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BIOCHEMICAL EFFECTS AND MORPHOLOGICAL CHANGES IN RAT LIVER MITOCHONDRIA EXPOSED TO Δ^1-TETRAHYDROCANNABINOL*

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SUMMARY

Δ^1-Tetrahydrocannabinol affects the configurational integrity of rat liver mitochondria to a degree dependent upon its concentration. When Δ^1-tetrahydrocannabinol concentration is increased, the mitochondria pass through three different morphological stages, which are visualized by electron microscopy. These stages are correlated with changes in oxygen uptake, ATPase activity and turbidity. Starting with control mitochondria, designated as Stage A, addition of 15 μg Δ^1-tetrahydrocannabinol per mg protein results in maximum stimulation of oxygen uptake (Stage B). In the presence of 50 μg Δ^1-tetrahydrocannabinol per mg protein this stimulation vanishes, the inner mitochondrial structure is disrupted and maximum values of swelling and ATPase activity are attained (Stage C). At higher Δ^1-tetrahydrocannabinol concentrations, up to more than 100 μg per mg protein, both stimulation of ATPase activity and swelling diminish. These effects are proposed to be associated with bonding of the hydrophobic Δ^1-tetrahydrocannabinol to some protein–phospholipid receptors in the mitochondrial membrane.

INTRODUCTION

Earlier work in this laboratory[1] has shown that Δ^1-tetrahydrocannabinol, one of the main psychoactive constituents of cannabis[2–4], causes a rise in ATPase activity; at larger doses of Δ^1-tetrahydrocannabinol the effect wanes. Similarly, it has been found (T. Bino and A. Chari-Bitron, unpublished results) that oxygen uptake is stimulated by low Δ^1-tetrahydrocannabinol concentrations, and is inhibited at higher ones, and that addition of Δ^1-tetrahydrocannabinol also causes a decrease in the P:O ratio. In the previous paper[1] we have assumed that the strongly hydrophobic Δ^1-tetrahydrocannabinol[5] interacts with certain receptors in the mitochondrial membranes, causing configurational changes in their molecular organization, to a degree depending upon concentration. This agrees with the assumption of Weinbach *et al.*[6] that normal mitochondrial morphology depends upon a continuing supply of high-energy intermediates generated by oxidative phosphorylation,

* Also designated as Δ^9-tetrahydrocannabinol.

whereas disruption of this biogenic process leads to a marked disorganization of normal mitochondrial structure.

For the purpose of verifying our assumption, turbidimetric measurements of Δ^1-tetrahydrocannabinol-induced mitochondria and concomitant electron-microscope studies were carried out, at various concentrations of the drug. The results are described in the present paper.

MATERIALS AND METHODS

Δ^1-tetrahydrocannabinol was supplied to us by Dr R. Mechoulam of the Hebrew University, and its solution in ethanol served for this investigation. The controls contained equivalent amounts of ethanol without Δ^1-tetrahydrocannabinol.

Male albino rats of our own stock, weighing about 150 g each, were used. The mitochondrial fraction in 0.25 M sucrose *plus* 0.01 M Tris–HCl (pH 7.4) was prepared from the livers[7] of the sacrificed animals. This mitochondrial fraction was resuspended in the sucrose solution and adjusted to contain 40 mg protein/ml.

For the turbidimetric measurements, small aliquots (20–30 μl) of mitochondrial suspension were introduced to either 0.125 M KCL *plus* 0.02 M Tris–HCl (pH 7.4) or to 0.22 M sucrose *plus* 0.02 M Tris–HCl(pH 7.4), each of them containing the appropriate amount of Δ^1-tetrahydrocannabinol; the volume was made up to 3 ml with one of these two solutions. The final concentration range of Δ^1-tetrahydrocannabinol, thus obtained, is $1 \cdot 10^{-5}$–$2 \cdot 10^{-4}$ M. The samples were incubated at 28 °C, and their light absorption at 520 nm was measured, every 0.5 min for a period of 10 min, with the aid of a Zeiss PMQ II spectrophotometer.

