Antineoplastic Activity of Cannabinoids

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SUMMARY—Lewis lung adenocarcinoma growth was retarded by the oral administration of Δ₉-tetrahydrocannabinol (Δ₉-THC), Δ₉-tetrahydrocannabinol (Δ₉-THC), and cannabidiol (CBD). Animals treated for 10 consecutive days with Δ₉-THC, beginning the day after tumor implantation, demonstrated a dose-dependent action of retarded tumor growth. Mice treated for 20 consecutive days with Δ₉-THC and CBD had reduced primary tumor size. CBD showed no inhibitory effect on tumor growth at 14, 21, or 28 days. Δ₉-THC, Δ₉-THC, and CBD increased the mean survival time (36% at 100 mg/kg, 25% at 200 mg/kg, and 27% at 50 mg/kg, respectively), whereas CBD did not. Δ₇-THC administered orally daily until death in doses of 50, 100, or 200 mg/kg did not increase the life-spans of (C57BL/6 × DBA/2)F₁ (BDF₁) mice hosting the L1210 murine leukemia. However, Δ₉-THC administered daily for 10 days significantly inhibited Friend leukemia virus-induced splenomegaly by 71% at 200 mg/kg as compared to 90.2% for actinomycin D. Experiments with bone marrow and isolated Lewis lung cells incubated in vitro with Δ₉-THC and Δ₇-THC showed a dose-dependent (10⁻⁴–10⁻¹) inhibition (80–20%, respectively) of tritiated thymidine and [¹⁴C]-uridine uptake into these cells. CBD was active only in high concentrations (10⁻¹).—J Natl Cancer Inst 55: 597–602, 1975.

Investigations into the physiologic processes affected by the psychoactive constituents of marihuana [Δ₉-tetrahydrocannabinol (Δ₉-THC) and Δ₉-tetrahydrocannabinol (Δ₉-THC)] purified from Cannabis sativa are extensive (1). However, only recently have attempts been made to elucidate the biochemical basis for their cytotoxic or cytostatic activity. Leuchtenberger et al. (2) demonstrated that human lung cultures exposed to marihuana smoke showed alterations in DNA synthesis, with the appearance of anaphase bridges. Zimmerman and McClean (3), studying macromolecular synthesis in Tetrahymena, indicated that very low concentrations of Δ₉-THC inhibited RNA, DNA, and protein synthesis and produced cytolyis. Stenchever et al. (4) showed an increase in the number of damaged or broken chromosomes in chronic users of marihuana. Δ₉-THC administered iv inhibited bone marrow leukopoiesis (5), and Kolodny et al. (6) reported that marihuana may impair testosterone secretion and spermatogenesis. Furthermore, Nahas et al. (7) showed that in chronic marihuana users there is a decreased lymphocyte reactivity to mitogens as measured by thymidine uptake. These and other (8) observations suggest that marihuana (Δ₉-THC) interferes with vital cell biochemical processes, though no definite mechanism has yet been established. A preliminary report from this laboratory (9) indicated that the ability of Δ₉-THC to interfere with normal cell functions might prove efficacious against neoplasms. This report represents an effort to test various cannabinoids in several in vivo and in vitro tumor systems to determine the kinds of tumors that are sensitive to these compounds and reveal their possible biochemical sites of action(s).

MATERIALS AND METHODS

The tumor systems used were the Lewis lung adenocarcinoma, leukemia L1210, and B-tropic Friend leukemia.

In vivo systems.—Lewis lung tumor: For the maintenance of the Lewis lung carcinoma, approximately 1-mm³ pieces of tumor were transplanted into C57BL/6 mice with a 15-gauge trocar. In experiments involving chemotherapy, 14- to 18-day-old tumors were excised, cleared of debris and necrotic tissue, and cut into small fragments (~1 mm³). Tumor tissue was then placed in 0.25% trypsin in Dulbecco's medium with 100 U penicillin/ml and 100 µg streptomycin/ml. After 90 minutes' incubation at 22° C, trypsin action was stopped by the addition of complete medium containing heat-inactivated fetal calf serum (final concentration, 20%). Cells were washed twice in complete medium, enumerated in a Coulter counter (Model ZB), or on a hemocytometer, and resuspended in serum-free medium at a concentration of 5 x 10⁶ cells/ml. Next 1 x 10⁴ cells were injected ip into the right hind gluteus muscle, and drugs administered as described in "Results." Standard regimens provided for 10 consecutive daily doses beginning 24 hours after tumor inoculation. Body weights were recorded before tumor inoculation and weekly for 2 weeks. Tumor size was measured weekly for the duration of the experiment and converted to mg tumor weight, as described by Mayo (10).

