

## Antineoplastic Activity of Cannabinoids<sup>1,2</sup>

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**SUMMARY**—Lewis lung adenocarcinoma growth was retarded by the oral administration of  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC),  $\Delta^8$ -tetrahydrocannabinol ( $\Delta^8$ -THC), and cannabinol (CBN), but not cannabidiol (CBD). Animals treated for 10 consecutive days with  $\Delta^9$ -THC, beginning the day after tumor implantation, demonstrated a dose-dependent action of retarded tumor growth. Mice treated for 20 consecutive days with  $\Delta^8$ -THC and CBN had reduced primary tumor size. CBD showed no inhibitory effect on tumor growth at 14, 21, or 28 days.  $\Delta^9$ -THC,  $\Delta^8$ -THC, and CBN 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.  $\Delta^9$ -THC administered orally daily until death in doses of 50, 100, or 200 mg/kg did not increase the life-spans of (C57BL/6 x DBA/2)<sub>F</sub><sub>1</sub> (BDF<sub>1</sub>) mice hosting the L1210 murine leukemia. However,  $\Delta^8$ -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  $\Delta^8$ -THC and  $\Delta^9$ -THC showed a dose-dependent ( $10^{-4}$ – $10^{-7}$ ) inhibition (80–20%, respectively) of tritiated thymidine and <sup>14</sup>C-uridine uptake into these cells. CBD was active only in high concentrations ( $10^{-4}$ ).—*J Natl Cancer Inst* 55: 597–602, 1975.

Investigations into the physiologic processes affected by the psychoactive constituents of marijuana [ $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) and  $\Delta^8$ -tetrahydrocannabinol ( $\Delta^8$ -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 marijuana 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  $\Delta^9$ -THC inhibited RNA, DNA, and protein synthesis and produced cytolysis. Stenchever et al. (4) showed an increase in the number of damaged or broken chromosomes in chronic users of marijuana.  $\Delta^9$ -THC administered iv inhibited bone marrow leukopoiesis (5), and Kolodny et al. (6) reported that marijuana may impair testosterone secretion and spermatogenesis. Furthermore, Nahas et al. (7) showed that in chronic marijuana users there is a decreased lymphocyte reactivity to mitogens as measured by thymidine uptake. These and other (8) observations suggest that marijuana ( $\Delta^9$ -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  $\Delta^8$ -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 adeno-

carcinoma, leukemia L1210, and B-tropic Friend leukemia.

***In vivo systems.***—Lewis lung tumor: For the maintenance of the Lewis lung carcinoma, approximately 1-mm<sup>3</sup> 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 ( $\approx 1$  mm<sup>3</sup>). Tumor tissue was then placed in 0.25% trypsin in Dulbecco's medium with 100 U penicillin/ml and 100  $\mu$ g 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 two times 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 10<sup>6</sup> cells/ml. Next 1 x 10<sup>6</sup> cells were injected im 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 10<sup>5</sup> cells derived from the peritoneal cavity. In these experiments, 10<sup>5</sup> leukemia cells were inoculated ip into (C57BL/6 x DBA/2)<sub>F</sub><sub>1</sub> (BDF<sub>1</sub>) 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-mm<sup>3</sup> sections of tumor to 0.25% trypsin at 22° C and stirring for 60–90 minutes. After trypsinization, the cells were centri-