Since, in all cases, the Δ^1-tetrahydrocannabinol effects depend on the amount of mitochondrial fraction present in the solution, the concentrations of Δ^1-tetrahydrocannabinol are expressed in μg per mg protein. The protein concentration for each determination is indicated in the legend of Fig. 1. The protein was determined according to the method of Lowry *et al.*[8].

Oxygen uptake was measured at 30 °C, using the Warburg manometric technique, in the presence of 10 mM glutamate as substrate. The medium contained: 1.3 mM $MgCl_2$; 2.6 mM ATP; 40 mM KCl; 30 mM Tris–HCl buffer (pH 7.4) and sucrose up to isotonicity. ATPase was determined at 30 °C, according to the method described in the previous paper[1].

For the electron-microscope examinations, the mitochondria were first suspended in 0.22 M sucrose *plus* 0.02 M Tris–HCl (pH 7.4) and exposed for 10 min to various doses of Δ^1-tetrahydrocannabinol. Thereafter, the mitochondria were sedimented by rapid centrifugation; the pellets were fixed in 2 % glutaraldehyde at 4 °C for 2 h, post-fixed in 1 % osmium tetroxide and embedded in Epon[9]. The sections were stained with uranyl acetate and lead citrate. Negative staining was carried out with 2 % phosphotungstic acid. All preparations were examined in the JEM 100 B electron microscope.

RESULTS AND DISCUSSION

Fig. 1 demonstrates the effects of various concentrations of Δ^1-tetrahydrocannabinol on oxygen uptake, on ATPase activity and on turbidity of the mito-

chondrial suspensions. On the basis of these effects of Δ^1-tetrahydrocannabinol, four mitochondrial stages may be distinguished, of which the intact one is designated as Stage A. The oxygen uptake increases with rising Δ^1-tetrahydrocannabinol concentration and reaches a maximum at 15 μg Δ^1-tetrahydrocannabinol per mg protein (Stage B). For these measurements, an incubation period of 20 min was chosen because, in all cases, the rise in oxygen uptake remained linear during this period (T. Bino and A. Chari-Bitron, unpublished results). Further increase in Δ^1-tetrahydrocannabinol content causes the rise in oxygen uptake to decrease monotonically until, at about 50 μg Δ^1-tetrahydrocannabinol per mg protein (Stage C) a complete abolishment was reached. Similar results were obtained with other substrates, such as succinate, β-hydroxybutyrate or α-ketoglutarate (T. Bino and A. Chari-Bitron, unpublished).