Friend leukemia: B-tropic Friend leukemia virus (FLV) was maintained in BALB/c mice, and drug evaluation performed in the same animals. Pools of virus were prepared from the plasma of mice given FLV and stored at −70° C. In experiments with FLV, 0.2 ml of a 1/20 dilution of plasma (derived from FLV-infected mice) in medium was inoculated ip into BALB/c mice. Cannabinoids were administered orally daily for 10 consecutive days beginning 24 hours after virus inoculation. Twenty-four hours after the last drug administration, the mice were killed by cervical dislocation, and the spleens removed and weighed. Mice not given FLV were treated as described above, to evaluate possible drug-induced splenomegaly.

L1210 leukemia: The murine leukemia L1210 was maintained in DBA/2 mice by weekly transfers of 10⁶ cells derived from the peritoneal cavity. In these experiments, 10⁶ leukemia cells were inoculated ip into C57BL/6 mice, and the mice were treated daily for 10 consecutive days beginning 24 hours after tumor cell inoculation. Mean survival time was used as an index of drug activity.

In vitro cell systems.—Lewis lung tumor: We obtained isolated Lewis lung tumor cells by subjecting 1-mm³ sections of tumor to 0.25% trypsin at 22° C and stirring for 60–90 minutes. After trypsinization, the cells were centrifu...
fuged (1,000 rpm for 10 min) and washed twice in Dul
becco's medium containing 20% heat-inactivated fetal calf
serum. They were then reconstituted to 10⁶ cells/ml in Dul
becco's medium containing, for every 500 ml, 5 ml of
200 mx glutamine, 5,000 U penicillin, and 5,000 µg strep
tomycin. Tumor cells (3e6 ml) were dispensed into 25-ml
Erlenmeyer flasks and preincubated with either the drug
or the drug vehicle for 15 minutes in a Dubnoff metabolic
shaker at 37°C in an atmosphere of 5% CO₂-95% O₂.
After preincubation, 10 µl tritiated thymidine (³H-TDR)
(10 µCi, 57 Ci/m mole; New England Nuclear Corp., Bos
ton, Mass.) was added to each flask and incubated for vari
ous times, after which 1-ml aliquots were removed and
placed in 10x75-mm test tubes containing 1 ml 10%
trichloroacetic acid (TCA) at 4°C. The TCA-precipi
tated samples were then filtered on 0.45-µ Millipore filters
and washed twice with 5 ml of 10% TCA at 4°C.
The filters were transferred to liquid scintillation vials
and counted in a toluene cocktail containing Liquidfluor
(New England Nuclear Corp.) (4 liters toluene to 160 ml
Liquifluor). Samples were then counted in a liquid
scintillator.

Bone marrow: Bone marrow cells were derived from
the tibias and fibulas of BDF1 mice. One ml Dubbecco's
medium containing 1 U heparin/ml was forced through
each bone by a 1-ml syringe with a 26-gauge needle. The
cells were washed three times, nucleated cells were enu
merated on a hemocytometer, and cell viability was ascer
tained by trypan blue exclusion. Cell number was ad
justed to 10⁶ cells/ml with heparin-free Dubbecco's
medium and incubated at 4°C for 15 minutes. Bone
marrow cells were then dispensed (3-5 ml) into 25-ml
Erlenmeyer flasks containing the test drug or the drug
vehicle. This preincubation period was followed by the
addition of 10 µl ³H-TDR and the procedures done as
outlined for the isolated Lewis lung cells.

L1210: L1210 cells were derived from DBA/2 mice as
described above. They were obtained from DBA/2 mice
and inoculated 7 days before the experiment by the
peritoneal cavity being flushed with 10 ml Dubbecco's
medium containing heparin (5 µ/ml). The cells were
washed three times in medium, and the final medium
wash did not contain heparin. The cells were resus
ded at 10⁶ cells/ml and treated as described above.
Cells were routinely counted with a hemocytometer for
the determination of cell viability with trypan blue; for
Lewis lung tumor and L1210 cells, a Coulter apparatus
(Mode ZB,) was also used.

All other reagents were of the highest quality grade
available. Actinomycin D, 5-fluorouracil (5-FU), and
cytosine arabinoside (ara-C) were provided by the Drug
Development Branch, National Cancer Institute (NCI).

