CHARACTERIZATION OF CYTOCHROME P-450
IN STUDIES OF INSECTICIDE RESISTANCE

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INTRODUCTION

The importance of cytochrome P-450 (P-450*)-dependent monooxygenase systems in the metabolism of xenobiotics, including insecticides, is well established and has been frequently reviewed (e.g., Estabrook et al., 1973; Hodgson and Tate, 1976; Hodgson and Dauterman, 1980; Kulkarni and Hodgson, 1980a; Nakatsugawa and Morelli, 1976; Ullrich, 1977). Although reviews are often restricted to investigations carried out on mammals, such systems have been described in many insects, and their importance is widely recognized (for references see reviews by Agosin and Perry, 1974; Agosin, 1976; Hodgson, 1976; Kulkarni and Hodgson, 1976d; Wilkinson and Brattsten, 1972). In one or two species of insect, such as the house fly, Musca domestica, and the southern armyworm, Spodoptera eridania, P-450 and its related enzymes have been described in some detail.

Since these foreign compounds enter the insect body by virtue of their lipophilicity, and since the lack of specificity of the microsomal P-450-dependent monooxygenase system is such that many lipophilic substrates are attacked, it is not surprising that this system is of primary importance for xenobiotic metabolism in insects. Since increased metabolism of the toxicant has long been known to be involved in resistance, it follows that this system would also be involved. While this should not be construed as decreasing the

*The following abbreviations are used: P-450 for cytochrome P-450; R for resistant; S for susceptible; EtNC for ethyl isocyanide; P-420 for cytochrome P-420.
Thus, for studies of resistance due to oxidative metabolism (essentially the comparative biochemistry of P-450 in closely related strains), we must not only exercise careful technique, but we must also question the underlying assumptions on which our techniques are based.

PREPARATIVE TECHNIQUES

Much has already been written on techniques for the preparation of insect microsomes (Hanson and Hodgson, 1971; Kulkarni and Hodgson, 1975), and this will not be pursued further except to stress that there are many variables that may affect the ultimate oxidative activity and the stability of P-450. These include not only the chemical nature and pH of the buffer, but also its ionic strength, as well as the temperature at which all operations are carried out and the nature of the homogenization technique.

By way of summary, the following points are worthy of emphasis:

1. Variables such as the above should be examined with each newly investigated species and each new strain, both susceptible and resistant.

2. These variables should also be examined in each organ and subcellular fraction in which P-450 is to be investigated.

3. The optimum conditions for homogenization may not be the same as those for resuspension and subsequent determination of enzyme activity.

4. The effect and optimum concentration of any co-factors or protectants should likewise be determined for each species, strain, organ and subcellular organelle.

Less frequently stressed is the need to observe particular care in the selection of living material. In this regard, we may stress the following:

1. Field-caught material is seldom useful. Induction, particularly in polyphagous lepidopterous larvae, is both rapid and related to feeding (Brattsten et al., 1977), and thus the levels of monooxygenase activity and P-450 are subject to unknown variables. Furthermore, the effects of disease and physiological stress are largely unknown, and it is almost impossible to determine the age of such material, relative to the previous molt, with any accuracy.

2. Since the monooxygenase activity and P-450 levels are usually close to zero at the molt, rise to a maximum during the intermolt period, and fall to a low level at the next molt...