Short communications

Brain levels of Δ^1-tetrahydrocannabinol and its metabolites in mice tolerant to the hypothermic effect of Δ^1-tetrahydrocannabinol

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Repeated voluntary consumption of cannabis in milk (approximately 8 mg per day for 15 days) produced in mice tolerance to the hypothermic effect of tritiated Δ^1-tetrahydrocannabinol ([^{3}H]-Δ^1-THC ; 2 mg/kg i.v.) but not to the effect of [^{3}H]-Δ^1-THC on immobility index. The development of tolerance was not accompanied by any detectable change in levels of radioactivity in the brain assigned to Δ^1-THC or its metabolites. It was concluded that changes in metabolism or distribution of Δ^1-THC are not responsible for tolerance at least to the hypothermic effect of Δ^1-THC in mice.

Numerous reports exist (Paton & Pertwee, 1973) describing how repeated treatment with cannabis extract or with Δ^1-tetrahydrocannabinol (Δ^1-THC) can cause a wide range of species to become tolerant to several of the effects of Δ^1-THC. Repeated treatment with Δ^1-THC *in vivo* has also been found to increase the capacity of rat liver to metabolize Δ^1-THC *in vitro* (Ho, Estevez & Englert, 1973). One of the metabolites of Δ^1-THC, 7-hydroxy-Δ^1-THC, is itself pharmacologically active (Christensen, Freudenthal, Gidley, Rosenfeld, Boegli, Testino, Brine, Pit & Wall, 1971) and contributes significantly towards the 'catalepsy' observed in mice injected with the parent compound (Gill, Jones & Lawrence, 1973). It is possible therefore that the development of tolerance to Δ^1-THC is caused, at least in some species and to some of the effects of Δ^1-THC, by alterations in patterns of metabolism or distribution, resulting in changes in the brain levels of Δ^1-THC and its metabolites. This possibility was explored by measurement of the effects of [^{3}H]-Δ^1-THC on the immobility index and body temperature of mice which had been repeatedly pretreated with cannabis and by determination of the brain levels of [^{3}H]-Δ^1-THC and its metabolites immediately after the bioassays had been completed.

Methods.—The experiment was carried out at an ambient temperature of 20 to 22° C with adult male white mice (Tuck No. 1 strain) initially weighing 20 to 25 grams. Each mouse was kept in a separate cage and received food and water *ad lib*. The experiment was divided into two phases. In phase 1, 12 mice were each supplied daily for 15 days with either 10 ml of milk or 10 ml of a freshly prepared dispersion of cannabis in milk. The solutions were taken voluntarily from drinking bottles. Six mice were assigned to each treatment. The dispersion was prepared (Paton unpublished) from the petrol-ether soluble fraction of an ethanolic tincture of cannabis (BPC 1949) containing by weight 6·4% of Δ^1-THC (Paton & Pertwee, 1972). The amount of petrol-ether soluble material present in each millilitre of milk was that obtained from 1·0 mg of whole extract. Phase 2 of the experiment took place on the 16th day. Each mouse received an intravenous injection of [^{3}H]-Δ^1THC. This had a specific activity of 584 mCi/mmole ($4{\cdot}13 \times 10^6$ (d/min)/μg) and was synthesized by Gill & Jones (1972a). The [^{3}H]-Δ^1-THC was dispersed in a mixture of Tween 80 and a 0·9% NaCl solution, the dispersion containing by weight 5 parts of Tween to 1 part of Δ^1-THC. The dose and volume of Δ^1-THC injected were respectively 2 mg/kg and 0·2 ml/25 gram.

The effect of [^{3}H]-Δ^1-THC on the immobility index was measured 15 min after the injection, by the ring test (Pertwee, 1972). In addition, rectal temperatures of mice were measured just before injection of [^{3}H]-Δ^1-THC and just after completion of the ring test. Temperature measurements were made with a thermistor which was inserted 3 cm into the rectum. Immediately after the second temperature measurement, the mice were killed with carbon monoxide. Levels of [^{3}H]-Δ^1-THC and its metabolites in individual whole brains and in blood samples were measured by a method described elsewhere (Gill & Jones, 1972b ; Jones & Pertwee, 1972). Two pooled

blood samples were used, each collected from 6 mice.

Limits of error were expressed as standard error of the means and differences between means were evaluated by Student's t test ($P<0.05$).

Results.—Both groups of mice consumed approximately the same volume of milk over 15 days. The group receiving cannabis took 8.3 ± 0.30 ml per mouse per day and the control group 8.7 ± 0.30 ml per mouse per day. The mean body weights of the two groups rose over the 15 days from 22 ± 0.6 g to 30.4 ± 0.6 g (cannabis) and from 23.5 ± 0.5 g to 31.9 ± 0.7 g (control group).