Cannabinoids.—The structures of the four compounds
are shown in text-figure 1. All occur naturally in mari
huana and were chemically synthesized. These drugs
were provided by the National Institute on Drug Abuse
or the Sheehan Institute for Research, Cambridge, Massa
chusetts. In the preparation of the drugs, the cannabi
noids were complexed to albumin or solubilized in
Emulphor-alcohol. Both preparations produced similar
antitumor activity. With albumin, the cannabinoids were
prepared in the following manner: A stock solution of
150 mg cannabinoid per ml absolute ethanol was made.
Six ml of this solution was placed in a 200-ml flask. The
ethanol was evaporated off under a stream of nitrogen
and 2,100 mg lyophilized bovine serum albumin (BSA)
added. After the addition of 20 ml distilled water, the
substances were stirred with a glass rod in a sonicator
until a good suspension was achieved. Sufficient distilled
water was then added to make the desired dilution. Con
centrations were routinely checked with a gas chromat
ograph. When Emulphor-alcohol was used as the vehi
cle, the desired amount of cannabinoid was solubilized in
a solution of equal volumes by absolute ethanol and
Emulphor (El-620; GAF Corp., New York, N.Y.) and
then diluted with 0.15 n NaCl for a final ratio of 1:1:4
(ethanol:Emulphor:NaCl).

RESULTS

Effects of Cannabinoids on Murine Tumors

Δ⁸-THC, Δ⁹-THC, and cannabinol (CBN) all inhibited
primary Lewis lung tumor growth, whereas cannabidiol
(CBD) enhanced tumor growth. Oral administration of
25, 50, or 100 mg Δ⁹-THC/kg inhibited primary tumor
growth by 48, 72, and 75%, respectively, when measured
12 days post tumor inoculation (table 1). On day 19,
mice given Δ⁹-THC had a 34% reduction in primary
tumor size. On day 30, primary tumor size was 76% that
of controls and only those given 100 mg
Δ⁹-THC/kg
survived significantly longer
(36%). Mice treated with Δ⁹-THC showed a slight weight loss
over the 2-week period (average loss, 0.3 g at 50 mg/kg
and 0.1 g at 100 mg/kg). This can be compared to cyclo
phosphamide, which caused weight loss approaching 20%
(table 2).

Δ⁹-THC activity was similar to that of Δ⁹-THC when
administered orally daily until death (table 2). However,
as with Δ⁹-THC, primary tumor growth approached
control values after 3 weeks. When measured 12 days post
tumor inoculation, all doses (50-400 mg/kg) of Δ⁹-THC
inhibited primary tumor growth between 40 and 60%.
Significant inhibition was also seen on day 21, which was
comparable to cyclophosphamide-treated mice. Although
this was not the optimum regimen for cyclophosphamide,
it was the positive control protocol provided by the NCI
(11). All mice given Δ⁹-THC survived significantly longer
than controls, except those treated with 100 mg/kg. Mice
given 50, 200, and 400 mg/kg Δ⁹-THC had an increased
life-span of 22.6, 24.6, and 27.2%, respectively, as com
pared to 33% for mice treated with 20 mg cyclophos-
Table 1.—Effect of Δ⁹-THC on tumor growth and survival time of mice hosting Lewis lung carcinoma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Body weight change (g)</th>
<th>Tumor weights (g) at</th>
<th>Mean survival (time)</th>
<th>Increased life-span, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 days b</td>
<td>19 days b</td>
<td>30 days b</td>
</tr>
<tr>
<td>Control (BSA 7.5%)</td>
<td>—</td>
<td>+1.5</td>
<td>892±150</td>
<td>3,456±252</td>
<td>5,883±673</td>
</tr>
<tr>
<td>Δ⁹-THC</td>
<td>25</td>
<td>+0.9</td>
<td>468±107 d</td>
<td>2,363±146 d</td>
<td>4,337±276 d</td>
</tr>
<tr>
<td>Δ⁹-THC</td>
<td>50</td>
<td>0.3</td>
<td>255±118 d</td>
<td>2,168±85 d</td>
<td>4,851</td>
</tr>
<tr>
<td>Δ⁹-THC</td>
<td>100</td>
<td>0.1</td>
<td>221±98 d</td>
<td>2,307±362 d</td>
<td>4,666±312 d</td>
</tr>
</tbody>
</table>

*Groups of mice were inoculated im with 1 X 10⁶ Lewis lung cells and treated orally for 10 days with Δ⁹-THC.

