Analytical and other results showed that the 1.2–1.8M-NaCl fractions were predominantly composed of chondroitin 4-sulphate and chondroitin 6-sulphate. The total uronic acid content in these fractions (expressed as a percentage of dry weight of cartilage) was fairly constant (3.4–4.5%) through childhood and adult life, but fell (to 2–3%) after the fifth decade. Changes were noted, however, in early life in the ease with which uronic acid-containing material was eluted from the Dowex 1 column. At birth up to 25% of the total uronic acid was eluted in the 1.2M fraction but during the first seven years this proportion fell to only 4%, reflecting an increase in the sulphation of the polymers. The amount of chondroitin 6-sulphate remained at about 55% of the total uronic acid containing material throughout life.

The predominant component of the fraction eluted by 2.5M-NaCl containing 8M-urea was an oversulphated keratan sulphate. The hexose content of this fraction (with D-galactose as standard), expressed as a percentage of dry weight of cartilage, ranged from negligible amounts in neonatal cartilage to 0.75% at 2 years and 1.6% at 17 years. Further increases up to a plateau of 2–2.5% during the fifth decade were noted but contents subsequently fell to about 1.5% after 65 years. Small quantities of keratan sulphate were observed in the early fractions from adult samples with a hexosamine/sulphate ratio closer to unity.

No consistent differences were observed in cartilages from different regions of the same bronchial tree.

The implications of these results will be discussed.

R. M. M. is grateful to the Medical Research Council for a Junior Research Fellowship.

Saltzman, H. A., Seizer, H. O. & Green, J. (1963). *J. Lab. clin. Med.* **62**, 78.

Scott, J. E. (1960). In *Methods of Biochemical Analysis*, vol. 8, p. 145. Ed. by Glick, D. New York: Interscience Publishers Inc.

Wusteman, F. S., Dodgson, K. S., Johnson, D. B. & Bell, D. (1968). *Life Sci.* **7**, 1281.

Conversion of Lycopene into β -Carotene by Chloroplasts of Higher Plants

By H. M. HILL and L. J. ROGERS. (*Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth*)

In the normal red tomato the major carotenoid is lycopene (87%) with smaller amounts of β -carotene (7%) also present (Ferrari & Benson, 1961). Some doubt still exists whether the β -carotene present is formed from lycopene, though Decker & Uehleke

(1961) claimed that chloroplasts isolated from carrot leaves carry out the conversion, an observation supported by Wells, Schelble & Porter (1964) from studies in which γ -, δ - and β -carotenes were identified after administration of [^{14}C]lycopene to isolated tomato plastids or spinach chloroplasts. However, in normal red fruit (Goodwin & Jamikorn, 1952), high temperature inhibits the formation of lycopene but not of β -carotene, suggesting that β -carotene is not synthesized from lycopene. There is evidence to support the suggestion (Tomes, 1963) that in tomato fruit two pathways exist for β -carotene synthesis: one, temperature-sensitive, involving lycopene, and the other, temperature-insensitive, in which β -carotene is formed by some alternative pathway. Because of the availability of [^{14}C]lycopene, the product of another investigation, we report here studies on the conversion of lycopene into β -carotene by plastid preparations from tomato fruit and French bean (*Phaseolus vulgaris*) seedlings.

Radioactive lycopene was prepared by administration of 50 μC of [^{14}C]mevalonic acid to whole half-ripened tomato fruit (120g.), which were then allowed to ripen for 4 days at 25° under artificial light. The red fruit were homogenized; lycopene was extracted by our standard procedures and recrystallized to constant specific radioactivity. The identification and purity of the lycopene were authenticated by thin-layer chromatographic behaviour and spectrophotometric studies.

[^{14}C]Lycopene (0.2mg.; 30 000 d.p.m./mg.) suspended in 0.5ml. of phosphate buffer, pH 7.2, containing 0.6% of Tween 80, was added to chloroplasts isolated by sucrose-density-gradient centrifugation (Leech, 1964) from French-bean leaves. After incubation at 25° in dim light for 15–24 hr., the β -carotene was extracted from the incubation mixtures, purified by standard procedures (see, e.g., Rogers, Shah & Goodwin, 1968), and recrystallized in the presence of unlabelled β -carotene after authentication by thin-layer chromatography and determination of spectra in three solvent systems.

It was found that incubation of [^{14}C]lycopene with disrupted chloroplasts resulted in incorporation of radioactivity into β -carotene. The specific radioactivity of the β -carotene isolated was in the range 12 000–13 000 d.p.m./mg. (representing incorporation of about 10% of the total radioactivity). On a specific radioactivity basis, similar results were obtained with plastids isolated from half-ripened tomato fruit, though total incorporation of ^{14}C in this case was only about 3%.

The incorporation of radioactivity into β -carotene appears to represent a small but real conversion from [^{14}C]lycopene, thus supporting the view that in plastids lycopene may be an intermediate in the biosynthesis of β -carotene.

