Table III. Effect of phenylbutazone and AHR-1911 on tissue hydroxyproline content during wound healing of the rat

Treatment	Tissue hydroxyproline content (μg/mg of dried tissue)[a]		
	skin	6th day	12th day
Control	4.30 ± 0.32	3.21 ± 0.18[b] (−25)	5.69 ± 0.21[b] (+32)
Phenylbutazone, 50 mg/kg	4.58 ± 0.20 (+6)	4.25 ± 0.32[c] (+32)	3.78 ± 0.23[b] (−34)
AHR-1911, 50 mg/kg	3.94 ± 0.14 (−8)	4.21 ± 0.12[b] (+31)	4.80 ± 0.12[d] (−16)

[a] Mean ± S.E. for 6 to 8 experiments. Values for treated groups are different from controls as follows, [b], $P < 0.001$; [c], $P < 0.01$ and [d], $P < 0.05$. Rest values are not significantly different, $P > 0.1$. Percent change of controls in parenthesis.

Results and discussion. Table I shows the effect of phenylbutazone, dexamethasone and AHR-1911, at equivalent anti-inflammatory doses, on the rate of wound healing in the rat. Phenylbutazone was the most potent drug in depressing the tensile strength of the wound (36–39%), whereas AHR-1911 was the least potent agent (18–19%). Dexamethasone was intermediate. Lower anti-inflammatory doses of AHR-1911 did not modify the rate of wound healing. The retardation potency on wound healing was as follows: phenylbutazone > dexamethasone > AHR-1911. This depression of the rate of wound healing by AHR-1911 seems to be due to a direct influence on skin and not through adrenal glands stimulation (Table II).

Table III shows that the hydroxyproline content in control rats decreased on 6th day of healing (−25%), while it increased on 12th day of healing (+ 32%). These findings are in agreement with those found by SORENSEN[13]. AHR-1911 as well as phenyl-butazone increased the hydroxyproline content on 6th day of healing to the levels of control rats, but they decreased it on 12th day. Phenylbutazone was twice as potent as AHR-1911 in lowering the levels of hydroxyproline of the wound.

Anti-inflammatory agents have been shown to stabilize the membrane of lysosomes[14] and to prevent the enzymes release from lysosomes during the inflammation phase of wound healing[15]. As a result of this membrane stabilization of lysosomes by anti-inflammatory agents, the hydroxyproline content of the wound increases relatively to levels of unwounded skin. Absolute values of hydroxyproline were decreased on 12th day of healing by both anti-inflammatory drugs (Table III); nevertheless, AHR-1911 had less effect than phenylbutazone on hydroxyproline levels of the wound.

Since hydroxyproline is a specific amino-acid of collagen[16], we assume that the collagen content of the wound is less modified by AHR-1911, and thus the rate of wound healing. These findings might be of importance in therapeutics.

Resumen. Varias drogas anti-inflamatorias incluyendo una droga antiinflamatoria no esteroidea nueva fueron investigadas en heridas producidas en ratas. AHR-1911 resultó ser la droga menos depresora de la cicatrización de las heridas cuando se comparó con la fenilbutazona y la dexametasona. Un menor contenido de colágeno en las heridas fue observado con el uso de AHR-1911.

M. VELASCO[17] and E. GUAITERO[18,19]

Càtedra de Farmacologîa, Escuela de Medicina J.M. Vargas, Universidad Central de Venezuela, Caracas (Venezuela), 16 April 1973.

[13] B. M. SORENSEN, in *Hormones and Connective Tissue* (Ed. G. ASBOE-HANSEN; Williams and Wilkins, Baltimore 1966), p. 106.
[14] G. WEISSMAN, Biochem. Pharmac. *14*, 525 (1965).
[15] J. C. HOUCK, J. JOHNSTON and R. A. JACOB, Biochem. Pharmac. *17*, Suppl., 69 (1968).
[16] E. W. DEMPSEY and A. I. LANSING, in *International Review of Cytology* (Ed. G. H. BOURNE and J. F. DANIELLI; Academic Press, Inc., New York 1954) vol. 3, p. 437.
[17] Present address: Emory University School of Medicine, Atlanta, Ga. USA.
[18] This study was in part presented at IV Latin-american Congress on Pharmacology and Therapeutics, Caracas, Venezuela, July 9–14, 1972.
[19] AHR-1911 was kindly supplied by Professor N. ERCOLI. Dexamethasone and phenylbutazone were supplied by Merck Sharp and Dohme and Geigy Laboratories, respectively, Caracas, Venezuela.

Open-Field Behavior and Acquisition of Discriminative Response Control in Δ^9-THC Tolerant Rats[1]

If complete tolerance to a drug occurs, no discriminative control based on the presence or absence of that drug in the CNS would seem possible. BUENO and CARLINI[2] have shown that discriminative responding is possible when using a cannabis extract, even after tolerance had been established. Discriminative training started when the rats negotiated a vertical rope as fast as did the controls (tolerance). Our working hypothesis was that if some tolerance occurred, then the cueing effects of tetra-hydrocannabinol (THC) should be weakened and discriminative response control should therefore develop more slowly in tolerant animals as compared to non-tolerant. The present study was undertaken to investigate this, and in addition open-field behavior before and after the presumed development of tolerance was studied.

Materials and methods. 18 male albino Sprague-Dawley rats (300–325 g), from a commercial breeder (Anticimex AB, Sollentuna, Sweden), were used. They

[1] Numbering system according to IUPAC rules. The drug was generously supplied by Dr. T. PETRZILKA, University of Zürich.
[2] O. F. A. BUENO and E. A. CARLINI, Psychopharmacologia *25*, 49 (1972).

