becomes hostile. Some of them now grasp the newly formed imago by its antennae, legs, wings and sting, while others proceed to sting it to death, much as they would do to any intruder in the nest (Figure 4). Wasps have never been observed to warm pupae of honeybees (inside or outside their cells), of ants or other insects at different stages, nor their own freeze dried or dummy pupae. Wasp pupae that had been cut in two or frozen to death, pupae kept in total darkness or wrapped in filter paper, do elicit heating behaviour. Filter paper strips impregnated with alcohol extracts of pupae alone, release the thermoregulatory behaviour: wasps cluster around the strip and proceed to heat it, for a short duration by typical abdominal pumping movements (Figure 5). Pupae incubated at 32 °C undergo metamorphosis to visible perfect adults, with fully-formed wings, those incubated at 22 °C or at room temperature grow to be mostly malformed, in the majority of cases with undeveloped (or wet) wings.

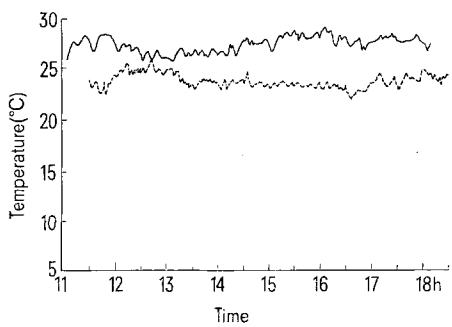

Fig. 5. Temperature recording (top plot) from a strip of filter paper heated by *Dolichovespula media* wasps, as compared to the surrounding temperature (bottom plot). The filter paper was impregnated with alcoholic extract of dark pupae of the same species.

Discussion. The results suggest that the volatile emanation of the pupae and not their color, shape or size, are responsible for the adults response and that the abdominal pumping movements of imagines near their pupae are triggered by pupal pheromone(s). The different intensity and duration of heating elicit by pupae proper, or their alcoholic extract, would seem to suggest a feedback mechanism, the pupa continuously stimulating the adult wasp to ventilatory movements. There can be little doubt that under the ecological conditions in which wasps develop, the active warming by the imagines of the stationary pupae contributes significantly to their successful maturation. However, I have not yet established whether the pupae of each species of wasps has its own pheronome to induce thermoregulatory behaviour, or whether the pupae of different species possess identical pheromone(s) which disappear(s) in the adult stage. The mechanism triggering thermoregulatory behaviour of adult wasps in clusters awaits also further elucidation[7].

Zusammenfassung. Beobachtung, dass Wespen und Hornissen auch nach deren Entnahme aus den Waben ihre Puppen erwärmen, da sie sich sonst zu verkrüppelten Imagines entwickeln. Die von den Puppen ausgeschiedenen volatilen Substanzen (Pheromone) fördern das Brüten der Arbeitswespen, was beweist, dass es sich um ein thermoregulatorisches Pheromon handelt.

J. Ishay

Department of Physiology and Pharmacology, Medical School, Tel Aviv University, Beilinson Hospital, P.O. Box 85, Petach-Tikva (Israel), 17 April 1972.

[7] This research has been done in the Institut für Bienenkunde, Oberursel/Ts., Department of Zoology, Frankfurt University, and supported by a grant from the Deutscher Akademischer Austauschdienst.

Suppression of Pup Retrieving Behavior in Rats Following Administration of L-Δ^9-Tetrahydrocannabinol

The behavioral effects of marihuana and its derivatives are currently the subject of intense experimental investigation. The present study extends these efforts to an examination of the effects of L-Δ^9-tetrahydrocannabinol(Δ^9-THC), the principal active component in marihuana, on maternal behavior in the rat.

Materials and methods. The subjects were 9 adult female rats, individually housed in cages 41 cm × 17.5 cm × 24 cm. The females arrived pregnant in the laboratory one week prior to parturition. After parturition, each litter was culled to 5 pups per dam.

Test conditions were similar to those described by Whalen[1]. Shortly before birth, shredded pieces of paper were placed in the home cages for the females to make nests with. Subjects were tested on days 7 and 10 after parturition. On day 7, the females were removed from their cages into which two wooden partitions were then inserted, thus dividing the cages into three equal sized sections. Each partition contained an opening of 10 cm through which the female could easily pass. In addition, each partition contained several holes of 2 cm in diameter. Immediately after i.m. injection of either 10.0 or 20.0 mg/kg Δ^9-THC in Triton X-100, or Triton X-100 only, in case of control animals, the females were returned to their litters. 2 h later, the females were again taken from their cages. During the following min, the pups were removed from the nest and were placed in the far end of the cage. The females were then returned to the area of the nest and were kept there for 1 min by blocking the opening in the partition. The obstruction was then removed and observations were made as to the latency for the female to enter the area into which the pups had been placed as well as the time taken to return all 5 pups to the nest. A limit of 5 min was imposed on the test period. On day 10, these treatments and observations were repeated with the exception that females previously receiving drug, now received placebo injections while previous placebo subjects now received 10.0 mg/kg Δ^9-THC. During the experiment, if a female failed to retrieve any pups during the test period, it was retested for an additional 5 min a few min later. If females retrieved during this second test session, the previous results for that animal were disregarded. Differences between groups were evaluated using the Kruskal-Wallis 'H' and the Mann-Whitney 'U' statistics[2].

[1] R. E. Whalen, in *Animal Behavior in Laboratory and Field* (Ed. A. W. Stokes; Freeman and Co., San Francisco 1968).

[2] S. Siegel, *Nonparametric Statistics* (McGraw Hill, New York 1956).

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 Δ^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 Δ^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-Δ^9-tetrahydrocannabinol. A des doses de 20 mg/kg il s'est complètement arrêté.

E. L. ABEL[4]

Department of Psychology, University of Toronto, Toronto (Canada), 4 April 1972.

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

[4] Present address: Dept. of Pharmacology, Swing Bldg., Univ. of North Carolina, Chapel Hill (North Carolina 27514, USA).

Tolerance to the Behavioral and Hypothermic Effects of 1-Δ^9-Tetrahydrocannabinol in Neonatal Chicks

The development of tolerance to the behavioral effects of marihuana and to its principle active ingredient, **1**-Δ^9-tetrahydrocannabinol (Δ^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 Δ^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 Δ^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 Δ^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 Δ^9-THC on photocell activity are presented in Table I. Δ^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, Δ^9-THC completely suppressed activity in subjects previously receiving placebo, but had no marked effect in subjects receiving prior injections of Δ^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 Δ^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 Δ^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

[1] D. E. MCMILLAN, W. L. DEWEY and L. S. HARRIS, Ann. N. Y. Acad. Sci. *191*, 83 (1971).

[2] J. M. FRANKENHEIM, D. E. MCMILLAN and L. S. HARRIS, J. Pharmac. exp. Ther. *178*, 241 (1971).

[3] D. E. MCMILLAN, L. S. HARRIS, J. M. FRANKENHEIM and J. S. KENNEDY, Science *169*, 501 (1970).

[4] S. SIEGEL, *Nonparametric Statistics* (McGraw-Hill, New York 1956).

[5] N. B. EDDY and LEIMBACH, J. Pharmac. exp. Ther. *107*, 385 (1953).

Table I. Tolerance to the effects of 10.0 mg/kg Δ^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 Δ^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] $p < 0.001$.