Table 2. Specificity of cytotoxicity for sarcoma cells in contrast to that for skin fibroblasts from the same patient. Parentheses indicate that the value is statistically significant.

Serum	Cytotoxic index	
	SA-13 (D.A. sarcoma line)	D.A. skin fibroblast line
D.A.*	.56	(.18)
T.A.	.86	(.04)
J.W.	.79	(.02)
B.S.	.34	(.18)
R.M.	.32	(0)
K.C.	.30	(.09)
N.C.	.21	(0)
J.T.	.94	.44
T.S.	.83	.26
C.H.	.44	.50
J.B.	.43	.37

* Autologous serum of patient from whom SA-13 sarcoma culture was obtained.

was found to be complement dependent in all serums except one serum with a high C.I. against SA-2 cells, which was found to be cytotoxic to a dilution of 1:32. When diluted 1 to 4, the complement dependency of this serum also became demonstrable.

Serum was fractionated to further establish the antibody character of the cytotoxic factor. The gamma globulin so obtained demonstrated complement dependent cytotoxicity in the microcytotoxicity test. Gamma globulin similarly extracted from three normal serums possessed no cytotoxic activity, indicating that the method of extraction introduced no cytotoxic substance.

Absorption studies were undertaken as another test of antibody specificity. One serum from a patient (D.A.) with osteogenic sarcoma was selected and absorbed with increasing numbers of sarcoma cells. Absorption with either 10^5 SA-1 or SA-2 cells, living or dead, removed the cytotoxicity from 0.1 ml of serum, whereas absorption of the serum with equal quantities of KB, HeLa, or Chang liver cells failed to remove the cytotoxicity for SA-2 cells.

The microcytotoxicity test as described possesses several advantages over classical techniques of cytotoxicity testing. It allows the detection of cytotoxicity directed against resistant sarcoma lines which show no reaction when tested by dye exclusion methods. By using only 100 cells per well, the test is applicable to nonestablished cell lines, such as SA-13, which are available in very limited numbers. The ratio of undiluted serum to this small number of cells is important to the sensitivity of the method, as was demonstrated by Boyse, Old, and Stockert (*5*) in a mouse system. The sensitivity of the test was further increased by incubation overnight because counts of viable cells beginning soon after the complement was added to the plates showed progressive cytotoxicity over a period of 4 to 6 hours.

Finally, the complement source was important because rabbit complement alone was frequently toxic for human sarcoma cells, producing nonspecific cytotoxicity. Human serum alone often failed to demonstrate cytotoxicity, but the combination was found to provide both specificity and cytotoxic potential.

It seems reasonable to conclude from the specificity of the cytotoxic effect that the serum factor responsible is an antibody. This conclusion is supported by the demonstration that this activity is complement dependent and that it resides in the gamma globulin fraction of the serum.

The high incidence of antibody against a single osteosarcoma target cell line found in the serum of patients with many diverse types of sarcoma indicates that the antigen against which this cytotoxic antibody is directed is shared by most sarcomas. This is consistent with our earlier studies in which other serologic methods were used. The existence of this common antigen suggests a common viral etiology for sarcomas by analogy with animal tumor systems. The existence of a sarcoma-inducing viral agent would help to explain the findings of a 58 percent incidence of antibody cytotoxic to sarcoma antigen among the relatives and close associates of sarcoma patients.

The sarcoma antigen is a tumor-specific antigen because only 8 percent of the normal blood donors, as compared with 70 percent of sarcoma patients, have cytotoxic antibody directed against it, and because the cytotoxic antibody is absorbed by sarcoma cells, but not by HeLa, KB, or Chang liver cells. Further evidence that the cytotoxic antibody is directed against a tumor-specific antigen rather than a histocompatibility antigen is provided by the observation that serum of patient D.A. was cytotoxic to his own sarcoma cells and by the failure of the majority of the serums which were cytotoxic to a sarcoma line (SA-13) to exhibit cytotoxicity to a normal cell line derived from the same patient.

Two implications of our data seem especially significant. First, the demonstration of a common antigen, specific to sarcoma cells, by this technique corroborates similar findings by immunofluorescence and complement fixation (*2*). These data suggest induction by a single viral agent. Second, this antigen is located at the cell surface and renders the tumor cell vulnerable to immune attack. This finding adds strength to the case being made for the importance of host immune defenses in human sarcomas.

