Biochemical Pharmacology, Vol. 27, pp. 1759–1765.

0006-2952/78/0701-1759$02.00/0

INFLUENCE OF Δ⁹-TETRAHYDROCANNABINOL ON CELL PROLIFERATION AND MACROMOLECULAR BIOSYNTHESIS IN HUMAN CELLS

MANUEL J. MON, ROBERT L. JANSING, STEPHEN DOGGETT, JANET L. STEIN and GARY S. STEIN

Department of Biochemistry and Molecular Biology (M. J. M., R. L. J., S. D. and G. S. S.), and Department of Immunology and Medical Microbiology (J. L. S.), University of Florida, Gainesville, FL 32610, U.S.A.

(*Received* 18 *June* 1977; *accepted* 11 *November* 1977)

Abstract—To elucidate the influence of Δ⁹-tetrahydrocannabinol (Δ⁹-THC) on proliferation of human cells and macromolecular biosynthetic events associated with the proliferative process, we have examined the following parameters of Δ⁹-THC-treated HeLa S_3 cells grown in suspension culture: (1) exponential population growth, (2) subcellular localization of the drug, (3) protein, DNA and RNA synthesis, (4) composition and metabolism of chromosomal proteins, and (5) chromatin transcription *in vitro*. Exponentially growing HeLa S_3 cells exhibit a dose-dependent depression in proliferative activity when exposed to concentrations of Δ⁹-THC ranging from 5 to 40 μM. Approximately 9 per cent of the compound added to suspension cultures went into the cells, and of this, approximately 99 per cent was recovered from the cytoplasmic fraction and approximately 1 per cent from the nuclear fraction. The total cellular amount of Δ⁹-THC was calculated to be 1.44 ng/10^3 cells. Treatment of cells with 10–40 μM Δ⁹-THC resulted in a dose-dependent decrease in the apparent synthesis of DNA and RNA as well as in the sizes of the radiolabeled intracellular acid-soluble nucleotide precursor pool. Similar results were observed in the protein-synthesizing ability of cells treated with 30–40 μM concentrations of Δ⁹-THC. Template activity *in vitro* of chromatin was not affected by treatment of the cells with 10–30 μM concentrations of the drug. Polyacrylamide gel electrophoretic analysis of chromosomal proteins pulse labeled with [³H]L-leucine suggests that Δ⁹-THC does not affect the composition, the rates of synthesis, or the turnover of histones and nonhistone chromosomal proteins.

Δ⁹-Tetrahydrocannabinol (Δ⁹-THC) is the major psychoactive cannabinoid present in marihuana [1]. During the past several years considerable attention has been focused on the overt psychological [2–4] and physiological [5, 6] effects of cannabinoids. The chemistry of the drugs has been extensively studied [7, 8].

Furthermore, some progress has been made toward establishing the mechanisms by which cannabinoids act at the cellular and molecular levels; the influences of Δ⁹-THC on cell proliferation [9, 10], macromolecular biosynthesis [10, 11], and formation of chromosomal aberrations [12, 13*] have been examined in several laboratories. The depressant effect of cannabinoids on protein and nucleic acid synthesis has been corroborated by others using different eukaryotic cell preparations [9, 11, 14–18]. But, the complexity of the biological systems studied makes the resolution of cellular and molecular actions of cannabinoids an extremely important, open-ended question.

We have selected continuously dividing HeLa S_3 cells *in vitro* as a system for studying the actions of Δ⁹-THC on proliferation and macromolecular biosynthetic events associated with the proliferative process in human cells. Additionally, since compelling evidence suggests that the complex and interdependent series of biochemical events which are prerequisite for DNA replication and mitosis require modifications in gene readout [19–22], we have examined the influence of Δ⁹-THC on chromosomal proteins, which dictate structural and transcriptional properties of the eukaryotic genome [20, 23, 24].

MATERIALS AND METHODS

Cell culture and influence of Δ⁹-THC *on log growth cultures.* Exponentially growing (log phase) HeLa S_3 cells, a hypotetraploid line of human cervical carcinoma cells, were maintained in suspension culture in Joklik-modified Eagle's minimal essential medium supplemented with 3.5% each of calf and fetal calf serum. In each experiment, Δ⁹-THC was added to final concentrations of 0.5, 5, 10, 15, 20 and 40 μM to duplicate cultures grown at 37°C. An equal amount of drug vehicle (95% ethanol) was added to one of the two control cultures. Cell viability was checked by trypan blue exclusion at 10 and 40 min after addition of Δ⁹-THC or vehicle. Periodic cell

* A. Morishima, in a statement before the subcommittee of the Internal Security Committee of the Judiciary, United States Senate, May 16, 1974.

density measurements were made by centrifugation of a culture aliquot at 500 *g* for 3 min in microcalibrated centrifuge tubes, a packed cell column of 0.02 ml/10 ml of culture being equivalent to 5.0×10^5 cells/ml. These calculations were verified by hemocytometer measurements. Results were plotted as per cent of initial cell density vs time incubated at the various Δ^9-THC concentrations. (−)-*trans*-Δ^9-THC (99 per cent pure by gas–liquid chromatography) was supplied by the National Institute on Drug Abuse.