Table 2.—Effect of Δ⁹-THC on tumor growth and survival time of BDF₁ mice hosting Lewis lung carcinoma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Body weight change (g)</th>
<th>Tumor weights (g) at</th>
<th>Mean survival (time)</th>
<th>Increased life-span, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 days b</td>
<td>21 days c</td>
<td></td>
</tr>
<tr>
<td>Control (BSA 7.5%)</td>
<td>—</td>
<td>—1.6</td>
<td>621±30</td>
<td>4,880±380</td>
<td></td>
</tr>
<tr>
<td>Δ⁹-THC</td>
<td>50</td>
<td>−0.9</td>
<td>238±46 d</td>
<td>3,104±274 d</td>
<td>37.4±1.7</td>
</tr>
<tr>
<td>Δ⁹-THC</td>
<td>100</td>
<td>−3.4</td>
<td>164±36 d</td>
<td>3,194±413 d</td>
<td>38.8±1.2</td>
</tr>
<tr>
<td>Δ⁹-THC</td>
<td>200</td>
<td>−1.6</td>
<td>174±33 d</td>
<td>2,940±194 d</td>
<td>40.6±1.8</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>20</td>
<td>−4.0</td>
<td>122±38 d</td>
<td>1,876±174 d</td>
<td>42.5±3.3</td>
</tr>
<tr>
<td>Pyran copolymer</td>
<td>50</td>
<td>+0.3</td>
<td>965±146</td>
<td>6,743±376</td>
<td>29.9±1.2</td>
</tr>
</tbody>
</table>

*Groups of male BDF₁ mice were inoculated im with 10⁶ Lewis lung carcinoma cells and treated orally daily with Δ⁹-THC until death. Cyclophosphamide and pyran copolymer were administered ip for 10 consecutive days beginning 24 hours after tumor inoculation.

Table 3.—Effect of CBN on tumor growth and survival time in BDF₁ mice hosting Lewis lung carcinoma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Body weight change (g)</th>
<th>Tumor weights (g) at</th>
<th>Mean survival (time)</th>
<th>Increased life-span, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>14 days c</td>
<td>24 days c</td>
<td></td>
</tr>
<tr>
<td>Control (BSA 7.5%)</td>
<td>—</td>
<td>+3.3</td>
<td>1,288±146</td>
<td>5,520±566</td>
<td>26.6±1.3</td>
</tr>
<tr>
<td>CBN</td>
<td>25</td>
<td>−0.6</td>
<td>965±146 d</td>
<td>6,743±376</td>
<td>29.9±1.2</td>
</tr>
<tr>
<td>CBN</td>
<td>50</td>
<td>−0.6</td>
<td>875±115 d</td>
<td>5,769±291</td>
<td>33.7±1.6</td>
</tr>
<tr>
<td>CBN</td>
<td>100</td>
<td>−2.6</td>
<td>296±98 d</td>
<td>4,843±462</td>
<td>27.8±0.9</td>
</tr>
</tbody>
</table>

*Groups of mice inoculated im with 1 X 10⁶ Lewis lung carcinoma cells and treated orally daily with Δ⁹-THC or CBN until death.

Cannabis, administered by gavage daily until death, demonstrated antitumor activity against the Lewis lung carcinoma when evaluated on day 14 post tumor inoculation (table 3). Primary tumor growth was inhibited by 77% at doses of 100 mg/kg on day 14 but only by 11% on day 24. At 50 mg/kg, CBN inhibited primary tumor growth by only 32% when measured on day 14, and no inhibition was observed on day 24; however, these animals did survive 27% longer.

CBD, administered at 25 or 200 mg/kg daily until death, showed no tumor-inhibitory properties as measured by primary Lewis lung tumor size or survival time (table 4). In this experiment, CBD-treated mice showed enhanced primary tumor growth. However, the control tumor growth rate in this experiment was decreased as compared to the previous studies.