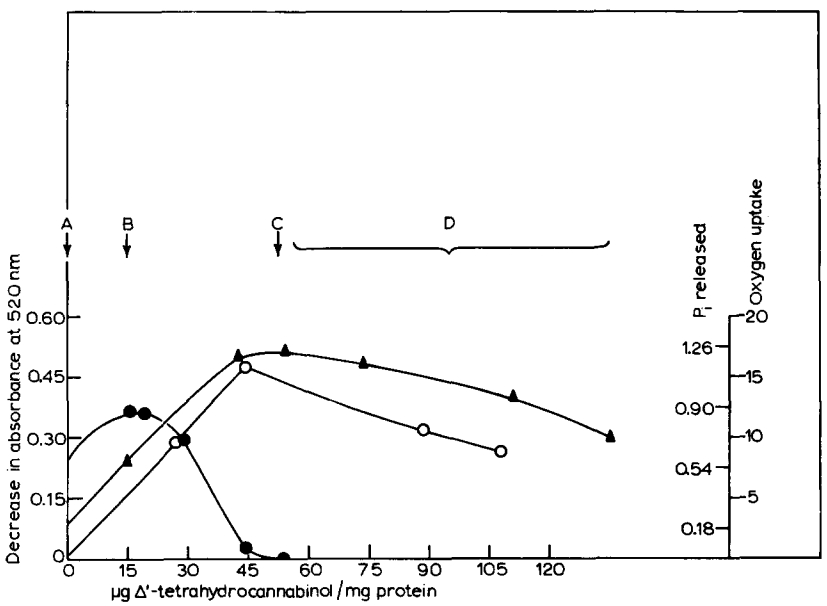
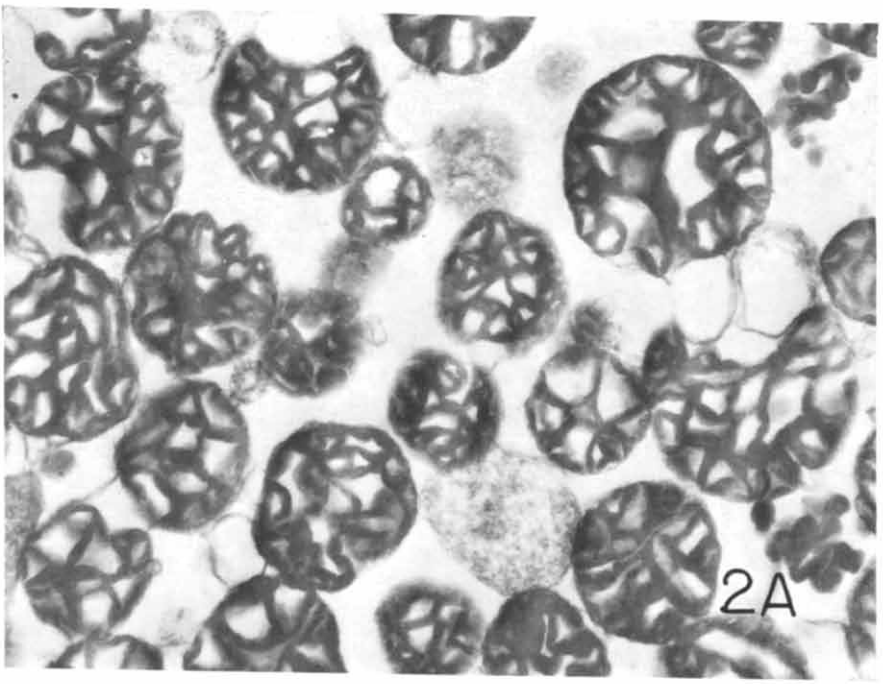


Fig. 1. Effect of Δ^1-tetrahydrocannabinol concentration on oxygen uptake, ATPase activity and turbidity of mitochondrial solutions. ●—●, oxygen uptake (μl O_2 per mg protein per 20 min), protein concentration 1 mg/ml. ○—○, ATPase activity (μmole P_i released per mg protein per 20 min), protein concentration 0.75 mg/ml. ▲—▲, mitochondrial turbidity (decrease in absorbance at 520 nm after 10 min), protein concentration 0.35 mg/ml. The medium contained 0.22 M sucrose *plus* 0.02 M Tris–HCl (pH 7.4).

