Effects of pesticides on hydroxylation of testosterone in hepatic microsomes of rabbits

	Control	EMP	MEP	BHC
Cytochrome P-450 (nmole/mg protein) [a]	0.900 ± 0.102	0.740 ± 0.082	0.554 ± 0.042	0.715 ± 0.158
Hydroxylated testosterones (nmole/mg protein) [a]	0.468 ± 0.188	0.244 ± 0.026 [b]	0.196 ± 0.022 [b]	0.214 ± 0.030 [b]
Hydroxylation (nmole/nmole cytochrome P-450) [a]	0.537 ± 0.124	0.330 ± 0.001	0.353 ± 0.013	0.304 ± 0.025
Unmetabolized testosterone (nmole/mg protein) [a]	6.036 ± 0.518 (69.4%) [c]	7.317 ± 0.044 [b] (85.5%) [c]	7.452 ± 0.048 [b] (86.1%) [c]	7.591 ± 0.013 [b] (87.3%) [c]

[a] Values are the mean ± S.D. obtained from four animals. [b] Different from the control at $P < 0.05$. [c] Percentage of the unmetabolized substrate to the substrate.

Results and discussion. The contents of cytochrome P-450 and the activities of testosterone hydroxylase in the hepatic microsomes of the control rabbits and the rabbits treated with the pesticides are shown in the Table. As the values in the 2 control groups were similar, they were combined. The content of cytochrome P-450 in the BHC- or EMP-treated rabbits was reduced to about 80% of that in the control animals and, in the MEP-treated rabbits, it was reduced to about 60%. The unmetabolized substrate, testosterone, was higher in the experimental group in comparison with that in the control group and the formation of hydroxytestosterones per mg protein or per cytochrome P-450 in the experimental groups was half as low as that in the control. These findings suggested that the pesticides inhibited steroid hydroxylase activity in liver microsomes.

STEVENS et al.[4] observed that malathion and parathion given 1 h prior to hexobarbital administration significantly prolonged hexobarbital sleeping time and suggested that the increase in sleeping time by these insecticides was not due to an inhibition of cholinesterase, but due to an impairment of hexobarbital metabolism. STEVENS and GREENE[2] found that a parallel relation between inhibition of ethylmorphine metabolism by these insecticides and the binding affinity of these agents to microsomal cytochrome P-450. The administration of chlorthion, organic phosphorothionate insecticides, to rats for 10 days inhibited the liver microsomal metabolism of testosterone, estradiol-17β, progesterone and deoxycorticosterone to highly polar metabolites, whereas chlorodane and DDT markedly stimulated the metabolism of these steroids by liver microsomes[3]. It was demonstrated that chlorinated insecticides can either stimulate or inhibit microsomal testosterone hydroxylation depending upon whether the agents are given chronically or acutely[3].

LUCIER et al.[9] suggested that the reduced content of cytochrome P-450 of rat livers occurred following the administration of methylmercury hydroxide for 2 days was due to increasing degradation of the fast-phase hemoprotein and/or decreasing synthesis of the slow-phase component and that aminopyrine demethylation, a mixed function oxidase reaction catalyzed by the cytochrome P-450, was decreased by reducing cytochrome P-450 levels.

Since no change was observed in the difference spectra (namely shift of the peak at 450 nm and appearance of the peak at 420 nm had not occurred) and the hydroxylase activity was not in parallel with the content of cytochrome P-450 in this study, the decrease in the hydroxylase activity was not only due to the decrease of cytochrome P-450 content, but also due to other factors, such as the impairment of the hepatic cells. In general, the pesticides seemed to have inhibitory effects on the drug and steroid metabolism in hepatic microsomes when given in a higher dose and/or for a long period.

Résumé. L'hydroxylation de la testostérone par les microsomes du foie de lapins adultes est inhibée par l'hexachlorobenzène, par le 0,0-diméthyle-0-(3-méthyle-4-nitrophényle) thiophosphate et par le phosphate éthylmercurique administrés par voie orale, tous les deux jours, pendant 5 mois.

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High Permeability of Insect Blood-Brain Barrier to Alcohols Demonstrated by an Electrophysiological Technique

Insects are alone among invertebrates in that their central nervous system possesses an ionic diffusion barrier between the extraneuronal spaces and the blood[1], analogous to that of vertebrates. The possible influence of this barrier on the movements of organic molecules is a complicating factor in pharmacological and toxicological studies, these being of extreme interest as many insecticides act primarily on the central nervous system[2]. Previous research using radioisotopes has suggested that the insect nerve cord is surprizingly impermeant to alcohols[3] and other organic molecules[4]. Here I describe use of the electrophysiological effects of the alcohols to measure their rate of arrival at the neurone surfaces, a technique which could be extended to other molecules

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and systems, and report that the barrier has little effcte on the movements of these substances, which are in fact very rapid.

