ent doses of clonidine. Thus, at a peripheral level, low doses of clonidine stimulate adrenergic receptors whereas high doses (1 mg/kg) cause adrenolytic effects [14].

Anden *et al.* [3] have shown that clonidine (100 μg/kg) reduced slightly but significantly DA disappearance from the whole brain after inhibition of its synthesis by α-methyltyrosine. Since clonidine had no stimulating effect on dopaminergic receptors [3, 15], it was suggested that NA receptor stimulation directly or indirectly may inhibit the neural activity in the central DA neurons. The present work does not support this hypothesis since our results indicate that clonidine does not alter significantly DA synthesis in the striatal dopaminergic neurons.

L. ROCHETTE and J. BRALET
Laboratoires de Physiologie pharmaceutique et Pharmacodynamie,
Facultés de Médecine et Pharmacie,
7 Bd. Jeanne d'Arc, 21 000 Dijon, France

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Δ^9-Tetrahydrocannabinol—Uptake by rat liver lysosomes*

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Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the main psychoactive constituent of marihuana and hashish [1]. The liver appears to be a major site for the accumulation of Δ^9-THC and its metabolites, which become associated with the particulate subcellular fractions [2]. We have recently shown that Δ^9-THC has a marked disruptive effect on rat liver lysosomes *in vitro* which causes the release of hydrolytic enzymes from this organelle [3]. To determine the distribution *in vivo* of Δ^9-THC and its metabolites within liver cells, we administered ^{14}C-Δ^9-THC to rats and have observed that liver lysosomes become highly labeled with ^{14}C-radioactivity compared to other subcellular fractions.

Female Wistar rats (150–200 g) were each given 1 μCi ^{14}C-Δ^9-THC in 0·05 ml of 95% ethanol by intravenous injection. In addition, each animal received an intraperitoneal injection of Triton WR 1339 (85 mg/100 g body wt) in sterile isotonic saline 4 days prior to sacrifice. At 5 min, 15 min, 1, 3, 18 and 122 hr after the injection of ^{14}C-Δ^9-THC, groups of four rats were sacrificed by decapitation.

Separate fractionations of subcellular particles were carried out on each rat liver by the procedure of Trouet [4] as modified by Leighton [5] *et al.* for the isolation of Triton WR 1339-filled rat liver lysosomes. Subcellular fractions were analyzed for protein [6] and for the lysosomal enzyme β-*N*-acetylglucosaminidase [7]. Samples were digested using NCS solubilizer (Amersham–Searle, Arlington Heights, Ill.) and diluted in Aquasol (New England Nuclear, Boston, Mass.) for determination of ^{14}C-radioactivity by liquid scintillation counting.

The Δ^9-THC radioactivity is taken up rapidly by the liver, so that the whole liver contains at 5 min 8·7% of the dose, at 15 min 15·5% at 1 hr 7·5%, at 3 hr 7·8%, at 18 hr 3·9% and at 122 hr 0·8%. Figure 1 shows that this radioactivity is concentrated in the lysosomes at all time periods studied. All other subcellular fractions show lower uptakes of radioactivity, not greatly differing in specific activity (dis/min/mg of protein) from the homogenate. Lysosomes, however, have a specific activity five times that of the homogenate within 5 min of the injection of ^{14}C-Δ^9-THC. This specific activity for the isotope in lysosomes increases to a maximum of 17-fold compared to the homogenate at 18 hr after the injection, and thereafter the specific activity declines slowly.

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Table 1. Recovery of radioactivity within lysosomes after ^{14}C-Δ^9-THC injection compared to the recovery and purification of the lysosomal marker enzyme β-N-acetylglucosaminidase*

Time	n	Radioactivity $\frac{^{14}C \text{ in lysosomes}}{^{14}C \text{ in homogenate}} \times 100$	Relative purification	β-N-acetylglucosaminidase $\frac{\text{Total activity in lysosomes}}{\text{Total activity in homogenate}} \times 100$
5 min	3	5·0 ± 0·3	25 ± 3	11 ± 2
15 min	3	4·6 ± 1·5	30 ± 1	26 ± 8
1 hr	4	3·7 ± 1·5	23 ± 9	14 ± 4
3 hr	4	5·0 ± 3·6	30 ± 11	17 ± 4
18 hr	4	9·3 ± 1·2	27 ± 3	15 ± 1
122 hr	4	5·0 ± 1·9	22 ± 4	12 ± 4

* The number of separate experiments at each time interval is given by n. The relative purification for the enzyme is the specific activity of the lysosomal fraction (L) divided by that of the homogenate.

Table 1 shows that the maximum percentage of the total liver radioactivity found within lysosomes corresponds to the time at which maximum ^{14}C-specific activity is observed, namely at 18 hr after the injection of ^{14}C-Δ^9-THC. Table 1 also shows that fraction L is rich in lysosomes as indicated by the degree of purification of the lysosomal marker enzyme β-N-acetylglucosaminidase. The mean relative purification of this enzyme for 22 lysosomal fractions compared to the homogenates was 26·0 ± 6·4, corresponding to a mean enzyme specific activity of 0·45 ± 0·14 μmole substrate hydrolyzed/min/mg of protein. The mean percentage recovery of the enzyme in the lysosomal fractions was 15 ± 6 per cent.

