Fig. 1 for subject GE, whose bile was collected most frequently, there was a delay before the radioactivity appeared in the bile samples. This delay may illustrate the approximate time needed for the excreted bile to flow from the liver to the sampling barrels. The mean value of bile flow during the sampling period was 11 ml/hr for GE, 27 ml/hr for ILE and 14 ml/hr for ÅL. Of the administered dose GE excreted 0·36% in bile within 24 hr, ILE 0·68% within 2·5 hr and ÅL 0·73% within 24 hr. The fraction of unchanged terbutaline in serum was of similar magnitude as in an earlier intravenous study in human volunteers.[7] Initially the fraction of unchanged drug in serum for GE and ILE was high; mean 89%, and then declined to a mean of 50% 90 min after injection. No serum was obtained from patient ÅL.

The radioactivities recovered in urine were 59% of the dose (GE) and 72% (ÅL) in 24 hr, of which unchanged drug constituted 50% and 63%, respectively. ILE excreted 66% of the dose in urine within 3 hr, of which 77% appeared as unchanged drug. These data are also in accordance with the results obtained in human volunteers free from disease.[7]

The amount of terbutaline in human bile is of a similar magnitude to that found in dog, where approximately 2% of the i.v. dose was excreted, but differs from rat, which excreted approximately 40% of i.v. terbutaline in the bile. The present result suggests that biliary excretion of terbutaline after parenteral administration is of minor importance for the fate of the drug in man.

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Effect of hashish compounds on rat liver lysosomes *in vitro*

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Δ^1-TETRAHYDROCANNABINOL (Δ^1-THC) is the major psychoactive compound of hashish. Its precursor in synthesis and probably also in biogenesis, cannabidiol, does not exhibit psychoactive effects.[1]

The effect of hashish compounds on rat and human erythrocytes[2,3] and on rat liver mitochondria[4,5] has been recently described. A paramount feature of the effect is an interaction between the hashish compounds and the membranes of these cells or organelles. It has been shown that 1·5 hr after intraperitoneal injection of ^{14}C-tetrahydrocannabinol to a rat, most of the radioactivity accumulates in the liver.[6] Thus it was of interest to study the effect of hashish components on rat liver lysosomes, with a particular emphasis on a possible effect on the integrity of the lysosomal membrane.

Male CR strain rats (100–300 g) were starved for 16–24 hr and killed by skull fracture. Livers were excised and rinsed with ice cold 0·45 M sucrose, 0·5 mM in EDTA, pH 7·2. The livers were minced and homogenized in 2·5 volumes of the same medium with a Teflon homogenizer. The homogenate was further diluted with the above medium to a final dilution of 1/8 (w/v), filtered through cheese cloth and subjected to differential centrifugation at 4°. The homogenate was centrifuged at 650 *g* for 10 min and the supernatant was centrifuged at 3300 *g* for 10 min. The 3300 *g* supernatant fraction was then centrifuged for 20 min at 16300 *g*. The resulting pellet was resuspended in ice cold 0·6 M sucrose, 0·5 mM in EDTA, pH 7·2, and centrifuged for 30 min at 5900 *g*. The 5900 *g* supernatant fraction was centrifuged again at 16300 *g* for 20 min and the pellet constituting the lysosomal fraction was resuspended in 0·25 M sucrose pH 7·0 to give a final concentration of 24 mg protein per ml.

The effect of hashish components on lysosomal integrity was followed by measuring the release to the medium of acid phosphatase. About 0·5 mg of lysosomal protein (20 μl of stock suspension) was diluted into 1 ml of 0·15 M sucrose (BDH), 0·01 M Na-Hepes pH 7·0 (Sigma) and the tested hashish component (obtained from Prof. R. Mechoulam, School of Pharmacy, The Hebrew University, Jerusalem) at the specified concentration. The hashish components were dissolved in methanol. All incubation mixtures including the controls contained 10 μl of methanol. The lysosomal suspensions were incubated for 15 min at 37°, cooled to 4°, centrifuged at 14,500 *g* for 20 min and the resulting supernatants were assayed for acid phosphatase activity. *p*-Nitrophenyl phosphate (Sigma) 100 μl of 0·1 M was added to each sample (0·9 ml of supernatant) and the rate of *p*-nitrophenol release at 37°, was followed at 420 nm in a Gilford Recording Spectrophotometer 2400-S. Acid phosphatase activity in the supernatants is expressed as percentage of total activity obtained in the presence of 0·1% of Triton X-100. The values were corrected for rates obtained at zero time incubation.