$\triangle^1$-THC produced a signficant degree of hypothermia in the control group, the mean fall in temperature being 2·9° C (Table 1). On the other hand, mice that had taken cannabis had become tolerant to the hypothermic effect. In this group, $\triangle^1$-THC produced a slight insignificant rise of 0·7° C in mean body temperature.

In contrast to these findings, tolerance to the effect of $\triangle^1$-THC on the immobility index had not completely developed in the cannabis group. The difference observed between the mean indices of the two groups of mice was not significant. The radioactive spots obtained by paper chromatography of blood and brain extracts were assigned provisionally to $\triangle^1$-THC, 7-hydroxy-$\triangle^1$-THC, 'polar metabolite' and 'non-extractable' material as described elsewhere (Gill & Jones, 1972b ; Jones & Pertwee, 1972). The table shows that with one apparent exception, the level of radioactivity in the brain and blood associated with each of these materials, was the same in the two groups of mice. The exception is the level of radioactivity assigned to 'non-extractable' material which differed in the two blood samples by a factor of 1·6. However, the wide limits of error associated with previous determination of the levels in blood of radioactivity assigned to 'non-extractable' material (Gill & Jones, 1972b) make it unlikely that this difference is significant.

The question arose as to whether the difference observed between the body temperatures of control and cannabis pretreated mice had itself influenced the rate of metabolism of $\triangle^1$-THC so as to mask any association between tolerance and the levels of $\triangle^1$-THC and its metabolites in the brain. Phase 2 of the experimental procedure described above was therefore repeated with two fresh groups of 6 mice. The first group was kept at an ambient temperature of 20–22° C throughout the experiment but the second, after injection of [^{3}H]-$\triangle^1$-THC was kept at 30–32° C, the thermoneutral zone for mice (Herrington, 1940). Following injection with [^{3}H]-$\triangle^1$-THC, the mean body temperature of the group at 20–22° C fell significantly by 1.3 ± 0.5° C. In contrast, the temperature of the second group rose by 1.0 ± 0.3 C. The final temperatures of the two groups, respectively 36.4 ± 0.3° C and 38.5 ± 0.3° C were significantly different from each other. This difference was not accompanied by any significant difference in the blood or brain levels of radioactivity assigned to $\triangle^1$-THC, 7-hydroxy-$\triangle^1$-THC, 'polar metabolite' or 'non-extractable' material.

Discussion.—The results show that repeated consumption of cannabis extract can produce tolerance to the hypothermic effect of $\triangle^1$-THC in mice. They also suggest that tolerance to hypothermia develops more readily than tolerance to

TABLE 1. *The metabolism of* [^3H]-$\triangle^1$-*tetrahydrocannabinol* ($\triangle^1$-*THC*) in vivo *and the effect of* [^3H]-$\triangle^1$-*THC* (2 *mg/kg* i.v.) *on body temperature* (t_R) *and immobility index* (*II*) *following repeated consumption of cannabis or milk by* 2 *groups of* 6 *mice*

Pretreatment	Mean t_R(°C)		Mean II	Mean levels in brain or blood (picomoles/g wet weight)				
	Before $\triangle^1$-THC	20 min after $\triangle^1$-THC		non-extractable	polar	7-OH-$\triangle^1$	$\triangle^1$-THC	Total
Cannabis/ milk	37·4±0·3	38·1±0·2	36±6	387±100	117±11	253±19	1641±98	2399±151 Brain
				1356	367	81	1716	3631 Blood
Milk	37·7±0.4	34·8±0·3*	49±4	590±129	123±14	241±27	1641±144	2672±166 Brain
				864	449	100	2043	3605 Blood

*Rectal temperature after injection significantly less than temperature before injection ($P<0.05$). Means are expressed ± S.E.M.

the effect of Δ^1-THC on the immobility index. It is also apparent from the results that a difference in body temperature of 2° C has no detectable effect on levels of Δ^1-THC or its metabolites in mouse brain. It is therefore unlikely that the fall in body temperature of 2·9° C which was observed only in the control group of mice exerted any effect on the brain levels of Δ^1-THC or its metabolites. This conclusion rules out the possibility that changes in the metabolism or distribution of Δ^1-THC accompanied the development of tolerance to Δ^1-THC but became masked because of a difference in body temperature between tolerant and non-tolerant mice. Finally, the results indicate that tolerance, at least to the hypothermic effect of Δ^1-THC in mice, does not depend on changes in the metabolism or distribution of Δ^1-THC leading to changes in the brain levels of the drug and its metabolites. This finding is consistent with results obtained recently from experiments with pigeons (Dewey, McMillan, Harris & Turk, 1973).

This work was supported by the Medical Research Council. One of us (DKL) was the recipient of an MRC scholarship for Training in Research Methods.

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(*Received June* 21, 1973)