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In Vivo Inhibition of Enterocyte Metabolism by Δ^9-Tetrahydrocannabinol (42285)

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Abstract. Mice were given 10 to 100 mg/kg by stomach tube of Δ^9-tetrahydrocannabinol (THC) in a single dose or for 4 consecutive days. [^{3}H]Thymidine or [^{3}H]glucosamine was given 3 or 24 hr before sacrifice. Enterocytes were isolated, and the incorporation of radioactivity into the acid insoluble fraction was measured. THC significantly inhibits in a dose-related fashion (from 10 to 90%) *in vivo* enterocyte metabolism. This inhibition is found in all enterocytes whatever their position in the intestinal tract; it is also independent of the state of differentiation of enterocytes. After a single ingestion of THC, crypt cells which synthesize DNA incorporate 37 to 45% less thymidine, and villus cells, which synthesize important amounts of glycoproteins, incorporate 15 to 39% less glucosamine. After 4 days of THC administration, the inhibition of thymidine incorporation is even more significant (up to 88%).

Δ^9-Tetrahydrocannabinol (THC) has been reported to impair cell division and macromolecular synthesis in eucaryotic cells (1, 2). It was suggested (3) that this effect might be related primarily to the liposolubility of THC in the double lipid bilayer of the plasma membrane, and that THC inhibits precursor transport through the plasma membrane (4).

Inhibition of cellular division is best observed in fast dividing cells such as lymphocytes (5, 6) and spermatocytes (7–9). It could therefore be assumed that anabolism of other fast dividing cells such as enterocytes might also be inhibited by THC. The purpose of the present study was to investigate the effect of this drug on differentiated and undifferentiated enterocytes.

Materials and Methods. Two series of experiments were performed on 40-day-old male mice OFl-Ico (Iffa Credo, France) weighing 25 to 30 g. In the first series, the animals fasted for 24 hr are administered by gastric tube 0.3, 1.5 or 3 mg of THC, with 100 μCi of [^{3}H]glucosamine. THC is diluted in a saline solution containing 10% Tween 80 and 7% ethanol. Total volume of the solution administered is 0.225 ml. A similar volume consisting of saline, Tween 80, and 7% alcohol is administered to control animals. The mice are sacrificed 3 hr after treatment, between 1:30 and 2 PM (Figs. 1a and 2a).

In the second series of experiments, THC (1.5 mg) is administered in a similar fashion daily for 4 days to a group of six mice. They were sacrificed the sixth day, 60 min after an intraperitoneal injection of [^{3}H]thymidine (Fig. 3a). The doses of THC used in these experiments correspond to 9.9–12, 49–60, and 99 to 120 mg/kg of body wt (one-fiftieth to one-fifth of LD_{50} (10)).

THC 20% (w/v) in an ethanol solution was kindly provided by the National Institute on Drug Abuse. [*Methyl*-^{3}H]Thymidine (25 Ci/mmole) and [1-^{3}H]glucosamine-hydrochloride (2.4 Ci/mmole) were obtained from Amersham, instagel from Packard, dithiothreitol from Sigma, and other chemicals from Prolabo.

The technique of intestinal cell isolation described by Weiser (12) and Lawson *et al.* (11) is used with some modifications. The intestine is swiftly removed and a 10-cm segment is excised, between the 5th and the 15th cm after the pylorus. In one series of experiments, the small bowel is divided in three equal parts of approximately 10 cm. The excised intestinal segments are gently flushed with ice-cold saline containing dithiothreitol (9 g/liter NaCl; 0.154 g/liter dithiothreitol) in order to remove extraneous luminal contents. Each tissue segment is inverted on to a glass rod and secured with surgical silk. The inverted intestine segments

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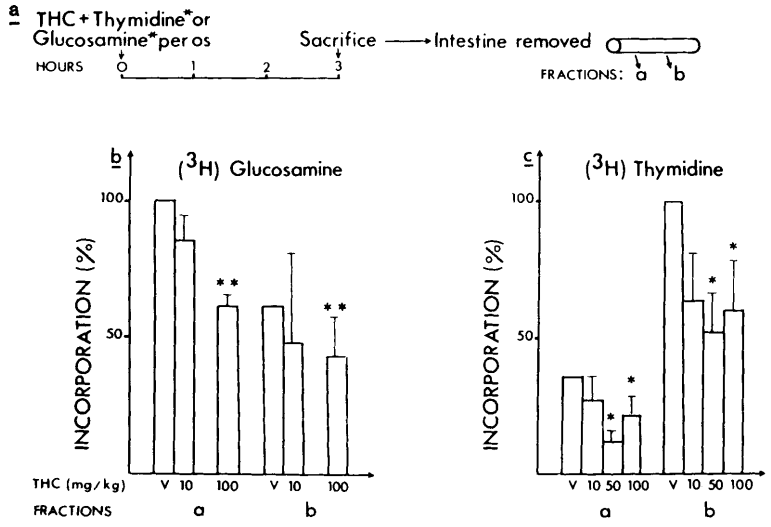

