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.

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The degree of plasma protein binding of sodium cromoglycate

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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.

from the difference in radioactivity present in each half of the cell. Tritium was determined by liquid scintillation counting.

For ultrafiltration, ^{3}H-DSCG was added to plasma in the concentrations shown (Table 1). Samples were pre-incubated for 1·5–2 h at 37° before forming ultrafiltrates (less than 15% of the starting volume) using Amicon Centriflo membrane cones (Amicon Ltd., High Wycombe, England). Aliquots of starting mixtures and ultrafiltrates were analysed for tritium and the percentage of DSCG which was protein-bound was calculated.

Results are in Table 1. The binding of ^{3}H-DSCG was moderate in all cases with marked species differences. Human plasma gave the highest and horse serum the lowest percentage binding. Species differences probably arise from differences in the concentrations and nature of competing substances present in plasma. This behaviour is consistent with a compound moderately bound to plasma proteins.

Table 1. *The binding of ^{3}H-DSCG to plasma or serum from various species.* Details are given in the text. In the experiments using equilibrium dialysis the ^{3}H-DSCG concentration was the final concentration inside the dialysis chamber. Results are given as mean with s.d. (n in brackets) or individual values when n was 3 or less. Plasma was separated from blood obtained from normal volunteers or from animals by venipuncture. Horse serum (Wellcome Reagents Ltd, Beckenham, England) was used.

Species	Method*	^{3}H-DSCG Concn M	% Protein bound
Horse serum	1	$1·1 \times 10^{-7}$	29, 13
		$2·2 \times 10^{-7}$	33, 40
		$1·1 \times 10^{-6}$	23 s.d. 4 (6)
		$1·9 \times 10^{-6}$	24 s.d. 8 (4)
		$3·7 \times 10^{-6}$	24 s.d. 2 (4)
Dog plasma (Beagle)	2	$3·6 \times 10^{-7}$	27, 30
		$3·6 \times 10^{-6}$	19, 20, 21
		$3·6 \times 10^{-5}$	21, 23
		$3·6 \times 10^{-4}$	16 s.d. 2 (4)
Rabbit plasma (Dutch)	1	$1·8 \times 10^{-4}$	34, 38
Rat plasma (Sprague-Dawley derived)	1	$7·9 \times 10^{-7}$	35, 43
		$1·2 \times 10^{-6}$	35, 36
Stump Tailed Macaque Monkey plasma (Macaca arctoides)	2	$3·6 \times 10^{-7}$	39
		$1·8 \times 10^{-6}$	37
		$3·6 \times 10^{-6}$	39
		$3·6 \times 10^{-5}$	34
Human plasma (pooled sample, male + female)	1	$1·3 \times 10^{-6}$	66 s.d. 7 (4)
		$2·1 \times 10^{-6}$	62 s.d. 3 (4)
		$4·2 \times 10^{-6}$	66 s.d. 3 (4)
Human plasma (individual male)	1	$4·2 \times 10^{-6}$	62, 65, 65
Human plasma (individual female)	1	$4·1 \times 10^{-6}$	57, 60, 61
Human plasma (four males + two females)	2	$3·6 \times 10^{-7}$	69 s.d. 5 (6)

*Method 1; equilibrium dialysis; Method 2; ultrafiltration.

Furthermore binding was largely independent of the DSCG concentration. The reason for this may be partly related to competition for binding sites by other substances present in the plasma. Similar effects were reported by Kucera & Bullock (1969) for the binding of salicylate. Similarly, digoxin was 70% bound to human serum proteins at all drug concentrations used (Ohnhaus, Spring & Dettli, 1972).

We believe that the observed binding was due to a readily reversible association with plasma albumin. The evidence for this is as follows. The plasma concentration of DSCG falls rapidly in man and animals (Moss & others, 1970; 1971; Ashton, Clark & others, 1973). Sephadex chromatography of albumin ^{3}H-DSCG mixtures on columns or thin-layers indicated reversible binding (unpublished work). Dilution studies with albumin solutions or with plasma indicated that the binding of ^{3}H-DSCG varied in a manner consistent with reversible association.

DSCG, being relatively weakly bound would not be expected to displace other protein-bound drugs from albumin. Sudlow, Birkett & Wade (1975) reported that DSCG did not displace warfarin from binding sites on human albumin in dilute solutions using a spectrofluorimetric method. We have carried out experiments with human plasma and [^{14}C]warfarin using thin-layer gel filtration (Clark & Kind, 1977) and failed to observe any displacement of warfarin at concentrations of DSCG from 2×10^{-6} to 3×10^{-3} M. DSCG is unlikely to result in adverse effects during clinical use due to this type of interaction.

In conclusion DSCG belongs to a large group of acidic drugs which are reversibly associated with plasma albumin in a non-specific way. The highly polar nature of the compound results in the binding being relatively weak.

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