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CHEMICAL INDUCTION OF RUBBER BIOSYNTHESIS IN GUAYULE: AN ELECTRON MICROSCOPE STUDY

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Chemical Induction of Rubber Biosynthesis in Guayule: an Electron Microscope Study.

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Abstract

Rubber biosynthesis in guayule (*Parthenium argentatum*) is stimulated by treatment with 2-(3,4-dichlorophenoxy)-triethylamine (DCPTA). Electron microscope examination of treated plants found that DCPTA causes the formation of new rubber globules in cells empty of rubber before treatment. DCPTA also causes changes in the chloroplasts of the leaves which suggest that the chloroplasts of the plant may be the source of rubber precursors, and that DCPTA may act by stimulating precursor production in these chloroplasts.

Introduction

Guayule (Parthenium argentatum) is a shrub of the Compositae family native to drylands of north-central Mexico and adjacent areas of Texas. The plant is currently receiving considerable attention because of its potential as a source of natural rubber (cis-1, 4-polyisoprene) (National Academy of Sciences, 1977). The plant can produce rubber in amounts of over twenty percent of its dry weight (NAS, 1977).

Unlike the majority of rubber producing plants, guayule has no latex vessels. In guayule, rubber is formed in otherwise apparantly ordinary cells of the cortex, pith, and vascular rays of the stems and roots (Artschwager, 1943).

The leaves of the plant contain very little rubber, but rubber formation in the stems and roots is largely dependent on precursors produced in the leaves (Arregiun and Bonner, 1950). The precursor(s) produced in the leaves is/are not sugars or other carbohydrates (Arreguin, 1950), and probably is/are one or more of the chemical species shown in figure 1 (Rogers, Shah, and Goodwin, 1968).

Neither the exact identity nor the specific source of the precursor(s) has been determined. The biological function of rubber in the plant is also unknown.

Yokoyama, Hayman, Hsu, Poling and Bauman (1977), have reported significant increases in rubber yield in guayule after application of 2-(3,4-dichlorophenoxy)-triethylamine (DCPTA). This result suggested that a study of the subcellular effects of DCPTA might produce useful insights into the mechanism of rubber production and the nature and source of the precursor(s).

Methods and Materials

For our experiment, both leaf and stem tissue were sampled before and at intervals after the application of DCPTA. DCPTA was applied by spraying the leaves with an aqueous solution containing 5000 parts per million DCPTA and 200 parts per million of Ortho X-77, a wetting agent. The Guayule plants used in the experiment were 18 month old strain 89 plants grown at the Los Angeles County Arboretum.

Tissues for electron microscope examination were fixed in gluteraldehyde and osmium, dehydrated in ethanol, and embedded in Spurr's low viscosity epoxy. Thin sections were stained with Reynolds lead stain.

The rubber content of tissues was determined by ^{13}C NMR analysis.

Results

As expected, none of the leaf samples taken before DCPTA treatment contained rubber. However, by 13 days after treatment small accumulations of rubber were found in the palisades cells, usually adjacent to chloroplasts (Plate 1A). Rubber accumulation continued in the leaf until a maximum was achieved at about 27 days after treatment. The rubber content of leaves sampled 55 days after the application of DCPTA averaged 2% of the dry weight.

The application of DCPTA also caused changes in the chloroplasts of the leaves. The plastoglobuli of the chloroplasts increased dramatically in size during the first 13 days after treatment, and then returned to normal pretreatment size by 55 days after treatment. Plates 1A and 1B are electron micrographs of palisades cells in leaf samples taken 13 and 55 days after application of DCPTA.

The green stems showed the most marked effect of DCPTA. While none of the cells in green stems sampled before DCPTA treatment contained a latex suspension of rubber globules, almost all of the parenchyma cells in green stems sampled 27 days after treatment contained a latex rich in rubber globules. Plate 1D is an electron micrograph of a typical parenchyma cell 27 days after treatment.

