in contact with water out of the vicinity of the metal centre, it would yield nitrite and nitrate ions, thus giving the appearance that the enzyme is actually converting nitrate to nitrite directly. The detection of nitrogen(IV) oxide in the natural systems will be difficult. ESR spectroscopy could be used, although the signal obtained from this free radical in solution can be broad and ill-defined[16]. The presence of an oxo group on the molybdenum(V) centre would be expected on chemical grounds[10] and this group would probably dominate the reaction pathway in two respects. First, in aiding initial substrate binding *trans* to the oxo group[17] and second in controlling the electronic structure at the metal. Facile electron transfer from an oxo-molybdenum(V) centre to a nitrate group will only occur if the latter is able to coordinate by way of one oxygen atom at a site *cis* to the oxo group with the plane of the NO_3 moiety containing the Mo=O axis (Fig. 1). Therefore, we suggest that, after the initial binding of the nitrate group, the enzymes must ensure that either suitable chelation or ligand rearrangement of this substrate is possible to enable one (and only one) of the oxygen atoms to be located *cis* to the oxo group. As there is strong evidence in favour of a molybdenum cofactor common to all the molybdenum enzymes[18] we suggest that the hydrophobic nature of the metal site and the facility for substrate binding *cis* to an oxo-molybdenum group may be important to the function of all the enzymes of this class.

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Lack of effect of *Avena sativa* on cigarette smoking

After his conclusion that an alcoholic extract of *Avena sativa* (the common oat) reduced craving for and consumption of cigarettes, Anand[1] recommended further investigation of its significance and role. We attempted the former by repeating his study in a situation less likely to prejudice participants than a chest ward and the latter by using a study design which would have cast light on the role of the treatment in altering smoking if such an effect had been evident.

The botanical state of his starting material was variously described as "selected just before harvest"[1] and presumably yellow, and "ripe and green"[2,3]. Thus contents of the putative active ingredient could differ. We therefore studied both a green plant with full sized ears and a ripe plant dug up one week before the main harvesting. Ethanolic extracts of the plant including its roots were made exactly as described by Anand[1]. In preliminary blind studies the extract was nearly always distinguished from dummy despite the resources of a pharmaceutical laboratory. Therefore, two differently tasting liquids though approximating the musty quality of the oat infusion were prepared with an identical alcohol content. All three were coloured deep red. Subjects knew two dummies were included in varying order so they could not conclude from a change in taste that a period of test material had begun.

Employees of The Wellcome Foundation, Crewe, volunteered in response to a notice requesting help from smokers who would not object if their smoking habits altered while testing the effect of a new drug on smoking. No direct appeal was made to those anxious to stop smoking. At the end of the study a reward of 50p was offered. Smokers bought their own cigarettes.

Subjects had diary sheets and recorded their daily cigarette consumption by joining up any two dots randomly from an array of dots for each cigarette, thereby hindering a too ready comparison of their daily consumption. They also recorded the doses of extract taken each day, a subjective enjoyment rating of the day's cigarettes (better, same, less, none), any unwanted symptoms or effects as, for example, those brought on by a cold and their opinion as to whether they had received dummy or oat extract. They knew no treatment would last for less than one week and that the first would be a dummy to provide a run-in period. Diary pages were collected from the subjects separately each week and the next week's liquid issued in exchange for the used bottle. Forty-three subjects participated in the first study and eighteen of them continued into the second study.

Treatments were of 1 ml of appropriate ethanolic liquid diluted with 4 ml of water for palatability and taken four times daily during waking hours. They were presented as shown in Table 1.

Table 1 Treatment schedules for smokers

Study 1	Extract immature oats (EIO) *v* dummy (D)			
week	Group 1	Group 2	Group 3	Group 4
1	D1	D2	D2	D1
2	D2	D1	D1	D2
3–6	EIO	EIO	D2	D1
7–10	D1	D2	EIO	EIO
11–12	No treatment			
No. of subjects	7	14	7	15

Volunteers had to decide whether they had taken oats or dummy infusion. Statistical analysis suggested that their decisions were guesses rather than detections of the oat preparation. Any persistent effects would have been observed in the period following the oats administration. The volunteers expected to learn at the end of the tenth week in which period

they took the test liquid. Actually all were misinformed and only correctly informed at the end of the twelfth week. If any real effect had operated by psychological rather than pharmacological means it might have diminished in volunteers of groups 3 and 4 during weeks 11 and 12.

Subjects' cigarette consumptions at a rate different from that of groups 1 and 2 during weeks 7 and 8, were compared for each week and period of the trial, allowing for different order of administration of preparations, by means of analysis of variance. The mean daily cigarette consumptions for each period in study 1 (43 subjects) were: run in (weeks 1 and 2) 23.5; EIO (weeks 3–6 or 7–10) 24.4; dummy (weeks 7–10 or 3–6) 24.0; run out (weeks 11 and 12) 25.4 (36 subjects). Individual subjects' mean daily consumption varied between 12 and 42 per day in the run in period, individual extreme consumptions being 6 and 54. Twenty-three subjects' mean daily consumptions were higher in the EIO period than the dummy period; fifteen consumed less in the EIO period and five did not change. The two largest individual changes in daily consumption between periods on EIO and dummy were respectively a rise from 21 to 27 (average 21 on run in) and a fall from 32 to 25 (26 on run in).

