# The effect of $\Delta^9$-tetrahydrocannabinol, cannabidiol and cannabinol on ether anaesthesia in mice

A number of reports have shown that both cannabis extracts and $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) are able to prolong barbiturate anaesthesia in laboratory animals (Loewe, 1944; Bose, Saifi & Bhagwat, 1963; Garriott, King & others, 1967; Gill, Paton & Pertwee, 1970; Paton & Pertwee, 1972; Chesher, Jackson & Starmer, 1974). Also, the effect of cannabis on the duration of ether anaesthesia in mice has been reported in two studies, with conflicting results. Whilst Gill & others (1970) found cannabis to be inactive, Chesher & others (1974) reported that cannabis prolonged ether anaesthesia in mice. However, in both studies, extracts prepared from cannabis leaf were employed. In recent years an increasing number of reports have appeared which indicate that complex interactions exist between the cannabinoids (cannabidiol (CBD), cannabinol (CBN) and $\Delta^9$-THC) and barbiturate anaesthesia (Krantz, Berger & Welch, 1971; Chesher & others, 1974) as well as interactions on other pharmacological parameters (see Anderson, Jackson & Chesher, 1974; Chesher, Dahl & others, 1973). Because of these various reports, we decided to eludicate the effect of the pure cannabinoids on ether anaesthesia in mice, and to see if cannabinoid interaction was evidenced in this parameter.

The cannabinoids were prepared in a suspension as previously described (Chesher & others, 1974) to provide in all cases a mixture containing the cannabinoid in 5% propylene glycol in Lissapol-Dispersol (ICI, Whittle, 1964), to provide a dose-volume of 1 ml 100 $g^{-1}$. QS strain female mice (25–32 g) were medicated by gavage with the appropriate cannabinoid (or the vehicle) 20 min before an injection (i.p.) of an ether–olive oil mixture (1 : 12·5 v/v), dose-volume 2 ml 100 $g^{-1}$. Immediately after the ether injection, the animals were placed in a constant temperature cabinet at 31 $\pm$ 1° to prevent hypothermia (Chesher & others, 1974). The duration of anaesthesia was taken as the interval between the time of administration of the ether and the recovery of the righting reflex, which was checked in each case by placing the mouse on its back once again and the end point recorded only if the animal regained an upright position within 30 s.

The results are summarised in Table 1. Of the 91 controls tested (vehicle administration only), 17 failed to be anaesthetized within 15 min (18·7%) and these data were rejected. $\Delta^9$-THC produced a dose-dependent prolongation of ether anaesthesia up to the highest dose of 40 mg $kg^{-1}$. At this dose 4 of the 20 animals tested became cyanotic and died. The prolongation of anaesthesia produced by CBN at doses of 10 mg $kg^{-1}$ and above was not dose-dependent, and the prolongation was less marked as the dose was increased. In contrast, the effect of CBD suggested a reversal of ether anaesthesia. At a dose of 20 mg CBD $kg^{-1}$, only 4 of 15 mice tested were anaesthetized with ether, (73% were not anaesthetized) and the mean duration of anaesthesia of these animals was similar to that of the controls. At the lower dose (10 mg $kg^{-1}$) CBD was without effect on the duration of anaesthesia and the number of mice not anaesthetized by ether was similar to that observed in the control group (i.e. 15·8 and 18·7% respectively).

An investigation of cannabinoid interactions suggested that the effect of combining $\Delta^9$-THC and CBD (10 mg $mg^{-1}$ of each) was no different from the effect of $\Delta^9$-THC (10 or 20 mg $kg^{-1}$) alone, and was not antagonistic, whereas the effect of combining CBD with CBN appeared to reverse the effect of CBN. Whilst the interaction between THC and CBN appears to be one of addition, the data presented suggest that the ability of $\Delta^9$-THC and CBN to prolong ether anaesthesia may be mediated by different mechanisms. The effect of $\Delta^9$-THC was dose-dependent whilst that of CBN was not

and the interactions of these cannabinoids with CBN were different. CBD apparently did not influence the duration of anaesthesia with $\Delta^9$-THC plus ether but it reversed the prolongation of the anaesthesia produced by CBN plus ether. Whether this reversal by CBD of CBN plus ether-induced anaesthesia is due to a CBD-CBN interaction or whether it is due to the ability of CBD itself to prevent ether anaesthesia and thus to reverse the CBN-induced prolongation of ether anaesthesia, cannot be adjudged from the present data. However, the data confirm the results of Chesher & others (1974) that cannabis is able to prolong ether anaesthesia, and provides a probable explanation for the failure of Gill & others (1970) to find the same phenomenon. The latter authors used an extract containing 6·4% $\Delta^9$-THC and 3·6% CBD with no specified CBN content, while Chesher & others (1974) used an extract containing 22·8% $\Delta^9$-THC, 43% CBD and 34% CBN.