Subcellular localization of Δ^9-THC. Exponentially growing HeLa S_3 cells in suspension culture were exposed for 30 min at 37° to a 30-μM concentration of [^{3}H]Δ^9-THC (sp. act. 20 μCi/mg). Cells were pelleted by centrifugation at 570 *g* for 5 min then washed in 20 vol. of Earle's balanced salt solution and pelleted again by centrifugation at 570 *g* for 5 min. The supernatant fractions were combined with 95% ethanol washings of the culture flask and will be referred to as the "culture flask and media" fraction. The nuclei were washed in 25 vol. of 0.15 M NaCl–10 mM Tris (pH 8.0) and pelleted by centrifugation at 570 *g* for 5 min. Both the supernatant fractions from the lysing medium and the salt washing were combined and will be referred to as the "cytoplasmic" fraction. Nuclei isolated in this manner are largely free of cytoplasmic material when examined by phase contrast microscopy. Nuclei were lysed by suspending the nuclear pellet in double glass-distilled water. The chromatin was allowed to swell at 4° for 30 min, and then pelleted at 20000 *g* for 15 min. The supernatant fraction was decanted and is referred to as the "nucleoplasmic" fraction; the chromatin was solubilized in 5 vol. of NCS^{tm} tissue solubilizer (Amersham & Searles) and will be referred to as the "chromatin" fraction. Aliquots of the various fractions were counted in a liquid scintillation counter along with appropriate internal standards used to calculate the per cent of total radioactivity present in each of the fractions. [1′,2′-^{3}H]Δ^9-THC (137 μCi/mg, > 95 per cent pure by gas–liquid chromatography) was supplied by the National Institute on Drug Abuse.

The possibility should be considered that detergents such as Triton X-100 might solubilize some of the [^{3}H]Δ^9-THC and thus bring about an apparent decreased level of the drug in our nuclear preparations.

Influence of Δ^9-THC *on protein, DNA and RNA synthesis.* Exponentially growing HeLa S_3 cells were exposed to 5, 10, 20, 30 and 40 μM concentrations of Δ^9-THC in culture media for 10 hr. In each experiment, two controls were used, one untreated and one treated with vehicle alone (95% ethanol). Four-ml aliquots (in triplicate) from each of the cultures (in the presence of Δ^9-THC) were pulse labeled for 15 min at 37° with 2 μCi [4,5-^{3}H]L-leucine (46 Ci/m-mole), 2 μCi [methyl-^{3}H]thymidine (13 Ci/m-mole), or 1 μCi [5-^{3}H]uridine (23 Ci/m-mole). Samples were continuously agitated during incubation with radioisotope. All radioisotopes were obtained from Schwarz/Mann, Orangeburg, NY. The samples were rapidly cooled to 4° by the addition of cold Earle's balanced salt solution and immersion in an ice-water bath. Cells were pelleted by centrifugation at 900 *g* for 5 min. The supernatant fraction was decanted and the cell pellets were resuspended in trichloroacetic acid. The acid-washed cell pellets were then centrifuged at 900 *g* for 5 min and supernatant fractions saved as the "acid-soluble fractions". The "acid-insoluble precipitate fractions" were solubilized in NCS^{tm} tissue solubilizer. Aliquots of both fractions were dissolved in a scintillation mixture (150 mg of 1,4-di[2-(5-phenyloxazolyl)]benzene, 240 mg napthalene, 15 g of 2,5-diphenylozazolyle, 1 liter toluene, 1 liter ethanol and 1 liter dioxane) and counted in a Beckman scintillation counter. The results were plotted as cpm × cell^{-1} vs Δ^9-THC concentrations for each of the isotopes incorporated into both the "acid-soluble" and "acid-insoluble" fraction.

Influence of Δ^9-THC *on composition and metabolism of chromosomal proteins.* Exponentially growing HeLa S_3 cells were exposed to 10, 20 and 30 μM concentrations of Δ^9-THC in culture media for 10 hr. In each experiment, two controls were used, one untreated and one treated with vehicle alone (95% ethanol). The cells were pelleted by centrifugation, resuspended in Earle's balanced salt solution containing 5 μCi/ml of [^{3}H]leucine (46 Ci/m-mole) and 2% fetal calf serum, and then pulse labeled for 15 min at 37°. Nuclei and chromatin were isolated at 4° as previously described [25]. Cells were harvested by centrifugation, washed three times with 80 vol. of 80 mM NaCl–20 mM EDTA–1% Triton X-100 (pH 7.2). Nuclei were pelleted by centrifugation at 1000 *g* for 3 min and washed twice in 0.15 M NaCl–10 mM Tris (pH 8.0) (collected by centrifugation at 1000 *g* for 3 min after each washing). Nuclei isolated in this manner are free of cytoplasmic contamination when examined by phase contrast microscopy. Nuclei were lysed in 60 vol. of double distilled water. The chromatin was allowed to swell in an ice bath at 4° for 20 min and was then pelleted by centrifugation at 12000 *g* for 15 min. Chromatin was homogenized into buffer containing 2% SDS–5% BME–0.0625 M Tris (pH 6.8) and then dialyzed against 0.1% SDS–0.1% BME–0.0625 M Tris (pH 6.8). Dialyzed samples were then subjected to SDS–8.75% polyacrylamide gel electrophoresis according to the method of Laemmli [26]. Gels were spectrophotometrically scanned at 590 nm, fractionated into 2-mm slices, placed in liquid scintillation counting vials and solubilized in 0.4 ml of 30% H_2O_2. Ten ml of scintillation mixture was added to each vial (2500 ml toluene, 1333 ml Triton X-100 and 168 ml liquifluor) and the fractions were counted in a Beckman liquid scintillation counter. Results were plotted as per cent of total counts per gel vs distance of migration.

