non-dormant buds (Tuan & Bonner, 1964). This observation has been interpreted to imply that there is a massive repression of the genome in dormant tissue. If this hypothesis is valid, then the role of hormones in regulating dormancy may involve the repression and de-repression of DNA. Thus it is possible that the occurrence of high concentrations of abscisic acid and low concentrations of gibberellins during the later stages of seed development may lead to repression of the genome, and that once the dormant state has been induced the maintenance of high concentrations of abscisic acid may not be necessary. Some evidence has, indeed, been obtained that abscisic acid affects the activity of chromatin isolated from radish hypocotyls (Pearson & Wareing, 1969). On the other hand, Ihle & Dure (1970) have suggested that in developing cotton seeds abscisic acid acts by preventing the premature translation of long-lived RNA.

By contrast, the removal of dormancy by chilling may involve de-repression of the genome by an increase in endogenous gibberellin concentrations. Thus it has been shown that the DNA-template availability of chromatin from hazel embryos is increased by pretreatment of the seeds with compound GA₃ (Jarvis *et al.* 1968).

Application of exogenous abscisic acid to dark-grown maize shoots and etiolated wheat leaves leads to decreased gibberellin concentrations (Wareing, Good & Manuel, 1969; Loveys & Wareing, 1971) and may indicate that abscisic acid can inhibit gibberellin biosynthesis (Ross & Bradbeer, 1971).

The controlling role of phytochrome in light-sensitive seeds is well known, but its mode of action remains unknown. Exposure to red light has been shown to lead to increased gibberellin concentrations in several types of tissue, but so far it has not been possible to demonstrate similar hormonal changes in light-requiring seeds.

- Frankland, B. & Wareing, P. F. (1966). *J. exp. Bot.* **17**, 596.
 Ihle, J. N. & Dure, L. (1970). *Biochem. biophys. Res. Commun.* **38**, 995.
 Jarvis, B. C., Frankland, B. & Cherry, J. H. (1968). *Planta*, **83**, 257.
 Kentzer, T. (1966). *Acta Soc. Bot. Pol.* **35**, 477.
 Khan, A. A. (1968). *Pl. Physiol., Lancaster*, **43**, 1463.
 Khan, A. A., Heit, C. E. & Lippold, P. C. (1968). *Biochem. biophys. Res. Commun.* **33**, 391.
 Loveys, B. & Wareing, P. F. (1971). *Planta* (in the Press).
 Pearson, J. A. & Wareing, P. F. (1969). *Nature, Lond.*, **221**, 672.
 Ross, J. D. & Bradbeer, J. W. (1968). *Nature, Lond.*, **220**, 85.
 Ross, J. D. & Bradbeer, J. W. (1971). *Planta* (in the Press).
 Rudnicki, R. (1969). *Planta*, **35**, 297.
 Sankhla, N. & Sankhla, D. (1968). *Z. PflPhysiol.* **58**, 402.
 Sinska, I. & Lewak, S. (1970). *Physiol. Vég.* **8**, 661.

- Sondheimer, E., Tzou, D. S. & Galson, E. C. (1968). *Pl. Physiol., Lancaster*, **43**, 1443.
 Tuan, D. Y. H. & Bonner, J. (1964). *Pl. Physiol., Lancaster*, **39**, 768.
 Wareing, P. F., Good, J. & Manuel, J. (1969). *Proc. 6th int. Cong. Plant Growth Substances, Ottawa*, p. 1561. Ottawa: Runge Press.
 Wareing, P. F. & Saunders, P. F. (1971). *A. Rev. Pl. Physiol.* **22**, 261.
 Wood, A. & Bradbeer, J. W. (1967). *New Phytol.* **66**, 17.

Gibberellins and Carbohydrate Mobilization in Germinating Barley Grains

By D. E. BRIGGS, V. J. CLUTTERBUCK and G. MURPHY. (*Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, U.K.*)

Of all the barley enzymes that degrade starch, α -amylase, which is believed to initiate the breakdown of the starch grains, has been most studied. The enzymes that break down the water-soluble gums and the cell walls of the endosperm and the remaining starch-degrading enzymes will not be considered, even though some at least behave very differently from α -amylase during germination.