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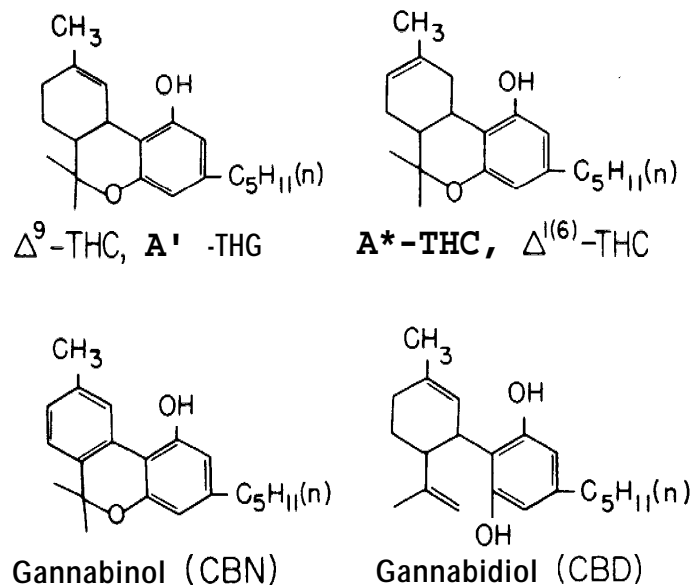
fused (1,000 rpm for 10 min) and washed twice in Dulbecco's medium containing 20% heat-inactivated fetal calf serum. They were then reconstituted to  $10^7$  cells/ml in Dulbecco's medium containing, for every 500 ml, 5 ml of 200 mM glutamine, 5,000 U penicillin, and 5,000  $\mu$ g streptomycin. Tumor cells (3-6 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  $37^\circ\text{C}$  in an atmosphere of 5%  $\text{CO}_2$ -95%  $\text{O}_2$ . After preincubation, 10  $\mu$ l tritiated thymidine ( $^3\text{H-TDR}$ ) (10  $\mu\text{Ci}$ , 57 Ci/mole; New England Nuclear Corp., Boston, Mass.) was added to each flask and incubated for various times, after which 1-ml aliquots were removed and placed in  $10 \times 75$ -mm test tubes containing 1 ml 10% trichloroacetic acid (TCA) at  $4^\circ\text{C}$ . The TCA-precipitated samples were then filtered on 0.45- $\mu$  Millipore filters and washed twice with 5 ml of 10% TCA at  $4^\circ\text{C}$ . The filters were transferred to liquid scintillation vials and counted in a toluene cocktail containing Liquifluor (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 BDF<sub>1</sub> mice. One ml Dulbecco'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 enumerated on a hemocytometer, and cell viability was ascertained by trypan blue exclusion. Cell number was adjusted to  $10^7$  cells/ml with heparin-free Dulbecco's medium and incubated at  $4^\circ\text{C}$  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  $\mu$ l  $^3\text{H-TDR}$  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 Dulbecco's medium containing heparin (5  $\mu$ /ml). The cells were washed three times in medium, and the final medium wash did not contain heparin. The cells were resuspended at  $10^7$  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 marijuana and were chemically synthesized. These drugs were provided by the National Institute on Drug Abuse or the Sheehan Institute for Research, Cambridge, Massachusetts. In the preparation of the drugs, the cannabinoids 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



TEXT-FIGURE 1.-Structures of the four major cannabinoids.

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. Concentrations were routinely checked with a gas chromatograph. When Emulphor-alcohol was used as the vehicle, the desired amount of cannabinoid was sonicated in a solution of equal volumes by absolute ethanol and Emulphor (EI-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

$\Delta^9$ -THC,  $\Delta^8$ -THC, and cannabimol (CBN) all inhibited primary Lewis lung tumor growth, whereas cannabidiol (CBD) enhanced tumor growth. Oral administration of 25, 50, or 100 mg  $\Delta^9$ -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  $\Delta^9$ -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  $\Delta^9$ -THC/kg had a significant increase in survival time (36%).

Mice treated with  $\Delta^9$ -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 cyclophosphamide, which caused weight loss approaching 20% (table 2).

