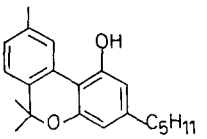
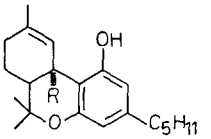
In vivo binding of Δ^1-tetrahydrocannabinol and cannabinol to rat serum proteins

N. K. McCallum, M. E. Eastwood, **Chemistry Division, DSIR, Private Bag, Petone, New Zealand*

It has been found that co-administration of cannabinol (CBN, I) with the main active constituent of marihuana Δ^1-tetrahydrocannabinol (Δ^1-THC, IIa) results in a greatly increased rate of clearance of the Δ^1-THC from the blood of rats (McCallum, 1975) and a modification of the action of Δ^1-THC (Fernandes, Schabarek & others, 1974; Takahashi & Karniol 1975). Highly protein-bound drugs can have their rates of action and clearance modified by competition from other drugs for available binding sites on the blood proteins (Gillette, 1973). The interactions between Δ^1-THC and CBN may be explained in these terms.



I



II

a. R=^{1}H; b. R=^{3}H

In vitro studies with human plasma have shown that greater than 80% of Δ^1-THC is bound to lipoproteins (Wahlqvist, Nilsson & others, 1970; Widman, Agurell & others 1974) although Klausner, Wilcox & Dingell (1971) in similar experiments reported that Δ^1-THC is also bound to albumin. Fehr & Kalant (1974), from *in vivo* experiments with rats, found the binding was to lipoproteins and albumin, the relative proportions being partly dependent on the mode of administration.

The avid binding of low concentrations of Δ^1-THC to glass or membranes for example, is well known (Garrett & Hunt, 1974) and the possibility that there is competition by these surfaces for Δ^1-THC during *in vitro* experiments cannot be ignored. As there is a possibility that CBN has an effect on the protein binding patterns of Δ^1-THC—even in relatively low proportions of CBN (McCallum, 1975)—it was felt studies should be made using Δ^1-THC which has been scrupulously separated from CBN, its aerial oxidation product. Apart from the work of Fehr & Kalant (1974), investigations have been *in vitro* and do not mention the purity of the Δ^1-THC used. Fehr & Kalant (1974) used Δ^1-THC of 96% purity for *in vivo* studies. No work has been done on the binding of CBN to plasma protein and the possibility that metabolically formed CBN (McCallum, Yagen & others, 1975) may confuse the Δ^1-THC measurements, does not appear to have been considered.

* Correspondence to Director at above address.

Previous work using the isotope effect was related to the production of CBN from Δ^1-THC by substitution of ^{3}H for ^{1}H at C(3) (IIb), (McCallum, Gugelmann & others, 1977) and it was noted that the production of CBN in rats could only be observed in the blood up to 40 s after administration of the Δ^1-THC. It was argued that only before the 40 s sampling was a significant amount of Δ^1-THC unbound and therefore available for metabolic dehydrogenation or for entering the rat brain. The pharmacokinetic interaction between Δ^1-THC and CBN is already pronounced by the 40 s sampling. It was therefore decided to examine the protein binding of CBN and Δ^1-THC after this interval. The use of Δ^1-THC labelled with ^{3}H at C(3) enabled detection of Δ^1-THC (both as the pure compound and in mixtures with unlabelled CBN) without confusion with metabolically formed CBN. Isotope effects (measured as an increase in the ratio of ^{3}H label at C(3) of Δ^1-THC to a ^{14}C label on the aromatic ring of Δ^1-THC) provide a measure of the progress of metabolic conversion of Δ^1-THC to CBN before protein binding occurs (McCallum & others, 1977) and therefore indicate the speed of the protein binding process. An *in vivo* comparison of the "averaged" propensity for binding of Δ^1-THC by individual blood proteins is thus also possible.

