The final concentration of the non-radioactive PgF$_{2a}$ was 0.1 µMoles NAD was applied as a co-factor in 4 mMoles final concentration. The incubation period was 2.5 min at 37 °C, under aerobic conditions. The intact PgF$_{2a}$ and the metabolites were dissociated by thin-layer chromatography, and Packard Radiochromatogram Scanner was applied to detect them (figure 1). The zones corresponding to PgF$_{2a}$ and the metabolites were scarped and the radioactivity was measured with a Nuclear Chicaco Liquid Scintillation Spectrometer. The protein content of the incubated substance was determined according to Lowry et al.[13]. Metabolism of PgF$_{2a}$ is expressed according to Carminati et al.[11] as pmoles of substrate metabolized h/mg protein.

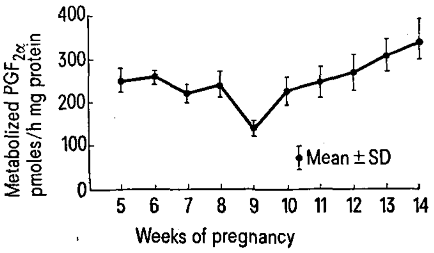

Fig. 2. Quantitative metabolism of PgF$_{2a}$ by human placenta during early pregnancy.

Results and discussion. The 15-OH-PGDH activity of the placenta shows a declining tendency from the 5 week of pregnancy till the 9 week when a minimum can be observed, then they rise gradually as pregnancy advances (figure 2). Placental prostaglandin metabolism per mg protein was significantly lower on week 9 of gestation ($p < 0.01$ for week 9 then all other weeks).

The experimental results reported here demonstrate that there is an important relationship between the stage of pregnancy and the metabolism of PgF$_{2a}$ in early human placenta. That the placenta could be a rich source of 15-OH-PGDH was demonstrated by Jarabak[8], and partial purifications of this enzyme from term placental tissue have been reported by Schlegel et al.[14]. It can be suggested that the high enzyme activities in the placenta is part of a mechanism by which the fetus is protected against potentially harmful effects of high concentrations of prostaglandins. The results of our studies may indicate that decrease of 15-OH-PGDH activity can result in the rise in the endogenous prostaglandin level, which makes a spontaneous contraction of the uterus possible. The highest probability of this is in the first trimester about the 9 week of the pregnancy.

[13] O. H. Lowry, N. J. Rosenburgh, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
[14] W. Schlegel, M. Laurence, M. Demers, H. E. Hildebrandt-Stark, H. R. Behrman and R. O. Greep, Prostaglandins *5*, 417 (1974).

Isotope effect studies on the dehydrogenation of Δ^1-tetrahydrocannabinol in the rat

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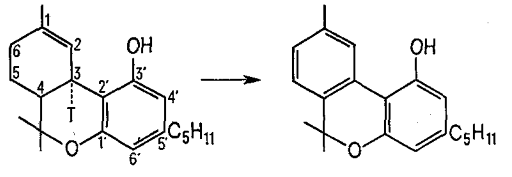
Summary. Isotope effect studies on the metabolic dehydrogenation of Δ^1-tetrahydrocannabinol in rats are described and it is shown that this process is confined to a very short period following i.v. administration. The implications of this finding are discussed.

Although the pharmacological potency of Cannabis sativa can be attributed mainly to its Δ^1-tetrahydrocannabinol (Δ^1-THC) content[4], recent work does indicate that other cannabinoids such as cannabinol (CBN) can modify the effects of this active constituent[5]. Thus, observations that CBN accelerates clearance of Δ^1-THC from the blood[6] and is also a rapidly formed metabolite of Δ^1-THC[7], suggests that the metabolic production of CBN may be involved with processes important to cannabis intoxication.



Δ^1-THC CBN

Since blood levels of metabolically produced CBN[6] are of little help in following its actual production it was decided to monitor the metabolism of Δ^1-THC by utilisation of the isotope effect. If tritium is substituted for the C(3) proton (figure 1) and this proton is involved in this metabolic process (as it appears likely), the reaction rate will be slower[8]. Thus in a mixture of the substituted and unsubstituted compound, a change in relative concentration can provide evidence of reaction involving the hydrogen isotope; and the rate of change can provide a concentration-independent measure of the nature and discrimination of this particular process.

