of THC approaching 0·2 mM most sperm cells ceased to move: individual sperm, however, were still observed in circular or twisting motion. A striking morphological change was visible in scanning electron microscopy; it consisted of a remarkable swelling of the mitochondrial helix in most of the sperm which has been exposed to 0·2 and 0·3 mM of THC (Fig. 1). No such swellings, however, were observed in the examined samples of untreated sperms.

These findings indicate that THC has a pronounced effect on fresh bull sperm. This effect consisted of damage to the mitochondrial helix, decrease of respiration and ATP content, and changes in sperm motility.

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Lysis of rat liver lysosomes *in vitro* by Δ^9-tetrahydrocannabinol*

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THE MAJOR psychoactive component of cannabis is Δ^9-tetrahydrocannabinol (THC; atoms numbered as in dibenzopyran); the metabolism and properties of this substance have been extensively investigated in recent years.[1–3] In rats the liver is a major site of accumulation of THC and its metabolites,[4] and these are associated with the particulate subcellular fractions.[5] The THC molecule is lipophilic and appears to have an affinity for biological membranes; it stabilizes erythrocyte membranes[6] but disrupts mitochondrial membranes by a detergent-like action.[7–9] The present study describes the lytic effect of THC on rat liver lysosomes which results in the loss of structural integrity and the release of acid hydrolases associated with this subcellular particle.

Male Wistar rats weighing 100–150 g were decapitated and their livers homogenised in 0·25 M sucrose–0·001 M EDTA (pH 7·4). The homogenisation procedure, and the isolation of a lysosomal mitochondrial pellet have been described previously.[10] The lysosomal–mitochondrial pellet was suspended in 0·15 M sucrose–0·001 M EDTA (pH 7·4) and the suspension divided into 1·35-ml aliquots. A solution of Δ^9-tetrahydrocannabinol in ethanol was diluted with dimethylsulfoxide and 0·15 ml was added to each aliquot of lysosomal–mitochondrial suspension. Replicate groups of six aliquots were used for the determination of each point in Fig. 1. Equivalent solutions of dimethylsulfoxide and of ethanol were added to control groups. Another control group contained 0·1 per cent Triton X-100 in place of the drug, to facilitate lysis

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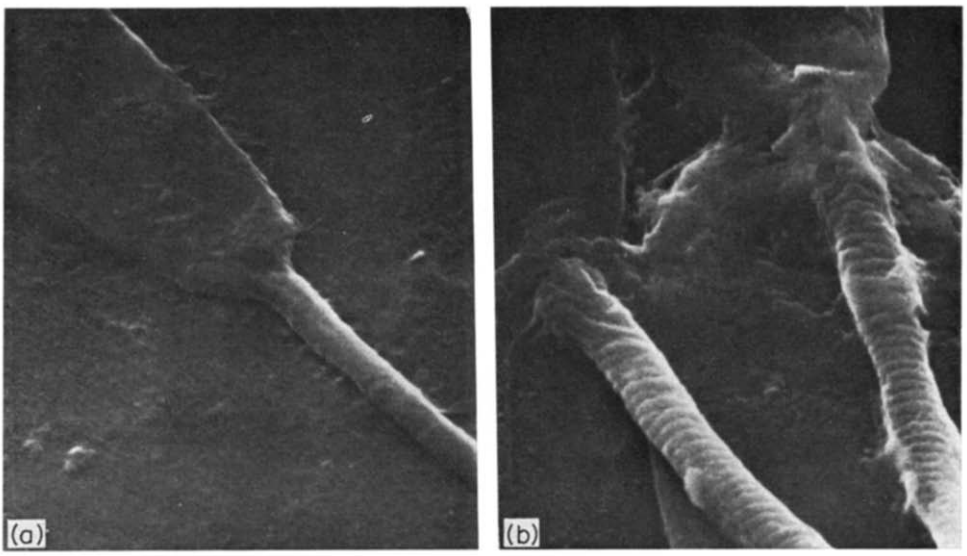

Fig. 1. Scanning electron microscopy of bull sperm: after 1 hr incubation at 37°. (a) Control magnification 10,000 × (b) 0·3 mM of THC magnification 15,000 ×. Note the swelling of the mitochondrial heliix.