During germination the breakdown of the starch and cell walls in the endosperm (modification) begins adjacent to the scutellum and progressively extends towards the apex of the grain, advancing most rapidly adjacent to the aleurone layer (Brown & Morris, 1890). Isolated embryos released α -amylase from the scutellum, but grain that is de-embryonated dry does not. Later the aleurone layer was shown to produce enzymes, but the quantities produced by separated embryos and endosperms never approached the amounts found in whole grains (Brown & Escombe, 1898). Hayashi (1940) added gibberellins to whole grain and demonstrated that malting was accelerated. Yomo (1958) showed that the embryo produced a soluble factor that induced the formation of α -amylase in the endosperm, and the factor had the properties of a gibberellin. Gibberellic acid (compound GA₃) could replace the embryo factor (Yomo, 1960*a,b,c,d*), and its action on separated endosperms was also recognized by Paleg (1960*a,b*). It seems that barley embryos produce compounds GA₃ and GA₁ (Radley, 1968), but as yet rigorous chemical characterization is lacking.

The site of production of α -amylase in the endosperm is the aleurone layer. The isolated tissue requires no external supply of sugars or amino acids (Briggs, 1964). Enzyme production is inhibited by many agents, including anoxia, 2,4-dinitrophenol, fluoroacetate, *p*-fluorophenylalanine, ethionine, 6-methylpurine, cycloheximide, actinomycin D and abscisic acid (Briggs, 1963; Yomo, 1960*a,b,c,d*;

Varner & Johri, 1968). The results of these inhibition studies and the fact that ^{14}C -labelled amino acids and oxygen of H_2^{18}O are incorporated into α -amylase indicate that it is synthesized *de novo* (Filner & Varner, 1967). Washing experiments show that compound GA_3 needs to be continuously present (Chrispeels & Varner, 1967). In response to compound GA_3 respiration is increased (Briggs, 1964) and RNA turnover is enhanced, and possibly a new species of RNA is formed (Ram Chandra & Duynstee, 1968).

α -Amylase is also formed and released by the embryonic scutellum, and up to 15% of the enzyme found in the grain may come from this source (Briggs, 1964). The contribution to enzyme production previously attributed to the scutellum itself, rather than to the adhering aleurone tissue (MacLeod & Palmer, 1966), was too small, owing to the failure to include suitable nutrients in the culture medium (V. J. Clutterbuck & D. E. Briggs, unpublished work). α -Amylase production by the scutellum differs from that occurring in the aleurone layer. Thus the embryo produces gibberellin(s), so that the scutellum does not need an exogenous source. Further, in contrast with the situation with the aleurone, scutellar production of α -amylase is strongly dependent on the nutrients supplied, either in the presence or the absence of added compound GA_3 . Enzyme production is enhanced by a mixture of amino acids, and in a medium containing amino acids the addition of sugars is inhibitory (Briggs, 1964). Further, sucrose appears to prevent gibberellin production by separated embryos (D. E. Briggs, unpublished work).

The contents of the aleurone cells alter markedly during germination or in response to added gibberellins (Van der Eb & Nieuwdorp, 1967; Paleg & Hyde, 1964; Jones, 1969*a,b,c*). Mitochondria increase and develop cristae; aleurone grains and their inclusions swell, dissolve and vacuolate; the spherosomes (lipid bodies) enlarge, and lose their contents; endoplasmic reticulum increases in amount and becomes 'rough' with ribosomes; material is formed within the endoplasmic reticulum and released into the space between the plasma-lemma and the cell wall. The aleurone cell walls begin to be degraded. Eventually the cell contents become highly vacuolated. It has been suggested that the gibberellins de-repress nuclear gene(s), allowing enhanced RNA metabolism leading to the synthesis of enzyme protein at the expense of the cell reserves.

There is substantial disagreement on the specificity of the aleurone response. In our experience only gibberellins, and less efficiently helminthosporol, helminthosporic acid and residues from some organic solvents, act as initiators (Briggs, 1966*a,b*). However, activity has been reported with kinetin,

ATP, cyclic AMP (adenosine 3':5'-cyclic monophosphate), glutamine, mevalonic acid and kaurene. Discrepancies may be caused by the use of different varieties of barley, or barley that is immature or pregerminated. Contamination by micro-organisms is a frequent problem, and often assays are used that are not specific for α -amylase (Briggs, 1967).