Untritiated Δ^1-THC was labelled with ^{14}C in the aromatic ring so that relative concentrations of the tritiated and untritiated species could be conveniently monitored by the ratio of the tritium and ^{14}C-activities.

Materials and methods. A mixture of these labelled compounds[9], unlabelled Δ^1-THC and unlabelled CBN were purified twice using preparative thin layer chromatography[10,11]. 3 studies were conducted. The first involved administration of a mixture of the 2 labelled Δ^1-THC species, the second involved administration of the 2 labelled Δ^1-THC species and unlabelled CBN, and the third involved administration of the labelled Δ^1-THC mixture to rats which had been pretreated twice daily for 5 administrations of unlabelled Δ^1-THC. The cannabinoids were administered to rats[12] in propylene glycol[13] as described previously[7]. The Δ^1-THC mixture was isolated from the blood[7] after further unlabelled Δ^1-THC had been added, and purified using thin layer chromatography[10]. Activies were determined by scintillation counting and the ratios were calculated[14] as $I = \frac{^{3}\text{H cpm}}{^{14}\text{C cpm}}$ and errors from counting are quoted as 1σ. Pure labelled compounds were periodically subjected to the experimental work-up during the course of these experiments, to confirm that the extraction and separation had no influence on the final ratio. Care was taken to ensure experimental conditions were identical in all details for comparisons[13]. Controls with the mixture of the labelled Δ^1-THC species being administered, were run at the same time as the experiments outlined in tables 2 and 3 to check that comparisons were valid.

Results and discussion. It has been demonstrated[7] that the production of CBN is not via non-metabolic dehydrogenation of Δ^1-THC and we also confirmed that the method used in these experiments was not responsible for any isotope ratio changes.

Isotope ratios of the radio-labelled Δ^1-THC isolated from the blood at differing intervals after injection of the labelled Δ^1-THC mixture are set out in table 1. It will be noted that there is an appreciable change of ratio less than 40 sec after injection, but no significant trend between the 40-sec-and 28-min-samplings which represents 42 times the initial period[15]. Results from the heart puncture sampling of a rat at 50 sec and following decapitation at 3 min were also the same within counting errors, and thus the transformation of Δ^1-THC would appear to be taking place very rapidly and prior to the 40-sec-sampling. This is in accordance with previous analytical data on metabolic CBN blood levels in rats[7].

The results clearly indicate that we are observing an isotope effect for the C(3)–H bond although it is not necessarily established that the attack is initially at this position. Since it is known that cannabinoids have an extremely high affinity for blood protein[16], the simplest explanation for the results in table 1 is that the advent of protein binding in the blood is responsible for halting the progress of this reaction. Thus production of cannabinol implies the presence of free Δ^1-THC, and a comparison of rates of change of isotope ratios at different times enables comparison of the proportion of unbound or free Δ^1-THC in the blood at these times.

It has been suggested by Oldendorf that free Δ^1-THC can be presumed to undergo complete clearance from the blood by the brain in a single passage[17], and it is known that with the more lipophilic drugs, rapid binding to plasma proteins can reduce availability of the free drug for crossing the BBB and result in an incomplete removal of drug to the brain[17,18].

Since published reports, as well as this data for isotope ratio changes, indicate that the proportion of unbound Δ^1-THC in the blood after 40 sec is extremely low[16] and low levels of drug (bound plus unbound) also prevail after this time[7] the amount of free drug reaching the brain more than 40 sec after the administration would appear to be

Table 1. Comparison of isotope ratios ($I = \frac{^{3}\text{H cpm}}{^{14}\text{C cpm}}$) of Δ^1-THC isolated from rat blood after varying intervals

Time elapsed	I
0 sec	3.4 ± 0.1
40 sec	4.7 ± 0.2
50 sec*	4.2 ± 0.4
3 min*	3.9 ± 0.4
10 min	4.9 ± 0.2
28 min	4.8 ± 0.3

* Sampled from the same rat.

Table 2. Isotope ratios after Δ^1-THC administration with cold CBN

Time elapsed	I
0 sec	3.4 ± 0.1
40 sec	3.82 ± 0.03
3 min	4.9 ± 0.15
7 min	4.2 ± 0.15

Table 3. Isotope ratios of Δ^1-THC after administration to pretreated rats

Time elapsed	I
0 sec	3.4 ± 0.1
40 sec (a)	5.4 ± 0.15
40 sec (b)	5.0 ± 0.15

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[4] R. Mechoulam, A. Shani, H. Edery and Y. Grunfeld, Science *169*, 611 (1970).