Δ^1-tetrahydrocannabinol also causes an increase in ATPase activity[1], up to a maximum at Stage C, concomitant with the disappearance of its effect on oxygen uptake. At Δ^1-tetrahydrocannabinol concentrations beyond that corresponding to mitochondrial Stage C, the ATPase activity decreases monotonically (Stage D), but the effect of Δ^1-tetrahydrocannabinol is not abolished even at concentrations exceeding 100 μg Δ^1-tetrahydrocannabinol per mg protein. Furthermore, Fig. 1 demonstrates that up to Stage C, the decrease in absorbance (swelling) of the mitochondrial suspension is augmented with rising Δ^1-tetrahydrocannabinol con-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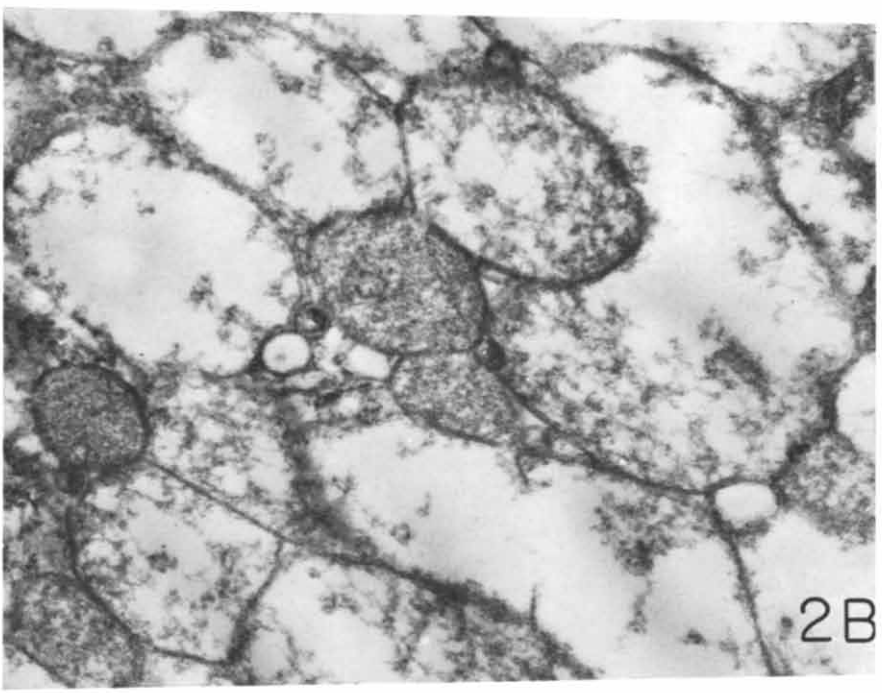
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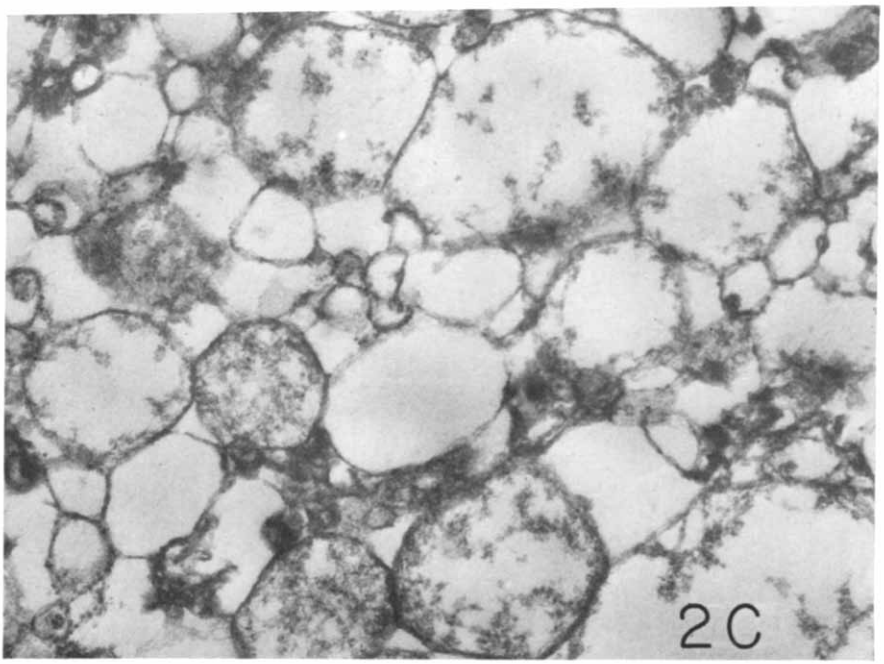


Fig. 2. Sectioned mitochondrial preparations. (A) Control (Stage A). (B) Mitochondria treated with 50 μg Δ^1-tetrahydrocannabinol per mg protein (Stage C) (C) Mitochondria treated with 100 μg Δ^1-tetrahydrocannabinol per mg protein (Stage D). × 27 000.

