

of flowering, and it remains to be seen if it will cause flower formation in an obligate long-day plant.

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J. LANGRIDGE

Division of Plant Industry,
Commonwealth Scientific and
Industrial Research Organization,
Canberra.
March 19.

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Metabolism of Acetate in *Hevea brasiliensis*

THERE is now considerable evidence to show that the synthesis of isoprenoid compounds in both plants and animals may proceed by the utilization of acetate as the sole primary carbon source^{1,2}, and that the pathways by which the isoprenoid monomer is derived from acetate may have a number of carboxylate intermediates in common, for example, $\beta\beta'$ -dimethyl acrylate and β -hydroxy- β -methyl glutarate^{1,3}. Before taking part in these metabolic reactions acetate first undergoes activation by coupling with coenzyme A, and this form of carboxyl activation is then transmitted through subsequent intermediates arising from acetate. An examination of the latex and tissues of *Hevea brasiliensis* for such activation systems is briefly reported here.

Freeze-dried serum, obtained from fresh latex which had been kept at -20° for 3 weeks, was used in much of the work. Samples of freeze-dried 'yellow-fraction' (the yellow sediment obtained by centrifuging fresh latex at high speed) were also tested. Tissue samples were derived either from young seedlings grown in a greenhouse, or from fresh fragments of the cambial layer of mature trees. These fragments were packed in polythene sachets surrounded by 'dry ice', and transported from Malaya in Dewar flasks. This method has been generally useful in the preservation of cellular enzyme systems.

Hydroxylamine was used as a trapping reagent for activated carboxyl compounds in an assay system similar to that described by Beinert *et al.*⁴. Water-soluble hydroxamates were determined as the red ferric complexes by the method of Kornberg and Pricer⁵. Hydroxamate extracts were prepared for chromatography by adding an equal volume of ethanol to portions (2-5 ml.) of the incubation mixture, and evaporating the filtrate to dryness under reduced pressure. The dry residue was extracted with warm ethanol (0.5 ml.), and spots analysed on Whatman No. 1 paper with a solvent system composed of *n*-butanol/water/acetic acid (63:27:10 by vol.)⁶.

Carboxyl activation was detected in freeze-dried serum one month after its preparation, hydroxamates being produced in incubation mixtures to which no acetate, adenosine triphosphate, or coenzyme A had been added. Addition of adenosine triphosphate and coenzyme A did not stimulate this inherent activity, but when extra acetate (10 μ moles) was supplied more hydroxamate was produced, and this reaction could be further increased by the addition

of adenosine triphosphate (7 μ moles), but not of coenzyme A. Activity was not detected in boiled serum, and rapidly decayed in solutions kept at room temperature. The dried serum powders were also very unstable in storage at 0° C., the activity falling by a half during the second month after preparation.

Chromatography of the hydroxamates resulted in three spots, that with R_F value 0.48 being markedly increased by the addition of acetate to incubation mixtures, and corresponding exactly with authentic acetylhydroxamic acid. The remaining spots with R_F values 0.67 and 0.81 were not positively identified.

Attempts to detect free acid intermediates of isoprenoid synthesis suggested that much of the acetate administered to latex was directed towards respiratory metabolism. General increases were observed for acids of the Krebs cycle, and more particularly for succinic and fumaric acids. However, it should be mentioned that these two acids, under the different chromatographic conditions employed, behaved identically with two possible isoprenoid intermediates, namely, β -hydroxy- β -methyl glutaric and β -methyl glutaconic acids, respectively, so that the possibility of mixtures could not be ruled out.

In most samples of freeze-dried yellow-fraction, carboxyl activation was barely detectable, but in a few cases the activity approached that of a comparable amount of whole serum. This suggests that the yellow-fraction particles may be important loci of carboxyl-activating systems.

The greatest difficulty in the use of cambial tissue for experiment was due to the high concentration of tannins present. No soluble protein could be extracted at pH values lower than 8.5, and carboxyl activation was not detected in homogenates. After grinding with sand in *tris* buffer of pH 9.2 (0.05 M), and washing with fresh buffer, the homogenates were active, and some inactive protein could be extracted by magnesium sulphate solution ($\frac{1}{4}$ sat.). The activity was associated entirely with the bulky cell debris, every attempt to obtain soluble systems being unsuccessful.

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A. D. PATRICK

Dunlop Research Centre,
Erdington,
Birmingham 24.
March 19.

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A Technique for the Measurement of Lead Tolerance in Plants

BRADSHAW reported¹ that root growth was inhibited when plants of *Agrostis tenuis* were grown in soil from the tip of an old Welsh lead mine, while plants originally growing on the tip produced normal roots. This suggested that some plants of *A. tenuis* were inherently more tolerant of the lead and zinc in the soil than others. On similar waste tips in Scotland, *A. tenuis* is replaced by *Festuca ovina* and *Deschampsia flexuosa*, and a method has been worked