The experiments were performed on isolated abdominal nerve cords of the cockroach (*Periplaneta americana*), and intracellular recordings were made from giant axons in the connectives between the IVth and Vth ganglia. Alcohol concentrations which were insufficient to bring about a complete conduction block caused a stable reduction in the action potential amplitude (Figure 1a). All changes were fully reversible and replicable within the range of concentrations used, hence it was possible to compile a dose-response curve from a series of such measurements (Figure 1b). By reference to this curve, the instantaneous concentrations at the giant axon surfaces during exposure to a high alcohol concentration were estimated from the time-course of the reduction in action potential amplitude.

The rate of arrival at the axon surfaces normally followed first-order kinetics fairly closely until about twice the half-time (Figure 1c), beyond which it was not possible to make accurate estimates from the dose-response curve. Analogous estimates for the rate of disappearance of the alcohol during recovery were very similar, demonstrating that the movements were essentially symmetrical (Figure 1d).

To avoid the effects of individual variation in sensitivity to the alcohols, a separate dose-response curve was compiled for each experiment. The effect of the ionic diffusion barrier on their rate of access was investigated by comparing the results obtained between intact connectives and those from which the nerve sheath had been surgically removed, a procedure which destroys the barrier[5]. The results, given in the Table, show that the access of the lower alcohols in particular is very rapid, and that the effect of de-sheathing is remarkably small.

The alcohols do not, however, even temporarily damage the ionic diffusion barrier in intact nerve cords. This has been demonstrated by exposing intact connectives to a high-potassium saline (which causes a rapid conduction block in de-sheathed connectives), and additionally applying a high alcohol concentration during part of this period (Figure 2). The subsequent recovery of the action potential in this saline shows the barrier to have been unaffected.

Radioisotope efflux experiments with *n*-butanol have shown that about 70% of the activity washes out from isolated connectives with a similarly rapid half-time (ca. 6 sec)[8], this component apparently having escaped detection by the previous investigators[3].

By analogy with permeability studies on other tissues[9,10], the rate of access of the alcohols into intact connectives is expected to be directly related to their liposolubility, but such an effect is seen only for the 2 butanol isomers (Table). The influx of ethanol is more rapid than expected, which may be explicable in terms of its low molecular weight, a similar effect for this size of molecule having been reported in other systems[9,10].

More surprizing is the slower access of hexanol and particularly octanol, the reduction in rate being grossly out of proportion to the increase in molecular weight. It is also of great interest that de-sheathing increases their rate of access far more than for the lower alcohols. The

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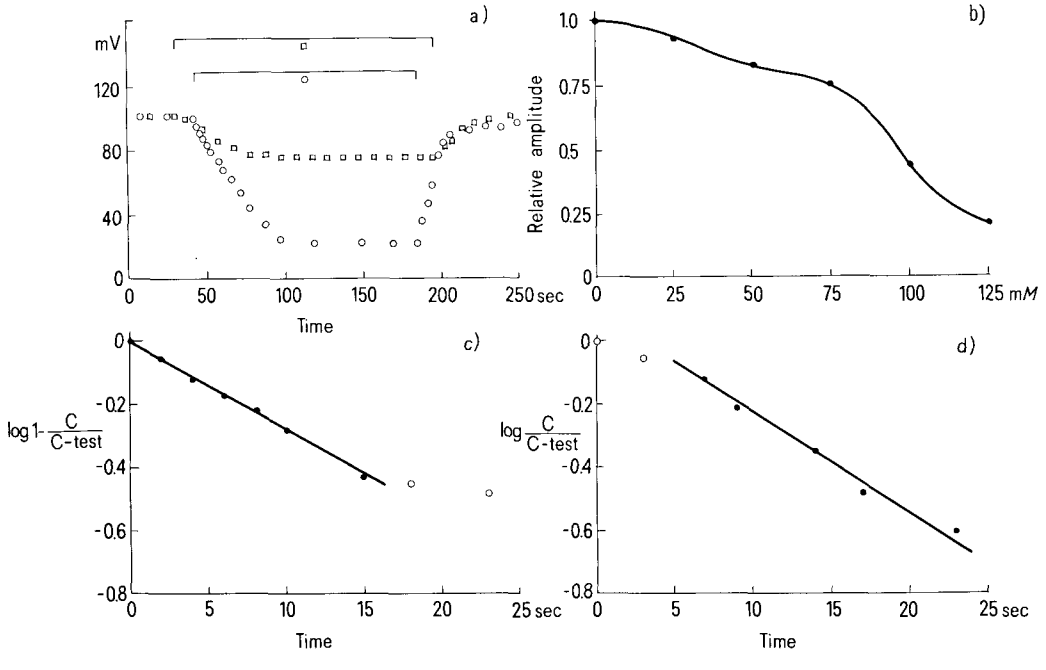

Fig. 1. This shows the results of a single experiment with *n*-butanol on an intact nerve cord. a) The effects of 75 m*M* (squares) and 125 m*M* (circles) butanol, applied for the periods indicated by the bars, on the amplitude of the action potential. b) Dose-response curve, compiled from the steady-state reductions in action potential amplitude observed in the presence of a series of butanol concentrations. c) The effect of 125 m*M* butanol (Figure a) has been used in conjunction with the dose-response curve, to calculate the time-course of the increase in concentration (C) at the neurone surface. The good approximation to a straight line suggests a simple first-order kinetic. Open circles were not included in the regression analysis, as estimates from this region of the dose-response curve were less reliable. d) As Figure c, but this being for the decrease in butanol concentration during recovery.