- Decker, K. & Uehleke, H. (1961). *Hoppe-Seyl. Z.* **323**, 61.
 Ferrari, R. A. & Benson, A. A. (1961). *Arch. Biochem. Biophys.* **93**, 185.
 Goodwin, T. W. & Jamikorn, M. (1952). *Nature, Lond.*, **170**, 104.
 Leech, R. M. (1964). *Biochim. biophys. Acta*, **79**, 637.
 Rogers, L. J., Shah, S. P. J. & Goodwin, T. W. (1968). *Photosynthetica*, **2**, 184.
 Tomes, M. L. (1963). *Bot. Gaz.* **124**, 180.
 Wells, L. W., Schelble, W. J. & Porter, J. W. (1964). *Fed. Proc.* **23**, 426.

ing seedlings although in the ungerminated seed it is present only in very small quantities in the free form (Schultze, 1898, cited by Chibnall, 1939).

We are indebted to the Science Research Council for the award of a Research Studentship to R. J. A. T.

Chibnall, A. C. (1939). *Protein Metabolism in the Plant*, p. 87. New Haven, Conn.: Yale University Press.

Tavener, R. J. A. & Laidman, D. L. (1968). *Biochem. J.* **109**, 9P.

Induction of Lipase Activity in the Starchy Endosperm of Germinating Wheat Grains

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

Early during the germination of wheat grains, lipase activity appears in the starchy endosperm, and coincidentally triglyceride metabolism in that tissue commences. When de-embryoed grains (endosperm halves) were incubated alone, no lipase activity appeared and no triglyceride breakdown took place in the starchy endosperm (Tavener & Laidman, 1968). None of the authenticated plant hormones, gibberellic acid, indolylacetic acid or kinetin, or any combination of them, was able to induce lipase activity in the endosperm halves.

This communication describes experiments that have elucidated the probable induction mechanism for this lipase activity. Aqueous extracts from embryos or diffusates from incubated embryos were able to induce lipase activity in the starchy endosperm. A factor emanating from the embryo axis or scutellum is therefore required for the induction. In contrast with the plant hormones, hydroxylamine or glutamine at 1.0 mM concentrations were very effective in inducing the enzyme activity. Several purine and pyrimidine nucleotides were effective to a lesser degree. Nitrate, nitrite and ammonium salts, and an amino acid mixture (casein hydrolysate) showed low inducing capacities. The induction of lipase activity in the endosperm by these compounds did not require the presence of aleurone tissue.

Actinomycin D, puromycin, cycloheximide and azaserine all strongly inhibited the induction by hydroxylamine. Mitomycin, hydroxyurea and chloroquine did not inhibit the process.

These data are consistent with the thesis that the induction of lipase activity in the wheat endosperm involves synthesis of RNA and protein, and that a suitable nitrogen source is the only requisite for the RNA synthesis to proceed.

It is pertinent to recall that glutamine accumulates in considerable quantities in several germinat-

Induction of Lipase and Phytase Activities in the Aleurone Tissue of Germinating Wheat Grains

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

The appearance of lipase activity and the increase in phytase activity in the aleurone tissue of germinating wheat grains is dependent on the presence of the embryo, but the authenticated plant hormones are unable to substitute for the embryo in this function (Tavener & Laidman, 1968; Eastwood & Laidman, 1968).

Although hydroxylamine or glutamine were effective in inducing lipase activity in the endosperm, they were unable to do so in the aleurone tissue of the grain. However, 1.0 mM-hydroxylamine + 10 μ M-indolylacetic acid or 1.0 mM-glutamine + 10 μ M-indolylacetic acid were able to induce lipase activity in the aleurone cells, and triglyceride metabolism then continued beyond that induced by cytokinins (Tavener & Laidman, 1968). Thus, in the aleurone cell there appear to be two triglyceride pools whose metabolisms are initiated by different hormonal systems during germination.

The induction of lipase activity by glutamine + indolylacetic acid was suppressed by inhibitors of RNA and protein synthesis and by azaserine, indicating the involvement of syntheses of RNA and protein. The efficacy of glutamine + indolylacetic acid to induce lipase activity in aleurone tissue was dependent on the prior hormonal action of cytokinin in that tissue. This dependence is analogous to the cytokinin-dependent induction of α -amylase activity by gibberellic acid (Eastwood, Tavener & Laidman, 1969).

In contrast, the increase in phytase activity in the aleurone tissue during germination appears to be the result of the activation of an existing, inactive form. Although 1.0 mM-hydroxylamine, 1.0 mM-glutamine, 1.0 mM-hydroxylamine + 1.0 μ M-indolylacetic acid, 1.0 mM-glutamine + 1.0 μ M-indolylacetic acid, or several purine and pyrimidine nucleotides, induced large increases in phytase activity in