Four studies were conducted. The first involved administration of a mixture of the ^{14}C and ^{3}H labelled Δ^1-THC species, the second involved administration of the ^{14}C and ^{3}H labelled Δ^1-THC species and unlabelled CBN, the third was the administration of ^{14}C labelled, pure CBN, and the fourth was the administration of ^{14}C labelled CBN with unlabelled Δ^1-THC. The cannabinoid purification, specific activities, administration and counting techniques were as described previously (McCallum & others, 1977). The ^{14}C and ^{3}H labelled Δ^1-THC was 99·6% pure (0·2% CBN, 0·15% cannabidiol) as determined by gas chromatography and had a radiopurity of 99·9%.

Male Sprague-Dawley rats (140 ± 10 g) were exsanguinated 40 s after administration of 1 mg of cannabinoid in propylene glycol as described previously (McCallum & others, 1975). The blood was allowed to clot, the supernatant serum (*ca* 200 μl) was drawn off and the proteins separated by electrophoresis on gradient porosity acrylamide gel (5–25%) (Margolis & Kenrick, 1968). A portion of stained gel (amido black) was removed for reference and the remainder was sliced into 35 fractions which were placed in separate scintillation vials. The appropriate cold cannabinoid (100 μg), added to inhibit the adsorption on the glass,

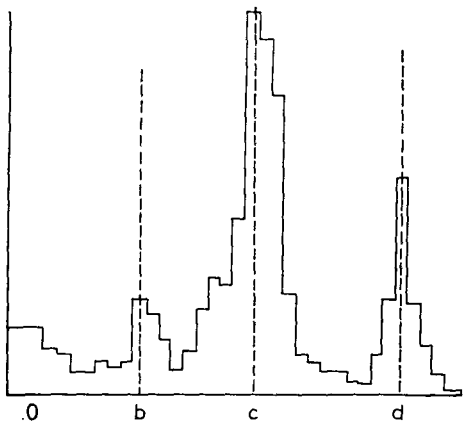


FIG. 1. Electrophoresis of rat serum sampled 40 s after intravenous administration of 1 mg ($^{14}C + {}^{3}H$)-Δ^1-tetrahydrocannabinol. The gel is visualized with amido-black and the distribution of radioactivity is shown as the histogram constructed from the activities of the individual slices. O: origin; b: β-lipoprotein; c: α-lipoprotein; d: albumin.

Soluene-350 (1 ml) and Insta-gel (5 ml) were added to the vials which were allowed to stand for a minimum of 24 h. Hydrochloric acid (1 M; 1 ml) followed by Insta-gel (5 ml) were then added to each vial which was subsequently counted after a further minimum 24 h.

Sample preparation was always the same so that ^{14}C and ^{3}H could be counted with constant efficiencies and these efficiencies were further controlled through the use of an external ^{137}Cs source (cf. Beckman Instruction booklet for LS-100c liquid scintillation counter). ^{3}H counts min^{-1} were corrected for ^{14}C spillover. Errors quoted are those for counting and are 1 standard deviation. The protein separation experiments were duplicated with the blood of a second rat and yielded the same results within counting errors.

The propensity of Δ^1-THC for binding to protein is extremely high (Garrett & Hunt, 1974) and competition for a particular binding site with CBN would result in Δ^1-THC being diverted or displaced to alternative sites. If the alternative sites have a lower affinity, the free Δ^1-THC in equilibrium with these sites will increase in a manner dependent on the reduced affinity and the population shift. For the competing compound (e.g. CBN) to compete effectively for the Δ^1-THC high-affinity sites in the manner previously described, it must have even higher affinity for these sites. Further, if the CBN is to have a significant effect at the observed low relative concentrations (McCallum, 1975), the higher affinity sites must have a low capacity. Thus our explanation of protein binding competition between CBN and Δ^1-THC should involve a binding model of high affinity, low capacity sites and low affinity, high capacity sites with the CBN diverting or displacing Δ^1-THC from the former to the latter (cf. Gill & Lawrence, 1974). This would provide a sufficient condition for observed increased rates of clearance of Δ^1-THC in the presence of CBN as well as for their pharmacological interaction.