The study finished just before a Christmas holiday when unusual social factors affect smoking habits. The study of the mature oats was therefore postponed. As the volunteers were no longer naïve (nor could any others from the same community be expected to be) dummies were not given during the run in periods. Instead, one-half took immature oats infusion over weeks 2 to 4 while the other took mature oats and in weeks 5 to 7 each group took the alternative. No significant differences between EIO and EMO were evident. Mean daily cigarette consumptions in study 2 (18 subjects) were run in 25.6, EIO 24.7 (14 subjects) and EMO 24.9. Four subjects stopped smoking after completing the second study because the 'budget' raised the cost of cigarettes. Regretfully we conclude that the views of our compatriot, Dr Samuel Johnson, on oats would not have been modified by our study, though by its lucky timing his views on excise might have been.

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Marijuana metabolites measured by a radioimmune technique

The recent surge of interest in the pharmacology of cannabinoids has created a need for a rapid assay for Δ^9-tetrahydrocannabinol (Δ^9-THC) and its metabolites in body fluids. A sensitive immune technique has proved elusive, and quantitation requires a multistep chromatographic and mass spectroscopic analysis[1]. Gas liquid chromatography with electron capture detection has also been used[2,3]. We describe here a simple rapid radioimmune assay for Δ^9-THC utilising goat antiserum obtained as previously described[4].

A standard antibody-binding curve was obtained as follows: 4.5 ng (5,000 c.p.m.) of ^{3}H-Δ^8-THC (specific activity 465 mCi mMol^{-1}, more than 98% pure, New England Nuclear Corp.) was incubated for 2 h at 4° C with increasing dilutions of antiserum (0.3 ml). The latter were made up with phosphate-buffered saline (PBS), 0.1 M in phosphate, *p*H 7.0 in 0.1% human γ globulin. After this initial incubation, 1 ml of a cold

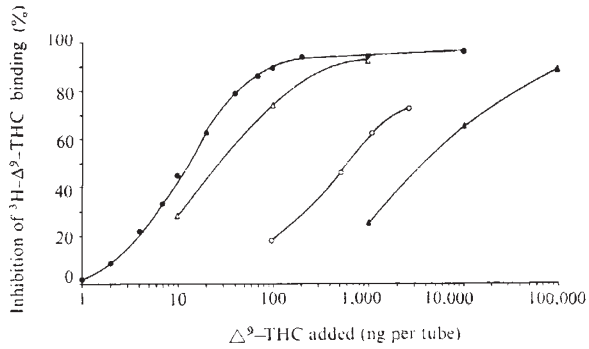

Fig. 1 Inhibition of ^{3}H-Δ^9-THC binding to antibody by increasing amounts of unlabelled THC metabolites. ●, Δ^9-THC; △, 11-hydroxy-Δ^9-THC; ○, 11-nor-9-carboxy-Δ^9-THC; ▲, cannabidiol.

dextran charcoal suspension (0.25% dextran–2.5% activated charcoal in PBS), was added and incubation continued for 15 min. The tubes were then centrifuged at 3,000 r.p.m. for 15 min at 4° C and the supernatant (1 ml) was counted in a Nuclear Chicago liquid scintillation spectrometer (70% tritium counting efficiency) with Aquasol (New England Nuclear Corp.), as the scintillation fluid. Binding of ^{3}H-Δ^8-THC by normal goat serum under the foregoing conditions was slightly above background. A maximum of 50% of marker was bound by a 1/60 early antiserum dilution. The latter serum dilution was used in the inhibition experiments described below.

To ascertain the specificity of the antiserum, various amounts of cold Δ^9-THC, 11-hydroxy-Δ^9-THC, 11-nor-9-carboxy-Δ^9-THC and cannabidiol were added to a 1/60 dilution of antiserum 30 min before addition of 4.5 ng ^{3}H-Δ^8-THC. The sensitivity of the inhibition assay was 1.2 ng per tube at a 95% confidence limit. A sample inhibition experiment at 50% binding is plotted in Fig. 1 and percentage cross reactivities are tabulated in Table 1.

Table 1 Cross reactions of cannabinoids with THC antiserum

	% Cross reaction
Δ^9-THC	100
11-Hydroxy-THC	47
11-Nor-9-carboxy-THC	2.6
Cannabidiol	0.5

*At 50% inhibition

The association constant for the antiserum was determined graphically from a Scatchard plot. In the range of dilutions tested, crude antiserum bound THC at $K_a = 4.0 \times 10^8$ M indicating a potential sensitivity of 300 pg per tube.

After a preliminary adsorption with charcoal[5], samples of male plasma treated with known quantities of Δ^9-THC (50–700 ng ml^{-1}) were measured. Samples of 1 ml were shaken with heptane ethanol (100:1.5), the extracts were dried and the residue was dissolved in 0.5 ml of 50% ethanol. This solution (50 μl) was used in inhibition assays. Ideally the assays should have shown 5, 10, 20, 50 and 70 μg per tube, respectively. Their deviation from these points on the standard curve (Fig. 2) was small and within the limits of experimental error.

Plasma samples were obtained from chronic users of marijuana who were part of a controlled marijuana research project and had used no other drug for at least 10 d previously. Blood was drawn 15 min after they had smoked a marijuana cigarette (0.9 g) containing 2.1% (18.9 mg) Δ^9-THC. Plasma was separated by centrifugation and stored frozen at −10° C. These samples (1–3 ml) were extracted with heptane–ethanol. Aliquots (0.25 ml) of reconstituted extract were used in the radioimmune assay system already described.

In each of six plasma samples Δ^9-THC was detected and quantitated 30 min before and 15 min after THC use. Pre-intoxication levels were 60–100 ng ml^{-1}. Postintoxication levels