Effects of some Components of *Cannabis sativa* on the Regenerating Planarian Worm *Dugesia tigrina*

The purpose of this note is to present a simple biological method of analysis of the toxic effects of the substances belonging to the series of cannabinoids. This would be useful because, tetrahydrocannabinols (THC) being insoluble in water, suitable concentrations are difficult to achieve. But we know from experience that regenerating pieces of planarian worms are able to absorb insoluble substances deposited on the base of the vessels in which they are cultured. We noted this, for instance, in the cases of thalidomide and of *p*-chlorophenylalanine[1]. With this new method we studied the toxicity of Δ^6 (or Δ^8)-THC-Δ^1 (orΔ^9)-THC and cannabidiol (CBD).

Some authors[2,3] suggested that THC affects the metabolism of serotonine (5HT) and that it shows some structural analogies with inhibitors of this neurohormone such as LSD[4]. One of us has already shown that 5HT, or a very closely related substance, plays an essential role in the process of regeneration in the planarians *Dugesia tigrina* and *Dugesia gonocephala*[1,5]. 5HT and certain of its precursors have a promoting effect in regeneration, while numerous substances with indole nucleus have an inhibiting one at low concentrations (of the order of $M\ 10^{-6}$).

We thought it would be of interest to study the effects of Δ^1-THC, Δ^6-THC, CBD, and an extract of *Cannabis*, on the regeneration of *Dugesia tigrina* and to test the protective effect of 5HT on the treated animals.

Material and method. Dugesia tigrina is well known for its facility of regeneration after the loss of a part of the body. The surgical experiments may be carried out without special precautions in connection with sterility. The operation consists of cutting a portion of the anterior part of the animal. The isolated piece forms a new head and a new tail within a few days. The tests for the diagnosis are the number of animals per population which heal 15–20 h after the operation, the number of dead animals 2 days after the beginning of the treatment, and the speed of the regeneration of the eyes in the living ones (in this last case diagnoses are performed 4 or 5 days after the beginning of the experiment and, if necessary, every day for a week). The tests are easy to observe, even the detection of the eyes is easy because they are pigmented, on the 4th day they resemble small dark round spots and the following day they resemble 2 commas. It is possible to work on a great number of planarians. We carried out 3 series of experiments using 3×20 pieces for each concentration of the substance to be tested and for the controls.

For determining THC, the vessels are prepared in the following manner: the substance is dissolved in chloroform and 1 ml of the solution is placed in a Petri dish of 10 cm diameter. Care must be taken to evaporate the solvent evenly over the surface of the base of the dish. After evaporation, 50 ml water are added and the pieces of planerian deposited therein. The culture is continued for 3 days at about 23 °C. Each day, the dead are counted and removed. On the 3rd day, the planarians are placed in baths of fresh water. If necessary, they are fixed for histological examination.

Results. When using the test for mortality, it was possible to establish the curves for the toxicity of Δ^6-THC, Δ^1-THC and CBD. Figure 1 indicates that the thresholds of toxicity were about the same in the 3 cases: between 0.02 and 0.06 mg of substances per 100 ml water ($6 \times 10^{-7}\ M$). With CBD, we noted variations of activity which are due to its degradation in the chloroform solution with time.

For testing the biological method we used a 10% petroleum ether extract of resin from *Cannabis*. The concentration of THC was 1 mg per ml as determined by chemical analysis[6]. The extract was diluted with chloroform to give various concentrations from $^1/_{10}$ to $^1/_{1000}$. At the concentration corresponding to 0.01 mg according to the chemical analysis, there were 42% deaths, a higher percentage than that corresponding to the toxicity curve (Figure 1). For this reason, the final experiment was made by comparison with control solutions of known concentrations of THC. These were $1.6 \times 10^{-7}M$, $3 \times 10^{-7}M$ and $16 \times 10^{-7}M$. The curves of toxicity as a function of time (Figure 2) show that the solutions of extract containing $6 \times 10^{-7}M$ to $3 \times 10^{-7}M$ had a slightly greater effect than the corresponding control solutions and the results of the analysis indicated that the extract contained about 1.25 mg per 1 ml. The excess of 0.25 may be due to the presence of substances similar in structure to THC such as CBD in the extract. This also contained fatty acids and chlorophyll[7].