FIG. 1. (a) Outline of the experimental procedure showing the timing of (i) THC and labeled precursor ingestion and (ii) sacrifice, removal of the intestinal section, and intestinal cell isolation. (b, c) [H]Glucosamine (b) and [^{3}H]thymidine (c) incorporation into the acid insoluble fraction of villus (a) and crypt (b) enterocytes isolated from intestines of mice treated with a single ingestion of THC (10–50 or 100 mg/kg) or with vehicle. $^*P < 0.05$, $^{**}P < 0.02$.

are washed for 15 min at room temperature in phosphate buffered saline (PBS, pH 7.3) containing 9.64 g/liter of sodium citrate. At the end of the incubation period the tubes containing PBS and 1.438 g/liter EDTA with 77 mg/liter dithiothreitol are shaken gently; the enterocytes detach spontaneously from the intestine and collect in the supernatant fluid. During this 1-hr incubation, villus cells which detach themselves more readily (and constitute fraction a in Figs. 1a and 2a) are harvested during the first 30 min. The crypt cells which

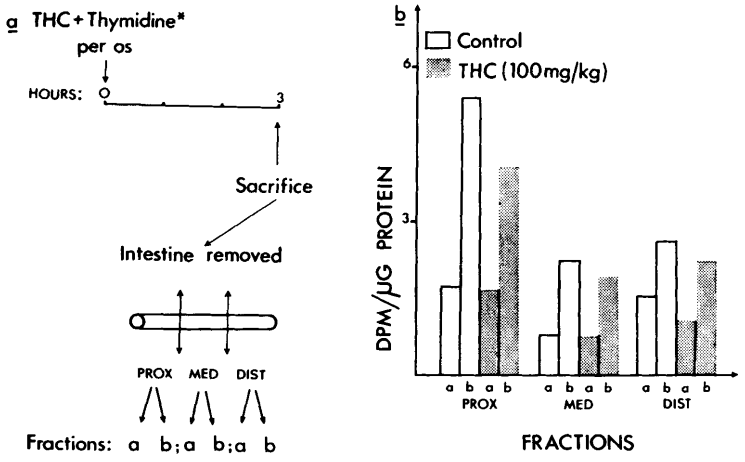

FIG. 2. (a) Outline of the experimental procedure showing the (i) THC and labeled thymidine ingestion and (ii) sacrifice and isolation of intestinal cells. (b) [^{3}H]Thymidine incorporation into the acid insoluble fraction of villus (a) and crypt (b) enterocytes, from proximal (prox), medial (med), or distal (dist) parts of the small intestine after a single ingestion of 100 mg/kg of THC or vehicle (control); $P < 0.01$.

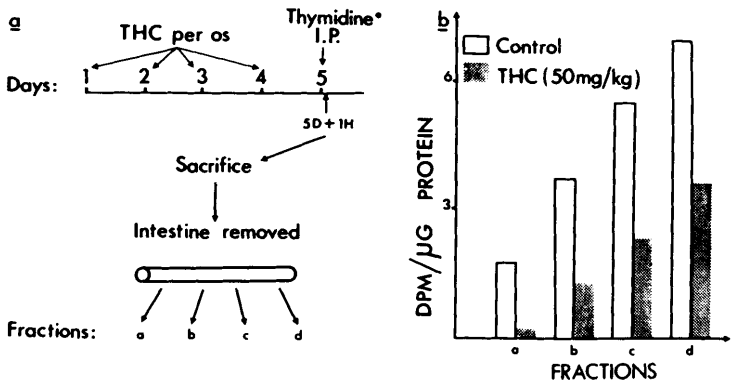

FIG. 3. (a) Outline of the experimental procedure showing the timing of (i) THC and labeled thymidine ingestion and (ii) sacrifice and intestinal cell isolation. (b) [^{3}H]Thymidine incorporation into the acid insoluble fraction of villus (a), crypt (d), or mixed population (b, c) of enterocytes, isolated from intestine of mice treated daily for 4 days with THC (50 mg/kg) or vehilce (control); $P < 0.03$.

detach themselves later from the intestine are harvested during the last 30 min of incubation (fraction b in Figs. 1a and 2a).

Aliquots of each fraction are studied by phase microscopy to check their morphology and also analyzed in triplicate for protein content and radioactivity. Cells are washed on a fiber-glass filter first with NaCl (9 g/liter) then with trichloroacetic acid 10%, and finally with methanol. Instagel (15 ml) is poured on the dried disk filters. Results are expressed in cpm/mg proteins. Three-way analysis of variance is used for statistical evaluation of the data.

Results. *Microscopic aspect and metabolism of enterocytes.* Aliquots from each cell fraction were analyzed by phase microscopy. The first fraction contained layers of aggregated cells, and simple columnar villus epithelial cells which displayed typical brush border morphology. The last fraction showed a mixed population of cuboidal cells and intact crypts of Lieber-Kuhn. Crypts were distinguished from aggregates of villus cells by their three-dimensional cylindrical structure.