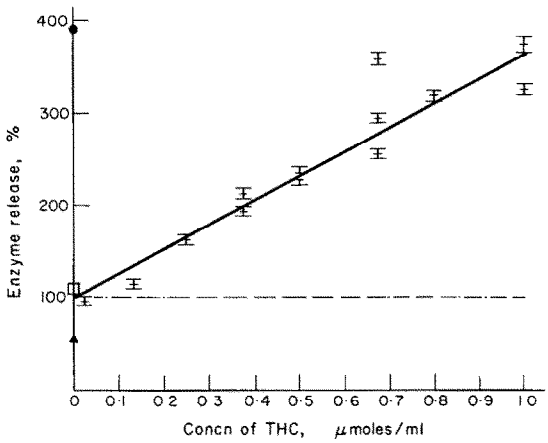

FIG. 1. Effect of THC on lysosomal latency. Each bar represents the mean ± standard deviation for six incubations of THC with rat liver lysosomes. Four independent preparations of lysosomal–mitochondrial pellets were used. The 100 per cent level at each concentration represents the mean of six controls from the same preparation incubated with the lipid solvent only. The enzyme activity of these controls fell within the range 57–131 nmoles phosphate liberated/ml/min. Other controls represent the mean of 24 incubations with 0·1% Triton in 0·15 M sucrose–0·001 M EDTA (●); the mean of six incubations with the maximal concentration of ethanol in dimethylsulfoxide plus 0·15 M sucrose–0·001 M EDTA (□); and the mean of six incubations containing only 0·15 M sucrose–0·001 M EDTA (▲). The straight line is computer-fitted by linear regression using the method of least squares. Differences between THC-treated groups and controls were highly significant ($P < 0·001$) at all concentrations of THC except the lowest, for which the difference was probably significant ($P < 0·05$).

of the lysosomes. The contents of the tubes were gently mixed and incubated for 30 min at 37° to allow release of lysosomal enzymes from the particulate suspension. All tubes were then centrifuged at 15,000 *g* for 10 min to sediment cell particles and the resultant supernatants were assayed for acid phosphatase using β-glycerophosphate as the substrate.[11] The assay was initiated by mixing 0·5 ml of each supernatant with 0·5 ml of 0·2 M β-glycerophosphate in 0·1 M acetate buffer (pH 5·0)–0·25 M sucrose to lower the pH from 7·4 to 5·0. The THC was provided by the Health Protection Branch, Health and Welfare Canada, Ottawa.

The results obtained show that THC has a marked lytic effect on lysosomes which results in the solubilisation of particulate acid phosphatase (Fig. 1). At high concentrations of THC (1×10^{-3}M), the lysis is close to that obtained with a detergent, 0·1 per cent Triton X-100. This effect is proportional to the concentration of THC used and is not due to the ethanol or dimethylsulfoxide which are used to disperse the lipid drug.

The THC concentration range used in our experiments is similar to that used to show mitochondrial disruption by THC[7–9] and the mechanism of membrane damage to mitochondria and lysosomes is probably similar. The major distinction is that in the case of lysosomes this damage may result in the release of hydrolytic enzymes: proteases, glycosidases, phospholipases and other enzymes capable of intracellular digestion. The lytic action of THC on lysosomes is analogous to the well studied action of vitamin A on this organelle.[12] Both THC and vitamin A are lipid alcohols and have membrane-modifying properties. The tissue regression seen in cartilaginous rudiments grown in the presence of high concentrations of vitamin A has been attributed to the vitamin A-induced lysis of lysosomes and the release of acid hydrolases.[13]

Though the concentrations of THC used here are clearly higher than physiological concentrations achieved via the use of cannabis, it may be reasonable to infer, as has been shown for vitamin A,[12] that THC will have some destabilizing effect on liver lysosomes *in vitro*. Such an effect may contribute to the hepatotoxicity and cirrhosis observed in regular users of cannabis.[14]

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