, P. F. (1963). In *Vistas in Botany*, vol. 3, p. 195. Ed. by Turrill, W. B. Oxford: Pergamon Press Ltd.
 Yomo, H. (1960). *J. Ferment. Ass. Japan*, **13**, 600; cited in *Chem. Abstr.* (1961), **55**, 26145.