protein synthesis [18, 19]. Thus, verification of enzyme saturation after prolonged drug exposure will require direct measurement of bound and free intracellular drug levels.

In summary, human SCLC cell lines are able to synthesize MTX polyglutamate derivatives with two to five additional γ-glutamyl groups. As has been reported in human breast cancer cell lines [11, 15], higher molecular weight derivatives with more than four glutamyl groups were selectively retained in the absence of extracellular drug, while MTX-Glu_1 and lower molecular weight polyglutamates effluxed rapidly. Polyglutamation of MTX is an important determinant of cytotoxicity, since prolonged drug retention above intracellular binding capacity was necessary for cytotoxic effect.

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Inhibition of mouse lymphocyte 5′-nucleotidase by cannabinoids: a physical toxicity effect

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Cannabinoids have very high partition coefficients for distribution between non-aqueous and aqueous phases. Their affinity for cell membranes results in the inhibition of a number of plasma membrane-bound enzymes when the drugs are present at relatively low concentrations in the aqueous phase. Among the membrane-bound enzymes inhibited are (Na^+–K^+)ATPases (EC 3.6.1.3) of mouse brain synaptosomes [1], human and rat erythrocytes, rat liver mitochondria [2] and rat ileum [3]. Other membrane enzymes inhibited include rat brain and heart mitochondrial NADH dehydrogenases [4], mouse neuroblastoma adenyl cyclase [5], mouse brain synaptosome and mouse lymphocyte acyl coenzyme A:lysophosphatidylcholine acyl transferase (LPC acyl transferase, EC 2.3.1.23) [6], and mouse lymphocyte acyl coenzyme A:lysophosphatidate acyl transferase (LPA acyl transferase) [7]. The ectoenzyme 5′-nucleotidase (EC 3.1.3.5) is present on the outer surface of mammalian plasma membranes, and the mouse lymphocyte enzyme can be inhibited by large hydrocarbons [8]. The widespread occurrence of this membrane enzyme suggests that it could be a useful model for the study of the inhibition of membrane-bound enzymes. In this study we have investigated the inhibition of 5′-nucleotidase by cannabinoids and other hydrocarbons to determine whether such inhibition is consistent with physical toxicity or if there are specific stereochemical requirements for inhibition.

Lymphocyte suspensions were prepared from spleens of 6 to 8-week-old male Swiss–Webster mice. The spleens were gently homogenized with a Teflon–glass homogenizer in 10 ml of cold Hanks' balanced salt solution (HBSS), and connective tissue was removed by filtering the homogenate through a fine mesh brass screen. The filtrate was centrifuged at 4° for 10 min at 280 *g*. Erythrocytes were removed according to the method of Boyle [9]. The resulting cell pellet was resuspended in 10 ml HBSS, and adherent cells were removed by passage through a short presoaked column of glass wool. The filtrate was centrifuged for 10 min at 280 *g* and 4°. This final pellet of lymphocytes was resuspended in RPMI 1640 medium with 25 mM HEPES (*N*-2-hydroxyethyl-piperazine-*N*′-2-ethanesulfonic acid) at

a concentration of 5.7×10^7 cells/ml.

Inhibition of 5′-nucleotidase by a variety of cannabinoids and other alcohols was assayed using [^{3}H]adenosine 5′-monophosphate as substrate. Aliquots of the cell suspension (175 μl) containing 10^7 cells were incubated with compounds dissolved in 5 μl dimethyl sulfoxide (DMSO). The final concentrations of compounds tested ranged from 7×10^{-6} M to 4×10^{-5} M. The lymphocytes were incubated with drugs or with DMSO only, for 30 min at 37°. 5′-Nucleotidase activity was assayed by the addition of 20 μl of a stock solution which gave final concentrations of 1 mM [^{3}H]-5′-AMP (0.2 μCi), 5 mM Mg^{2+} and 5 mM *p*-nitrophenylphosphate, an alternate substrate for non-specific phosphatases. Substrate hydrolysis was carried out for 30 min at 37° and the reaction was stopped with 0.15 ml of 0.15 M $ZnSO_4$. Protein and remaining 5′-AMP were precipitated by addition of 0.15 ml of 0.15 M $Ba(OH)_2$. After centrifugation at 8500 *g* for 1 min, the supernatant fractions containing free [^{3}H]adenosine were counted by liquid scintillation spectrometry.