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References

1. L. J. Old and E. A. Boyse, *Fed. Proc.* **24**, 1009 (1965); G. Klein, *Ann. Rev. Microbiol.* **20**, 223 (1966).
2. D. L. Morton and R. A. Malmgren, *Science* **162**, 1279 (1968); F. R. Eilber and D. L. Morton, *Cancer* **26**, 588 (1970).
3. D. L. Morton, R. A. Malmgren, W. T. Hall, G. Schidlovsky, *Surgery* **66**, 152 (1969).
4. F. R. Eilber and D. L. Morton, *J. Nat. Cancer Inst.* **44**, 651 (1970).
5. E. A. Boyse, L. J. Old, E. Stockert, *Ann. N.Y. Acad. Sci.* **99**, 574 (1962).

14 August 1970

Marihuana: Studies on the Disposition and Metabolism of Delta-9-Tetrahydrocannabinol in Man

Abstract. *Δ^9-Tetrahydrocannabinol (the major active component of marihuana) administered intravenously to normal human volunteers persists in plasma for more than 3 days ($t_{1/2}$ = 56 hours). Its metabolites appear in plasma within 10 minutes after administration and persist along with the precursor compound. Δ^9-Tetrahydrocannabinol is completely metabolized in man, and the radioactive metabolites are excreted in urine and feces for more than 8 days.*

Marihuana and hashish are psychoactive plant materials prepared from *Cannabis sativa*. The active component of *Cannabis* in animals (*1*) and in man (*2*) has been reported to be Δ^9-tetrahydrocannabinol (Δ^9THC). Until recently there has been little information regarding the metabolism and disposition of Δ^9THC because of the difficulty in synthesizing and assaying this com-

pound. The availability of ^{14}C-labeled Δ^9THC of relatively high specific activity has made possible studies of its physiologic disposition and metabolism in rabbits (*3*) and rats (*4*). However, studies on the physiologic disposition and metabolism in man have not been reported. We now report the levels of Δ^9THC in plasma after its intravenous injection, its retention in body stores, and the excretion of its metabolites in man.

Three normal volunteers (one male and two females) between the ages of 18 and 22 who professed no previous exposure to *Cannabis* (*5*) were given 0.5 mg of [^{14}C]Δ^9THC (*6*) intravenously. Blood samples were obtained at intervals thereafter, and urine and feces were collected for up to 10 days after injection of the labeled compound. The unchanged Δ^9THC was measured by extraction at *p*H 6.5 to 7.5 into four volumes of heptane containing 1.5 percent isoamyl alcohol.

The radioactivity in the organic phase was assayed by liquid-scintillation spectrometry. Of the Δ^9THC added to plasma or urine 95 ± 5 percent was recovered. Total radioactivity in aliquots of plasma and urine and in a methanol extract of feces was determined by liquid-scintillation spectrometry. After extraction of Δ^9THC with heptane, polar metabolites which remained in the aqueous phase were extracted first with ether and then with ethyl acetate. The most polar metabolites and conjugates remained in the aqueous phase.

After intravenous administration of [^{14}C]Δ^9THC the amount of this compound in plasma declined rapidly during the first hour (with a half-life of about 30 minutes) (Fig. 1). After 1 hour the Δ^9THC fell much more slowly (with a half-life of 50 to 60 hours). To establish the identity of the apparent Δ^9THC measured in the heptane extract of plasma, samples of plasma collected during the first hour, during the remainder of day 1, and during days 2 and 3 were pooled separately and extracted with heptane. After evaporation at reduced pressure, the residue was taken up in a small volume of ethanol and applied to an Eastman silica-gel chromatogram sheet for development in a hexane : acetone system (3:1). Most of the radioactivity (about 85 percent) in the extract obtained during the first hour is chromatographically identical with authentic Δ^9THC (Fig. 2). A small portion of the radioactivity in the heptane extract had the mobility of authentic 11-OH-THC (*7*). Almost all of the radioactivity in the heptane extract of plasma obtained during the remainder of day 1 and during days 2 and 3 had the same mobility on chromatography as synthetic Δ^9THC.

