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].

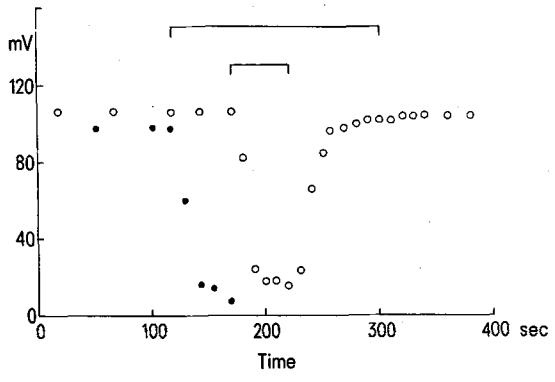

Fig. 2. This shows that n-butanol does not damage the ionic diffusion barrier. High-potassium saline (applied for period indicated by upper bar) rapidly abolishes the action potential in de-sheathed connectives filled circles, but has no effect on intact ones (open circles). Temporary exposure to 200 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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Methods. Groups of 8 CFLP female mice (a hysterectomy-derived strain of Swiss origin), 50 days old at start of treatment when the hair growth cycle is in the telogen phase, were painted on 3 consecutive days with each compound at concentrations of 1, 0.5 or 0.1% (w/v or v/v) in acetone. A control group of 16 mice was treated with acetone alone. 3 days after the final application, the mice were killed and a sample of skin from the centre of the treated area was excized and immediately frozen in hexane precooled to −75 °C.

Three 22 μm transverse cryostat sections were cut at 100 μm intervals from each skin sample. The sections were air-dried at 6 °C and fixed in formol-calcium-gum-sucrose. Non-specific esterase activity was demonstrated in the sections by the method of HOLT[7] using 5-bromo-indoxyl acetate as substrate.

Sections of skin from every group were included in each incubation procedure to allow subsequent correction for incubation variations. The sections were then mounted in 90% (w/v) aqueous solution of polyvinylpyrrolidone. The area of non-specific esterase activity in the sebaceous glands, as indicated by the reaction product, was measured in each skin sample using a 'Quantimet B' image analyser (Image Analysing Computers Ltd., Melbourn, Royston, Herts, U.K.).

Results. An analysis of variance of the results obtained (Figure) showed that non-specific esterase activity in sebaceous glands was significantly suppressed ($p < 0.001$) by treatment with 0.5% and 1.0% *o*-chlorobenzylidenemalononitrile and β-nitrostyrene (Figure), but not by the other compounds tested when compared with that of the acetone-treated controls. The suppression produced by β-nitrostyrene was apparently linear with respect to dose, but further doses intermediate to 0.1% and 0.5% need to be studied before the dose response with *o*-chlorobenzylidenemalononitrile can be assessed.

Discussion. All the compounds studied react readily with thiol groups[8] but this alone is not the explanation for the marked suppression produced by 2 of the compounds when none was produced by the others, or by thiol reagents such as 1-fluoro-2,4-dinitrobenzene[9], which is a potent tumor-promoting agent, but not a carcinogen der se[10]. Thus as only *o*-chlorobenzylidenemalononitrile and β-nitrostyrene suppressed enzyme activity, this test does not merely detect electrophilic agents. β-Nitrostyrene is chemically related to reactive nitro-olefins, some of which are carcinogenic and can occur as air pollutants probably being formed by reaction of unsaturated hydrocarbons in petrol with nitrogen oxides produced during combustion[11].

Interpretation of suppression of sebaceous gland non-specific esterase activity by foreign compounds is difficult, but the results obtained so far, suggest that this bioassay system is worth thorough investigation as a possible rapid means of assessing the tumorigenicity of large series of related compounds. It may prove more useful than some of the short-term tests for tumorigenicity[12,13] currently employed. It is intriguing that sebaceous glands contain relatively large amounts of aryl hydrocarbon hydroxylase activity[14], an enzyme system which may be implicated in the production of the ultimate carcinogenic forms of polycyclic hydrocarbons[15].

Summary. Sebaceous gland non-specific esterase activity was suppressed following application to mouse skin of 2 electrophilic αβ-unsaturated compounds, *o*-chlorobenzylidenemalononitrile and β-nitrostyrene, but not by three others.

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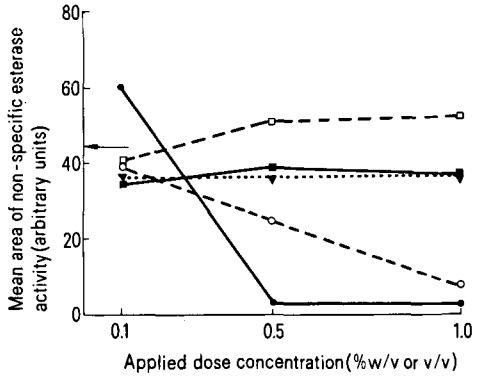

Effect of treatment by αβ-unsaturated compounds on skin sebaceous gland non-specific esterase activity in mice. Menadione (□- - - -□), hex-2-en-1-al (▼....▼), cyclohex-2-en-1-one (■——■), β-nitrostyrene (○——○) and *o*-chlorobenzylidenemalononitrile (●——●) were applied to mouse skin as described in the text. The control level of non-specific esterase activity (44 arbitrary units) is arrowed.

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