Influence of Δ^9-THC *on transcription of chromatin* in vitro. Exponentially growing HeLa S_3 cells were exposed to 15 and 30 μM concentrations of Δ^9-THC in culture media for 10 hr. A control culture was treated with vehicle alone (95% ethanol). Another control culture was not treated with drug or vehicle. Chromatin was isolated as described above [25] and then transcribed *in vitro* using fraction V *Escherichia coli* RNA polymerase according to the method of Berg *et al.* [27]. Transcription was carried

out with varying amounts of DNA as chromatin for 10 min at 37°. The incubation mixture in a final volume of 0.25 ml contained: 40 mM Tris (pH 8.0), 4 mM $MgCl_2$, 1 mM $MnCl_2$, 0.008% β-mercaptoethanol, 0.4 mM each of ATP, UTP, GTP and CTP, 0.1 μCi [^{14}C]ATP (50 μCi/m-mole, from Schwarz/Mann, Orangeburg, NY), varying amounts of DNA as chromatin ranging from 1.5 to 65 μg/ml and 4.3 units of RNA polymerase. The reaction was stopped by addition of cold 15% trichloroacetic acid and the samples were placed in an ice-water bath at 4° for 10 min. The acid-insoluble precipitates were collected by vacuum filtration on 0.45 μm Millipore filters and each filter was washed with 30 ml of 10% trichloroacetic acid. Filters were dissolved in 1 ml of Cellosolve in a liquid scintillation vial. Ten ml of scintillation mixture (2365 ml toluene, 788 ml Cellosolve and 132 ml liquifluor) was added to each vial and the samples were counted in a Beckman liquid scintillation counter. Results were plotted as cpm of [^{14}C]ATP vs μg DNA in chromatin.

RESULTS AND DISCUSSION

Influence of Δ⁹-THC *on cell proliferation in exponentially growing HeLa S_3 cells.* To examine the influence of Δ⁹-THC on cell proliferation in human cells, we initially assayed the effect of various concentrations of the drug on the growth of continuously dividing HeLa S_3 cells. Δ⁹-THC was present in the cultures throughout the experiments. At the start of each experiment, the cell concentration was adjusted to 2.5×10^5 cells/ml to insure the potential

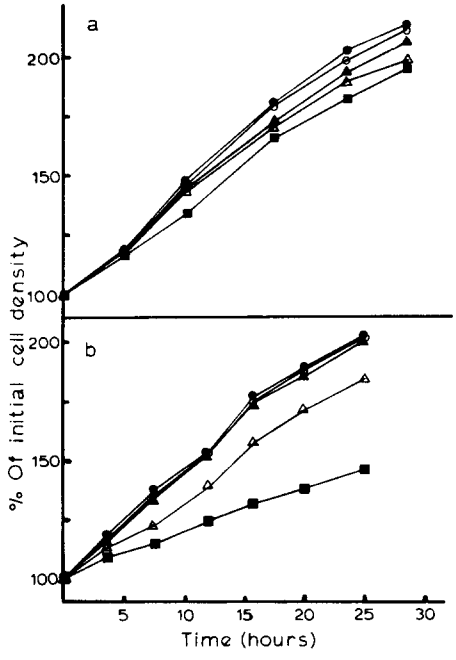

Fig. 1. Effects of varying concentrations of Δ⁹-THC on exponentially growing HeLa S_3 cells in suspension cultures. Packed cell volumes were used to determine the per cent of initial cell density as a function of time in the presence of: (a) control (●), vehicle (○), 5 μM Δ⁹-THC (▲), 10 μM Δ⁹-THC (△) and 15 μM Δ⁹-THC (■), and (b) control (●), vehicle (○), 0.5 μM Δ⁹-THC (▲), 20 μM Δ⁹-THC (△) and 40 μM Δ⁹-THC (■).

for exponential growth. Cell viabilities (ascertained by trypan blue exclusion) were greater than 98 per cent for the control, vehicle-treated, and Δ⁹-THC-treated cultures at 10 and 40 min after initiation of the growth curves; this rules out the possibility that Δ⁹-THC and/or the drug vehicle may have produced toxic nonspecific cell destruction giving an incorrect initial "viable" cell number. As indicated in Fig. 1, Δ⁹-THC depresses the exponential growth of HeLa S_3 cells in a dose-dependent fashion with concentrations of 5, 10, 15, 20 and 40 μM causing depressions of approximately 8, 12, 20, 31 and 56 per cent respectively. The values plotted represent the mean of two independent determinations. The above concentrations of Δ⁹-THC fall within the range at which other investigators have observed similar results in the growth kinetics of other eukaryotic cells [10, 13, 28]. A control with 95% ethanol (used as drug vehicle) and a Δ⁹-THC concentration of 0.5 μM did not exhibit inhibition of the exponential growth of HeLa cells when compared to that of untreated cells.

Subcellular localization of Δ⁹-THC. Two important questions which arise are how much, if any, of the Δ⁹-THC enters the cell and what is its subcellular distribution? Defining the drug level present in various intracellular components is immediately relevant to evaluating the site of action of cannabinoids. Since Δ⁹-THC exhibits a high affinity for nonspecific components of serum in the culture media and for glassware, the concentration of cannabinoids in the growth medium may not be a reliable reflection of the amount of drug available to the cells. To compensate for nonspecific sequestering of Δ⁹-THC, all experiments were carried out in the same volumes of culture media. Knowing the intracellular concentrations of Δ⁹-THC is also essential for the execution of experiments in which the drug is utilized in cell-free systems to insure that drug levels accurately represent conditions *in vivo*.

