Mutagenicity testing of 3 hallucinogens: LSD, psilocybin and Δ^9-THC, using the micronucleus test

G. F. Van Went[1]

National Institute of Public Health, Laboratory of Pharmacology, P.O. Box 1, Bilthoven (The Netherlands),
5 August 1977

Summary. Using the micronucleus test as a screening method for mutagenic activity, no significant increase in the number of micronuclei was found when LSD, psilocybin or Δ^9-THC were administered in 3 logarithmically increasing doses to mice. Azathioprine (Imuran®), given as a positive control, caused a statistically significant increase in micronucleated cells.

Considering the application of lysergic acid diethylamide (LSD) and psilocybin in psychotherapy and the use of *Cannabis* as a hallucinogen, the testing of the mutagenic activity of these substances is of great importance. One of the methods recommended nowadays for testing the mutagenic potential of chemical agents is the micronucleus test. Schroeder[2] first mentioned the presence of micronuclei in nucleated bone marrow cells and suggested scoring as a reflection of chromosome-lagging. The development into the mutagenicity test in use now was done by Schmid et al.[3, 4]. The rationale of the test procedure is the anaphase-lagging of acentric chromosome fragments, apparently caused by chromosome break, while the centric elements move to the poles of the cell. Chromosome loss due to partial impairment of the spindle fibres, can also give rise to the presence of micronuclei. It is assumed that entire chromosomes or chromosome parts, which stay behind, form one or more small fragments of nuclear material. These fragments usually are much smaller than normal nuclei – they have a diameter of $^1/_{20}$–$^1/_5$ of an erythrocyte – and therefore are called micronuclei. Micronuclei are found in an increased frequency in bone marrow cells which have been exposed to certain chemical agents (e.g. cytostatic agents). The micronuclei are preferably counted in young erythrocytes, because erythroblasts expelling their nucleus retain the micronucleus.

In the micronucleus test, demonstration of a statistically significant increase in the number of micronuclei is an indication for the chromosome-breaking potential of the agent under investigation. Here we report experiments on LSD, psilocybin and Δ^9-THC. The latter compound was chosen instead of *Cannabis* itself, being the main active component of the preparation.

Material and methods. Young adult Swiss mice, 7–8 weeks old, were used in the experiments. For each dose 5 animals were chosen, 2 females and 3 males. LSD (Sandoz) was given in doses of 80, 160 and 320 µg/kg b.wt; psilocybin (Sandoz) in doses of 4, 8 and 16 mg/kg b.wt and Δ^9-THC (Batch UNC 441, United Nations, Narcotic Drugs) in doses of 5, 10 and 20 mg/kg b.wt. For the positive control experiment azathioprine (Imuran®, Burrough's Wellcome) was applied in a dose of 50 mg/ kg b.wt. The doses used for LSD and psilocybin were derived from the human dose in psychotherapy; for THC doses effective in animal behaviour pharmacology were chosen. LSD, psilocybin and azathioprine were dissolved in buffered saline, for Δ^9-THC 4% Tween 80 in 0.9% NaCl was used as the vehiculum. All agents were injected twice i.p. with an interval of 24 h; and 24 h after the last injection the animals were killed and bone marrow smears were made. The smears were stained in May-Grünwald Giemsa stain according to the procedure of Schmid[4], with the modification that Sørensen buffer of pH 6.8 was used instead of distilled water. In the slides 1000 polychromatic erythrocytes were screened for the presence of micronuclei. The values given in the table for each dose are the means corresponding to 5 animals, obtained using the transformation of Freeman and Tukey. For the transformed data analyses of variance were calculated.

Results and discussion. The results of the experiments are given in the table. No statistically significant increase in the number of cells with micronuclei was found after treatment with different doses of LSD, psilocybin or Δ^9-THC. As expected, the mice treated with 50 mg/kg azathioprine, known to be positive in the micronucleus test, did show a significantly increased number of cells with micronuclei in comparison to control values. After administration of Tween 80, the vehiculum for Δ^9-THC, the number of cells with micronuclei was in the same range as the control values of animals receiving buffered saline only.

Experiments concerning the mutagenic activity of LSD have led to conflicting results. In recent studies dominant lethals, the frequency of which was dose-dependent, were induced in ♀ and ♂ mice, using the dominant lethal test[5, 6]. Structural chromosome abnormalities in primary spermatocytes of rats and mice were sporadic, but a significant increase in the number of spermatocytes with univalents did occur[7]. No chromosome abnormalities were found after in vivo application of the drug in humans[8, 9] and Syrian hamsters[10]. However, an increase in structural chromosome aberrations was found when LSD was added to the lymphocytes of the same patients in vitro[8].

