incubated wheat bran, the induction was not inhibited by actinomycin D, chloramphenicol, puromycin or cycloheximide. Further, when homogenates of the bran from ungerminated grain were incubated alone for several hours at 30°, their phytase activities steadily increased. This situation is similar to that described for β -amylase by Rowsell & Goad (1964).

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The Effects of Plant Hormones on the Efflux of Inorganic Ions from Wheat Aleurone Tissue

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During germination the macro-nutrient elements potassium, phosphorus, magnesium and calcium stored in the aleurone tissue of the quiescent grain are translocated to the developing seedling. Undoubtedly, the permeability of the membranes of the aleurone cells plays an important role in controlling this process. Some permeability characteristics of these membranes in the aleurone cells of ungerminated grain were reported by Eastwood & Laidman (1968).

Bran isolated from ungerminated, quiescent grains rapidly released its entire reserves of macro-nutrient elements into aqueous incubation media. In contrast, bran from grains germinated for 48 hr. released inorganic ions at rates less than half of the values with ungerminated grain. Further, about 50% of the aleurone content of potassium, phosphorus, magnesium and calcium was now unavailable for efflux from the tissues. After further periods of germination, efflux rates from the isolated, incubated brans increased again to a maximum on the fifth day of germination.

De-embryoed grains (endosperm halves) were allowed to imbibe water for 48 hr. under germination conditions. The bran of these endosperm halves was found to have undergone a decrease in ion-efflux capacity identical to that occurring in the intact germinating grain. However, when the bran from ungerminated grain was incubated alone under conditions of minimal mineral loss, no reduction in its ion-efflux capacity occurred. Applications of

starchy endosperm extracts with known cytokinin activity (Tavener & Laidman, 1968) to the bran induced a decrease in efflux rates almost as great as that induced by the changes *in vivo*. Authentic cytokinins at 0.05 μ M were also active in inducing the reduction in efflux rate. The other plant hormones were completely inactive. The cytokinin action was strongly suppressed by inhibitors of DNA transcription and protein synthesis. These observations suggest that a cytokinin from the endosperm of wheat induces changes in the permeability of membranes in the aleurone cells early during germination. Protein synthesis appears to be involved in the induction process.

When cytokinin-treated bran was subsequently incubated for up to 3 days with 10 μ M-gibberellic acid, an increase in ion-efflux rates resulted. Cycloheximide and 1.0 μ M-abscissic acid completely inhibited the gibberellic acid action, although a number of other inhibitors of DNA transcription and protein synthesis were without effect.

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The Dehydrogenation Sequence leading to Coloured Carotenoids in Photosynthetic Bacteria

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The sequence of reactions in which phytoene undergoes a stepwise dehydrogenation through phytofluene, ζ -carotene and neurosporene to lycopene was first suggested by Porter & Lincoln (1950) and is now established as the basic pathway of carotenoid formation in a wide variety of organisms. The structures of the intermediates were established by unambiguous synthesis (Davis, Jackman, Siddons & Weedon, 1961, 1966); the symmetrical structure of ζ -carotene (7,8,7',8'-tetrahydrolycopene) implies that the dehydrogenation steps occur alternately to the left and to the right of the central chromophore.

A conjugated heptaene, assumed to be ζ -carotene, was isolated from diphenylamine-inhibited cultures of *Rhodospirillum rubrum* by Goodwin & Osman (1954). Jensen, Cohen-Bazire, Nakayama & Stanier (1958) showed that this compound, together with other precursors, disappeared when the inhibition was removed and they deduced that the Porter &

Lincoln sequence operates in photosynthetic bacteria.

ζ -Carotene from tomato fruit or carrot root has absorption maxima in light petroleum at 378, 400 and 425nm., but the corresponding values for the *R. rubrum* heptaene are 376, 396 and 418nm., suggesting that the two heptaenes are different.

Chromatographic studies have eliminated the possibility of one of the heptaenes either having a different number of carbon atoms in the molecule or containing two β -ionone rings, and isomerization studies show that both have the all-*trans* configuration. The possibility that the *R. rubrum* heptaene has its chromophore located unsymmetrically in the molecule is strengthened by the close agreement between its absorption spectrum and that reported for a synthetic unsymmetrical isomer of ζ -carotene (Davis *et al.* 1966).

Mass spectrometry confirms that both the natural heptaenes are $C_{40}H_{60}$ and that the ζ -carotene from carrot root is symmetrical (7,8,7',8'-tetrahydrolycopene), showing fission at the C-3-C-4 and C-7-C-8 bonds. The heptaene from *R. rubrum*, however, shows additional fission at the C-11-C-12 bond, confirming that it is the unsymmetrical isomer (7,8,11,12-tetrahydrolycopene).

Thus, in these bacteria, there is a novel dehydrogenation sequence, involving two consecutive dehydrogenations at one side of the central chromophore followed by two at the other, so that phytofluene, 7,8,11,12-tetrahydrolycopene and neurosporene are the intermediates in the formation of lycopene from phytoene.

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Trisporic Acid Biosynthesis and Carotenogenesis in *Blakesleea trispora*

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Trisporic acids form only in mixed cultures of (+) and (-) strains of *B. trispora* and related Phyco-

mycetes, where they mediate increased carotenoid synthesis by an inducer-like action on the (-) strain. They are oxidized unsaturated derivatives of 1,1,3-trimethyl-2-(3-methyloctyl)cyclohexane, and biogenetically they could be classed as elaborated sesquiterpenes, or as degraded diterpenes, or as degraded carotenoids. However, synthesis of the trimethylcyclohexyl system has so far only been demonstrated to occur at the C_{40} level, i.e. in cyclic carotenoids.

With pre-mated cultures on malt-extract media, to optimize trisporic acid yields, added $[2-^{14}C]$ -mevalonate was incorporated into both β -carotene and trisporic acids. Degradation of the latter by reductive ozonolysis revealed four equally labelled C_5 units in the C_{18} molecule, which must therefore be derived either from a diterpenoid or from carotenoid precursor. Plausible mechanisms can be proposed for the formation of trisporic acids from a dicyclic diterpene or from β -carotene but currently we prefer the latter suggestion for the following reasons.

Labelled β -carotene is a better precursor of trisporic acids than is $[2-^{14}C]$ mevalonate. Moreover, when the conversion of phytoene into β -carotene is blocked with diphenylamine the trisporic acids are not formed. This suggests that the trisporic acids are formed from the carotenoids whose synthesis they promote. Similar biogenetic origins seem equally probable for related trimethylcyclohexane derivatives in other organisms, such as β -ionone, the abscisins, and various insect products.

New Pathways of Methoxy Carotenoid Formation in *Rhodospirillum rubrum*

By B. H. DAVIES and E. ANN HOLMES. (*Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth*)

In *Rhodospirillum rubrum*, spirilloxanthin is normally formed from lycopene by a series of consecutive reactions that involves the hydration of an isopropylidene end group to form a tertiary alcohol, the introduction of a double bond in conjugation with the polyene chain at the hydroxylated end, and the methylation of the tertiary hydroxyl group. These reactions convert lycopene first into anhydorrhodovibrin and then, by occurring at the unchanged end of the molecule, convert anhydorrhodovibrin into spirilloxanthin (Jensen, 1961; Jensen, Cohen-Bazire & Stanier, 1961).

In the present work on the pigments of diphenylamine-inhibited cultures of *R. rubrum*, two carotenoids that were described by Goodwin & Osman (1954) and designated P450 and P412 by Jensen,