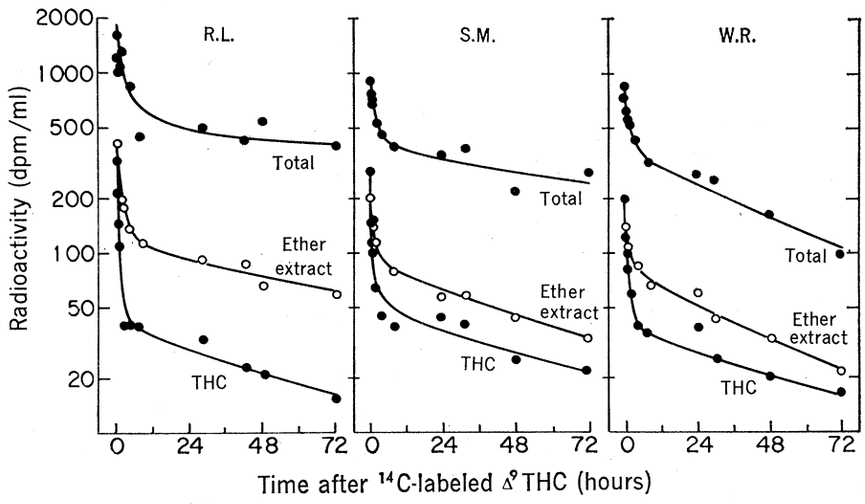

Fig. 1. Plasma levels of Δ^9THC, total radioactivity, and ether-extractable radioactivity after the intravenous injection of [^{14}C]Δ^9THC (0.5 mg in 1 ml of ethanol) to three normal volunteers. The radioactive solution was injected during an interval of 1 minute into the tubing of a rapidly flowing intravenous infusion of 5 percent dextrose in water. The dose ranged from 80 to 146 nc/kg (5.6 μg/kg to 7.9 μg/kg). Blood samples were drawn in heparinized syringes from the opposite arm at various times. Plasma was assayed for Δ^9THC, total radioactivity, and ether-extractable radioactivity by liquid-scintillation spectrometry.

The decline of total radioactivity and the more polar ether-extractable metabolites in plasma was similar to that of Δ^9THC. A rapid initial decline preceded a much slower phase of disappearance from the plasma. Polar metabolites were formed rapidly and were present in plasma at higher concentrations than Δ^9THC.

About 30 percent of the administered radioactivity was excreted in the urine (Fig. 3); most appeared during day 1, but metabolites continued to be excreted into the urine for more than 1 week. Less than 1 percent of the urinary radioactivity was unchanged Δ^9THC, and ^{14}C-labeled 11-OH-THC did not appear to account for more than a small percentage of the metabolites. Even after hydrolysis of conjugates with Glusulase (*8*), which increased the proportion of labeled 11-OH-THC in the

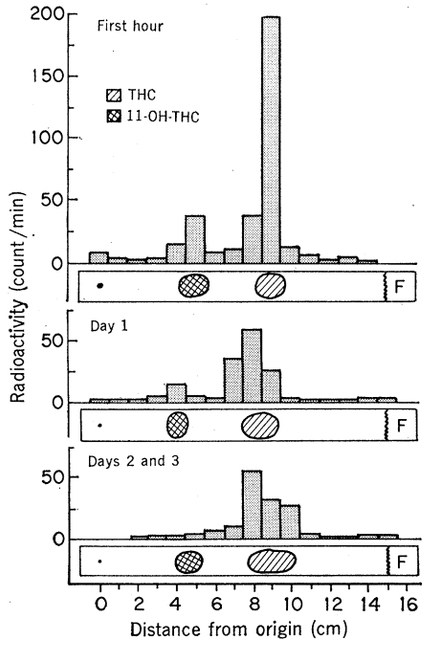

Fig. 2. Histogram of thin-layer chromatography of ^{14}C-labeled Δ^9THC extracted from plasma at various times. R.L. represents a typical subject. Samples of plasma from the first hour, from the remainder of day 1, and from days 2 and 3 were pooled separately and extracted with four volumes of a solution of heptane containing 1.5 percent isoamyl alcohol. The extract was evaporated to dryness at reduced pressure, dissolved in a small volume of ethanol, applied to an Eastman silica-gel chromatogram sheet, and developed in a hexane : acetone (3 : 1) system. Authentic Δ^9THC and 11-OH-Δ^8THC were cochromatographed with the heptane extract. The sheet was cut into 1-cm strips from the origin to the solvent front and placed in vials containing scintillation solution, and radioactivity was determined by liquid-scintillation spectrometry.