To determine the amounts of Δ⁹-THC associated with the various cellular compartments of HeLa S_3 cells, we incubated the cells in the presence of [^{3}H]Δ⁹-THC for 30 min. Table 1 shows that, at a total Δ⁹-THC concentration of 30 μM in culture media, 9.13 per cent of the radiolabeled Δ⁹-THC was associated with the cellular fraction. This corresponds to 1.44 ng Δ⁹-THC/10^3 cells. In agreement with previous observations in which 85–95 per cent of THC is bound to serum lipoproteins [29, 30], we observed that 90.8 per cent of the radiolabeled Δ⁹-THC remained associated with the culture flask and medium containing 7% calf serum. The cells were washed three times in order to remove any nonspecifically adhering [^{3}H]Δ⁹-THC from the cells. Of the compound entering the cells, 98.7 per cent was recovered in the "cytoplasmic" fraction and 1.27 per cent in the "nuclear" fraction. The total recovery of radiolabeled Δ⁹-THC was 86 per cent. At a final glance our results appear to be inconsistent with those previously obtained from subcellular fractionation procedures in which crude nuclear preparations were made from mouse brain [31]. The crude mouse brain nuclei contained 20–25 per cent of the total intracellular label, a higher percentage of the

Table 1. Δ^9-THC (at a 30 μM concentration) in the subcellular fraction of HeLa S_3 cells in suspension culture*

Fraction	Per cent of total [^{3}H]Δ^9-THC	Per cent of cellular [^{3}H]Δ^9-THC	Δ^9-THC/10^3 cells (pg)
Culture flask + media	90.8 ± 0.9	—	—
Cellular	9.13 ± 0.16	100.00 ± 1.8	1436 ± 26
Cytoplasmic	9.01 ± 0.16	98.7 ± 1.8	1418 ± 25
Nuclear	0.116 ± 0.003	1.27 ± 0.03	18 ± 0.5
Nucleoplasmic	0.028 ± 0.001	0.307 ± 0.007	4 ± 0.2
Chromatin	0.088 ± 0.002	0.963 ± 0.024	14 ± 0.3

* Exponentially growing HeLa S_3 cells in suspension cultures were exposed for 30 min at 37° to a 30 μM concentration of [^{3}H]Δ^9-THC with a specific activity of 20 μCi/mg. The various cellular fractions were isolated as described in Materials and Methods. Results were calculated as per cent of total and of cellular [^{3}H]Δ^9-THC as determined from internal standards appropriate for each fraction isolated and are expressed as mean ± average deviation.

cellular Δ^9-THC than we find associated with the HeLa cell nuclear fraction. To resolve the differences in the representations of [^{3}H]Δ^9-THC in nuclei from mouse brain and HeLa cells, we prepared a crude nuclear fraction from [^{3}H]Δ^9-THC-treated HeLa S_3 cells by a sucrose procedure according to the method of DeRobertis *et al.* [32, 33]. In the HeLa nuclei prepared by the sucrose method, 86 per cent of the labeled cannabinoid was associated with the cytoplasmic fraction and 14 per cent with the nuclear fraction. The level of Δ^9-THC in sucrose prepared HeLa nuclei compares quite favorably with that in mouse brain nuclei isolated by a similar procedure [31]. Since the HeLa nuclei prepared by the sucrose procedure are associated with significant amounts of cytoplasmic material (as observed by phase contrast microscopy), and the nuclei of detergent prepared HeLa nuclei are not, it seems reasonable to conclude that the elevated levels of Δ^9-THC found in sucrose prepared nuclei from HeLa or mouse brain cells are most likely attributable to drug present in cytoplasmic contaminants.

Influence of Δ^9-THC *on protein, DNA and RNA synthesis.* Cell proliferation involves a complex and interdependent series of biochemical events requiring differential gene expression. Such modifications in gene readout are prerequisite for DNA replication and mitotic division. We examined the possibility that treatment of human cells with cannabinoids results in perturbation of the macromolecular biosynthetic events essential for cell proliferation. We assayed the influence of Δ^9-THC on the biosynthesis of protein, DNA and RNA in continuously dividing HeLa S_3 cells by pulse labeling the cells with [^{3}H]leucine, [^{3}H]thymidine, or [^{3}H]uridine and determining the incorporation of these radioactive protein and nucleic acid precursors into acid-insoluble material. The effect of Δ^9-THC on the cellular uptake of the radiolabeled acid-soluble precursors was also monitored since it would limit their incorporation into the acid-insoluble fractions and thus any fluctuations in precursor uptake would yield misleading data as to the actual direct effect of Δ^9-THC on macromolecular synthesis. As shown in Figs. 2 and 3, a dose-dependent depression in the apparent rates of DNA and RNA synthesis was observed in HeLa