Discussion

The observed extensive *de novo* formation of rubber in stem cells, together with the formation of rubber in the plant's leaves, indicates that most of the cells of guayule are capable of producing rubber if provided sufficient precursors. Thus the rate limiting step in rubber biosynthesis in guayule would appear to be the production of precursors.

The effect of DCPTA on the chloroplasts of the leaves supports our earlier results, which suggest that guayule's chloroplasts may be the source of the rubber precursors utilized in the stems and roots. A chloroplast source for rubber precursors contradicts the well established "compartmentalization hypothesis" regarding terpene biosynthesis, and so we did not originally consider guayule's chloroplasts as a possible source for the rubber precursors.

The compartmentalization hypothesis states that the membrane envelope surrounding the chloroplasts is impermeable to the terpene precursors listed in figure 1 (Rogers et al., 1968). This membrane barrier allows the independent regulation of the biosynthesis of intrachloroplastic terpenoids (carotenoid pigments, plastoquionones, etc.) and extrachloroplastic terpenoids (steriods, ubiquinones, etc.) (Rogers, et al., 1968).

However, autoradiographic experiments with exogenously supplied tritium labeled mevalonate demonstrated extensive labeling of the chloroplasts of guayule (Bauer, 1977), and the administration of high concentrations of mevalonate produced large plastoglobuli-like structures within the chloroplasts (Bauer, 1977). Since the soluble enzymes necessary for converting mevalonate to isopentenyl pyrophosphate exist in the plant's cytoplasm, we are not certain whether mevalonate or one or more of the phosphoralated compounds actually penetrated the chloroplasts' envelopes. But it does seem certain that guayule's chloroplast envelopes are permeable to one or more of these terpene precursors.

As shown in Plate 1A, DCPTA causes rapid growth of plastoglobuli, which are at least partially terpenoid in composition (Lichtenthaler, 1969). This suggests that DCPTA may achieve its effect by stimulating terpene precursor production in Guayule's chloroplasts. It seems reasonable to suppose that the rate of plastoglobuli formation is a positive function of available precursors, and stimulated production of precursors should result in an increased outflow of precursors and an increased availablility of precursors within the chloroplasts.

The history of the discovery of the rubber production enhancing effects of DCPTA provides some additional evidence that the chemical works by stimulating precursor production in guayule's chloroplasts. Dr. Henry Yokoyama, who discovered the effects of DCPTA, was originally working with similar compounds which promoted pigment formation in the rinds of citrus fruits (Coggins, Henning and Yokoyama, 1970). The pigment formation enhanced was of pigments present in chromoplasts of the cells of the fruit rinds. Chromoplasts are plastids very closely related to chloroplasts, and the pigments they contain are terpenoids (Coggins, 1970).

Further evidence of a chloroplast source for rubber precursors in guayule was found in green stem tissue. Most of the cells in the green stems sampled before application of DCPTA were empty of rubber, and none of the cells contained a latex suspension of rubber globules. However, a few cells were found which were almost packed with rubber (Plate 1C). Besides rubber, the cell in Plate 1C contains what appear to be chromoplasts or chloroplasts in transition to chromoplasts. There is experimental evidence that the membrane envelope surrounding chloroplasts becomes more permeable to terpene precursors during the transition of chloroplasts to chromoplasts (Schneider, Hampp, and Ziegler, 1977). The appearance of Plate 1C suggests that guayule's chloroplasts follow this pattern and that the resultant increased outflow of precursors has produced large amounts of rubber.

If, as appears likely, the chloroplasts of guayule are the source of the rubber precursors, variations in rubber producing ability may be primarily the result of variations in chloroplast structure and/or function.

Acknowledgments

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Figure 1

The biosynthesis of the terpenes from Acetyl CoA.