$\Delta^8$ -THC activity was similar to that of  $\Delta^9$ -THC when administered orally daily until death (table 2). However, as with  $\Delta^9$ -THC, primary tumor growth approached control values after 3 weeks. When measured 12 days post tumor inoculation, all doses (50-400 mg/kg) of  $\Delta^8$ -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 (II). All mice given  $\Delta^8$ -THC survived significantly longer than controls, except those treated with 100 mg/kg. Mice given 50, 200, and 400 mg/kg  $\Delta^8$ -THC had an increased life-span of 22.6, 24.6, and 27.2%, respectively, as compared to 33% for mice treated with 20 mg cyclophos-

TABLE 1.—Effect of Δ<sup>9</sup>-THC on tumor growth and survival time of mice hosting Lewis lung carcinoma <sup>a</sup>

Treatment	Dose mg/kg	Body weight change (g) <sup>b</sup>	Tumor weights (g) at			Mean survival time (days)	Increased life-span, %
			12 days <sup>c</sup>	19 days <sup>c</sup>	30 days <sup>c</sup>		
Control (BSA 7.5%)	—	+1.5	892±1 (8)	3,456±252 (8)	5,883±673 (3)	25.8f1.3	—
Δ <sup>9</sup> -THC	2 5	+0.9	468±107 <sup>d</sup> (8)	2,363±146 <sup>d</sup> (8)	4,337±276 <sup>d</sup> (3)	30.3±2.0	17.4
Δ <sup>9</sup> -THC	5 0	-0.3	253±118 <sup>d</sup> (8)	2,168±195 <sup>d</sup> (8)	4,851 <sup>d</sup> (4)	27.4f0.6	6.2
Δ <sup>9</sup> -THC	1 0 0	-0.1	221±98 <sup>d</sup> (7)	2,307±362 <sup>d</sup> (7)	4,666±312 <sup>d</sup> (7)	35.0±1.1 <sup>d</sup>	36

<sup>a</sup> Groups of mice were inoculated im with 1×10<sup>6</sup> Lewis lung cells and treated orally for 10 days with Δ<sup>9</sup>-THC.  
<sup>b</sup> Whole body weight changes after 10 days of treatment.  
<sup>c</sup> Post tumor implants; tumor weights were derived from measurement of major and minor axes. Values are means±SE; number of mice are indicated in parentheses.  
<sup>d</sup> P<0.05 as compared to controls.

TABLE 2.—Effect of Δ<sup>9</sup>-THC on tumor growth and survival time of BDF<sub>1</sub> mice hosting Lewis lung carcinoma <sup>a</sup>

Treatment,	Dose mg/kg	Body weight change (g) <sup>b</sup>	Tumor weights (g) at		Mean survival time (days)	Increased life-span, %
			12 days <sup>c</sup>	21 days <sup>c</sup>		
Control (BSA 7.5%)	—	-1.6	521±30 (30)	4,880±380 (30)	30.5f0.9	
Δ <sup>9</sup> -THC	50	-0.9	238±46 <sup>d</sup> (7)	3,104±274 <sup>d</sup> (7)	37.4±1.7 <sup>d</sup>	22.6
Δ <sup>9</sup> -THC	100	-3.4	164±36 <sup>d</sup> (7)	2,299±236 <sup>d</sup> (7)	34.3zJz1.9	12.4
Δ <sup>9</sup> -THC	200	-1.6	174±53 <sup>d</sup> (6)	3,188±389 <sup>d</sup> (6)	38.0f1.9 <sup>d</sup>	24.6
Δ <sup>9</sup> -THC	400	-3.3	235±78 <sup>d</sup> (6)	3,194±413 <sup>d</sup> (6)	38.8±1.2 <sup>d</sup>	27.2
Cyclophosphamide	20	-4.0	(6)	2,940±194 <sup>d</sup> (6)	40.6±1.8 <sup>d</sup>	33.0
Pyran copolymer	50	+0.3	122±38 <sup>d</sup> (8)	1,876±174 <sup>d</sup> (8)	42.5±3.3 <sup>d</sup>	39.3

<sup>a</sup> Groups of male BDF<sub>1</sub> mice were inoculated im with 10<sup>6</sup> Lewis lung carcinoma cells and treated orally daily with Δ<sup>9</sup>-THC until death. Cyclophosphamide and pyran copolymer were administered ip for 10 consecutive days beginning 24 hours after tumor inoculation.  
<sup>b</sup> Whole body weight changes after 10 days of treatment.  
<sup>c</sup> 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.  
<sup>d</sup> P<0.05 as compared to controls.