Survival time of BDF₁ mice hosting L1210 leukemia...
TABLE 4.—Effect of CBD on tumor growth and survival time in BDF₁ mice hosting Lewis lung carcinoma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Body weight change (g) b</th>
<th>Tumor weights (g) at</th>
<th>Mean survival time (days)</th>
<th>Increased life-span, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(day 0–7, 0–14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (BSA 7.5%)</td>
<td>—</td>
<td>+0.9 (6)</td>
<td>1,005±108</td>
<td>30.7±3.3</td>
<td>—</td>
</tr>
<tr>
<td>CBD</td>
<td>25</td>
<td>+0.9 (6)</td>
<td>1,274±219</td>
<td>7.709±711</td>
<td>28.4±2.3</td>
</tr>
<tr>
<td>CBD</td>
<td>200</td>
<td>+0.7 (8)</td>
<td>3,890±261</td>
<td>26.3±1.64</td>
<td>0</td>
</tr>
</tbody>
</table>

* Groups of mice were inoculated im with 1 X 10⁶ Lewis lung cells and treated orally daily until death with CBD.
* Whole body weight changes after 10 days of treatment.
* Post tumor implants; tumor weights were derived from measurement of major and minor tumor axes. Values are means±SE; number of mice are indicated in parentheses.

Longer incubations (i.e., 60 min) did not significantly change the uptake pattern for control and Δ⁹-THC-treated tumor cells.

The effect of several cannabinoids on the uptake of ³H-TDR into cells incubated in vitro indicated that Δ⁹-THC, Δ⁸-THC, and CBN produced a dose-dependent inhibition of radiolabel uptake in the three cell types (table 7). These results, presented as percent inhibition of radiolabel uptake as compared to control, represented an effect of cannabinoids on one aspect of macromolecular synthesis. CBD was the least active of the cannabinoids, but showed its greatest activity in the L1210 leukemia cells. Other data (not shown) indicate that these

TABLE 5.—Δ⁹-THC vs. leukemia L1210 a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean survival time (days) b</th>
<th>Increased life-span, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>—</td>
<td>8.6±0.2</td>
<td>0</td>
</tr>
<tr>
<td>Δ⁹-THC</td>
<td>50</td>
<td>8.5±0.2</td>
<td>0</td>
</tr>
<tr>
<td>Δ⁸-THC</td>
<td>100</td>
<td>7.8±0.7</td>
<td>0</td>
</tr>
<tr>
<td>Δ⁹-THC</td>
<td>200</td>
<td>8.6±0.3</td>
<td>0</td>
</tr>
</tbody>
</table>

a BDF₁ mice were inoculated with 10⁶ L1210 cells and treated orally daily until death.

b Values are means±SE; 8 mice per group.

c Emulphor diluent administered orally at 0.01 ml/g.

Effect of Cannabinoids on Isolated Cells In Vitro

Isolated cells incubated in vitro represent a simple, reliable, and, hopefully, predictive method for the monitoring of the effects of agents on several biochemical parameters at the same time. The incorporation of ³H-TDR into TCA-precipitable counts in isolated Lewis lung cells is shown in text-figure 2. Similar types of curves were seen for bone marrow and L1210 cells. In all instances, for 15–45 minutes there was a linear increase in ³H-TDR uptake into the TCA-precipitable fraction. Qualitatively, similar data (not shown) were seen after a pulse with ¹⁴C-uridine. Actinomycin D (1 µg/ml) preferentially inhibited ¹⁴C-uridine incorporation, whereas it only affected ³H-TDR incorporation after uridine uptake had decreased to less than 30% that of control (data not shown). This is indirect evidence that we were measuring RNA synthesis. Experiments (data not shown) done with 5-FU (10⁻⁴ M) indicated that, in isolated bone marrow cells, both thymidine and uridine uptake were markedly inhibited, whereas the isolated Lewis lung cells showed marked insensitivity to 5-FU at this concentration. Inhibition of thymidine uptake with time by Δ⁹-THC (10⁻⁵ M) on Lewis lung cells is depicted in text-figure 2. In this experiment, Δ⁹-THC caused a nonlinear uptake of ³H-TDR. At 30 minutes, uptake of ³H-TDR into the acid-precipitable fraction was about 50% that of control.

TEXT-Figure 2.—Lewis lung tumor cells were prepared as described in "Materials and Methods." Incubation conditions were the same as described in the footnote of table 7. One-ml samples were removed every 5 minutes, and radioactivity in TCA-precipitable fraction was determined. Each point represents mean±SE of four observations.
compounds similarly effect the uptake of ¹³C-uridine into the acid-precipitable fraction. Ara-C markedly inhibited the uptake of ¹³C-TDR uptake more dramatically than did the cannabinoids (table 7). Note that Δ⁹-THC exhibited inhibitory properties in the isolated Lewis lung tumor and L1210 cells at concentrations that did not interfere with thymidine uptake into bone marrow cells. At certain concentrations of CBD (2.5 X 10⁻⁶ and 2.5 X 10⁻⁵ M), radiolabel uptake was consistently stimulated in bone marrow cells and in several experiments with the isolated Lewis lung cells.

