PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 515th Meeting of the Society was held at the University College of North Wales, Bangor, Caernarvonshire, on Thursday and Friday, 20 and 21 May 1971, when the following papers were presented:

COLLOQUIUM ON 'BIOCHEMISTRY OF GERMINATION'

The Control of Seed Dormancy

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The term 'dormancy' is applied where the growth of an organ or other structure, such as an embryo or bud, is temporarily arrested. In many instances a seed may fail to germinate even though conditions of temperature, moisture and aeration appear favourable and indeed allow germination and growth at other phases of the life-cycle. In such cases we speak of 'innate dormancy' or 'rest'. There are several forms of innate dormancy in seeds, and these may be distinguished by the conditions necessary to overcome it; thus certain seeds, including those of many woody plants, require a period of chilling at 0–5°C for several weeks in the imbibed condition. Many seeds are dormant only when the intact coats are present, and if these are removed, cut or perforated the embryos rapidly germinate. Evidently, in such cases, dormancy is due to some inhibitory effect of the coat, and most evidence suggests that this effect is due to the limitation of oxygen uptake, but there is also evidence that in some species the coat may mechanically restrain growth of the embryo. Although, in most cases, seed dormancy is due to the coats, in a number of species the embryo itself is dormant and will not readily germinate even if the coats are removed. However, the boundary between 'coat-imposed' and embryo dormancy is not a sharp one. It is clear that where the embryo itself is dormant there must be some block to metabolism and growth, but the nature of this block remains obscure. Earlier comparative studies of respiratory metabolism in dormant and non-dormant seeds revealed no clear-cut differences. Most recent work has been concerned with nucleic acid metabolism and the role of growth hormones (for a review see Wareing & Saunders, 1971).

Gibberellic acid (compound GA₃) will stimulate the germination of many types of dormant seed, and gibberellins GA₄ and GA₇ have been found to be even more effective in several cases, whereas in certain species of seed cytokinins are active. In

lettuce seed it is found that the primary effect of compound GA₃ is to stimulate root growth, whereas cytokinins, such as kinetin, stimulate growth of the cotyledons. On the other hand, abscisic acid inhibits the germination of many non-dormant seeds. In lettuce (Sankhla & Sankhla, 1968) and barley (Khan, 1968) the effects of abscisic acid can be overcome by kinetin, but not by compound GA₃.

In several species it has been found that chilling leads to an increase in the concentrations of endogenous gibberellins. In some seeds there appears to be an increase in gibberellin concentrations during the chilling period itself (Frankland & Wareing, 1966; Kentzer, 1966; Sinska & Lewak, 1970), but Ross & Bradbeer (1968) have reported that in hazel seed most of the increase in gibberellin concentrations occurs, not during the chilling period, but when the seeds are transferred to warm conditions at the end of the chilling period. Thus, according to these workers, chilling leads to the removal of some block to gibberellin biosynthesis and the observed increase in gibberellin concentrations after chilling causes of the breaking of dormancy.

Abscisic acid has been shown to be present in several species of seed with a chilling requirement and its concentration appears to decline during chilling in *Fraxinus americana* (Sondheimer, Tzou & Galson, 1968) and apple (Rudnicki, 1969). These studies have led to the concept that in seeds having a chilling requirement dormancy may be regulated by an interaction between growth-promoting hormones, such as gibberellin and cytokinins, and inhibitors, such as abscisic acid. However, it seems doubtful whether the state of dormancy depends directly on the relative concentrations of promoters and inhibitors existing in the tissues at any given time. Several studies have indicated that nucleic acid synthesis and protein synthesis are diminished in dormant seeds (Jarvis, Frankland & Cherry, 1968; Khan, Heit & Lippold, 1968; Wood & Bradbeer, 1967), and it has been shown that the capacity for DNA-dependent RNA synthesis of 'chromatin' prepared from dormant potato buds is significantly lower than that of 'chromatin' from

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.

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Gibberellins and Carbohydrate Mobilization in Germinating Barley Grains

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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*;