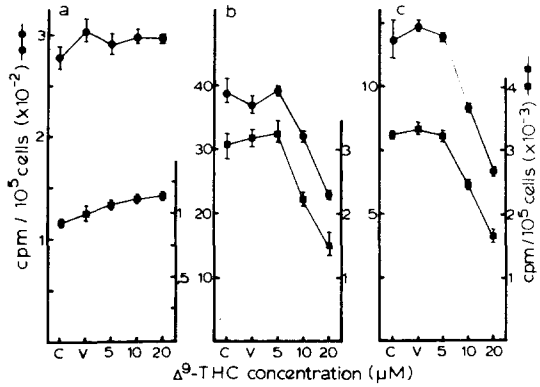

Fig. 2. Effects of Δ^9-THC on the incorporation of (a) [^{3}H]leucine, (b) [^{3}H]thymidine and (c) [^{3}H]uridine into protein, DNA and RNA respectively. Exponentially growing HeLa S_3 cells in suspension cultures were pulse labeled for 15 min with the appropriate radioactive precursor. The incorporation of radioactivity into the acid-insoluble (●) and acid-soluble (■) fractions was determined for cells pretreated for 10 hr with 5, 10 and 20 μM concentrations of Δ^9-THC. C = control; V = vehicle-treated cells.

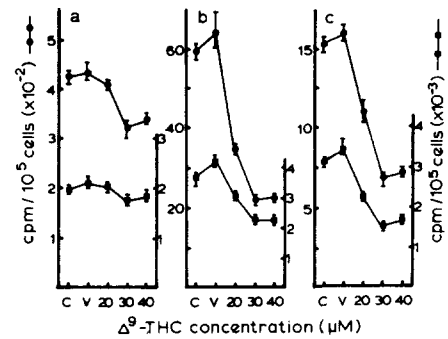

Fig. 3. Effects of Δ^9-THC on the incorporation of (a) [^{3}H]leucine, (b) [^{3}H]thymidine and (c) [^{3}H]uridine into protein, DNA and RNA respectively. Exponentially growing HeLa S_3 cells in suspension cultures were pulse labeled for 15 min with the appropriate radioactive precursor. The incorporation of radioactivity into the acid-insoluble (●) and acid-soluble (■) fractions was determined for cells pretreated for 10 hr with 20, 30 and 40 μM concentrations of Δ^9-THC. C = control; V = vehicle-treated cells.

S_3 cells after 10 hr of exposure to 10, 20, 30 and 40 μM concentrations of Δ⁹-THC. A depressant effect on protein synthesis was observed at 30 and 40 μM concentrations of Δ⁹-THC. Exposure to a 5 μM concentration of Δ⁹-THC did not appear to affect DNA or synthesis, while exposure to 5, 10 and 20 μM concentrations of Δ⁹-THC did not significantly affect protein synthesis. The results obtained for the acid-soluble fractions closely parallel those obtained for the acid-insoluble fractions, indicating some interference by Δ⁹-THC with incorporation of labeled exogenous precursors into the intracellular precursor pool. These results could arise from at least two possible mechanisms of action for the molecular effects of Δ⁹-THC: (1) direct interference with the macromolecular synthesizing mechanisms, and (2) interference, possibly at the cell membrane level, with the incorporation of exogenous protein and nucleic acid precursors into the intracellular pool. Our results, along with the previous observations of other investigators [11, 34–36], suggest that the inhibition of macromolecular synthesis seen in the pulse-labeling experiments may be due to the effect of Δ⁹-THC limiting the access of the radiolabeled precursor into the cell. Several investigators have observed a reduction in the radioisotopically measured precursor pool sizes which could account for the decrease in macromolecular synthesis in the presence of Δ⁹-THC in cultured human diploid fibroblasts, human and mouse neuroblastoma cells [11], in cultured human lymphocytes [34, 35], and in Lewis lung tumor cells [37, 38]. Similar inhibitory effects on macromolecular synthesis have been reported with Δ⁹-THC concentrations varying from 1 μM [9, 39, 40] to 100 μM [41], depending on the serum content of the culture medium [34, 35] and the cell type used [42].

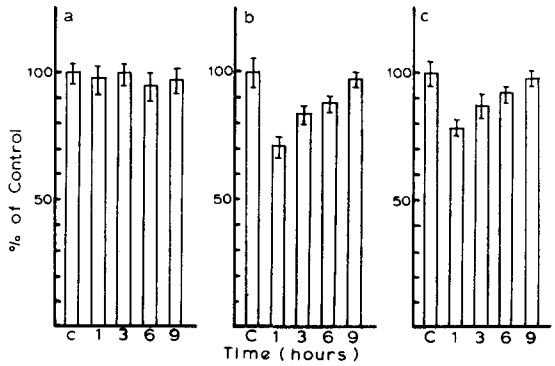

Fig. 4. Effect of drug vehicle (95% ethanol) on the incorporation of (a) [³H]leucine, (b) [³H]thymidine and (c) [³H]uridine into protein, DNA and RNA respectively. Exponentially growing HeLa S_3 cells in suspension cultures were pulse labeled for 15 min with the appropriate radioactive precursor. The incorporation of radioactivity into the acid-insoluble fractions was determined for cells pre-treated with 71 μl of 95% ethanol/50 ml of culture media for 1, 3, 6 and 9 hr. Appropriate untreated controls were obtained for each radioactive precursor at each time interval. Results are plotted as per cent incorporation of control for each radioactive precursor at 1, 3, 6 and 9 hr of preincubation with drug vehicle.