TABLE 3.—Effect of CRN on tumor growth and survival time in BDF<sub>1</sub> mice hosting Lewis lung carcinoma <sup>a</sup>

Treatment	Dose mg/kg	Body weight change (g) <sup>b</sup>	Tumor weights (g) at		Mean survival time (days)	Increased life-span, %
			14 days <sup>c</sup>	24 days <sup>c</sup>		
Control (BSA 7.5%)		+3.3	1,288±146 (21)	5,520±566 (21)	26.6±1.3	
CBN	25	-0.6	965±146 <sup>d</sup> (8)	6,743±376 (8)	29.9±1.2	12
CBN	50	-0.6	875±115 <sup>d</sup> (6)	5,769±291 (6)	33.7±1.6	27 <sup>d</sup>
CBN	100	-2.6	296±98 <sup>d</sup> (7)	4,843±462 (7)	27.8±0.9	3.5

<sup>a</sup> Groups of mice inoculated im with 1×10<sup>6</sup> Lewis lung cells and treated orally daily with Δ<sup>9</sup>-THC or CBN until death.  
<sup>b</sup> Whole body weight changes after 10 days of treatment.  
<sup>c</sup> 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.  
<sup>d</sup> P<0.05 as compared to controls.

phamide/kg. Pyran copolymer, an immunopotentiator (12) when administered at 50 mg/kg, also significantly increased the survival time of the animals (39.3%).

CBN, 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 BDF<sub>1</sub> mice hosting L1210 leukemia

TABLE 4.—Effect of CBD on tumor growth and survival time in BDF<sub>1</sub> mice hosting Lewis lung carcinoma<sup>a</sup>

Treatment	Dose mg/kg	Body weight change (g) <sup>b</sup> (day 0-7, 0-14)	Tumor weights (g) at			Mean survival time (days)	Increased life-span, %
			14 days <sup>c</sup>	21 days <sup>c</sup>	28 days <sup>c</sup>		
Control (BSA 7.5%)	—	+0.9 (6) +2.4 (6)	1,005 ± 108	2,813 ± 224	4,283 ± 139	30.7f3.3	—
CBD	2 5	+0.9 +1.6	1,274 ± 219 <sup>(6)</sup>	4,172 ± 525 <sup>d</sup> <sup>(6)</sup>	7,709 ± 711 <sup>d</sup> <sup>(4)</sup>	28.4f2.3	0
CBD	2 0 0	+0.7 +1.9	1,284 ± 128 <sup>(8)</sup>	3,890 ± 261 <sup>d</sup> <sup>(8)</sup>	6,872 ± 1,173 <sup>d</sup> <sup>(3)</sup>	26.3f1.6 <sup>d</sup>	0

<sup>a</sup> Groups of mice were inoculated im with 1 × 10<sup>6</sup> Lewis lung cells and treated orally daily until death with CBD.

<sup>b</sup> Whole body weight changes after 10 days of treatment.

<sup>c</sup> 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.

<sup>d</sup> P < 0.05 as compared to controls.

was not prolonged by Δ<sup>9</sup>-THC treatment (table 5). Mice treated with Δ<sup>9</sup>-THC at doses of 50, 100, and 200 mg/kg, administered orally daily until death, survived 8.5, 7.8, and 8.6 days, respectively, as compared to 8.6 days for mice treated with the diluent. However, Δ<sup>9</sup>-THC inhibited FLV-induced splenomegaly by 71% at 200 mg/kg as compared to 90.2% for the positive control actinomycin D (0.25 mg/kg). Although there was a dose-related inhibition, only the high dose was statistically significant (table 6).