Some of the radioactivity within the liver and its lysosomes will be in the form of 11-hydroxy-Δ^9-THC, the major metabolite of Δ^9-THC in animal tissues [8]. Assuming that all of the radioactivity is due to these two compounds, we can calculate that their combined mean molar concentration 15 min after the injection of 4·8 moles Δ^9-THC is 4·5 pmoles/mg of protein in the liver, and 28·3 pmoles/mg

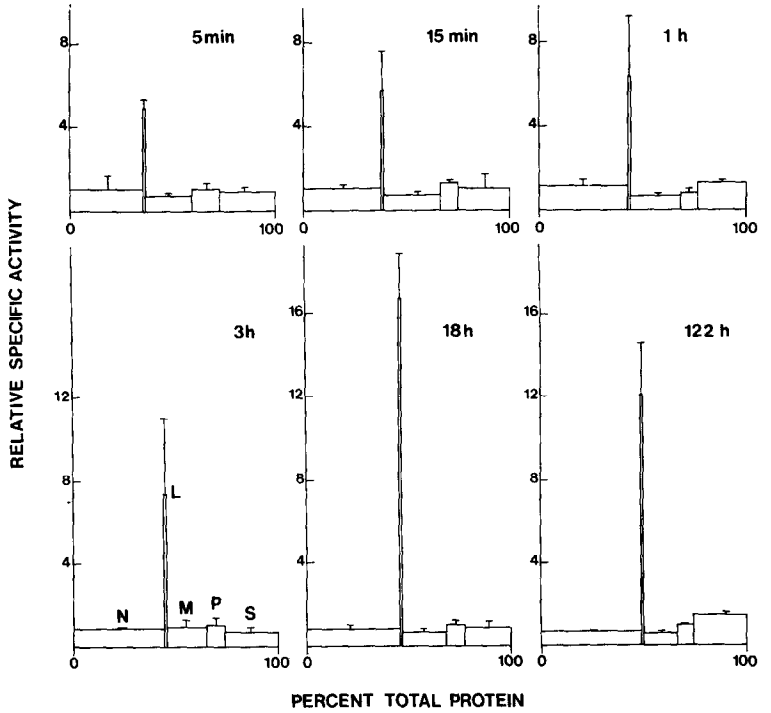

Fig. 1. Distribution of ^{14}C-radioactivity within rat liver subcellular fractions at various times after the administration of ^{14}C-Δ^9-tetrahydrocannabinol. The relative specific activity is the specific radioactivity of each fraction divided by that of the homogenate. Means are shown, with bars for the standard deviation, for three or four separate subcellular fractionations at each time period as specified in Table 1. Fractions are labeled as follows: N, nuclear and cell debris; L, Triton WR 1339-filled lysosomes; M, remaining protein from the sucrose density gradient consisting mainly of mitochondria; P, endoplasmic reticulum; and S, soluble fraction.

of protein in the lysosomal fraction. These are low concentrations resulting from very small doses of Δ^9-THC. It has been estimated that a chronic user of marihuana or hashish may ingest several hundred mg of Δ^9-THC/day [9]. We would expect from the present study that substantial quantities of cannabinoid metabolites would accumulate within tissue lysosomes in these individuals. Our previous studies have indicated that Δ^9-THC and related cannabinoids have a disruptive effect on rat liver lysosomes *in vitro* at concentrations from 25 μM to 1·0 mM [3].

These observation may explain some of the clinical evidence that chronic marihuana use leads to hepatotoxicity and cirrhosis [10]. Damage to lysosomes by cannabinoids may also be the basis of the reduced cellular immunity seen in chronic users of marihuana and hashish [11], since cellular immunity is mediated in part through lysosomal involvement in the immune response [12].

Department of Chemistry,
University of Guelph,
Ontario N1G 2W1, Canada

JEAN E. IRVIN
ALAN MELLORS

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Comparison of the inhibitory effects of propargylamine and pargyline on brain and liver monoamine oxidase activity*

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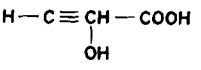
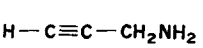
Studies on the mechanism of action of flavoproteins have led to the conclusion that substrate analogs containing an acetylenic function α,β to the carbon which undergoes oxidation are irreversible inactivators [1, 2]. We have shown that α-OH-butynoic acid (I) inactivates lactate oxidase [1], and that propargylamine (II) inactivates beef mitochondrial amine oxidase. These compounds form a covalent complex between the inactivator and the enzyme-bound flavin.

Pargyline (III) is a well-established inactivator of mitochondrial amine oxidase [3], and it has recently been shown that this compound forms an adduct with flavin [4]. It is very probable that propargylamine and pargyline act through the same mechanism and that this mechanism involves the reactivity of the acetylenic group. Our previous studies have shown that propargylamine is a potent inhibitor of monoamine oxidase (MAO) activity in intact pituitary cells in culture [5]. The purpose of the present investigation was to extend these observations on propargylamine to the intact animal, and to determine if the structural differences between pargyline and propargylamine affect their activities *in vivo* on mouse brain and liver MAO.



(I)



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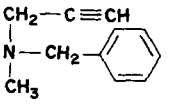

(III)

Albino mice (Swiss Webster strain) of both sexes, weighing 23–30 g, were obtained from Charles River Laboratory. Propargylamine or pargyline was injected intraperitoneally at doses of either 250 or 500 μg/mouse. Nine separate experiments were performed, and similar results were obtained in

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