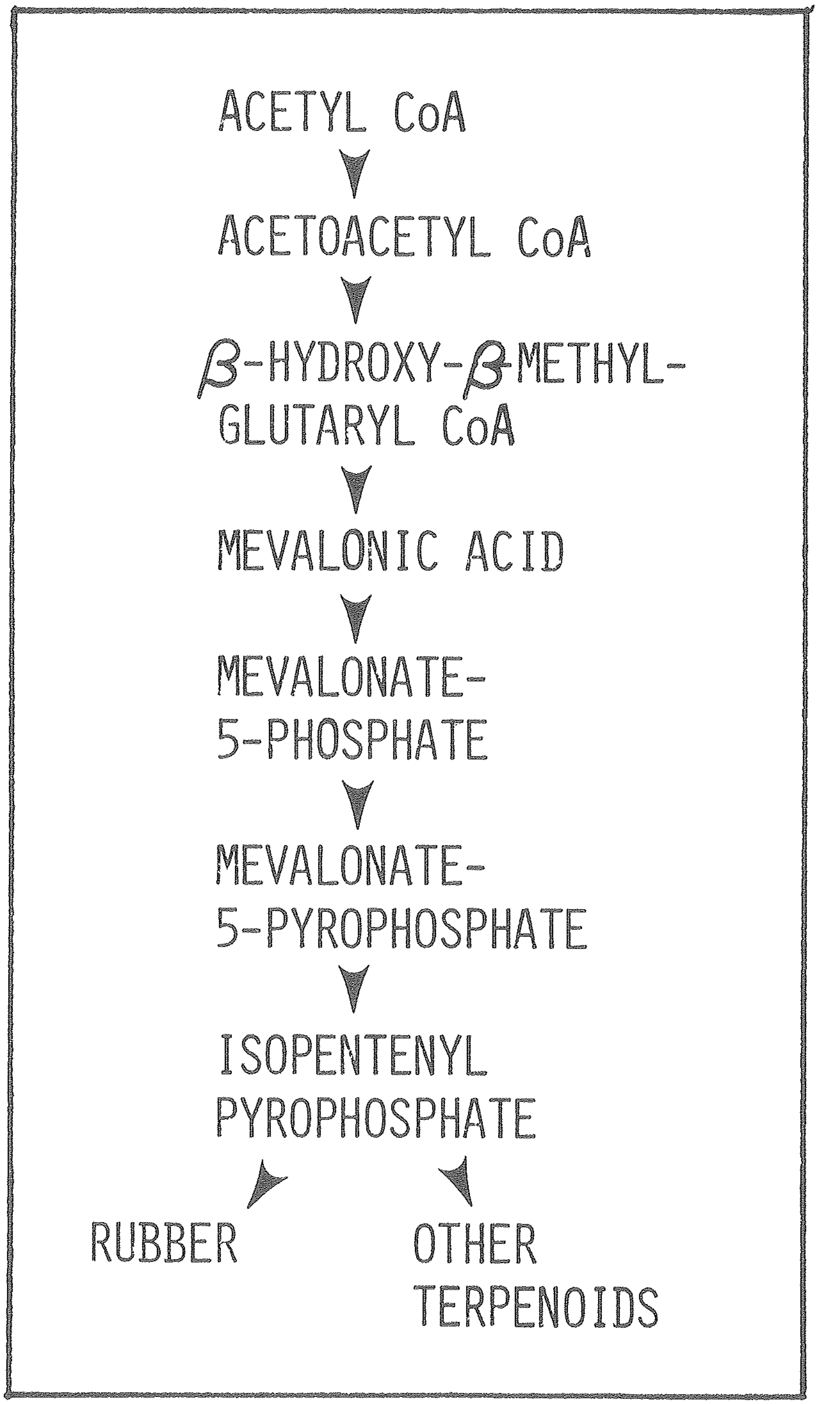

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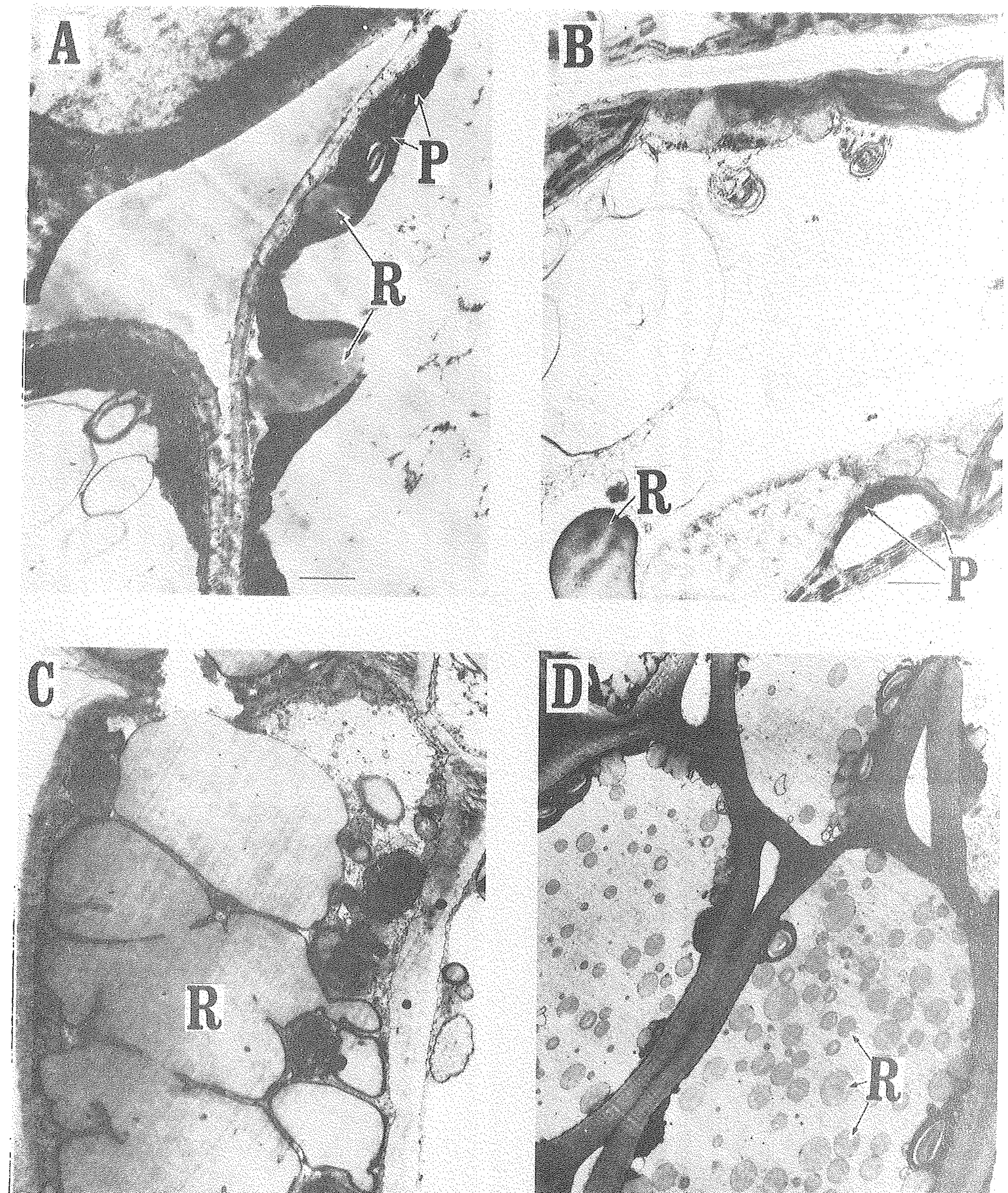
Plate 1

Electron micrographs showing effects of DCPTA on Guayule. A. Leaf palisade cells 13 days after treatment contain small accumulations of rubber and chloroplasts with greatly enlarged plastoglobuli. B. Leaf palisade cell 55 days after treatment containing a large rubber globule and plastoglobuli reduced to almost pretreatment size. C. Stem parenchyma cell before treatment. Most of the stem cells contained no rubber before treatment, but a few like this one were almost filled with rubber. D. Stem parenchyma cells 27 days after treatment. Almost all the stem parencyma cells contained a latex rich in rubber globules by 27 days after treatment.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