Table 1. *^{3}H min^{-1}/^{14}C min^{-1} ratios determined for Δ^1-tetrahydrocannabinol on individual proteins after administration of 1 mg unlabelled CBN and/or 1 mg labelled Δ^1-THC (isotope ratio 3·4 before administration).*

	β-Lipoprotein %	α-Lipoprotein %	Albumin %
Δ^1-THC*	4·5	4·7	6·2
	3·4	4·3	8·0
Δ^1-THC* + CBN	3·8	4·2	5·0

Table 2. *The binding of labelled cannabinol to rat plasma after administration of 1 mg unlabelled Δ^1-THC and/or 1 mg ^{14}C-CBN.*

	β-Lipoprotein %	α-Lipoprotein %	Albumin %
CBN*	26 ± 1·8	35 ± 2·5	39 ± 2·7
CBN* + Δ^1-THC	15 ± 0·9	45 ± 2·1	40 ± 2·8

Fig. 1 illustrates an electrophoretic separation of blood proteins and the radioactive histogram. Table 1 represents isotope ratios determined for the three major proteins. As we have discussed, changes in isotope ratio provide an indication of the length of time the Δ^1-THC has taken to equilibrate with its binding sites and assuming qualitatively similar binding processes, we would expect the kinetics of the binding process to reflect avidity of binding. The ^{14}C activity includes that from ^{14}C-CBN as a metabolite of the ^{14}C-Δ^1-THC and the quoted isotope ratios therefore will be depressed according to the amount of metabolic CBN present. However, our earlier work has demonstrated that the metabolically produced CBN is at relatively low concentrations (McCallum & others, 1975) and these amounts (*ca* 4% of the total Δ^1-THC), indicate that the presence of metabolically produced CBN would not affect the conclusions drawn from Table 1. Thus overall, albumin appears to bind Δ^1-THC much less avidly than α-lipoprotein which in turn shows a

Table 3. *The binding of labelled Δ^1-tetrahydrocannabinol to rat plasma after administration of 1 mg unlabelled CBN and/or 1 mg ^{3}H-Δ^1-THC.*

	β-Lipoprotein %	α-Lipoprotein %	Albumin %
Δ^1-THC*	14·5 ± 0·9	59 ± 3·1	27·5 ± 1·2
Δ^1-THC* + CBN	18 ± 1·1	52 ± 2·7	30 ± 1·4

slightly reduced avidity compared with β-lipoprotein.

Tables 2 and 3 present the distributions of sorbed CBN and Δ^1-THC between the three proteins under the different experimental conditions. As might be expected, the pure cannabinoids distribute similarly. The small differences in protein binding found between the cannabinoids administered pure and as a mixture may be significant according to the quoted counting errors but are certainly not adequate to exclude other variables (e.g. a simple concentration effect).

According to these results therefore, protein binding competition between CBN and Δ^1-THC is not apparent. However, the situation where "low" and "high" affinity sites do in reality differ markedly in their affinity for Δ^1-THC, would require only small population changes to produce the desired effects and establishing this would lie outside the capabilities of this type of experimentation.

The authors wish to thank Dr M. Braude of the NIDA and Dr S. Burstein of the Worcester Foundation for Experimental Biology for generous gifts of labelled material. They also wish to thank Messrs C. A. M. Brenninkmeijer and A. Gugelmann of the Institute of Nuclear Sciences (N.Z.) for their assistance with this work.