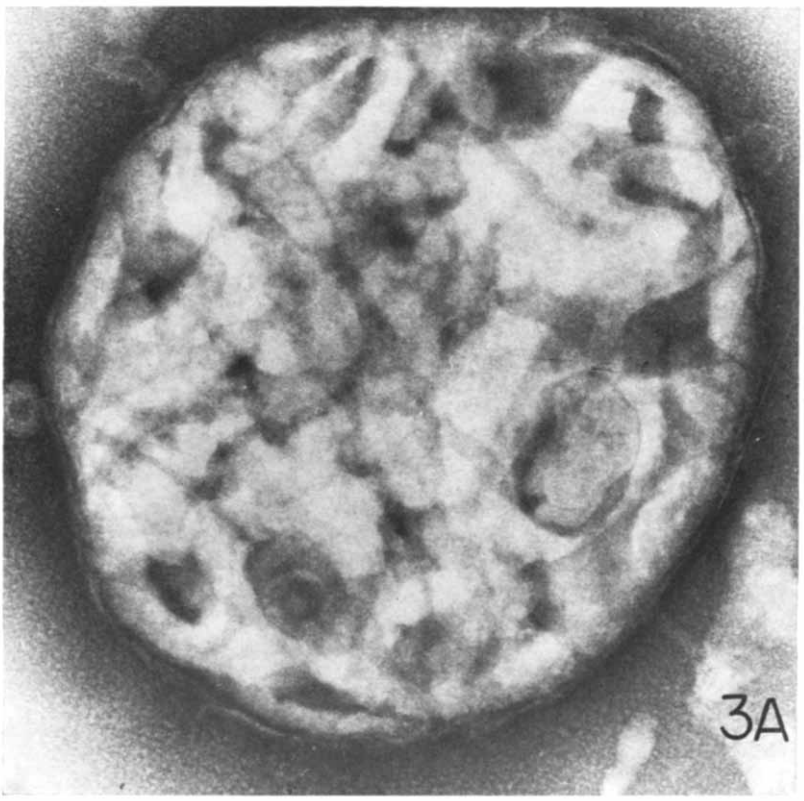
centration. At higher concentrations of the drug, corresponding to mitochondrial Stage D, the swelling decreases monotonically with increasing Δ^1-tetrahydrocannabinol concentrations, but it does not vanish even at 135 μg per mg protein. The two curves describing the changes in swelling and ATPase activity are parallel to each other in the whole range of Δ^1-tetrahydrocannabinol concentrations investigated. It is to be pointed out that addition of ATP does not reverse Δ^1-tetrahydrocannabinol-induced swelling, contrary to the case of thyroxine-induced swelling[10].