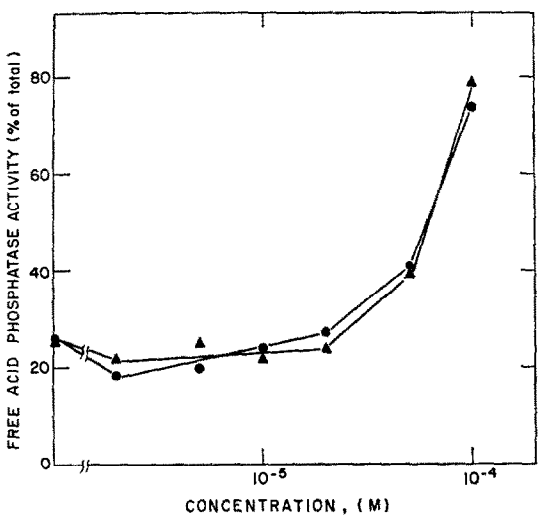

FIG. 1. Effect of Δ^1-THC (●) and of Cannabidiol (▲) on the release of acid phosphatase from lysosomes. Experimental details are given in the text.

Figure 1 shows that both Δ^1-THC and cannabidiol at a concentration of 2×10^{-6} M decrease the extent of acid phosphatase release from lysosomes suspended in hypoosmotic medium. Relative to the total acid phosphatase activity this decrease appears to be small, but when related to the control level, the extent of decrease is indeed appreciable. At concentrations exceeding 2×10^{-5} M both compounds damage lysosomal integrity releasing up to 80% of lysosomal acid phosphatase into the medium. The data given in Fig. 1 are representative of five experiments, all showing essentially an identical pattern.

At hypoosmolar conditions lysosomes are more labile than at isoosmotic conditions. Hashish components were found to have the same damaging effect on the integrity of lysosomes when the latter were suspended in 0·25 M sucrose as under hypoosmolar conditions. Lysosomal turbidity often serves as an indication of their integrity. Upon incubation with hashish components a rapid (less than 10 min) decrease in lysosomal turbidity (measured at 540 nm) was observed.

No significant difference in either the concentration range needed or the damaging effect of the two components tested could be detected under the experimental conditions employed. Both stabilization

and rupture of lysosomal membranes by other fat soluble compounds including vitamins and steroids are well documented.[7]

Δ^1-THC and cannabidiol interact with the erythrocyte membrane in a way leading to protection against hypotonic hemolysis at a concentration range[3] at which they cause total rupture of lysosomal integrity. The marked difference in the effect of the two components on the two membrane limited structures might be explained by a higher expending capacity of the erythrocyte membrane (inferred from measurements of increase in volume as a result of the interaction with hashish components)[3] as compared to that of the lysosomal membrane.

Though the action of Δ^1-THC and cannabidiol does not seem to be specific, the irreversible damage and release of lysosomal enzymes may be of biological significance. A high concentration of these components in the liver or the nervous system may lead, aside from the psychomimetic effect, to permanent damage on the cellular level. When testing Δ^1-THC on cells and subcellular organelles and inferring from its action on the psychomimic phenomenon it seems worthwhile to test also non psychoactive components as cannabidiol as it gives an indication to the degree of specificity of the biological effect.

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Effect of an amphetamine derivative on rat adipose tissue lipolysis and glycerol utilization *in vitro*

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NUMEROUS synthetic analogues of the naturally occurring sympathomimetic amines have been shown to possess varying capacities for mobilizing fat.[1] One of these analogues, amphetamine, may exert its fat-mobilizing action by releasing endogenous catecholamines or through an effect on serotonin receptors.[2–4] We have studied the effect of an amphetamine derivative, the chlorhydrate of [(methyl-1 phenyl-2) ethylamine]-3 propionitrile (CMPEP) on adipose tissue metabolism *in vitro*. As we have observed previously that glycerol utilization by adipose tissue is considerably higher than previously thought,[5,6] in this study we have determined the effect of CMPEP on the *in vitro* production and utilization of glycerol by rat epididymal fat-pads.

Pieces of epididymal fat pads from fed rats were incubated at 37° for 120 min in Krebs–Ringer bicarbonate buffer, pH 7·4 in the presence of 10 mg/ml of bovine albumin purified according to Chen,[7] 0·5 μCi/ml of (1-^{14}C) glycerol (15·3 mCi/mmole) and different concentrations of CMPEP (gift of Roussel-Amor Gil laboratorios). Details of the incubation and the processing of the samples have already been described.[5,6] As it is shown in Table 1, the amount of glycerol formed by the