From the present data, and from other cannabinoid interaction studies (Chesher & others, 1973; Anderson & others, 1974; Chesher & others, 1974) it is clear that the ratio of cannabinoids present will influence the response with a particular pharmacological parameter. The interaction of the cannabinoids with ether is different to their interactions with pentobarbitone. Whilst both ether and pentobarbitone anaesthesia are potentiated by $\Delta^9$-THC (see above references), CBN is inactive in potentiating pentobarbitone anaesthesia but is active in prolonging ether anaesthesia. Moreover, whilst CBD is more potent than $\Delta^9$-THC in prolonging pentobarbitone anaesthesia (Loewe, 1944; Gill & others, 1970; Chesher & others, 1974), it is able to inhibit ether anaesthesia. Within-cannabinoid interactions vary also with the different anaesthetics; e.g. CBN antagonizes $\Delta^9$-THC + pentobarbitone anaesthesia in mice (Krantz & others, 1971) but is additive to $\Delta^9$-THC + ether-induced anaesthesia in the same species (Table 1).

Table 1. *The effect of $\Delta^9$-tetrahydrocannabinol, cannabinol and cannabidiol on ether anaesthesia in mice.* For procedural details, see text. The data presented are the mean anaesthetic times ± the standard error of the mean. n is the number of animals which were anaesthetized at each dosage-regimen and the % anaesthetized represents the percentage of animals given ether which were anaesthetized with the various cannabinoid pretreatments.

| Group & dose (mg kg$^{-1}$) | n | % anaesthetized | Mean ± s.e.m. min |
|---|---|---|---|
| Controls | 74 | 81% | 43·2 ± 1·54 |
| THC 5 | 15 | 75% | 45·0 ± 3·01 |
| THC 10 | 29 | 91% | 76·5 ± 4·94 |
| THC 20 | 14 | 74% | 75·1 ± 7·46 |
| THC 40 | 16 | 80% | 109·8 ± 8·09 |
| CBN 5 | 15 | 100% | 49·9 ± 3·04 |
| CBN 10 | 25 | 86% | 64·0 ± 5·07 |
| CBN 20 | 15 | 75% | 59·1 ± 5·43 |
| CBN 40 | 16 | 80% | 51·0 ± 2·70 |
| CBD 10 | 16 | 84% | 45·5 ± 4·15 |
| CBD 20 | 4 | 27% | 49·1 ± 9·12 |
| THC + CBN 5 + 5 | 17 | 68% | 58·9 ± 4·64 |
| THC + CBN 10 + 10 | 15 | 100% | 90·0 ± 5·42 |
| THC + CBD 10 + 10 | 14 | 70% | 88·0 ± 5·65 |
| CBD + CBN 10 + 10 | 15 | 100% | 49·0 ± 3·77 |

From our present data we are unable to provide an explanation for these differences but a number of possibilities might be considered. Firstly there is the possibility of competition for lipid storage sites between ether and the cannabinoids, however, from structural considerations the different cannabinoids would be expected to behave similarly. Secondly there is the possibility of an inhibition by the cannabinoids of the metabolism of pentobarbitone, but not of ether which is not metabolized (Paton & Pertwee, 1972) and thirdly, different interactions in the central nervous system between the cannabinoids and the different anaesthetics might occur. Such possibilities deserve further investigation because of their potential clinical and social significance.

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# Effect of *in vitro* changes in urinary pH on the enzymatic measurement and daily variation in excretion of D-glucaric acid

Latham (1974a) suggested that the measurement of urinary D-glucaric acid, by an enzymatic assay adapted from that of Marsh (1963), was a reliable test of hepatic enzyme induction after the administration of anticonvulsant drugs to man. Preceding these studies, several workers (Mowat, 1968; Hunter, Carrella & others, 1971; Hunter, Maxwell & others, 1972; Latham, Millbank & others, 1973) have used this or a similar technique (Marsh, 1963) without establishing limitations of the methodology. Recently, Simmons, Davis & others (1974) suggested that the procedure of Marsh (1963) allowed an increase in urinary pH on boiling in acid. This effect was thought to interfere with the final D-glucaric acid concentration obtained because the original assay relied on the conversion of D-glucaric acid to the $\beta$-glucuronidase inhibitor D-glucaro-(1–>4)-lactone, the lactonization being pH dependent. Confirmation of these observations could impair the validity of the method previously described by Latham (1974a), which used identical buffers to those described by Marsh (1963), unless a satisfactory alternative explanation could be established.

The effect of the inherent buffering properties of different urine specimens on the daily variation in the measured excretion of urinary D-glucaric acid in normal