

# Cytogenetic effects of delta-9-tetrahydrocannabinol ( $\Delta^9$ -THC) on hamster bone marrow<sup>1</sup>

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**Summary.** Single s.c. injections of 10 or 1000 mg/kg of  $\Delta^9$ -THC did not induce discernable chromosomal damage but caused significant mitotic inhibition in the bone marrow of Syrian hamsters.

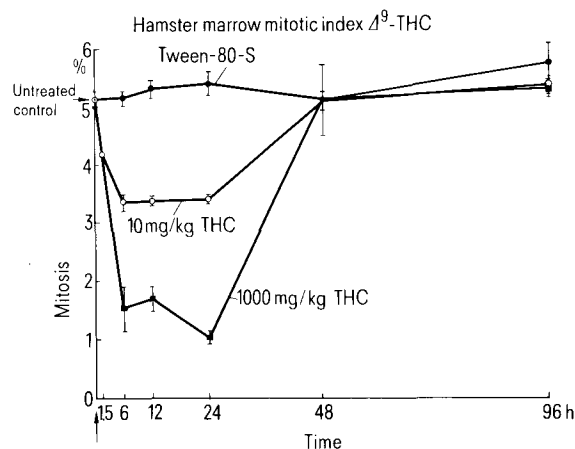
The increase in illicit consumption of *Cannabis* and its derivatives by young adults over the past several years<sup>2</sup> has generated a considerable interest in the effects of these drugs on genetic material. The majority of experiments on the effects of *Cannabis* have employed human tissues exposed to various cannabinoids in vivo<sup>3-8</sup> or in vitro<sup>8-10</sup> and then grown in culture prior to cytogenetic analysis. However, studies on human subjects have not been reliable due to several difficulties of experimental design, such as impurities in drug samples, multiple drug abuse and the lack of accurate dose estimates. Recently, the synthetic form of  $\Delta^9$ -THC, the major psychoactive component of *Cannabis* has become easily available, but only a few experiments have been reported on the effects of this compound on chromosomes of cultured human leukocytes<sup>10,11</sup> and in-vivo studies using  $\Delta^9$ -THC alone have not been extensively performed<sup>12</sup>. In this communication, we report the in-vivo cytogenetic effects of pure synthetic  $\Delta^9$ -THC on hamster bone marrow.

$\Delta^9$ -THC prepared by Arthur D. Little Inc. was received through the Department of National Health and Welfare, Health Protection Branch, Ottawa. The drug used was certified to be 96% pure and was from Lot. No. 79124. Random bred male Syrian hamsters (*Mesocricetus auratus*) weighing  $100 \pm 5$  g, purchased from High Oak Ranch Ltd, Goodwood, Ontario, were used in this study. Test animals were given a single s.c. injection of 10 or 1000 mg/kg b.wt of  $\Delta^9$ -THC dissolved in Tween-80-saline. Controls were either untreated or received equivalent volumes of the vehicle. As positive controls, an additional group of 5 hamsters was injected with 10 mg/kg of mitomycin C, a compound known to cause extensive chromosomal damage. Groups of 5 animals were sacrificed at 1.5, 6, 12, 24, 48 and 96 h after treatment, except mitomycin injected hamsters which were killed 24 h after drug administration. Each hamster was given  $10 \mu\text{g}$  per g b.wt of colchicine i.p. 90 min prior to sacrifice. Bone marrow was flushed from both femurs and the cells were pretreated with 1% sodium citrate at  $37^\circ\text{C}$  for 20 min. The cells were then fixed in glacial acetic acid: methanol (1:3) and 2-3 drops of the resulting suspension were placed on chilled glass slides. The slides were air-dried and stained routinely with Giemsa.

The results of chromosomal and karyotype aberrations are summarized in the table. Only isochromatid or chromatid

gaps and breaks were found in vehicle-injected control and  $\Delta^9$ -THC groups. Chromosome exchanges were frequent in the mitomycin-treated samples which had a high overall frequency of aberrations (78.2%), as expected. The untreated, or vehicle-injected controls and  $\Delta^9$ -THC groups did not reveal any significant differences in the frequencies of chromosome aberrations; a majority of the groups showed no discernable damage to their metaphase chromosomes. Chromosome counts of at least 100 metaphase plates in each group showed a very low frequency of numerical deviations (aneuploids) in several  $\Delta^9$ -THC treated samples but these values remained comparable to those of controls and were not higher than 2.0% in any group. 10 karyotypes prepared from each group did not show any structural alterations in chromosomes.

Mitotic indices were determined in control and  $\Delta^9$ -THC exposed specimens, scoring at least 4000 cells for each sample. The data presented in the figure show that the untreated control specimens had an average mitotic index



Mitotic index in the bone marrow of hamsters. ○ Untreated, ● Tween-80-saline (vehicle) injected controls, □ 10 mg/kg  $\Delta^9$ -THC, ■ 1000 mg/kg  $\Delta^9$ -THC. Each point represents an average value  $\pm$ SD for 5 hamsters.