A 10-hr exposure was chosen since, as shown in Fig. 4, treatment of HeLa cells with a concentration of ethanol (23 mM) equivalent to that used as drug vehicle brought about an initial decrease in the apparent nucleic acid synthesis which was not evident by 9–10 hr after the addition of ethanol. Further results in our laboratory (unpublished) have shown this initial decrease not to be due to a reduction of the incorporation of exogenous radiolabeled precursor into the intracellular pool.

Δ⁹-THC is very fat soluble [43] and binds to the double lipid layer of the plasma membrane [44]. There already exists evidence for the action of Δ⁹-THC at the membrane level on mitochondria [44–46], microsomal drug-metabolizing systems [46, 47], and erythrocytes [40]. This action of Δ⁹-THC on cell membranes could be responsible for a reduction in uptake of exogenous precursors into the intracellular pool leading to the observed apparent decrease in macromolecular synthesis.

Influence of Δ⁹-THC *on composition and metabolism of chromosomal proteins.* Accumulating evidence suggests that chromosomal proteins play both structural and regulatory roles in genetic material of eukaryotic cells [20, 21]. Chromosomal proteins have been divided into two groups: (1) the histones which appear to be involved in determining the structure of chromatin [48–50], and (2) the non-histone protein components which appear to exert a regulatory role in gene transcription [20]. Since alterations in gene readout are necessary for the normal progression of events in the cell cycle and since some of these changes have been associated with changes in the metabolism of chromosomal proteins, we have studied the composition and metabolism of chromosomal proteins after exposure of HeLa S_3 cells to varying concentrations of Δ⁹-THC (10, 20 and 30 μM) for 10 hr. Cells were then pulse labeled with [³H]leucine. The ³H-labeled chromosomal proteins were extracted as explained in Materials and Methods and then electrophoresed in SDS-polyacrylamide gels. Figure 5 shows the patterns of [³H]leucine incorporation into total chromosomal proteins obtained by SDS-polyacrylamide gel electrophoresis. No significant change from the control was observed in this pattern when HeLa S_3 cells were exposed to either the vehicle (95% ethanol) or Δ⁹-THC concentrations of 10, 20 and 30 μM. The electrophoretic banding patterns of the stained proteins in control and in drug-treated cells were identical. These results indicate that Δ⁹-THC does not appear to affect the composition and metabolism of chromosomal proteins, and thus may not directly affect chromatin structure or function by these means.

Chromatin alterations have been observed in histochemical studies ultilizing anionic and cationic staining techniques specific for basic proteins (such as histones) and for nuclear acidic phosphoproteins. A decrease in the staining of basic nuclear proteins, along with changes in the staining of arginine-rich histones and an increase in the staining of nuclear acidic phosphoproteins were observed in the leukocytes of chronic marihuana users [51]. These observed histochemical changes of nuclear proteins may be due not to a quantitative change in the com-

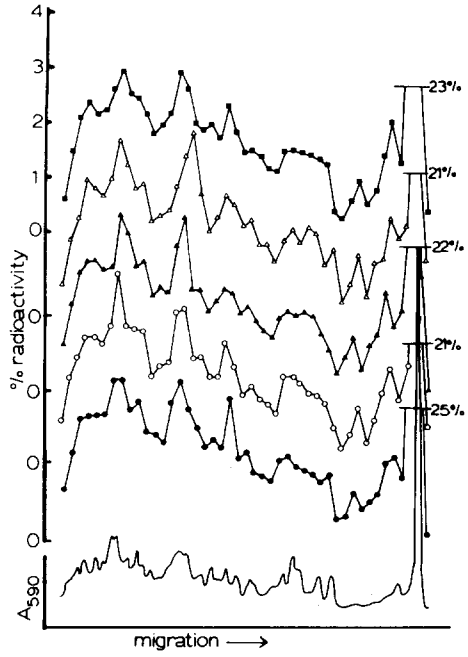

Fig. 5. Effect of Δ^9-THC on the incorporation of [^{3}H]leucine into total chromosomal proteins. Exponentially growing HeLa S_3 cells were exposed to 10 μM (▲), 20 μM (△) and 30 μM (■) Δ^9-THC in suspension cultures for 10 hr and then pulse labeled for 15 min with 5 μCi/ml of [^{3}H]leucine. Chromosomal proteins were isolated and electrophoresed in SDS-8.75% polyacrylamide slab gels as described in Materials and Methods. Control (●) and vehicle-treated (○) samples were also studied. Radioactivity in each gel fraction is plotted as per cent of total radioactivity in the gel. A_{590} (——) profiles of the stained protein bands were similar for each sample gel.

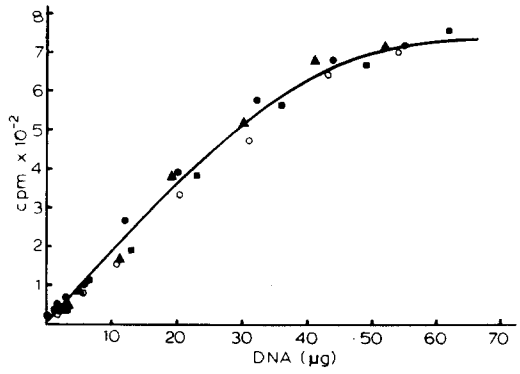

Fig. 6. Effect of Δ^9-THC on the transcription *in vitro* of chromatin isolated from exponentially growing HeLa S_3 cells pretreated for 10 hr with 15 μM (▲) and 30 μM (■) Δ^9-THC in suspension culture. Control (●) and vehicle-treated (○) samples were also studied. Transcription *in vitro* of isolated chromatin was carried out using *E. coli* RNA polymerase in the presence of [^{14}C]ATP, as described in Materials and Methods. Results are plotted as cpm of incorporated [^{14}C]ATP vs μg of DNA in chromatin.