#### 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 <sup>3</sup>H-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 1545 minutes there was a linear increase in <sup>3</sup>H-TDR uptake into the TCA-precipitable fraction. Qualitatively, similar data (not shown) were seen after a pulse with <sup>14</sup>C-uridine. Actinomycin D (1 μg/ml) preferentially inhibited <sup>14</sup>C-uridine incorporation, whereas it only effected <sup>3</sup>H-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<sup>-6</sup>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 Δ<sup>9</sup>-THC (10<sup>-5</sup>M) on Lewis lung cells is depicted in text-figure 2. In this experiment, Δ<sup>9</sup>-THC caused a nonlinear uptake of <sup>3</sup>H-TDR. At 30 minutes, uptake of <sup>3</sup>H-TDR into the acid-precipitable fraction was about 50% that of control.

TABLE 5.—Δ<sup>9</sup>-THC vs. leukemia L1210<sup>a</sup>

Treatment	Dose mg/kg	Mean survival time (days) <sup>b</sup>	Increased life-span, %
Vehicle <sup>c</sup>	—	8.6 ± 0.2	—
Δ <sup>9</sup> -THC	50	8.5 ± 0.2	0
Δ <sup>9</sup> -THC	100	7.8 ± 0.7	0
Δ <sup>9</sup> -THC	200	8.6 ± 0.3	0

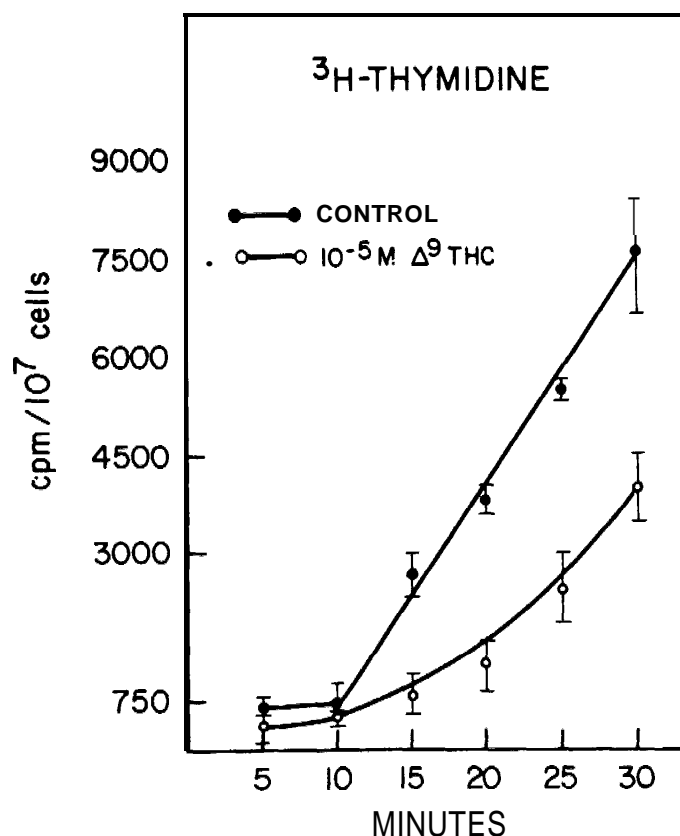
<sup>a</sup> BDF<sub>1</sub> mice were inoculated with 10<sup>6</sup> L1210 cells and treated orally daily until death.

<sup>b</sup> Values are means ± SE; 8 mice per group.

<sup>c</sup> Emulphor diluent administered orally at 0.01 ml/g.

Longer incubations (i.e., 60 min) did not significantly change the uptake pattern for control and Δ<sup>9</sup>-THC-treated tumor cells.

The effect of several cannabinoids on the uptake of <sup>3</sup>H-TDR into cells incubated in vitro indicated that Δ<sup>9</sup>-THC, Δ<sup>8</sup>-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



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.