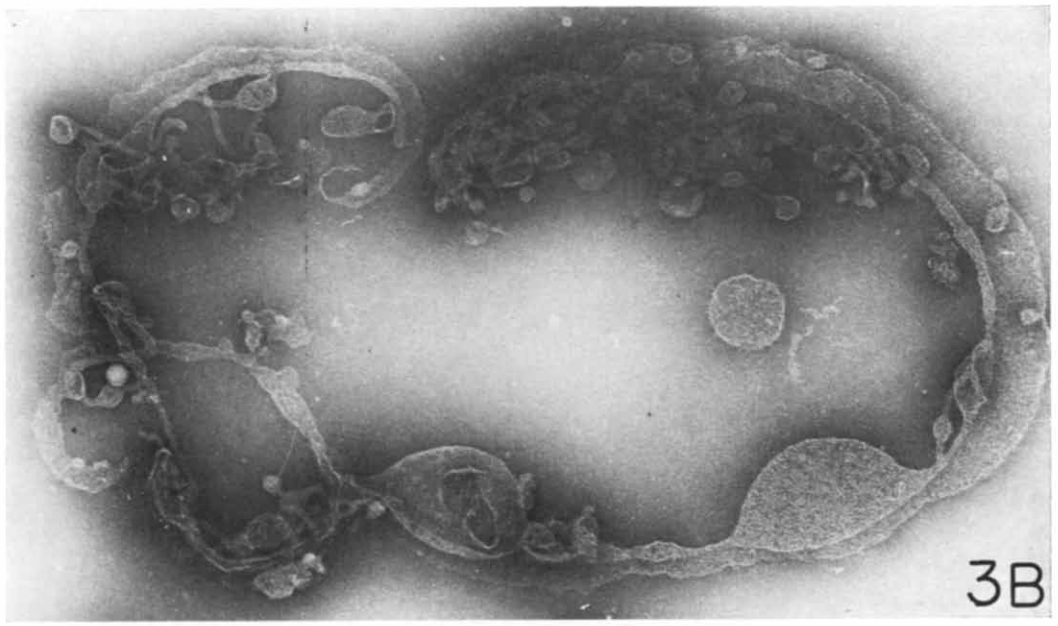
The complex structure of the mitochondrion, with its outer and inner membranes and its characteristic arrangement of cristae, may be associated with different types of swelling, particularly if there is difference in permeability between the outer and inner membrane. Therefore, in order to gain a more accurate knowledge about the morphological changes induced by Δ^1-tetrahydrocannabinol, electron-microscopic studies were conducted on a large number of thin sections and negatively stained specimens, representative pictures of which are shown in Fig. 2.

Freshly isolated mitochondrial preparations in state 1 of Chance's terminology[11], containing ethanol as control, were compared to similar preparations containing various concentrations of Δ^1-tetrahydrocannabinol. Fig. 2 demonstrates sectioned mitochondrial preparations. The mitochondria in Stage A (Fig. 2A) are regular in shape and the rounded outer and inner membranes, as well as the cristae, are clearly visible. Mitochondria treated with 15 μg Δ^1-tetrahydrocannabinol per mg protein (Stage B), showed no clear morphological differences in comparison to those in Stage A. It is to be noted that the mitochondrial suspensions were never

homogeneous and even among the normal mitochondria structural variations were observed. This heterogeneity escaped observation when volume changes of mitochondria were small. However, mitochondria in Stage C showed distinct structural alterations (Fig. 2B). The mitochondria were observed in two morphological stages differing in size and shape. The major one consisted of swollen mitochondria with "flocculent" matrix; cristae were damaged or residual and the outer membrane was ruptured. A few mitochondria still maintained an intact outer membrane. No significant differences are observed between Stage C (Fig. 2B) and Stage D (Fig. 2C). However, in Stage D the mitochondria appear to be less swollen with dense matrix and, in addition, hollow mitochondria are noticeable. These findings may clarify why in the light absorption measurements (Fig. 1) a decrease in absorbance was observed between Stages A and C and an increase between Stages C and D.

The corresponding negatively stained preparations (Fig. 3) reveal similar phenomena to those of sectioned preparations. In the intact mitochondria of Stage A (Fig. 3A), outer membrane and cristae are clearly visible. Since no clear differences have been observed between A and B, the latter is not displayed. However, in mitochondria in Stage C (Fig. 3B), residual cristae tend to aggregate into clusters attached to the inner part of the thickened outer membrane. In Fig. 3C, which corresponds to Stage D, most mitochondria lack an outer membrane and clusters of cristae aggregate so as to expose subunit particles. It is possible that these subunit particles are artifacts of negative staining[12].