To characterize the endogenous enzyme initiator further we bioassayed the gibberellin content and measured α -amylase in 'malting' decorticated barley (Groat & Briggs, 1969). Under malting conditions enzyme breakdown does not occur, unlike α -amylase in grain germinating with water (Briggs, 1968*a,b,c*). The rate of α -amylase formation rose and fell about 20 h after the rise and fall in the concentration of endogenous gibberellins. Other samples were de-embryonated at different times, to remove the source of 'initiator', and the quantities of α -amylase formed during further periods of incubation, the 'enzyme-forming potentials' of the endosperms, were determined. The quantities of gibberellin and the 'enzyme-forming potentials' of endosperm samples rose and fell together. As the endosperm gained most of its 'enzyme-forming potential' in a few hours it appeared that a single 'instantaneous' dose of compound GA_3 applied to de-embryonated grains might induce a response similar to that of the natural initiator, if that were a gibberellin. In dosed endosperms α -amylase increased with time in a sigmoid pattern, as in whole grains. Different doses of compound GA_3 were applied at the appropriate times to endosperms that had been imbibed with embryos *in situ*. The doses of compound GA_3 (0.3 and 0.6 ng) that induced quantities of α -amylase equal to those found in the endosperms of whole grain were surprisingly similar to the measured amount of gibberellins (0.6 ng of compound GA_3 equivalents) found there. This suggests strongly that the endogenous enzyme-initiating hormone is gibberellin in nature, but inherent experimental uncertainties prevent this from being taken as unequivocal proof.

- Briggs, D. E. (1963). *J. Inst. Brew.* **69**, 13.
 Briggs, D. E. (1964). *J. Inst. Brew.* **70**, 14.
 Briggs, D. E. (1966*a*). *Nature, Lond.*, **210**, 418.
 Briggs, D. E. (1966*b*). *Nature, Lond.*, **210**, 419.
 Briggs, D. E. (1967). *J. Inst. Brew.* **75**, 361.
 Briggs, D. E. (1968*a*). *Phytochemistry*, **7**, 513.
 Briggs, D. E. (1968*b*). *Phytochemistry*, **7**, 531.
 Briggs, D. E. (1968*c*). *Phytochemistry*, **7**, 539.
 Brown, H. T. & Escombe, F. (1898). *Proc. R. Soc.* **63**, 3.
 Brown, H. T. & Morris, G. H. (1890). *J. chem. Soc.* **57**, 458.
 Chrispeels, M. J. & Varner, J. E. (1967). *Pl. Physiol., Lancaster*, **42**, 1008.
 Filner, P. F. & Varner, J. E. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 1520.
 Groat, J. I. & Briggs, D. E. (1969). *Phytochemistry*, **8**, 1615.

- Hayashi, T. (1940). *J. agric. Chem. Soc. Japan*, **16**, 531.
 Jones, R. L. (1969a). *Planta*, **85**, 359.
 Jones, R. L. (1969b). *Planta*, **87**, 119.
 Jones, R. L. (1969c). *Planta*, **88**, 73.
 MacLeod, A. M. & Palmer, G. H. (1966). *J. Inst. Brew.* **72**, 580.
 Paleg, L. G. (1960a). *Pl. Physiol., Lancaster*, **35**, 293.
 Paleg, L. G. (1960b). *Pl. Physiol. Lancaster*, **35**, 902.
 Paleg, L. G. & Hyde, B. (1964). *Pl. Physiol., Lancaster*, **39**, 673.
 Radley, J. M. (1968). *Monog. Society of Chemical Industry* no. 31: *Plant Growth Regulators*, p. 53.
 Ram Chandra, G. & Duynstee, E. E. (1968). In *Biochemistry and Physiology of Plant Growth Substances*, p. 723. Ed. by Wightman, F. & Setterfield, G. Ottawa: Runge Press Ltd.
 Van der Eb, A. A. & Nieuwdorp, P. J. (1967). *Acta bot. neerl.* **15**, 690.
 Varner, J. E. & Johri, M. M. (1968). In *Biochemistry and Physiology of Plant Growth Substances*, p. 793. Ed. by Wightman, F. & Setterfield, G. Ottawa: Runge Press Ltd.
 Yomo, H. (1958). *J. Ferment. Ass. Japan*, **16**, 444.
 Yomo, H. (1960a). *J. Ferment. Ass. Japan*, **18**, 494.
 Yomo, H. (1960b). *J. Ferment. Ass. Japan*, **18**, 500.
 Yomo, H. (1960c). *J. Ferment. Ass. Japan*, **18**, 600.
 Yomo, H. (1960d). *J. Ferment. Ass. Japan*, **18**, 603.