Protein contents of each fraction decreased from the first fraction to the last one. In the collection divided into four fractions 11.8, 4.9, 3.4, and 1.6 mg of protein were recovered per fraction. In the collection divided into two fractions 14.4 and 9.4 mg (mean of eight animals, triplicate) were measured, respectively.

Labeled thymidine incorporation in DNA was significantly larger in the fraction rich in undifferentiated crypt cells than in the fraction rich in differentiated villus cells: 5.2 and 1.8 $\times 10^3$ dpm/mg proteins, respectively (mean of seven animals, triplicate). This difference of incorporation is even more evident in a collection of four fractions (Fig. 3).

Labeled glucosamine incorporation in glycoproteins showed an opposite trend: the higher incorporation occurred in the fraction rich in differentiated villus cells: 1.83×10^3 versus 1.06×10^3 dpm/mg of proteins (mean of four animals, triplicate; Fig. 1b).

Effect of THC on intestinal cell isolation. THC did not affect cell collection, qualitatively nor quantitatively ($P = 0.153$). A slight enhancement of protein in both fraction a (+11%) and fraction b (+2%) was observed but was not statistically significant ($P = 0.074$).

Effect of THC on [^{3}H]thymidine incorporation. In the first series of experiments, a single dose of THC (100 mg/kg) inhibited tritiated thymidine incorporation in all parts of the intestine: proximal, medial, or distal and in either fraction ($P = 0.006$). In villus cells of proximal and medial fractions inhibition was minimal (Fig. 2b).

After a single dose of THC (10, 50, or 100 mg/kg) the incorporation of thymidine in DNA is inhibited in fractions a and b of proximal intestine cells ($P = 0.037$ for 100 mg/kg, $P = 0.033$ for 50 mg/kg, and $P = 0.109$ for 10 mg/kg Fig. 1c). This inhibition is not related to the experiment ($P = 0.369$) nor to the differentiation state of the cells ($P = 0.239$).

In the second series of experiments the cu-

mulative effect of THC, after 4 days of daily ingestion of 50 mg/kg, is observed. There is a significant inhibition of incorporation ($P < 0.03$, Fig. 3b).

Effect of THC on [^3H]glucosamine incorporation. Glucosamine incorporation into the acid insoluble fraction is measured to evaluate glycoprotein synthesis in enterocytes. After a single dose of THC, 10 or 100 mg/kg, there is a dose-related inhibition of the incorporation of tritiated glucosamine in glycoproteins (Fig. 1b). The three-way analysis of variance of all the results gives $P = 0.0187$. The inhibition is neither related to the experiments ($P = 0.295$) nor to the differentiation state of the cells ($P = 0.167$).

Discussion. The technique of intestinal cell isolation that is used in the present experiments produced results similar to those obtained by others (10, 11) for cytological characteristics, protein content, [^{3}H]thymidine, and [^{3}H]glucosamine incorporation. The low incorporation of thymidine fraction a could be accounted for by the low note incorporation in villus cells which are numerous in this fraction while crypt cells with a high rate of incorporation are not.

The effects of THC were exerted on all parts of the small intestine. There is a variability in sensitivity to THC from one lot of animal to the next. Therefore the effects of the drug were expressed in terms of percentage change from measurements performed in a control group studied in parallel. The doses of 10 and 50 mg/kg were tested in the same experiments and compared with the same control group. A dose-dependent effect was observed. In a subsequent experiment, a group of animals treated with 100 mg/kg were tested and compared to a parallel control group. In this series, 100 mg had an effect which was smaller than that observed with 50 mg in a previous experiment. The variability in THC response of different groups of animals may also account for the difference in results observed in two series of experiments where the effects of the drug were tested on thymidine incorporation of villus cells from proximal and medial sections of the intestine (Fig. 2b): in one experiment, incorporation was low in the other it was high (up to 68% of control).

While Lawson *et al.* (11) isolated intestinal cells into six fractions, two fractions are enough to differentiate the two populations of cells. The division rate of undifferentiated cells is measured by DNA incorporation of thymidine and the biosynthesis of differentiated cells is measured by macromolecular incorporation of glucosamine. Although the significance of these measurements has been debated, in the present studies they do reflect the metabolism of these different cell types.

Some drugs are more active on undifferentiated cells, others on differentiated ones (13). THC inhibits cell metabolism whatever their state of differentiation.

Other studies have reported that THC inhibits the plasma membrane transport of macromolecule precursors (4) in human lymphocytes thereby decreasing cellular anabolism. The mechanism of biosynthesis inhibition by THC seems to be a general one exerted on all fast growing cells.

The inhibitory effects of THC on enterocytes may account for the decrease in growth of animals (rats, mice, rabbits, and dogs) chronically treated with this drug (14–17). The intestinal mucosa of mammals is markedly exposed to THC and its metabolites which are mostly (80%) eliminated via the fecal route, and also undergo enterohepatic recirculation (18–20).

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