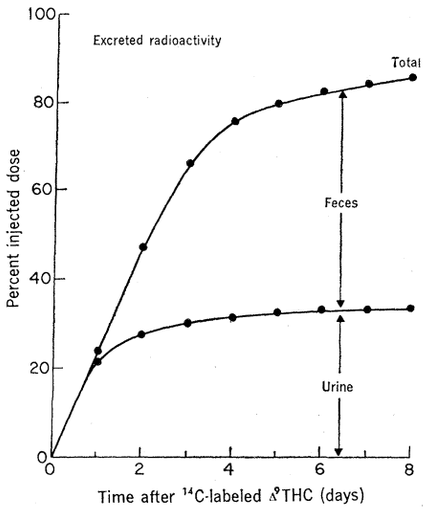

Fig. 3. Cumulative excretion of radioactivity after the intravenous injection of [^{14}C]Δ^9THC. W.R. represents a typical subject. Urine and feces were collected for at least 8 days after the intravenous administration of [^{14}C]Δ^9THC. Urine and feces were frozen until analyzed. The feces were suspended in three volumes of methanol and vigorously shaken for 10 minutes on a mechanical shaker. The material was centrifuged, and an aliquot of the methanol extract was assayed for total radioactivity. Urine was assayed directly for total radioactivity by liquid-scintillation spectrometry.

ether extract, about 80 percent of the metabolites remained uncharacterized. The extraction and chromatographic properties of this portion suggested that the radioactivity might be in the form of polar compounds.

About one-half of the radioactivity administered as [^{14}C]Δ^9THC is recovered in the feces (Fig. 3). There was almost no radioactivity in the feces collected on day 1, presumably because the compounds were excreted in the bile and their passage through the gastrointestinal tract delayed excretion in the feces. A methanol extract of feces containing the radioactivity was evaporated at reduced pressure, and the residue was dissolved in phosphate buffer (0.1*M*, *p*H 6.5). The heptane extract of this aqueous solution contained most of the radioactivity. Almost none had the chromatographic characteristics of Δ^9THC, however; and only 10 percent appeared to be the 11-hydroxy derivative. Hydrolysis of conjugates by incubation with Glusulase increased the apparent 11-OH-[^{14}C]THC to about 20 percent of the radioactivity. Most of the remaining ^{14}C stayed at the origin, presumably in the form of more polar metabolites.

The initially rapid decrease of [^{14}C]Δ^9THC in the plasma represents redistribution of the Δ^9THC from the intravascular compartment into tissues (including brain) and metabolism. In man the effects of marihuana are maximum within 15 minutes, diminished between 30 minutes and 1 hour, and largely dissipated by 3 hours (*9*). This would be consistent with the finding that, after intravenous administration of [^{14}C]Δ^9THC, the concentration in plasma rapidly declines, with a half-life of about 30 minutes, and that over two-thirds of the total radioactivity excreted in the urine is present during day 1. During this initial phase, metabolites of Δ^9THC are present in higher concentrations in the plasma than the parent drug (Fig. 1).

The slower decline of Δ^9THC in plasma ($t_{1/2} = 56$ hours) and of total radioactivity ($t_{1/2} = 67$ hours) presumably represents retention and slow release of the drug from its stores. Since Δ^9THC is a nonpolar compound, it may accumulate in fat or other tissues such as lung which have an affinity for drugs. It has been reported that, in animals (*3, 10*) soon after the intravenous administration of labeled Δ^9THC, higher levels of radioactivity were present in lung than in other tissues. If, indeed, the Δ^9THC is bound in lung, then in man this would be even more significant since inhalation is the usual route of administration.

The finding that Δ^9THC and its metabolites persist in humans for long periods indicates that the drug and its metabolites accumulate in tissues when administered repeatedly. It may explain in part the phenomenon of "reverse tolerance" seen in chronic users of marihuana. Possibly a critical degree of tissue saturation must be attained before effective threshold levels of Δ^9THC can be achieved. On the other hand, long-term administration of marihuana may induce enzymes which convert the drug to an active metabolite of Δ^9THC. In animals it appears that 11-OH-THC is as active as Δ^9THC (*7*). In the present study of naive subjects, 11-OH-THC appears to be only a minor metabolite of the Δ^9THC. However, the more polar metabolites present in urine and feces may represent further metabolic products of 11-OH-THC.