Table 1 shows the concentrations of alcohols and cannabinoids that gave 50% inhibition of mouse spleen lymphocyte 5′-nucleotidase. With an increase in the molecular volume, V_x, of the alcohol, there is an increase in the concentration of the compound in the cell membrane lipids (biophase). At high molecular volumes there was an insignificant amount of the drug in the aqueous phase, and so a 50% inhibition is given at similar concentrations for all compounds, in the absence of specific interactions. There was no evidence of specific inhibition by any of the compounds tested. The psychoactive cannabinoids Δ^9-tetrahydrocannabinol (Δ^9-THC), (−)-Δ^8-THC, synhexyl Δ^9-THC, and dimethylheptyl Δ^9-THC did not show any greater potency in inhibition of the membrane enzyme than the other large alcohols, including the non-psychoactive cannabinoids. The psychoactive stereoisomer (−)-Δ^8-THC

Table 1. Molar volumes and biological activities*

Compound	(36,000 $V_x - E_B$)	K_i (M)
1 Butanol	1.43	7.58×10^{-2}
2 Hexanol	2.45	5.75×10^{-3}
3 Octanol	3.46	5.75×10^{-4}
4 Nonanol	3.97	2.45×10^{-4}
5 Decanol	4.55	1.15×10^{-4}
6 Dodecanol	5.48	6.92×10^{-5}
7 Transretinol	8.38	7.58×10^{-5}
8 11-Hydroxy Δ^9-THC	6.88	1.46×10^{-5}
9 Cannabinol	7.57	1.58×10^{-5}
10 Δ^9-THC	7.87	1.65×10^{-5}
11 (−)-Δ^8-THC	7.87	1.76×10^{-5}
12 (+)-Δ^8-THC	7.87	2.24×10^{-5}
13 Synhexyl Δ^9-THC	8.38	2.8×10^{-5}
14 Cannabidiol	8.71	1.71×10^{-5}
15 Cannabigerol	9.11	1.33×10^{-5}
16 Dimethylheptyl Δ^9-THC	9.90	1.21×10^{-5}

* Values of K_i for compounds 1–7 were determined by Sharom and Mellors [8]; estimates of molecular volume (V_x) and interaction terms (E_B) were made as described previously [8, 10].

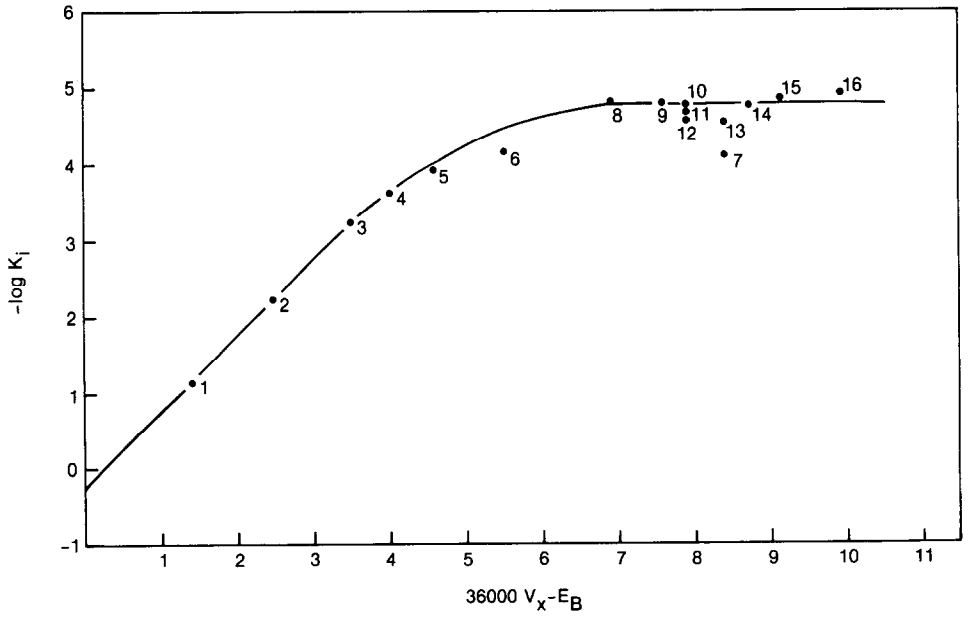

Fig. 1. Molar volume and inhibition of lymphocyte 5′-nucleotidase by cannabinoids and other lipophilic compounds. Numbers refer to specific compounds listed in Table 1.