Frequency of metaphase plates with chromosomal aberrations or aneuploid karyotypes in the bone marrow of controls or hamsters injected with 10 or 1000 mg/kg of  $\Delta^9$ -THC or 10 mg/kg mitomycin C\*

Time after injections (h)	Untreated controls		Vehicle-injected controls***		$\Delta^9$ -THC (10 mg/kg)		$\Delta^9$ -THC (1000 mg/kg)		Mitomycin C (10 mg/kg)	
	Aberr.** (%)	Aneu. (%)	Aberr. (%)	Aneu. (%)	Aberr. (%)	Aneu. (%)	Aberr. (%)	Aneu. (%)	Aberr. (%)	Aneu. (%)
0	1.8	1.7								
1.5					0.0	2.0				
6			0.0	1.0	0.0	0.0	0.0	1.0		
12			0.0	0.0	0.0	1.0	2.0	2.0		
24			1.0	0.0	0.0	0.0	1.9	1.0	78.2	
48			0.0	0.0	0.0	1.0	0.0	1.6		
96			0.0	0.0	0.0	0.0	0.0	1.2		

\* Minimum 100 metaphase plates per sample. \*\* Aberr.: Chromosome aberrations; Aneu.: aneuploids. \*\*\* Tween-80-saline.

of 5.16% and the vehicle injected controls did not exhibit large deviations from this value during the 4-day period. However, a statistically significant decrease in the mitotic index ( $p < 0.05$ ) was observed in the marrow of hamsters receiving 1000 mg/kg of  $\Delta^9$ -THC in samples examined up to 24 h post-treatment. The values returned to control levels in those examined at 48 and 96 h after treatment. A much smaller dose of 10 mg/kg of  $\Delta^9$ -THC also produced a significant drop in the mitotic indices of marrow at corresponding times, but the effect was less marked than in samples receiving the larger dose.

There has been conflicting evidence as to whether or not *Cannabis* or its derivatives cause chromosomal damage. In the present study, pure  $\Delta^9$ -THC did not cause any significant effects on chromosome structure or karyotype stability in the marrow of hamsters receiving even a very large dose of 1000 mg/kg of  $\Delta^9$ -THC. However, the possibility of minute undetected chromosomal aberrations or point mutations cannot be completely excluded. It is possible that the aberrations observed by some investigators<sup>4,5,7,8</sup> may have been caused by impurities or other cannabinoids present in the preparations used. Our findings on the reduction of mitotic indices after  $\Delta^9$ -THC treatment in vivo are consistent with previous reports which employed exposure to marihuana smoke<sup>9</sup>, *Cannabis* resin<sup>6</sup> or  $\Delta^8$ - and  $\Delta^9$ -THC<sup>11</sup> in in vitro systems. The reduced mitotic activity in the bone marrow could have significant effects on the

pattern of proliferation and differentiation of hematopoietic cells.

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## Alkaline phosphatase activity in normal and denervated skeletal muscle

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**Summary.** Changes in the specific activity of alkaline phosphatase in the normal and denervated skeletal muscle have been studied both histochemically as well as biochemically for a maximum period of 8 weeks of its postnatal development. In the normal muscle, a heterogeneous population of fibres with respect to the enzyme distribution is observed. Relatively higher levels of enzyme in the denervated muscle and also the proliferation of extrafibrillar connective tissue in the diseased muscle show its specific association with the lytic processes.

The role of acid phosphatase in different myopathies including various neuromuscular disorders has been a subject of extensive investigation<sup>1-5</sup>, and very little attention has been paid to alkaline phosphatase which is an equally important enzyme functioning at a different pH optimum. Kar and Pearson<sup>6</sup> have associated the proliferation of noncontractile connective tissue with higher levels of alkaline phosphatase activity in the diseased human muscle and have assigned a lytic role to this enzyme. On the other hand, Dubowitz<sup>7</sup> rules out the presence of this enzyme in normal muscle, whereas Pennington<sup>8</sup> does not consider the possibility of the 2 enzymes functioning at very different pH optima in the same cell at one time. In light of these conflicting views, it was considered desirable to study the sequential changes in the levels of this enzyme during the growth of normal as well as denervated skeletal muscle since both lysis and an increase in the noncontractile connective tissue are observed in the denervated muscle. The present investigation has been carried out on the gastrocnemii muscles of chick and includes quantitative estimation of the enzyme at weekly intervals for a maximum period of 8 weeks, in the normal as well as denervated muscle. Histochemical localization of the enzyme has been made to determine the sites of activity change during different stages of muscle growth.

**Material and methods:** Male chicks of *Gallus domesticus* (white Leghorn variety) were procured from Government Poultry Farm at Simla (India). The animals were divided into 2 groups and maintained under normal laboratory hygienic conditions. 1 group served as controls, whereas members of the other group were subjected to unilateral sciectomy on the 5th day of their postnatal life. Denervation procedure is described elsewhere<sup>9</sup>. Autopsies were performed at weekly intervals for a maximum of 8 weeks after denervation and the 3 gastrocnemii viz; pars externa, media and interna were excised immediately and processed as follows. The enzyme was localized histochemically by Gomori's technique<sup>10</sup> at pH 8.9 with minor modifications using unfixed, fresh-frozen air-dried sections and employing sodium-B-glycerophosphate as the substrate. The quantitative estimation of the enzyme was made as by Weil and Russel<sup>11</sup> and Fiske and Subbarow<sup>12</sup>. Optical density was read in Carl Zeiss VSU-2 spectrophotometer at 600 nm. Standard curve was plotted using different concentrations of  $\text{KH}_2\text{PO}_4$ .

**Results.** The positive fibre staining in all the 3 gastrocnemii at pH 8.9 confirms the presence of alkaline phosphatase in the normal muscle, and a distinct heterogeneity in the fibre population<sup>13</sup> is observed (figure 1). The narrow red fibres are richer in the enzyme concentration than the white type.