January 20, 1978

REFERENCES

FEHR, K. O. & KALANT, H. (1974). *Eur. J. Pharmac.*, **25**, 1–8.
FERNANDES, M., SCHABAREK, A., COPER, H. & HILL, R. (1974). *Pyschopharmacologia (Berl.)*, **38**, 329–338.
GARRETT, E. R. & HUNT, C. A. (1974). *J. pharm. Sci.*, **63**, 1056–1064.
GILL, E. W. & LAWRENCE, D. K. (1974). *Biochem. Pharmac.*, **23**, 1140–1143.
GILLETTE, J. R. (1973). *Ann. N.Y. Acad. Sci.*, **226**, 6–17.
KLAUSNER, H. A., WILCOX, H. G. & DINGELL, J. W. (1971). *Acta pharm. suecica*, **8**, 705–706.
MCCALLUM, N. K. (1975). *Experientia*, **31**, 957–958.
MCCALLUM, N. K., GUGELMANN, A., BRENNINKMEIJER, C. A. M. & MECHOULAM, R. (1977). *Ibid.*, **33**, 1012–1013.
MCCALLUM, N. K., YAGEN, B., LEVY, S. & MECHOULAM, R. (1975). *Ibid.*, **31**, 520–521.
MARGOLIS, J. & KENRICK, K. G. (1968). *Analyt. Biochem.*, **25**, 347.
TAKAHASHI, R. N. & KARNIOL, I. G. (1975). *Psychopharmacologia (Berl.)*, **41**, 277–284.
WAHLQVIST, M., NILSSON, I. M., SANDBERG, F., AGURELL, S. & GRANDSTRAND, B. (1970). *Biochem. Pharmac.*, **19**, 2579–2584.
WIDMAN, M., AGURELL, S., EHRENBO, M. & JONES, G. (1974). *J. Pharm. Pharmac.*, **26**, 914–916.

The degree of plasma protein binding of sodium cromoglycate

B. CLARK*, G. F. MOSS, M. G. NEALE, *Fisons Limited, Pharmaceutical Division, Research & Development Laboratories, Bakewell Road, Loughborough, Leicestershire, LE11 0QY, U.K.*

Sodium cromoglycate (DSCG) (disodium salt of 1,3-bis-(2-carboxychromon-5-yloxy)-2-hydroxypropane) after inhalation, gives only low plasma concentrations of the order of 2×10^{-8} to 10^{-7} M (Moss, Jones & others, 1971). But because of the unusually acidic nature of the carboxyl groups (pKa <2), and because other acidic drugs have been shown to be associated reversibly with plasma albumin to varying degrees (Meyer & Guttman, 1968), the interaction of DSCG with plasma proteins has been examined.

[^{3}H]Sodium cromoglycate (^{3}H-DSCG) was prepared as described by Moss, Jones & others (1970) and had a radiochemical purity greater than 98% and a specific activity of 33–38 μCi mg^{-1}. Preliminary experiments established that ^{3}H-DSCG, when added *in vitro* to heparinized blood of a number of species, was recovered entirely in the plasma. For example ^{3}H-DSCG was added to six normal human blood samples at a final concentration of $7{\cdot}3 \times 10^{-6}$ M, incubated at 37° for 2 h and the plasma separated. Tritium in each plasma sample was measured by liquid scintillation counting. 98·2 s.d. 1·2% of the ^{3}H-DSCG was recovered in the plasma.

The interaction of ^{3}H-DSCG with plasma or sera from a number of species was studied by equilibrium dialysis or by ultra-filtration. For equilibrium dialysis ^{3}H-DSCG was added to heparinized plasma or horse serum in concentrations from $7{\cdot}8 \times 10^{-7}$–$7{\cdot}8 \times 10^{-6}$ M (Table 1 gives the concentrations after dialysis). The mixtures were equilibrated at 37° for 0·5 h and samples (1 ml) were dialysed against 0·154 M NaCl (1 ml) for 16 h in a water bath with shaking at 37°. Custom made Perspex dialysis cells with two compartments (5 ml volume) separated by a Cellophane (Visking tubing) membrane were used. The percentage of ^{3}H-DSCG bound to plasma proteins was calculated

* Correspondence.