**DISCUSSION**

We investigated four cannabinoids for antineoplastic activity against three animal tumor models in vivo and for cytotoxic or cystostatic activity in two tumor cell lines and bone marrow cells in vitro. The cannabinoids (Δ⁹-THC, Δ⁴-THC, and CBN) active in vivo against the Lewis lung tumor cells are also active in the in vitro systems. The differential sensitivity of Δ⁹-THC against Lewis lung cells versus bone marrow cells is unique in that Δ⁹-THC and CBN are equally active in these systems. Johnson and Wiersma (5) reported that Δ⁴-THC administered i.v. caused a reduction in bone marrow myelocytes and an increase in lymphocytes. It is unclear from the data whether this is a depression of myelopoiesis or if it represents a lymphocyte infiltration into the bone marrow. The use of isolated bone marrow cells, which represent a nonneoplastic rapidly proliferating tissue, enables the rapid evaluation and assessment of drug sensitivity and specificity, and thereby may predict toxicity related to bone marrow suppression. CBD showed noninhibitory activity either against the Lewis lung cells in vivo or Lewis lung and bone marrow cells in vitro at 10⁻⁶ M and 10⁻⁵ M, respectively. Indeed, the tumor growth rate in mice treated with CBD was significantly increased over controls. This may, in part, be the consequence of the observation made in vitro (i.e., 10⁻⁷ M CBD stimulated thymidine uptake), which may be reflected by an increased rate of tumor growth.

One problem related to the use of cannabinoids is the development of tolerance to many of its behavioral effects (13). It also appears that tolerance functions in the chemotherapy of neoplasms in that the growth of the Lewis lung tumor is initially markedly inhibited but, by 3 weeks, approaches that of vehicle-treated mice (table 2). This, in part, may reflect drug regimens, doses used, increased drug metabolism, or conversion to metabolites with antagonistic actions to Δ⁹-THC. It may also represent some tumor cell modifications rendering the cell insensitive to these drugs. Of further interest was the lack of activity of Δ⁹-THC against the L1210 in vivo, whereas the in vitro L1210 studies indicated that Δ⁹-THC could effectively inhibit thymidine uptake. The apparent reason for this discrepancy may be related to the high growth fraction and the short doubling time of this tumor. The in vitro data do not indicate that the cannabinoids possess that degree of activity; e.g., ara-C, which "cures" L1210 mice, is several orders of magnitude more potent on a molar basis than Δ⁹-THC in vitro.

Inhibition of tumor growth and increased animal survival after treatment with Δ⁹-THC may, in part, be due to...
to the ability of the drug to inhibit nucleic acid synthesis. Preliminary data with Lewis lung cells grown in tissue culture indicate that $10^{-5} \text{M} \Delta^9$-THC inhibits by 50% the uptake of $^3\text{H}$-TDR into acid-precipitable counts over a 4-hour incubation period. Simultaneous determination of acid-soluble fractions did not show any inhibitory effects on radiolabeled uptake. Therefore, $\Delta^9$-THC may be acting at site(s) distal to the uptake of precursor. We are currently evaluating the acid-soluble pool to see if phosphorylation of precursor is involved in the action of $\Delta^9$-THC.

These results lend further support to increasing evidence that, in addition to the well-known behavioral effects of $\Delta^9$-THC, this agent modifies other cell responses that may have greater biologic significance in that they have antineoplastic activity. The high doses of $\Delta^9$-THC (i.e., 200 mg/kg) are not tolerable in humans. On a body-surface basis, this would be about 17 mg/m² for mice. Extrapolation to a 60-kg man would require 1,020 mg for comparable dosage. The highest doses administered to man have been 250–300 mg (14). Whether only cannabinoids active in the central nervous system (CNS) exhibit this antineoplastic property is not the question, since CBN, which lacks marhuana-like psychoactivity, is quite active in our systems (15). With structure-activity investigations, more active agents may be designed and synthesized which are devoid of or have reduced CNS activity. That these compounds readily cross the blood-brain barrier and do not possess many of the toxic manifestations of presently used cytotoxic agents, makes them an appealing group of drugs to study.

REFERENCES