position of the proteins, but to an alteration of the state of the chromatin. African Green Monkey kidney cells exposed to a 500-μM concentration of Δ^9-THC have displayed marked chromatin alterations as observed by fluorescence microscopy of cells stained with acridine-organe [16]. Any effect that Δ^9-THC may have on post-translational modifications of chromosomal proteins or their association with nucleic acids could lead to structural and functional alterations of chromatin. Modifications of this type have been implicated in the transcriptional control of gene expression. We are presently investigating the effects which Δ^9-THC may have on the post-translational acetylation and phosphorylation of chromosomal proteins.

Influence of Δ^9-THC *on the transcription of chromatin* in vitro. A reduction in the polyribosomal content of Δ^9-THC-treated cells has been previously reported in the protozoan Tetrahymena [10], and this reduction has been linked to a concomitant decrease in cellular mRNA. This decrease in cellular mRNA may be due either to increased degradation of polyribosomal RNA or to a decrease in the rate of synthesis of mRNA. The previously observed RNA values *in vivo* are difficult to interpret definitively due to concomitant changes in the cellular precursor pool brought about by exposure to Δ^9-THC. To look directly at transcription and to circumvent some of the complications encountered with labeling RNA in intact cells, we have proceeded to study the effect of Δ^9-THC on the transcriptional capacity of chromatin. Chromatin, isolated from HeLa S_3 cells previously exposed to varying concentrations of Δ^9-THC, was transcribed *in vitro* using *E. coli* RNA polymerase in the presence of [^{14}C]ATP. It should be noted that the isolated chromatin contained no significant level of endogenous RNA polymerase activity, and that the *E. coli* RNA polymerase did not exhibit any exotransferase activity. Figure 6 shows no difference in the transcriptional activity of isolated chromatin among control, vehicle-treated, and Δ^9-THC-treated cells at concentrations of 15 and 30 μM of Δ^9-THC. These results indicate that Δ^9-THC does not appear to have an effect on the translational capacity *in vitro* of chromatin isolated from pretreated cells.

In conclusion, we have observed that Δ^9-THC causes a dose-dependent inhibition of proliferation in HeLa S_3 cells. This decrease is accompanied by comparable inhibition in the apparent synthesis of protein, DNA and RNA in intact cells. But this depression in macromolecular synthesis may in part be due to a limitation by Δ^9-THC of the access of the radiolabeled precursor into the cell. Chromosomal protein synthesis does not appear to be affected by Δ^9-THC under the conditions studied. However, this study does not rule out any probable effect of Δ^9-THC on the post-translational modifications of chromosomal proteins or their interaction with nucleic acids. These results are consistent with low concentrations of a principal psychoactive cannabinoid bringing about perturbations of key biochemical events required for division of human cells. However, the specific molecular sites remain to be resolved.

Acknowledgement—These studies were supported by Grant DA-01188 from the National Institute on Drug Abuse.