showed no more inhibition of 5′-nucleotidase than the non-psychoactive (+)-Δ^8-THC. The very lipophilic synthetic cannabinoid dimethylheptyl Δ^9-THC was not significantly more inhibitory than the other cannabinoids.

It has been observed that the partition coefficient (x) of a compound between an organic phase and water can be estimated from the characteristic volume V_x by the relationship

$$\log_{10}x = kV_x - E_B \quad (1)$$

where k is a constant and E_B is an interaction term to account for specific interactions (usually by polar groups) which increase the solubility of the compound in one or both liquid phases [11]. The constant k is close to 36,000 moles M^{-3} at 298° K for most organic liquids and is largely determined by the properties of water. The relationship (1) has been used to estimate partition coefficients of steroids between ether and water [10]. From Equation 1 an expression has been derived [12] relating the toxic dose C_t of drug to its characteristic volume V_x:

$$C_t = A + [B \times 10^{-36{,}000\ V_x} + E_B] \quad (2)$$

In Equation 2 A and B are constants for the system and the ratio A/B is equal to the ratio of volume of non-aqueous phase/volume of aqueous phase.

In Fig. 1 the $-\log K_i$ values for the inhibition of 5′-nucleotidase have been plotted as a function of the characteristic volume V_x. The plot indicates that the inhibition follows that expected for physical toxicity with the curve having the form of Equation 2. Only dodecanol and trans-retinol show much deviation from the curve, in both cases giving less inhibition than expected for physically toxic compounds. When Equation 2 is applied to the curve of Fig. 1, the value of the constant A is 1.78×10^{-5} moles/l and the value of the constant B is 1.78 moles/l, this being the concentration of the drugs required in the biophase for 50% inhibition. The value of $A/B = 1 \times 10^{-5}$, which from Equation 2 is the ratio of the volume of the biophase to the volume of cell suspension, so that 10 μl of biophase is present in 1 liter of cell suspension. This is in accord with the calculated volume of plasma membrane lipid in lymphocyte suspensions [8].

The lack of specificity in the inhibition of mouse lymphocyte 5′-nucleotidase by cannabinoids does not exclude the possibility that neuronal 5′-nucleotidase may be susceptible to specific effects. Similar physicochemical correlations may be applied to the inhibition of other membrane-bound enzymes such as brain ATPases or 5′-nucleotidase by cannabinoids to determine whether such effects are related to the psychoactivity of these compounds.

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Quipazine and induction of adrenal enzymes

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Two enzymes of the rat adrenal medulla, tyrosine hydroxylase (TH, EC 1.14.16.2), the rate-limiting enzyme in the biosynthesis of catecholamines, and ornithine decarboxylase (ODC, EC 4.1.1.17), the first enzyme in the polyamine biosynthetic pathway, are induced following repeated administration of dopaminergic agents [1–5]. These drugs act supraspinally. There is also evidence that serotonin-containing fibers originating in the medial raphe nucleus play a role in this process [4, 6].

Another type of drug that acts supraspinally in the rat to cause an increase in adrenal TH activity is the piperazine-substituted quinoline derivative quipazine [7]. This substance has a variety of actions relating it to central dopamine functions [8–10]. Nevertheless, the stimulatory action of quipazine on adrenal TH activity does not immediately fit with its other effects that are related to serotonin functions: binding to postsynaptic sites that mediate inhibitory action of this amine [11, 12]; facilitation of serotonin release [13]; inhibition of its uptake [14]; and inhibition of type A monoamine oxidase [15], the form of this enzyme that acts physiologically on serotonin.

To resolve this apparent contradiction in the action of quipazine, we have tested this drug in animals previously given a serotonin-receptor blocking agent in order to leave the dopamine-sensitive sites available. The blocker we have used is methiothepin, a substance shown earlier [2] to cause