With the same test we studied the protective action of 5HT against Δ^6-THC, Δ^1-THC and CBD.

Δ^6-THC: 3 experiments were carried out. In the first, the control was a solution of THC $6 \times 10^{-7}M$ and the test for protection was made by the addition of $6 \times 10^{-7}M$ 5HT to this solution. A 3rd group of animals was treated with a mixture where the amount of 5HT was $12 \times 10^{-7}M$. In this last case, protection was achieved (Figure 3). In the 2nd experiment, the amount of THC was slightly increased. The control solution contained $9 \times 10^{-7}M$. For protection, $9 \times 10^{-7}M$ 5HT was added (Figure 3b). In the 3rd experiment, the solution of THC contained $15 \times 10^{-7}M$. This was more toxic than the two other solutions and we did not obtain protection by adding an amount equal to or greater than $16 \times 10^{-7}M$ (Figure 3, c)).

It should be noted that the addition of relatively large amounts of 5HT, far from protecting the organism,

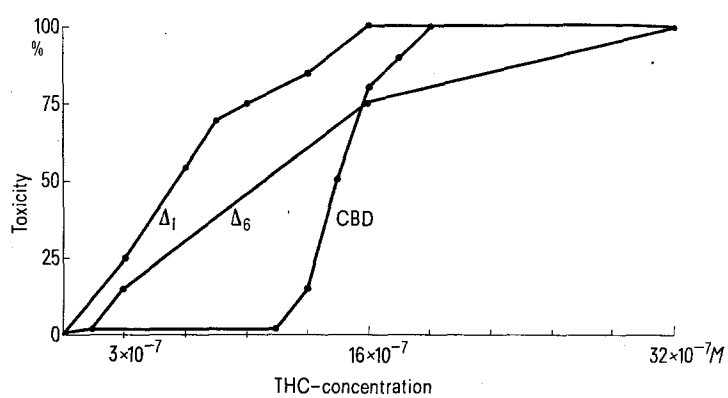

Fig. 1. Toxicity curve for Δ^6-THC, Δ^1-THC, CBD: ordinate, toxicity as percentage of mortality; abscissa, concentration in *M* of THC. All the controls were in good health.

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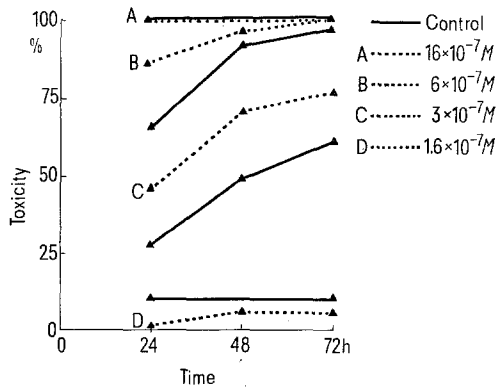

Fig. 2. Biological determination of THC in *Cannabis* extract. The control solutions (—) were made with synthetic THC; dilutions of extract (---).

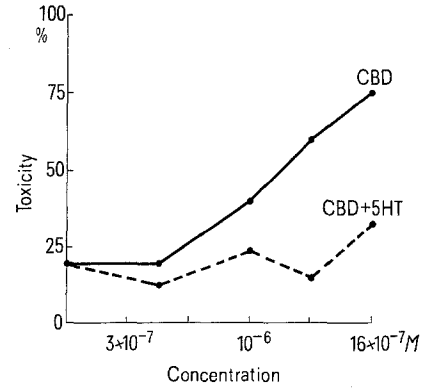

Fig. 4. Effect of CBD on the regeneration of the eyes as function of the concentration and protection by addition of 5HT. Ordinate: toxicity as percentage of animals without eye. Abscissa: concentration of the substances.

