Transformation of Fischer Rat Embryo Cells by the Combined Action of Murine Leukemia Virus and (-)-*Trans*-Δ^9-Tetrahydrocannabinol[1] (36478)

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Huebner and Todaro have postulated that the oncogenes of the C-type virus serve as specific gene determinants of cancer induction (1). The tumor expressions of these oncogenes appear to be controlled by host cell repressors which can be derepressed by different agents such as radiation and chemicals. We have applied this hypothesis to the development of *in vitro* transformation systems, in which cultures chronically infected with a C-type leukemia virus had increased sensitivity to transformation by chemicals. Using these systems, we and our colleagues have been able to show the transforming potential of diethylnitrosamine (2), 3-methylcholanthrene (3), dimethylbenzanthracene (4), benzo(a)pyrene (5), extracts of city smog (5), extracts of tobacco smoke condensate (6), and a series of other agents (7). The purpose of this paper is to present our data regarding the oncogenic potential of the four major cannabinoids found in marihuana.

Materials and Methods. High passage Fischer rat embryo cells (F1706 P_{88}) were inoculated with Rauscher leukemia virus (RLV), and after three subcultures treated in duplicate with 1.0 μg or 0.01 μg per ml of either (-)-trans-Δ^8-tetrahydrocannabinol (Δ^8THC), trans-cannabidiol (CBD), (-)-trans-Δ^9-tetrahydrocannabinol (Δ^9THC), or cannabinol (CBN). Cultures treated with 0.5 μg per ml of 3-methylcholanthrene (3MC), acetone (1:100), or virus alone were used as controls. The cannabinoids were supplied through the courtesy of Dr. John Scigleano of the National Institute of Mental Health, who showed, by gas chromatography, that the CBD and CBN were 99% pure, Δ^8THC 98% pure, and Δ^9THC 95.4% pure. The 3MC and the cannabinoids were diluted in acetone to 1000 μg/ml, and further diluted to the desired concentrations in complete growth medium. The rat embryo cells were fed with chemical every other day for 6 days and then passaged serially at weekly intervals in the absence of chemical. The duplicate cultures were carried each as individual series. At each passage, one set of flasks was set aside to hold indefinitely (holding series) and the other set was subdivided to provide two new sets of cultures, one for holding indefinitely and the other for biweekly subdivision (vertical series). The holding series was refed twice a week, and the vertical series three times a week, with Eagle's minimal essential medium (EMEM) supplemented with 10% fetal bovine serum, 2 m*M* L-glutamine, 0.1 m*M* nonessential amino acids, and antibiotics.

Our standard assay has been based on the transformation of RLV infected F1706 cells by 3MC, which usually occurs three passages after chemical treatment. Once the positive 3MC control has become transformed, we continue to test negative controls and experimental unknowns for an additional three vertical subdivisions. Those compounds which cause transformation are considered to be carcinogenic. In order to monitor the point at which spontaneous transformation occurs in our cell system, a number of untreated controls and selected transforming and non-transforming agents have been tested for extended periods of time (20 vertical passages, 40 weeks). Those chemicals which induce trans-

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formation in this extended assay are considered to be weak or questionable carcinogens needing further study.

Results. In each of two standard assay experiments, the cannabinoids under study failed to cause any visible cell transformation after six subdivisions, although the 3MC positive control became transformed after three subdivisions. Thus, by our standard test, the cannabinoids were not transforming agents. In two extended assay experiments, however, cultures treated with either 1.0 μg or 0.1 μg Δ^9THC did produce transformed foci, but not until the 13th subculture, when macroscopic foci became evident after three weeks in the holding series. Cultures treated with 1 μg/ml produced an average of one focus/flask; cultures treated with 0.01 μg/ml produced an average of six foci-flask. The control cultures and cultures treated with Δ^8THC, CBN, or CBD showed no transformed foci after 20 vertical passages and 6 weeks of holding at each passage level.

A large focus, about the area of a dime, was removed from a 0.01 μg Δ^9THC culture (13 subdivisions, 5 weeks of holding) and a cell line initiated. Another cell line was started from a similar large focus found in a sister series treated with 3MC. A random scraping from the RLV control which had been treated only with acetone was also cultured from the 5-week holding series of the 13th vertical subpassage.

Cultures initiated from the transformed foci of the 3MC and 0.01 μg Δ^9THC cultures, and from the random scraping of the RLV control, were inoculated subcutaneously into newborn Fischer rats at a concentration of 1.5×10^6 cells/0.05 ml. Progressively growing sarcomas appeared at the site of inoculation in five days in nine out of nine rats inoculated with the 3MC transformed cultures, and in six out of six rats inoculated with 0.01 μg Δ^9THC culture. No tumors were seen in eleven rats inoculated with the RLV–acetone culture after 90 days (Table I).

TABLE I. Transformation of Rat Embryo Cells by Δ^9THC In an Extended Assay System.

Chemical	Transformed Foci	Tumors
1.0 μg Δ^8THC	– (P_{20})	ND
0.01 μg Δ^8THC	– (P_{20})	ND
1.0 μg Δ^9THC	+ (P_{13})	ND
0.01 μg Δ^9THC	+ (P_{13})	+ (6 of 6) (5 days)
1.0 μg CBD	– (P_{20})	ND
0.01 μg CBD	– (P_{20})	ND
1.0 μg CBN	– (P_{20})	ND
0.01 μg CBN	– (P_{20})	ND
0.5 μg 3MC	+ (P_3)	+ (9 of 9) (5 days)
Acetone 1:100	– (P_{20})	– (0 of 11) (90 days)

Discussion. Our data indicate that the cannabinoids, except for Δ^9THC, are inactive as transforming agents in either our standard assay or extended assay test systems. The Δ^9THC does have transforming activity which can be shown only in the extended assay. The fact that 0.01 μg/ml Δ^9THC produced more foci than 1.0 μg/ml indicates that the lower concentration was close to the optimal concentration for transformation in this system. It is quite common in this type of experiment that there is an optimal concentration, and that levels above and below it produce fewer or even no foci (3).

It is extremely difficult to interpret these results in terms of the potential danger of Δ^9THC as a carcinogen. First of all, we must emphasize that what we tested were the plant extracts, not smoke condensates. Secondly, we do not know how to weigh the factors involved in the transformation systems. On the one hand, considering the extreme sensitivity of the test system and the relative length of time needed to see transformation, it would seem that the drug is of little or no importance as a carcinogen. On the other hand, considering the low dose needed to cause transformation and the high malignancy of the cells once transformed, it would seem that the drug is worthy of further study as a potential carcinogen.

Summary. The cannabinoid, Δ^9THC [(-)-trans-Δ^9-tetrahydrocannabinol], was found to transform high passage Fischer rat cells chronically infected with Rauscher leukemia virus, but the transformation could be demonstrated only after extended subculture (13 passages) of the cells. Under the same condi-

tions, control cultures and cultures treated with Δ^8THC [(-)-trans-Δ^8-tetrahydrocannabinol], CBN [cannabinol], or CBD [transcannabinol], were not transformed (20 passages). In contrast, cultures treated with 3MC [3-methylcholanthrene] were transformed in three subpassages. Thus, Δ^9THC is the most active of the major cannabinoids as a transforming agent, but this activity is weak as compared to 3MC.

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