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April 26, 1974

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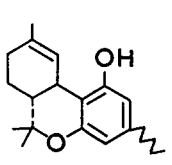
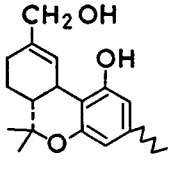
Binding of (+)- and (−)-Δ^1-tetrahydrocannabinols and (−)-7-hydroxy-Δ^1-tetrahydrocannabinol to blood cells and plasma proteins in man

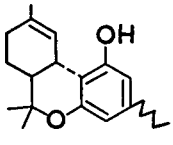
Previously we have studied the binding of (−)-Δ^1-tetrahydrocannbinol [(−)-Δ^1-THC*; I], the major psychoactive constituent of *Cannabis sativa* L. to human plasma proteins by using different electrophoretic techniques (Wahlqvist, Nilsson & others, 1970). We found that (−)-Δ^1-THC was associated 80–90% with lipoproteins, while Klausner, Wilcox & Dingell (1971), using ultracentrifugation, reported a binding of (−)-Δ^1-THC to both lipoproteins and albumin. Fehr & Kalant (1974) using electrophoresis also found a similar protein binding pattern of Δ^1-THC in rat serum. (−)-7-Hydroxy-Δ^1-tetrahydrocannabinol [(−)-7-hydroxy-Δ^1-THC†, II], which is a major primary metabolite of (−)-Δ^1-THC, with pharmacological activity, showed a binding of 94–99% to both lipoproteins and albumin by equilibrium dialysis, ultrafiltration, electrophoresis and ultracentrifugation (Widman, Nilsson & others, 1973).



I



II



III

We have now examined the distribution of (−)-Δ^1-THC and its metabolite in whole blood using equilibrium dialysis and centrifugation. We have also investigated the binding properties of the enantiomorphic (+)-Δ^1-THC (III) which does not occur naturally and has little pharmacological activity in rhesus monkeys and mice (Edery, Grunfeld & others, 1971; Jones, Pertwee & others, 1974).

Fresh human blood was collected in heparinized tubes and plasma was obtained by centrifugation. A suspension of washed blood cells was prepared according to McArthur, Dawkins & Smith (1971). After centrifugation, the blood cell fraction

* Designated as Δ^9-THC using the dibenzopyran numbering system.
† 11-Hydroxy-Δ^9-THC.

was washed six times with approximately the same volume of phosphate buffer, pH 7·35 (Ehrnebo, Agurell & others, 1974). Finally the volume was adjusted with buffer to that of the original whole blood sample. This suspension is referred to as the "blood cell suspension".

[^{3}H]-(−)-Δ^1-THC with a specific activity of 0·18 mCi mg^{-1} was used (Agurell, Gustafsson & others, 1973). [^{3}H]-(−)-7-Hydroxy-Δ^1-THC (specific activity 0·18 mCi mg^{-1}) was prepared by microsomal oxidation of [^{3}H]-(−)-Δ^1-THC as described by Nilsson, Agurell & others (1970). [^{3}H]-(+)-Δ^1-THC (specific activity 4·1 mCi mg^{-1}) was prepared as described by Jones & others (1974). A radiochemical purity of these compounds greater than 95% was verified by thin-layer chromatography (Jones, Widman & others, 1974).

Equilibrium dialysis was used to determine the total binding of the drugs to human plasma proteins (Ehrnebo, Agurell & others, 1971). The compounds were dissolved in buffer and dialysed against human plasma at 37° for 20 h. After equilibration, aliquots from both sides of the dialysis membrane (Standard Membranes No. 105–10, Technicon Ltd., Ireland) were withdrawn and the concentration of drug in plasma and buffer determined in duplicate by liquid scintillation counting.