Since a considerable percentage of the metabolites of Δ^9THC are excreted in urine during day 1 after its administration, it should be possible, by means of solvent extraction and thin-layer chromatography, to develop a sensitive assay for the detection of metabolites of Δ^9THC in human urine.

From our results it can be concluded that Δ^9THC persists for a long time in normal volunteers and that it is completely metabolized in man and excreted as polar metabolites in urine and feces.

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References and Notes

1. Y. Gaoni and R. Mechoulam, *J. Amer. Chem. Soc.* **86**, 1646 (1964); C. L. Scheckel, E. Boff, P. Dahlem, T. Smart, *Science* **160**, 1467 (1968); R. Mechoulam, A. Shani, H. Edery, Y. Grunfeld, *ibid.* **167**, 611 (1970).
2. H. Isbell, C. W. Gorodetzky, D. Yasinski, U. Claussen, F. von Spulak, F. Korte, *Psychopharmacologia* **11**, 184 (1967); L. E. Hollister, R. K. Richards, H. K. Gillespie, *Clin. Pharmacol. Ther.* **9**, 783 (1968).
3. S. Agurell, I. M. Nilsson, A. Ohlsson, F. Sandberg, *Biochem. Pharmacol.* **19**, 1333 (1970).
4. ———, *ibid.* **18**, 1195 (1969); H. A. Klausner and J. V. Dingel, *Pharmacologist* **12**, 259 (1970); B. T. Ho, G. E. Fritchie, P. M. Kralik, L. F. Englert, W. M. McIsaac, J. I. Heikkila, *J. Pharm. Pharmacol.* **22**, 538 (1970).
5. The volunteers were in residence at the Clinical Center, National Institutes of Health, for periods of at least 1 month prior to the study and received no medications either during this period or during our study.
6. The [^{14}C]Δ^9THC was synthesized by Research Triangle Institute (Research Triangle Park, North Carolina; sample JW-III-47A; specific activity (17.5 μc/mg, 5.5 mc/mmole) is labeled on the 2- and 4-positions of the benzene ring. Purity was shown to be greater than 99 percent by chromatography. The radioactive material was subjected to pyrogen and sterility tests and then prepared sterilely in single-dose vials in ethanol by the NIH Radiopharmacy.
7. 11-Hydroxytetrahydrocannabinol has been synthesized independently by several investigators: E. B. Truit, *Fed. Proc.* **29**, 619 (1970); R. L. Foltz, A. F. Fentiman, Jr., E. G. Leighty, J. L. Walter, H. R. Drewes, W. E. Schwartz, T. F. Page, E. B. Truitt, Jr., *Science* **168**, 844 (1970); M. E. Wall, D. R. Brine, G. A. Brine, C. G. Pitt, R. I. Freudenthal, H. D. Christensen, *J. Amer. Chem. Soc.* **92**, 3466 (1970); Z. Ben-Ziv, R. Mechoulam, S. Burstein, *J. Amer. Chem. Soc.* **92**, 3468 (1970); I. M. Nilsson, S. Agurell, J. L. G. Nilsson, A. Ohlsson, F. Sandberg, M. Wahlqvist, *Science* **168**, 1228 (1970). In our study 11-hydroxy-Δ^8THC was used as a reference compound since 11-hydroxy-Δ^9THC is not commercially available.
8. Glusulase (Endo Laboratories) is a mixture of β-glucuronidase and sulfatase. Urine and feces were adjusted to *p*H 5 and incubated at 37°C for 18 hours.
9. A. T. Weil, N. E. Zinberg, J. M. Nelsen, *Science* **162**, 1234 (1968).
10. H. A. Klausner and J. V. Dingel, *Pharmacologist* **12**, 259 (1970); B. T. Ho, G. E. Fritchie, P. M. Kralik, F. L. Englert, W. M. McIsaac, J. I. Heikkila, *J. Pharm. Pharmacol.* **22**, 538 (1970).
11. The ^{14}C-labeled Δ^9THC, the unlabeled Δ^9THC, and the unlabeled 11-OH-Δ^8THC were obtained from Drs. J. A. Scigliano and M. Braude, Center for Studies of Narcotics and Drug Abuse, National Institute of Mental Health, Chevy Chase, Maryland.

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