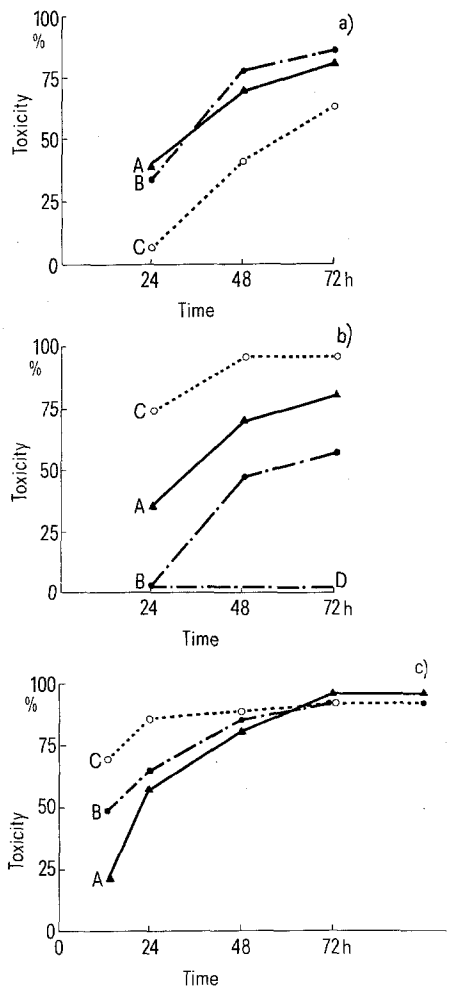

Fig. 3. Protective effect by the addition of 5 HT to the solution of THC. a) curve A: THC = $6\times10^{-7}M$; curve B: THC = $6\times10^{-7}M$ and 5HT $6\times10^{-7}M$; curve C: THC = $6\times10^{-7}M$ and 5HT $12.10^{-7}M$: in this last case there is protection by serotonine (5HT). b) curve A: THC = $9\times10^{-7}M$; curve B: THC = $9\times10^{-7}M$ plus 5HT = $9\times10^{-7}M$; curve C: THC = $9\times10^{-7}M$ plus 5HT = $4{,}5\times10^{-6}M$; curve D: 5HT = $4{,}5\times10^{-6}M$. The serotonine at $9\times10^{-7}M$ protected the animal against THC, at the higher dose it increased its toxicity. c) curve A: THC = $16\times10^{-7}M$. curves B and C: THC = $16\times10^{-7}M$ plus 5HT, respectively, $3\times10^{-6}M$ and $4{,}5\times10^{-6}M$; there was no protection against this dose of the poison. On the contrary, the 'high' doses of 5HT increased the toxicity as in experiment B, group C.

increased the toxic effects of the substance (Figure 3, b), curve C). We had already observed this phenomenon on several occasions, e.g. in the case of reserpine. At the concentrations used in the present study, 5HT was not itself toxic (Figure 3, b), curve D). It is, therefore, the combined action of the 2 substances which is concerned.

Δ^1-THC: the addition of $6\times10^{-7}M$ of 5HT had a protective effect against the equal dose of Δ^1-THC, but there was no protection when using higher doses of these substances.

CBD: 5HT had a high protective effect against CBD at concentrations of $6\times10^{-7}M$ and $16\times10^{-7}M$.

The animals which were not killed by the treatment with THC or CBD had a longer time of healing and of regeneration. As shown by Figure 4, the addition of 5HT protected the treated animals.

Conclusion. The biological method described above is interesting for determining the activity of the components of *Cannabis*. It enables the determination of very small amount of THC and CBD. We were also able to observe an effect of some components of *Cannabis sativa* on the metabolism of serotonin. It will be possible, with this method, to extend this study to secondary components of *Cannabis* whose physiological effects are still not well known[8,9].

Résumé. Une méthode simple d'analyse biologique des effets toxiques des substances de la série des cannabinoïdes est présentée. Avec cette méthode, il est montré que les tetrahydrocannabinols $\Delta1$ et $\Delta6$ ainsi que le cannabidiol agissent sur le métabolisme de la sérotonine.

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