



Monooxygenases responsible for the metabolism of chlorotoluron in wheat microsomes

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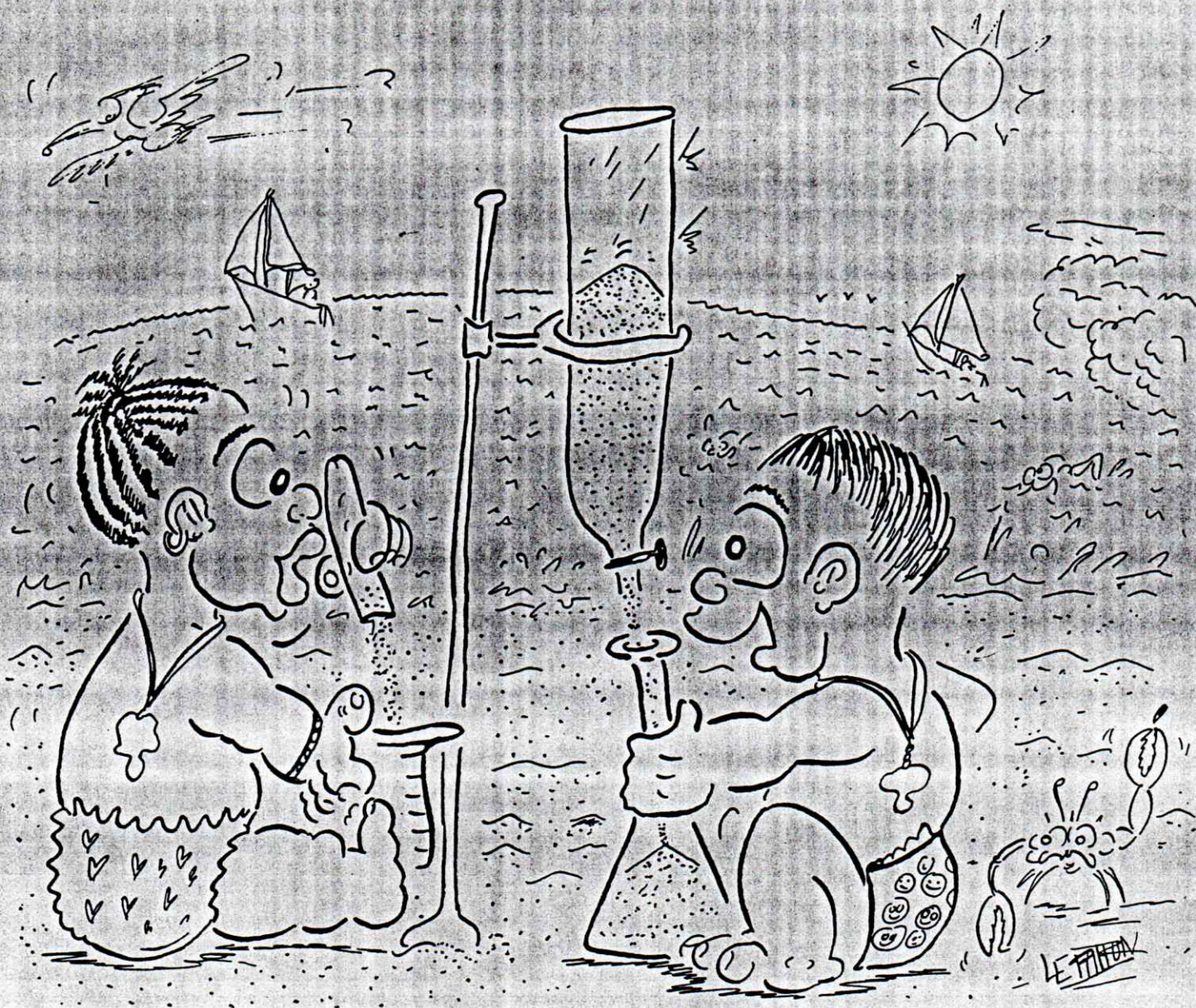
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MONOOXYGENASES RESPONSIBLES FOR THE METABOLISM OF CHLOROTOLURON IN WHEAT MICROSOMES

Microsomes isolated from achlorophyllous cells of wheat (*Triticum aestivum* L. var. Koga II) metabolized the herbicide chlorotoluron by two enzymatic reactions, namely a ring-methyl hydroxylation and a N-demethylation. These activities, and also lauric acid in-chain hydroxylation and concentrations of cytochrome P-450, were increased following cell treatment with cyometrinil or 2,4-D. The last compound was also an inducer of cinnamic acid 4-hydroxylase.

Cofactor requirements and susceptibility to inhibitors of the reactions lead to the assumption that chlorotoluron ring-methyl hydroxylase and N-demethylase probably belong to the family of cytochrome P-450 monooxygenases. However, the properties of the N-demethylase appeared to be somewhat different from those of ring-methyl hydroxylase. N-demethylation was not sensitive to CO and could be supported by cumene hydroperoxide acting as oxygen donor.

Other extraction conditions and buffer compositions, as well as incubations of microsomes with chlorotoluron in the presence of inhibitors of peroxidases, peroxygenases, flavin-containing monooxygenases and cytochromes P-450 confirmed the involvement of cytochrome P-450(s) in the oxidative metabolism of chlorotoluron. However, no inhibition of ring-methyl hydroxylation and N-demethylation could be obtained with polyclonal antibodies prepared against NADPH cytochrome P-450 reductase purified from Jerusalem artichoke.

Substrate specificity of enzymes oxidizing chlorotoluron was investigated. Wheat cell microsomes were not able to metabolize diclofop, which has been shown to be hydroxylated in wheat plants by a P-450 monooxygenase, nor to transform diuron, a structural analog of chlorotoluron.

Until now, cell treatments with classical inducers of P-450(s) systems such as phenobarbital and aminopyrine did not lead to any specific induction of chlorotoluron metabolism. Microsomes from wheat seedlings exhibited only basal rates of ring-methyl hydroxylation and N-demethylation without any detectable effects of inducers.

Cellular localisation of activities showed that they belong to the endoplasmic reticulum. Improvement of specific activities is in progress through enrichment of the endoplasmic reticulum in the microsomal fraction.