*Results.* The mean latency to enter the area containing the pups was 4.9, 5.5, and 10.8 sec for the 0, 10.0 and 20.0 mg/kg groups respectively. These differences were not significant (H = 0,7, n.s.). None of the 3 females in the 20.0 mg/kg group returned any pups to the nest. Two of the 6 females in the 10.0 group and all of the 9 females in the control group did return their pups to the nest. The mean time to retrieve the first pup was 6.0 sec for the control group compared with 208.3 and 300.0 sec for the 10.0 and 20.0 mg/kg groups, respectively (H = 10.3, $p < 0.01$). The difference between the control and the 2 drug groups was significant (U = 8, $p < 0.05$ for the 0 and 10.0 and 0 and 20.0 groups, respectively), while that between the 2 drug groups was not significant (U = 12, n.s.).

*Discussion.* Since the total test period was 300 sec, it is apparent that these differences did not emerge as a result of any effects of $\Delta^9$-THC on motor activity. All animals had more than enough time to retrieve pups as indicated by their latencies to enter the area into which the pups had been placed. At present, no explanation is available for the failure of animals given $\Delta^9$-THC to retrieve their young[3].

*Résumé.* Le comportement des femelles rat rapportant ses petits au nid a été fortement influencé par le L-$\Delta^9$-tetrahydrocannabinol. A des doses de 20 mg/kg il s'est complètement arrêté.

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[3] This study was supported by a grant from the Medical Research Council of Canada and by NIMH grant No. MH 17001.

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## Tolerance to the Behavioral and Hypothermic Effects of 1-$\Delta^9$-Tetrahydrocannabinol in Neonatal Chicks

The development of tolerance to the behavioral effects of marihuana and to its principle active ingredient, 1-$\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) has been demonstrated in various species of adult animals including rats, mice, pigeons, and monkeys[1-3]. Our experiments with chicks show that tolerance develops not only to the behavioral effects of $\Delta^9$-THC but to its physiological effects as well, and that this is also observable in neonatal animals.

Approximately 12 h after hatching, chicks ($n = 10$ per group) were injected i.m. with either 10 mg/kg $\Delta^9$-THC suspended in 2.5% Triton X-100 or placebo (1 cc/kg). One-half hour later, they were placed in a single photocell activity cage (General Controls Corp.). The apparatus was activated 1 min later and the number of interruptions of the light beam during the following 30 min was recorded. The procedure was repeated daily until no differences appeared for 6 days between the activity counts of experimental and control subjects. On day 17, all subjects were injected with 10 mg/kgm $\Delta^9$-THC and tested as before.

Twelve hours after the last injection on day 17, all birds were weighed, then sacrificed and their livers excised and weighed to determine whether the chronic drug treatment had affected the weight of this organ. Differences between groups were evaluated using the Mann-Whitney 'U' Statistic[4].

The effects of $\Delta^9$-THC on photocell activity are presented in Table I. $\Delta^9$-THC markedly suppressed activity but with continued drug administration, the degree of suppression diminished considerably. By day 11, the differences between groups were not significant (U = 43, n.s.). However, activity never increased to the maximum observed in control subjects receiving only placebo injections. On day 17, $\Delta^9$-THC completely suppressed activity in subjects previously receiving placebo, but had no marked effect in subjects receiving prior injections of $\Delta^9$-THC (U = 6, $p < 0.001$).

Mean body weights for control and experimental animals were 169.3 g and 140.7 g, respectively. Mean liver weight for control subjects was 5.56 g compared with 5.50 g for experimental subjects. The ratio of (liver weight/body weight) $\times 100$ was 3.27 and 3.95, respectively, for control and experimental subjects.

The differences in body weight were significant (U = 20, $p < 0.05$) as was the ratio between liver weight and body weight (U = 13, $p < 0.01$), whereas the differences between absolute liver size were not (U = 49, n.s.). The latter finding suggests that chronic injections of $\Delta^9$-THC had no effect on liver, but did lead to lowered body weight and it is this factor which resulted in the significant increase in the ratio of liver weight/body weight.

Using a modification of the hot plate test of EDDY and LEIMBACH[5], we investigated tolerance to the effects of $\Delta^9$-THC on escape responding to a heat stimulus. One-half hour after injection subjects were placed on the hot plate (75 °C) and the time to jump from the plate was determined. A maximum of 60 sec was imposed. This procedure was used on days 1, 3, and 5. On days 3 and 4

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Table I. Tolerance to the effects of 10.0 mg/kg $\Delta^9$-THC on the spontaneous activity[a] of neonatal chicks

| Days | 1 | 2 | 3 | 4 | 5 | 10 | 11 | 13 | 15 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control ($n = 10$) | 3.2 | 30.3 | 53.3 | 39.7 | 41.0 | 75.7 | 62.4 | 19.4 | 7.3 | 1.2 |
| Drug ($n = 10$) | 1.2 | 0.3[d] | 1.0[d] | 0.7[d] | 4.4[c] | 18.5[b] | 26.0 | 13.0 | 16.0 | 17.6 |

All subjects received 10.0 mg/kg $\Delta^9$-THC on day 17. [a] Activity is expressed as mean square root number of photocell interruptions per 30 min test period. [b] $p < 0.05$. [c] $p < 0.01$. [d] $p < 0.001$.