Triglyceride Mobilization in the Germinating Wheat Grain

By D. L. LAIDMAN and R. J. A. TAVENER. (*Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caerns., U.K.*)

The central role of gibberellins in initiating the mobilization of carbohydrate reserves in the germinating barley grain has been unambiguously demonstrated (see Briggs, 1971), and investigations with wheat have indicated that in this cereal the situation is similar (Eastwood, Tavener & Laidman, 1969a). Besides the major carbohydrate reserves of the starchy endosperm, however, the wheat grain also contains appreciable reserves of triglycerides in all of its tissues. Chemical analysis of the wheat bran reveals that, in this part of the grain at least, triglycerides are a major reserve material (Bechtel, Geddes & Gilles, 1964). Electron micrographs of the aleurone cells from the wheat bran (A. J. Colborne, G. Morris & D. L. Laidman, unpublished work) show large numbers of membrane-bound fat-bodies arranged peripherally around the aleurone bodies within the cytoplasm. During germination these triglycerides, and those of the starchy endosperm, are metabolized, and it is important to know whether or not this metabolism is initiated by the same hormonal system as that for the mobilization of the carbohydrate reserves. In this laboratory we have used incubated excised tissues from the wheat grain to investigate the situation (Tavener &

Laidman, 1968, 1969; Eastwood, Tavener & Laidman, 1969b).

In the quiescent grain lipase activity was not detectable in either the starchy endosperm or the bran tissues. Within 12h of the commencement of germination, however, lipase activity appeared in the starchy endosperm and soon afterwards in the bran. The activity in each tissue rose to a maximum by the sixth day of germination. Concomitant with the appearance of lipase activity in these tissues, triglyceride metabolism commenced and the triglyceride reserves were progressively mobilized throughout the experimental period of 8 days.

When endosperm halves (de-embryonated grain) were incubated alone no lipase activity appeared in the starchy endosperm and no triglyceride metabolism occurred. In the bran, however, an unexpected pattern of triglyceride metabolism occurred. In this tissue no lipase activity was detected, but some 22% of the tissue reserves of triglycerides were metabolized within the first day though no metabolism occurred thereafter. The nature of this non-lipase triglyceride metabolism has not yet been elucidated. Extracts of quiescent embryos and diffusates from germinated embryos were effective in inducing lipase activities in both the starchy endosperm and the bran tissues of the incubated endosperm halves. The same extracts and diffusates initiated triglyceride metabolism in the starchy endosperm and extended the metabolism in the bran, so that the patterns of metabolism in each tissue now resembled those occurring in the tissues of the whole germinating grain. These results demonstrate the major role played by the embryo in initiating and controlling the mobilization of triglyceride reserves in the endosperm tissues.

The effects of embryo extracts and diffusates on the incubating endosperm halves could not be simulated by any of the authentic phytohormones, either singly or in combination. On the other hand, a number of nitrogen-containing compounds at substrate concentrations were very effective in inducing lipase activity in the starchy endosperm of the endosperm halves. Most notable among these compounds were glutamine and hydroxylamine. The induced lipase activity was accompanied by the metabolism of triglyceride in that tissue, suggesting a cause-and-effect relationship between the two.

In contrast with the situation in the starchy endosperm, neither hydroxylamine nor glutamine induced lipase activity in the bran of the endosperm halves. Lipase activity in this tissue could, however, be induced by a mixture of glutamine at substrate concentration and indol-3-ylacetic acid at hormonal concentration. The same components together also induced a pattern of triglyceride metabolism similar to that occurring in the bran of germinating grain.