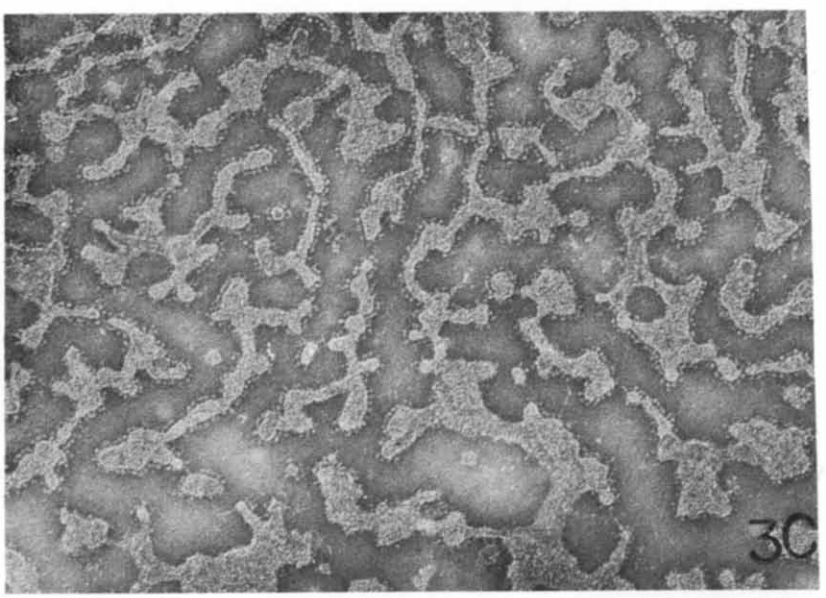


Fig. 3. Negative stained preparations. (A) Control (Stage A). (B) Mitochondria treated with 50 μg Δ^1-tetrahydrocannabinol per mg protein (Stage C). (C) Mitochondria treated with 100 μg Δ^1-tetrahydrocannabinol per mg protein (Stage D). A, $\times$ 13 500; B and C, $\times$ 48 000.

The observations reported in the present study indicate that Δ^1-tetrahydrocannabinol, which is very hydrophobic, affects the configurational integrity of rat liver mitochondria in three different stages, to a degree depending upon the drug concentration. The ultrastructural examinations of the mitochondrion con-

firmed the existence of configurational alterations, as assumed from the observed changes in absorbance, ATPase activity and oxygen uptake. These alterations were shown to consist mainly of a derangement of the inner mitochondrial structure.

At high concentrations of Δ^1-tetrahydrocannabinol, inhibition of both ATPase activity and oxygen uptake occur, a finding which points to the appearance of membrane-disruptive effects of Δ^1-tetrahydrocannabinol. The concentration-dependent (enhancing and inhibitory) action of Δ^1-tetrahydrocannabinol resembles the effect of detergents upon (Na^+–K^+)-ATPase in microsomal fraction[13]. Furthermore, such biphasic effects have been detailed explicitly for both erythrocyte and mitochondrial membranes[14]. In this connection it is also of interest to note that similar concentrations of Δ^1-tetrahydrocannabinol have no disruptive effect upon erythrocyte membranes[15].

Although this study substantiates the assumption that Δ^1-tetrahydrocannabinol reacts with mitochondrial membrane, it remains unclear whether the primary sites are specific enzymes. To answer this question, more has to be known about the mechanisms of oxidative phosphorylation and the role of mitochondrial membranes. It might be postulated that since mitochondrial membrane is considered to be composed of protein–phospholipid complexes, and, taking into account the findings of Wahlqvist *et al.*[5] that 80–95 % of Δ^1-tetrahydrocannabinol is adsorbed by lipoprotein fractions, Δ^1-tetrahydrocannabinol interacts with such specific sites in the membrane. These, in turn, might be responsible for the mitochondrial structure or its permeability and thus affect electron transport. More work is in progress concerning the effect of Δ^1-tetrahydrocannabinol on enzymatic properties of the inner and outer membranes of rat liver mitochondria treated by Δ^1-tetrahydrocannabinol.

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