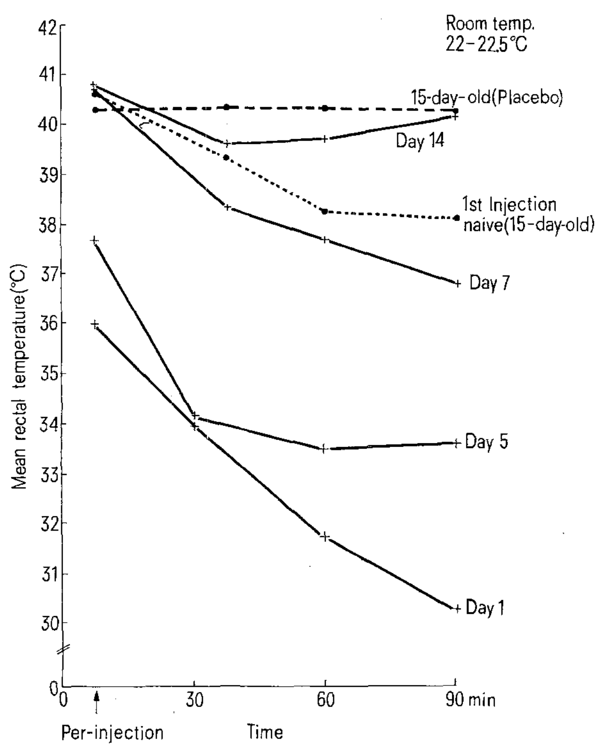
and 8 the exact same procedure was employed except that the hot plate was not heated. This procedure permitted evaluation of possible escape learning as a factor during test days with the heat on.

The results are shown in Table II. The latency to escape decreased as a function of tolerance and not maturation or learning since previously naive birds who responded immediately to the heat from the hot plate, had much greater latencies following an initial administration of $\Delta^9$-THC on day 10, compared with the latencies for chronically injected subjects which continue to decrease. Although the difference in latencies appears greater on day 5 than day 3, this difference was not significant since it was due to one subject in the drug group whose latency on that day was the maximum 60 sec. The decreases in latency on day 8 when no heat was applied to the hot plate were due to one subject in each group jumping from the hot plate within 10 sec after tests began. The other subjects in each group had near maximal latencies.

Table II. Tolerance to the effects of 3.0 mg/kg $\Delta^9$-THC on the heat escape response in neonatal chicks

| Days | 1 | 3 | 5 | 7 | 9 | 10 |
|---|---|---|---|---|---|---|
| Expt'l | 44.8[a] | 9.6 | 18.4 | 11.8 | 17.6 | 14.6[a] |
| Control | 10.0 | 6.6 | 5.7 | 7.8 | 14.3 | 33.5 |
| Mean latency to jump from hot plate (no heat) | | | | | | |
| Days | 2 | 4 | 8 | | | |
| Expt'l | 58.0 | 53.8 | 49.0 | | | |
| Control | 60.0 | 60.0 | 44.7 | | | |

All subjects received 3.0 mg/kg $\Delta^9$-THC on day 10. [a] $p < 0.05$.




Tolerance to the hypothermic effects of $\Delta^9$-THC: The dashed lines represent the changes in body temperature of 15-day-old chicks following either i.m. injection of lcc/kg Triton-X-100 (·—·) or 10.0 mg/kg $\Delta^9$-THC (·····) for the first time. The (+ — +) lines represent the changes in body temperature produced by daily/i.m. injections of 10.0 mg/kg $\Delta^9$-THC in a group of chicks from day 1 to day 15 after birth.

To determine if tolerance develops to the hypothermic effect of $\Delta^9$-THC[6] as suggested by LOMAX[7], we studied the effects of chronic injections of $\Delta^9$-THC on young chicks. At the ages being examined, differences in body temperature due to sex and size of animals are negligible[8] so that the preceding observation of reduced body weight following chronic administration of $\Delta^9$-THC probably is not an important factor in evaluating the results of the following experiment.

Rectal temperatures of the subjects ($n = 5$) were determined daily using a Yellow Springs rectal telethermometer (Model No. 34TD). Rectal temperatures were examined immediately prior to and at 30, 60 and 90 min after i.m. injection of 10 mg/kg $\Delta^9$-THC or placebo. This injection procedure was continued daily until day 15, at which time there was less than a 1°C change in mean body temperature after injection compared to pre-injection body temperature. Two other naive groups of chicks were also examined at this time. One group ($n = 5$) was injected for the first time with 10 mg/kg $\Delta^9$-THC while the second ($n = 4$) received a placebo injection. Temperature changes were measured as in chronically treated subjects.

The results are shown in the Figure. Adult thermoregulation is not completely developed in chicks until they are about 15 days of age[8], accounting for the differences in the pre-injection body temperatures. However, by day 7, the body temperature of newly hatched chicks is well within the range of body temperature in adult chickens.

The greatest lowering of body temperature by $\Delta^9$-THC occurred on day 1 (approximately 5.5°C after 90 min). However, by day 15, the decrease in body temperature induced by $\Delta^9$-THC was less than 1°C. In contrast, hypothermia (approximately 2.5°C) after $\Delta^9$-THC was still observable in naive 15 day old chicks (U difference = 0, $p < 0.05$) thus indicating that tolerance had indeed occurred to the hypothermic effects of $\Delta^9$-THC.

These observations on the development of tolerance in chicks are of importance because they extend the previous observations on the development of tolerance to neonatal as well as adult animals. In addition, they also extend the generality of $\Delta^9$-THC tolerance to physiological measures as indicated by LOMAX[7].

*Résumé*. La tolérance de l'effet du **1**-$\Delta^9$-tétrahydrocannabinol sur l'activité, sur l'échappement d'un stimulant de chaleur et sur la température du corps a été examinée sur des poussins néonataux. La tolérance s'est produite dans les trois conditions examinées.

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