In our experiments with the micronucleus test, no indication was found for a chromosome-breaking activity of

Survey of the number of cells with micronuclei in 1000 polychromatic erythrocytes in bone marrow of mice after 2 i.p. administrations of LSD, psilocybin, Δ^9-THC and vehiculum respectively. Azathioprine is used as a positive control

Treatment	Number of cells with micronuclei*	Range of number of cells with micronuclei
Saline	3.3	1–7
LSD 80 γ/kg	1.7	0–9
LSD 160 γ/kg	3.2	2–4
LSD 320 γ/kg	4.5	2–6
Saline	2.5	1–5
Psilocybin 4 mg/kg	2.0	1–3
Psilocybin 8 mg/kg	1.2	0–2
Psilocybin 16 mg/kg	2.4	2–3
4% Tween 80	2.5	1–5
Δ^9-THC 5 mg	2.9	2–5
Δ^9-THC 10 mg	2.5	1–4
Δ^9-THC 20 mg	3.5	2–5
Azathioprine 50 mg/kg	30.1	26–33

* In each animal 1000 polychromatic erythrocytes were analyzed; the values are the means corresponding to 5 animals, obtained using the transformation of Freeman and Tukey.

LSD. The absence of the metabolic activity of the liver and the greater sensitivity of cells in vitro may be causes for the discrepancy in results between in vivo and in vitro systems. Psilocybin has not been investigated as extensively as LSD. Addition of psilocybin to human lymphocytes in vitro led to an increase of chromosome gaps[11]. This type of chromosome abnormality, however, is usually not seen as representative for chromosome breakage. In contrast to the publication of Eberle and Leuner[12], where an increased number of chromosome breaks was found in 4 patients treated with psilocybin, results of our experiments with humans on psychotherapy and Chinese hamsters[20] gave no indication for an in vivo chromosome-damaging activity of psilocybin and are in good agreement with the negative findings in the micronucleus test. The results with THC in the micronucleus test correspond well with literature findings. Neither Δ^8- nor Δ^9-THC induced chromosome aberrations when added to human lymphocytes in vitro[13, 14]. Δ^9-THC taken orally or smoked in different dosages in cigarettes by humans, did not cause an increase in chromosome breaks[15, 16], nor did s.c. application in Syrian hamsters[10]. In vitro addition of *Cannabis* resin to embryonic rat fibroblasts or human lymphocytes, in vivo application to pregnant rats or smoking by humans did not lead to an increase in chromosome abnormalities[17]. In a large group of marihuana users, on the other hand, a statistically significant increase in chromosome aberrations was found[18, 19]. The conflicting data from marihuana smokers may be explained by assuming a component, other than THC, being the cause of the chromosome damage. In mutagenecity testing it is not possible at present to prove the mutagenic potential of a compound in a single test system. Results of other tests will be needed to confirm our negative results with the micronucleus test.

1 Acknowledgment. The expert technical assistance of Mr Joop Branger is greatfully acknowledged. We thank Prof. Dr C. A. Salemink, University of Utrecht, for the supply of the THC, and Dr J. Fokkens, National Institute of Public Health, for preparing the solutions.