Half-times of arrival of the aliphatic alcohols to the neurone surfaces, as estimated by the technique shown in Figure 1

Alcohol	C-test (mM)	Mean half-time ± SE		P (t-test)	Octanol/water partn. coeff.
		Intact	de-sheathed		
Ethanol	1000	15.9 ± 0.7	13.5 ± 1.3	0.2	0.48
n-Butanol	125	7.5 ± 1.0	7.7 ± 0.5	0.9	7.6
tert. Butanol	500	29.8 ± 1.8	21.3 ± 1.8	0.02	2.3
n-Hexanol	10	20.2 ± 1.0	11.7 ± 0.6	<0.001	110
n-Octanol	1.2	157 ± 12	71.2 ± 3.1	<0.001	1400

n = 5 for the hexanol experiments, and 4 for the others. Octanol/water partition coefficients are taken from Ref.[6]; the membrane/saline coefficients are likely to be about 5 times lower[7].

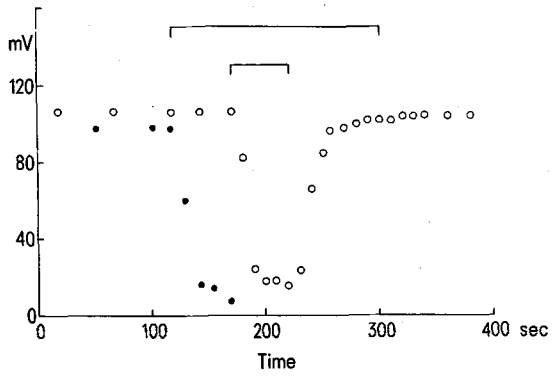

Fig. 2. This shows that n-butanol does not damage the ionic diffusion barrier. High-potassium saline (applied for period indicated by upper bar) rapidly abolishes the action potential in de-sheathed connectives filled circles, but has no effect on intact ones (open circles). Temporary exposure to 200 mM butanol in this saline (lower bar) causes a reversible abolition of the action potential, demonstrating that the barrier to ionic diffusion remains intact.

most plausible explanation for these phenomena is that the high partition coefficients of these alcohols cause the lipid phase of the nerve cord to have a significant reservoir effect, buffering any changes in their aqueous concentration and thereby increasing the time required for equilibration. The effect of de-sheathing can be interpreted in terms of an approximately 50% reduction in the size of this reservoir, which is reasonable in view of the quantity of tissue removed. This theory, in conjunction with that of a lipophilic barrier, predicts that molecules having partition coefficients near unity will have the highest rate of access.

Summary. Using the anaesthetic effects of the alcohols to measure their concentration within the cockroach central nervous system, it is shown that the lower homologues have access half-times of only a few seconds. Slower access of the higher homologues is interpreted in terms of a reservoir effect resulting from their higher liposolubility.

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Suppression of Sebaceous Gland Non-Specific Esterase Activity by Electrophilic $\alpha\beta$-Unsaturated Compounds

A rapid bioassay system involving determination of the degree of suppression of non-specific esterase activity in sebaceous glands has proved to be particularly useful in ranking tobacco smoke condensates and other materials in terms of their potential tumorigenicity[1]. The bioassay system involves the application of the test compound to mouse skin, followed by the measurement of the area of skin sebaceous gland non-specific esterase activity[1,2] with an image analysing computer. The degree of suppression has been shown to correlate with the known potency of the tumorigenic compound[1].

Following the observation that treatment with the riot control agent o-chlorobenzylidenemalononitrile suppressed non-specific esterase activity to a similar extent as the potent carcinogen 7,12-dimethylbenz[a]anthracene[3], it was of interest to examine the effects of other reactive $\alpha\beta$-unsaturated compounds, which may be regarded as electrophilic agents.

The compounds chosen for study were menadione (vitamin K_3), hex-2-en-1-al, a naturally occurring flavour component[4], cyclohex-2-en-1-one and β-nitrostyrene which possesses antibacterial properties[5]. o-Chlorobenzylidenemalononitrile[6] was used as the positive control[3].

Materials. The compounds studied were commercially available and where necessary, the purity was established.

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