[5] M. Fernandes, A. Schabarek, H. Coper and R. Hill, Psychopharmacologia *38*, 329 (1974); R. N. Takahashi and I. G. Karniol, Psychopharmacologia *41*, 277 (1975); and cited references.

[6] N. K. McCallum, Experientia *31*, 957 (1975).

[7] N. K. McCallum, B. Yagen, S. Levy and R. Mechoulam, Experientia *31*, 520 (1975).

[8] S. Aronoff, in: Techniques and Radiobiochemistry, p. 9. Iowa State College Press, 1956.

[9] ^{3}H-Δ^1-THC labelled at C(3), was the kind gift of Dr S. Burstein of the Worcester Foundation for Experimental Biology; ^{14}C-Δ^1-THC labelled at C(6′) and C(4′) in the aromatic ring was kindly supplied by Dr M. Braude of the National Institute on Drug Abuse, Maryland, USA.

[10] J. A. Vinson and J. E. Hooyman, J. Chromatogr. *106*, 196 (1975).

[11] Purities of the cannabinoids were: Δ^1-THC contained 0.4% CBN by standard glc techniques and 0.1% CBN using varied exposures of a thin layer chromatogram to photographic plates; the CBN was 99.5% pure with ca. 0.1% Δ^1-THC by glc.

[12] Male Sprague-Dawley rats (130–150 g) were used with 1 mg CBN and/or 1 mg ^{3}H, ^{14}C-Δ^1-THC (ca. 4 μCi in total) per administration.

[13] The propylene glycol showed a tendency to absorb moisture from the air causing the solution to form a cloudy, microsuspension of cannabinoid. Results using such a solution tended to be erratic (generally higher) unless the cannabinoid was solubilized by further warming or addition of traces of acetone and remixed.

[14] Donald L. Horrocks, Applications of Liquid Scintillation Counting. Academic Press, New York and London 1974.

[15] On the basis of blood level data[6] it can be argued that up to 35 sec after administration the injection plug is moving from the tail to the heart, and thus mixing (and therefore metabolism) can only take place after it reaches the heart, i.e. between 35 and 40 sec after administration. If this is the case the latter period represents not 42, but over 300 times the initial period during which metabolism is taking place.

[16] E. R. Garrett and C. A. Hunt, J. pharm. Sci. *63*, 1056 (1974).

[17] W. H. Oldendorf, Proc. Soc. exp. Biol. Med. *147*, 813 (1974).

[18] A. Goldstein, L. Aronow and S. M. Calman, Principles of Drug Action: The basis of Pharmacology, 2nd ed., p. 190. John Wiley & Sons Inc., 1974.

minimal. The data also demonstrates – not unexpectedly – that relatively high proportions of unbound molecules are available to the brain for an initial short period when high overall concentrations[7] of the drug prevail. This suggests that under these experimental conditions at least, the bulk of the Δ^1-THC enters the brain within 40 sec of i.v. administration. This short period encompasses the unstable situation prior to the equilibration of free drug with blood proteins. Once inside the brain, avid binding of the Δ^1-THC to protein and membranes and possible slow metabolism to 7-OH-Δ^1-THC[19] would be expected to contribute to a slow release and prolonged duration of action[20].

It is possible that an enhancement of the transformation of Δ^1-THC to CBN could be responsible for observed pharmacokinetic interactions between Δ^1-THC and CBN[6]. Table 2 illustrates the isotope ratios obtained from rats treated with pure labelled Δ^1-THC, and with labelled Δ^1-THC and cold CBN under identical experimental conditions to those used above and no enhancement of the dehydrogenation of Δ^1-THC is to be noted.

Table 3 illustrates the results of another experiment to simulate regular users; the rats were pretreated twice daily for 5 administrations of unlabelled Δ^1-THC before the pure labelled Δ^1-THC was finally administered. Again the difference does not appear to be sufficient to be the basis of differences in metabolism rates [21], but extended experiments should be undertaken to confirm this.

Thus the production of CBN from Δ^1-THC in rats may be viewed as competition for available free Δ^1-THC, the effect of which on the overall intoxication, is yet to be evaluated. Enhancement of this transformation does not appear to be the basis of either the THC/CBN pharmacokinetic interaction[6] or increased clearance rates of 'chronic users'[21].