Binding of cannabinoids to blood cells was determined by incubation with whole blood and with blood cell suspension followed by centrifugation. Glassware used in the incubation was previously silanized to minimize binding of the drugs to the vessel walls. Whole blood (2 ml) and blood cell suspension (2 ml) were incubated at 37° with labelled (−)-Δ^1-THC, (+)-Δ^1-THC or (−)-7-hydroxy-Δ^1-THC. After 30 and 60 min incubation, aliquots were withdrawn and centrifuged. The supernatants, plasma and buffer, were assayed by liquid scintillation counting after addition of Insta-Gel. The concentrations of the drugs in whole blood and blood cell suspension were determined as follows. Samples (100 μl) were digested with 1·5 ml of Soluene 350–isopropanol (1:1) and 0·6 ml 30% hydrogen peroxide and were allowed to stand at room temperature (20°) for 10 min before addition of 15 ml Insta-Gel–0·5 M aqueous HCl (9:1) scintillator. The recovery of radioactivity after digestion was 93% (range 92–95%, n = 6). Corrections were made for the loss of activity. The hematocrit values (0·39–0·46) were determined on an Adams Autocrit centrifuge (Clay-Adams, New York). The distribution of drug to blood cells, plasma proteins and plasma water was calculated according to Ehrnebo & others (1974).

Table 1 shows the results of the dialysis and incubation experiments. In the dialysis experiments equilibrium was achieved in 20 h. These highly lipophilic drugs showed a marked binding to the dialysis membrane, especially in control experiments with the drug dissolved in buffer and dialysed against buffer. When 20–50 ng mg^{-1} of (+)-Δ^1-THC in buffer was dialysed against buffer, about 95% of the activity was bound to the membrane or walls of the cell when equilibrium was achieved but with increasing amount of drug the binding became less. About 70% was bound when

Table 1. *Distribution of* (−)-Δ^1-*THC*, (−)-7-*hydroxy*-Δ^1-*THC and* (+)-Δ^1-*THC in whole blood cell suspension at* 37° in vitro.

Compound	% bound in plasma	% in whole blood distributed to: plasma water	plasma proteins	blood cells	% in blood cell susp. distributed to blood cells
(−)-Δ^1THC	97	3	88	9	96
(−)-7-hydroxy-Δ^1-THC	99	1	90	9	98
(+)-Δ^1-THC	97		87	10	97

Since the results agreed closely only mean values of 2–3 experiments are given. Drug concentrations in plasma and blood: 0·3–0·5 μg ml^{-1}.

500 ng ml^{-1} of (+)-Δ^1-THC dissolved in buffer was dialysed against buffer, while about 40% was bound when the drug was dialysed against plasma.

Concentration dependence was tested with (+)-Δ^1-THC (1–300 ng ml^{-1}) in the dialysis experiments and binding of the drug to the plasma proteins was found to be constant (96–98%) over this concentration range. In the incubation experiments the concentration dependence was examined with (−)-Δ^1-THC. When (−)-Δ^1-THC (40–500 ng ml^{-1}) was incubated with whole blood at these concentrations there was a binding of only 7–9% to the red blood cells whereas when incubated with blood cell suspension, as much as 96–98% was found to be bound to the red blood cells suspended in buffer. Hence, the binding to plasma proteins and blood cells seemed to be independent of concentration in this range.

As evident from Table 1, all compounds are highly bound with a total binding of 97–99% to the plasma proteins, This is in agreement with earlier findings in humans (Widman & others, 1973) and with Garrett & Hunt (1974) who have studied the binding of (−)-Δ^1-THC to dog plasma proteins. The distribution of the compounds in whole blood showed a binding to the red blood cells of only 9–10%. When the cannabinoids were incubated with washed red blood cells in the absence of plasma proteins they showed an extensive binding to the cells. These results indicate that in a cannabis smoker most of the (−)-Δ^1-THC and (−)-7-hydroxy-Δ^1-THC in blood is present in the plasma and is associated there with the proteins.

This work was supported by the Swedish Medical Research Council. G. J. gratefully acknowledges the support of a Wellcome-Swedish Travelling Research Fellowship.

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June 30, 1974

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