TABLE 6.—Effect of  $\Delta^9$ -THC on splenomegaly induced by FLV<sup>a</sup>

Treatment	Dose mg/kg	FLV	Spleen weight (mg)	Inhibition (%)
Emulphor	—	—	112±6	—
Emulphor	—	+	710±85	—
$\Delta^9$ -THC	50	—	101±8	—
	50	+	437±49	44
$\Delta^9$ -THC	100	—	103±6	—
	100	+	504±93	33
$\Delta^9$ -THC	200	—	117±14	—
	200	+	295±62	71 <sup>b</sup>
Actinomycin D (positive control)	0.025	—	62±5	—
	0.025	+	127±37	90.2 <sup>b</sup>

<sup>a</sup> Groups of BALB/c mice were inoculated ip with FLV(1/10wt/vol of 9,000 ×g supernatant from mice infected with FLV for 10 days).  $\Delta^9$ -THC was administered orally daily for 10 days. Spleen weight was determined 15 days after virus inoculation.

<sup>b</sup> P < 0.05.

TABLE 7.—In vitro effects of cannabinoids on <sup>3</sup>H-TDR uptake in Lewis lung tumor, bone marrow, and L1210 leukemia cells<sup>a</sup>

Cells	Treatment	Percent inhibition of radiolabel uptake as compared to control <sup>b</sup>			
		2.5×10 <sup>-4</sup> M	2.5×10 <sup>-5</sup> M	2.5×10 <sup>-6</sup> M	2.5×10 <sup>-7</sup> M
Lewis lung tumor	$\Delta^9$ -THC	26	39	55	91
	$\Delta^8$ -THC	14	21	31	66
	CBD	51	70	80	93
	CBN	3	22	63	69
	Ara-C	3	7	22	—
Bone marrow	$\Delta^9$ -THC	—	64	103	102
	$\Delta^8$ -THC	24	22	24	70
	CBD	58	95	120	178
	CBN	17	38	63	—
	$\Delta^9$ -THC	34	42	62	78
L1210	C B D	30	36	101	92
	Ara-C	5	3	8	24

<sup>a</sup> Lewis lung tumor, L1210 leukemia, and bone marrow cells were prepared as described in "Materials and Methods." Cells were incubated in Dulbecco's medium at a concentration of 10<sup>7</sup> cells/ml in Dubnoff shaker bath at 37° C under 95% O<sub>2</sub>-5% CO<sub>2</sub>. Drugs were incubated with tumor cells for 15 minutes before addition of 10  $\mu$ Ci <sup>3</sup>H-TDR.

<sup>b</sup> Calculated 30 minutes after addition of <sup>3</sup>H-TDR.

compounds similarly effect the uptake of <sup>14</sup>C-uridine into the acid-precipitable fraction. Ara-C markedly inhibited <sup>3</sup>H-TDR uptake more dramatically than did the cannabinoids (table 7). Note that  $\Delta^9$ -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<sup>-6</sup> and 2.5 x 10<sup>-7</sup>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 ( $\Delta^9$ -THC,  $\Delta^8$ -THC, and CBN) active in vivo against the Lewis lung tumor cells are also active in the in vitro systems. The differential sensitivity of  $\Delta^9$ -THC against Lewis lung cells versus bone marrow cells is unique in that  $\Delta^8$ -THC and CBN are equally active in these systems. Johnson and Wiersema (5) reported that  $\Delta^9$ -THC administered iv caused a reduction in bone marrow metamyelocytes 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 tox-

icity 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<sup>-5</sup>M and 10<sup>-6</sup> 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<sup>-7</sup>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 (tables 1, 3). This, in part, may reflect drug regimens, doses used, increased drug metabolism, or conversion to metabolites with antagonistic actions to  $\Delta^9$ -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  $\Delta^9$ -THC against the L1210 in vivo, whereas the in vitro L1210 studies indicated that  $\Delta^9$ -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  $\Delta^9$ -THC in vitro.

Inhibition of tumor growth and increased animal survival after treatment with  $\Delta^9$ -THC may, in part, be due

to the ability of the drug to inhibit nucleic acid synthesis. Preliminary data with Lewis lung cells grown in tissue culture indicate that  $10^{-5}$  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/m<sup>2</sup> 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 marijuana-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.

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