[19] Pharmacology of Marihuana, p. 63. Ed. M. C. Braude and S. Szara. Raven Press, New York 1975.

[20] Thiopental [A. Goldstein and L. Aronow, J. Pharmac. exp. Ther. *128*, 1 (1960) provides an analogy with Δ^1-THC. In this case lower lipophilicity results in less protein binding and would be expected to result in much less retention of the drug by the brain.

[21] L. Lemberger, N. R. Tamarkin, J. Axelrod and I. J. Kopin, Science *173*, 72 (1971).

Spin label studies of ATP phosphoribosyltransferase of E. coli

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Summary. Covalently bound bromoacetamide nitroxides have been used to detect the conformational changes and enzyme association induced by its feedback inhibitor, histidine.

ATP phosphoribosyltransferase (EC2.4.2.17) is the first enzyme of the histidine biosynthetic pathway[2] and is allosterically inhibited by the end product, histidine[3]. It catalyzes the reversible reaction of ATP and 5-phosphoribosyl α-1-pyrophosphate to yield phosphoribosyl-ATP and pyrophosphate. Although the primary structure of the active site is unknown, Bell and Koshland showed that an SH group is necessary for catalytic activity from studies on iodoacetate inactivation in the Salmonella typhimurium enzyme[4].

Studies in our laboratory with the E. coli enzyme have dealt with conformational changes and association-dissociation effected by substrates and other ligands. The action of histidine on this enzyme has been studied by several techniques like fluorescence[5], gel filtration[6], equilibrium sedimentation[7] and steady state kinetics of the inhibition of the reaction catalyzed by the enzyme[8]. However, both processes induced by histidine, namely binding of the effector to the enzyme and enzyme association, could not be discriminated by any of the techniques mentioned above. In the work presented here, at very low histidine concentration the correlation time, τ, decreases, suggesting a conformational change in which the unpaired spin becomes a little freer. When the histidine concentration increases, τ also increases, apparently due to the enzyme aggregation induced by histidine.

Materials and methods. The spin labels 3-[(2-Bromoacetamido)methyl]-2, 2, 5, 5-tetramethyl-1-pyrrolidinyloxyl (No. 131), 3-[2-(2-Bromoacetamido)acetamido]-2, 2, 5, 5-tetramethyl-1-pyrrolidinyloxyl (No. 132), and 3-[3-(2-Bromoacetamido)propyl]carbamoyl) 2, 2, 5, 5-tetramethyl-1-pyrrolidinyloxyl (No. 133), were purchased from Synvar, Palo Alto, California.

ESR parameters of the nitroxide spectra

	ΔH_1 (G)	ΔH_0 (G)	ΔH_{-1} (G)	$a_{1,0}$ (G)	$a_{-1,0}$ (G)	τ (ns)
Free label (No. 132)	1.2	1.2	1.3	16.2	16.2	0.07
Adsorbed label (No. 131)	2.4	2.3	2.8	16.2	16.3	1.14
Adsorbed label (No. 132)	2.0	2.0	2.3	16.1	16.3	1.06
Adsorbed label (No. 133)	1.6	1.5	1.9	16.3	16.4	0.69

–1, 0 and 1 denote the 3 lines of the nitroxide spectrum assigned to ^{14}N nuclear spin quantum states; M = –1 is the line corresponding to the higher field. a is the hyperfine splitting constant. ΔH is the peak-to-peak width.

[1] We are indebted to J. F. García de la Banda for his support and encouragement, and to E. T. Kaiser, V. M. Fernández and M. Rico for critical reading of the manuscript.

[2] M. Brenner and B. N. Ames, in: Metabolic Pathways, vol. V, p. 349. Ed. D. M. Greenberg and H. J. Vogel. Academic Press, New York 1971.

[3] R. G. Martin, J. biol. Chem. *238*, 257 (1963).

[4] R. M. Bell and D. E. Koshland, Jr, Bioorg. Chem. *1*, 409 (1971).

[5] A. R. Tébar, V. M. Fernández, R. Martín Del Río and A. Ballesteros, FEBS Lett. *50*, 239 (1975).

[6] A. R. Tébar, V. M. Fernández, R. Martín Del Río and A. Ballesteros, Experientia *29*, 1477 (1973).

[7] L. Klungsöyr and H. Kryvi, Biochim. biophys. Acta *227*, 327 (1971).

[8] A. R. Tébar and A. Ballesteros, Mol. cell. Biochem. *11*, 131 (1976).