REFERENCES

1. Y. Gaoni and R. Mechoulam, *J. Am. chem. Soc.* **86,** 1646 (1964).
2. L. Lemberger, *Am. Soc. Pharmac. exp. Ther.* **1,** 461 (1973).
3. W. D. M. Paton and R. G. Pertwee, *Acta pharm. suecica* **8,** 691 (1971).
4. I. G. Karniol and E. A. Carlini, *J. Pharm. Pharmac.* **24,** 833 (1972).
5. C. Leuchtenberger and R. Leuchtenberger, *Nature, Lond.* **234,** 227 (1971).
6. C. Leuchtenberger, R. Leuchtenberger and A. Schneider, *Nature, Lond.* **241,** 137 (1973).
7. E. R. Garrett and C. A. Hunt, *J. pharm. Sci.* **62,** 1211 (1973).
8. E. R. Garrett and C. A. Hunt, *J. pharm. Sci.* **63,** 1056 (1974).
9. A. M. Zimmerman and D. K. McClean, in *Drugs and the Cell Cycle* (Eds A. M. Zimmerman, G. M. Padilla and I. L. Cameron), p. 67. Academic Press, NY (1973).
10. A. M. Zimmerman and S. B. Zimmerman, in *Marihuana: Chemistry, Biochemistry and Cellular Effects* (Ed. G. G. Nahas), p. 195. Springer, NY (1976).
11. R. D. Blevins and J. D. Reagen, in *Marihuana: Chemistry, Biochemistry and Cellular Effects* (Ed. G. G. Nahas), p. 213. Springer, NY (1976).
12. D. G. Gilmour, A. D. Bloom, K. P. Lele, E. S. Robbins and C. Maximilian, *Archs gen. Psychiat.* **24,** 268 (1971).
13. M. A. Stenchever, T. J. Kunyszy and M. A. Allen, *Am. J. Obstet. Gynec.* **118,** 106 (1974).
14. S. Bram and P. Brachet, in *Marihuana: Chemistry, Biochemistry and Cellular Effects* (Ed. G. G. Nahas), p. 207. Springer, NY (1976).
15. R. A. Carchman, W. Warner, A. C. White and L. S. Harris, in *Marihuana: Chemistry, Biochemistry and Cellular Effects* (Ed. G. G. Nahas), p. 329. Springer, NY (1976).
16. J. Huot, in *Marihuana: Chemistry, Biochemistry and Cellular Effects* (Ed. G. G. Nahas), p. 313. Springer, NY (1976).
17. A. Jakubovic and P. L. McGeer, in *Marihuana: Chemistry, Biochemistry and Cellular Effects* (Ed. G. G. Nahas), p. 223. Springer, NY (1976).
18. C. Leuchtenberger, R. Leuchtenberger, V. Ritter and N. Inui, *Nature, Lond.* **242,** 403 (1973).
19. G. S. Stein and R. Baserga, *Adv. Cancer Res.* **15,** 287 (1972).
20. G. S. Stein, T. C. Spelsberg and L. J. Kleinsmith, *Science, N.Y.* **183,** 817 (1974).
21. G. S. Stein and J. L. Stein, *Bioscience* **26,** 488 (1976).
22. J. L. Stein, C. L. Thrall, W. D. Park, R. J. Mans and G. S. Stein, *Science, N.Y.* **189,** 125 (1975).
23. S. C. R. Elgin and H. Weintraub, *A. Rev. Biochem.* **44,** 725 (1975).
24. G. S. Stein and L. J. Kleinsmith (Eds), *Chromosomal Proteins and Their Role in the Regulation of Gene Expression.* Academic Press, NY (1975).
25. G. S. Stein and J. Farber, *Proc. natn. Acad. Sci. U.S.A.* **60,** 2918 (1972).
26. V. K. Laemmli, *Nature, Lond.* **227,** 680 (1970).
27. D. Berg, K. Barrett and M. Chamberlin, *Meth. Enzym.* **21,** 506 (1971).
28. G. G. Nahas, N. Suciu-Foca, J-P. Armand and A. Morishima, *Science, N. Y.* **183,** 419 (1974).
29. H. A. Klausner and J. V. Dingel, *Life Sci.* **10,** 49 (1971).
30. M. Wahlavist, I. M. Nilsson, F. Sandberg and S. Agurell, *Biochem. Pharmac.* **19,** 2579 (1970).
31. W. L. Dewey, B. R. Martin, J. S. Beckner and L. S. Harris, in *Marihuana: Chemistry, Biochemistry and Cellular Effects* (Ed. G. G. Nahas), p. 349. Springer, NY (1976).
32. E. DeRobertis, A. Pellegrino de Iraldi, G. Rodriguez de Lores Arnaiz and L. Salganicoff, *J. Neurochem.* **9,** 23 (1962).
33. E. DeRobertis, G. Rodriguez de Lores Arnaiz, L. Salganicoff and A. Pellegrino de Iraldi, *J. Neurochem.* **10,** 225 (1963).
34. G. G. Nahas, B. Desoize, J. Hsu and A. Morishima, in *Marihuana: Chemistry, Biochemistry and Cellular Effects* (Ed. G. G. Nahas), p. 195. Springer, NY (1976).
35. G. G. Nahas, A. Morishima and B. Desoize, *Fedn Proc.* **36,** 1748 (1977).
36. B. Desoize and G. G. Nahas, *C. hebd. Seanc. Acad. Sci., Paris* **281,** 475 (1975).
37. A. E. Munson, L. S. Harris, M. A. Friedman, W. L. Dewey and R. A. Carchman, *J. natn. Cancer Inst.* **55,** 597 (1975).
38. A. C. White, J. A. Munson, A. E. Munson and R. A. Carchman, *J. natn. Cancer Inst.* **56,** 655 (1976).
39. G. G. Nahas, J. P. Armand and J. Hsu, *C. hebd. Seanc. Acad. Sci., Paris* **278,** 679 (1974).
40. A. Raz, A. Schurr and A. Livne, *Biochim. biophys. Acta* **274,** 269 (1972).
41. G. G. Nahas, B. Desoize, J. P. Armand, J. Hsu and A. Morishima, *C. hebd. Seanc. Acad. Sci., Paris* **279,** 785 (1974).
42. R. A. Carchman, L. S. Harris and A. E. Munson, *Cancer Res.* **36,** 95 (1976).
43. D. S. Kreuz and J. Axelrod, *Science, N.Y.* **179,** 391 (1973).
44. A. Chari-Bitron, *Life Sci.* **10,** 1273 (1971).
45. A. Chari-Bitron and T. Bino, *Biochem. Pharmac.* **20,** 473 (1971).
46. G. M. Cohen, D. W. Peterson and G. J. Mannering, *Life Sci.* **10,** 1207 (1971).
47. J. M. Mahoney and R. A. Harris, *Biochem. Pharmac.* **21,** 1217 (1972).
48. V. G. Allfrey, in *Histones and Nucleohistones* (Ed. D. M. Phillips), p. 241. Plenum Press, NY (1971).
49. L. S. Hnilica, M. E. McClure and T. S. Spelsberg, in *Histones and Nucleohistones* (Ed. D. M. Phillips), p. 187. Plenum Press, NY (1971).
50. A. J. Louie and G. H. Dixon, *Proc. natn. Acad. Sci. U.S.A.* **69,** 1975 (1972).
51. C. N. Stefanis and M. R. Issidorides, in *Marihuana: Chemistry, Biochemistry and Cellular Effects* (Ed. G. G. Nahas), p. 533. Springer, NY (1976).