2 T. M. Schroeder, in: Chemical Mutagenesis in Mammals and Man, p. 214. Ed. F. Vogel and G. Röhrborn. Springer Verlag, Berlin 1970.
3 K. Boller and W. Schmid, Hum. Genet. *11*, 35 (1970).
4 W. Schmid, in: Chemical Mutagens, vol. 4, p. 31. Plenum Publ. Corp., New York 1976.
5 R. J. Šrám, Z. Zudová and P. Goetz, Mutat. Res. *26*, 517 (1974).
6 R. J. Šrám and P. Goetz, Mutat. Res. *26*, 523 (1974).
7 P. Goetz, R. J. Šrám and Z. Zudová, Mutat. Res. *26*, 513 (1974).
8 L. F. Jarvik, F. S. Yen, C. C. Dahlberg, J. L. Fleiss, J. Jaffe, T. Kato and E. Moralishvili, Dis. nerv. Syst. *35*, 399 (1974).
9 Fernandez, I. W. Browne, J. Cullen, T. Cullen, T. Brennan, H. Matheu, I. Fischer, J. Masterson and E. Law, Ann. hum. Genet. *37*, 81 (1973).
10 M. T. Nicholson, H. B. Pace and W. M. Davis, Res. Commun. chem. Path. Pharmac. *6*, 427 (1973).
11 H. Marquez-Monter, E. Ruiz-Ramirez and J. Carranza-Acevedo, Patología *13*, 276 (1975).
12 P. Eberle and H. Leuner, Hum. Genet. *9*, 281 (1970).
13 R. L. Neu, H. O. Powers, S. King and L. I. Gardner, J. clin. Pharmac. *10*, 228 (1970).
14 M. A. Stenchever and M. Allen, Am. J. Obstet. Gynec. *114*, 819 (1972).
15 W. W. Nichols, R. C. Miller, W. Heneen, C. Bradt, L. Hollister and S. Kanter, Mutat. Res. *26*, 413 (1974).
16 S. S. Matsuyama, F. S. Yen, L. F. Jarvik, R. S. Sparkes, T. K. Fu, H. Fischer, N. Reccius and I. M. Frank, Mutat. Res. *48*, 255 (1977).
17 P. A. Martin, M. Thorburn and S. A. Bryant, Teratology *9*, 81 (1974).
18 M. A. Stenchever, T. J. Kunysz and M. A. Allen, Am. J. Obstet. Gynec. *118*, 106 (1974).
19 T. H. Maugh, Science *185*, 683 (1974).
20 G. F. Van Went, to be published.

Chromosomal polymorphism caused by supernumerary chromosomes in *Rattus rattus* ssp. *frugivurus* (Rafinesque, 1814) (Rodentia, Muridae)

M. A. Pretel and G. R. Diaz de la Guardia

Departamento de Genetica, Facultad de Ciencias, Universidad de Granada (Spain), 15 August 1977

Summary. A chromosomal numeric polymorphism 2n = 38, 39, 40 and 41 in the species *Rattus rattus* ssp. *frugivurus* (Rafinesque, 1814) is reported for the first time for this subspecies. The numbers 2n = 39, 40 and 41 are new for the species. The polymorphism is due to the presence of 1, 2 or 3 B-chromosomes, which are all small metacentrics of the size and shaped very close to the other autosomes of the normal complement, and whose character of being supernumeraries is shown in Meiosis.

While chromosomal polymorphism due to supernumerary or B-chromosomes is widely extended in plants and among some groups of insects[1], its incidence among the mammals is rather rare. And yet, some cases have been described, among which stands out one reported for the marsupial *Schoinobates volans*[2], which contains in its cells a variable number of small additional metacentric chromosomes (microchromosomes), ranging from 1 to 3. In *Echymipera kalabu* was pointed out by Hayman[3], in parallel with a sex chromosome mosaicism. Certain extra or supernumerary chromosomes were also reported in other mammalian species, such as *Vulpes vulpes*[4-6]. In rodents, *Reithrodontomys megalotys*[7], *Rattus rattus* ssp. *diardii*[8], *Rattus rattus*[9] and *Apodemus giliacus*[10].

The numeric polymorphism of *R. rattus* ssp. *diardii*, described by Yong[8] in the individuals with standard karyotype 2n = 42 of Malaysia, is due to the presence of some small additional chromosomes, which appeared all to be small metacentrics, indistinguishable from small metacentrics of the normal complement. Gropp et al.[11] found in

1 M. J. D. White, Animal Cytology and Evolution, 3rd ed. Cambridge Univ. Press, 1973.
2 D. L. Hayman and P. G. Martin, Aust. J. biol. Sci. *18*, 1081 (1965).
3 D. L. Hayman, P. G. Martin and P. F. Waller, Chromosoma 27, 371 (1969).
4 I. Gustavson and C. O. Sundt, J. Hered. *58*, 75 (1967).
5 M. Sasaki, H. Shimba, M. Itoh, S. Makino and G. Shiota, Proc. Japan Acad. *44*, 847 (1968).
6 K. E. Buckton and C. Cunningham, Chromosoma *33*, 268 (1971).
7 H. S. Shellhammer, Chromosoma *27*, 102 (1969).
8 H. S. Yong and S. S. Dhaliwal, Chromosoma *36*, 256 (1972).
9 K. L. Satya Prakash and N. V. Aswathanarayana, J. Mysore Univ. *25*, 23 (1972/73).
10 I. Hayata, Chromosoma *42*, 403 (1973).