Table I. Median values and mean deviation (within brackets) of ambulation (A), rearing (R), defecation (D), urination (U), latency (L), grooming (G), and circling (C) for rats treated with Δ^9-THC, 5 mg/kg (group 1), and controls (group 2) for two open field (O-F) sessions

Group	O-F nr.	O-F measures A	R	D	U	L	G	C
1	1	4.0 (7.0) [a]	0.0 (0.3) [a]	0.0 (0.0)	0.0 (4.3)	59.5 (64.2) [d]	0.0 (0.0) [d]	13.5 (5.2) [a]
(Δ^9-THC, 5 mg/kg)	2	13.5 (6.6) [d]	1.0 (2.9) [d]	1.0 (0.8) [c]	0.0 (4.3)	7.4 (8.3) [b]	0.0 (0.0)	4.5 (2.5) [a]
2	1	43.0 (8.3)	13.5 (5.8)	0.0 (0.3)	0.0 (4.3)	8.6 (7.7)	1.0 (1.1)	0.0 (0.0)
(Vehicle, 1 ml/kg)	2	24.0 (14.1)	5.5 (2.2)	0.0 (0.3)	0.0 (0.0)	1.0 (0.5)	0.0 (0.3)	0.0 (0.0)

[a] $p < 0.001$; [b] $p < 0.01$; [c] $p < 0.025$; [d] $p < 0.05$ (group 1 significantly different from group 2; Mann-Whitney U-test, one-tailed). The interval between the two O-F testings was 18 days. The behavioral categories are defined in the text.

were individually housed and maintained ad libitum on food and water. The animals were randomly divided into 3 groups with 6 rats in each. Group 1 was given an i.p. injection (1 ml/kg) of Δ^9-THC (5 mg/kg), suspended in saline plus Tween-80 (1%) and propylene glycol, 30 min prior to the first open-field (O–F) test. Group 2 received the vehicle (1 ml/kg) and was then subjected to the same test as group 1. Group 3 was included to find out if our conventional procedure[3] for establishing response control would yield different results than those obtained for groups 1 and 2. This group therefore was subjected to discriminative tranining only (see below).

The O–F was an acrylate box (60 × 60 × 26 cm) with an open top, white walls and a brown floor, divided into 16 squares (15 × 15 cm) and 1 additional square (15 × 15 cm) was marked in the centre of the field. The O–F was illuminated by a frosted 60 W bulb, positioned 150 cm above the centre of the field. One rat at the time was placed in the centre square and was allowed to explore the field for 4 min. Records were kept of the following measures: ambulation (A): the number of squares crossed with all 4 feet; rearing (R): the number of times the rat stood on its hind feet; defecation (D): the number of fecal boli deposited; urination (U): the number of times the animals urinated in the O–F box; latency (L): time in sec to leave the start square upon placement in the O–F; grooming (G): the number of cleaning bouts, including washing of the face and trimming of the fur; circling (C): the number of times the animal turned around its vertical axis, 1 point given for each complete 360 degree turn.

After the first O–F test, group 1 received daily oral administrations of Δ^9-THC, 5 mg/kg for 18 days. Group 2 received the vehicle only. Hereafter these 2 groups were again O–F tested, the procedure being the same as described above.

The animals were then subjected to discriminative training in a T-shaped water maze, and in this stage group 3 was included. The drug, Δ^9-THC, 5 mg/kg, constituted the first state for half the number of *Ss* in each group and the others received the vehicle. The injections were given 30 min prior to the sessions. The imposed states, drugged and non-drugged, which were changed from day to day, were the only cues from which arm escape from the water was possible. The *Ss* were trained 10 trials per day for 5 days a week. A response was recorded when the animal had left the choice area, the 15 cm^2 junction of the 3 arms, with its whole body excluding the tail. A self-correcting procedure was used. The water in the maze had a temperature of 20 °C.

Results and discussion. From Table I it can be seen that all behavioral categories, except urination, differentiated group 1 and 2 in the O–F test. A comparison between the groups shows that Δ^9-THC depressed ambulation, rearing, and washing. Much longer latencies were found in the drugged rats. Defecation, a presumed index of emotionality, was only seen in drugged *Ss* at the second O–F test. This is in line with previous findings[4,5], and in fact the former research group[4] has suggested that the stressful component of THC is not evident until tolerance is disclosed. Circling was only seen in group 1. This was also reported by SJÖDÉN et al.[5].

A Wilcoxin sign test[6] did not reveal significant changes ($p > 0.05$) for the two O–F sessions. Nevertheless, an inspection of the results from the second O–F test suggests that the drugged *Ss* ambulated more, showed more rearing and less circling than at the first O–F test. The latency scores were significantly reduced ($p < 0.05$, t-test).

The mean number of correct first-trial responses during the 12 discriminative training sessions are shown in Table II. The development of differential responding was faster for group 2 than for group 1 ($t = 5.13$; $df = 10$; $p < 0.001$). The 2 non-tolerant groups (2 and 3) did not differ statistically ($p > 0.05$). These results are consistent with the hypothesis that tolerance to THC would render the *Ss* less sensitive to the cueing effects of the drug. If our conclusion is correct, it seems that the cueing effects of THC should appear in a dose-related manner, and recent work in our laboratory confirms such a notion when employing THC doses of 5 mg/kg and less. Furthermore, THC doses, lower than the one used for establishing differential responding, yield a progressive decline in the number of drug responses[7]. These data add additional support to our conclusion.

Table II. Mean number and ± SD of correct first-trial responses during 12 discriminative training sessions in a T-shaped water maze for 3 groups of rats

Group	1	2	3
($n = 6$)	6.70 ± 1.05 [a]	8.70 ± 1.05	8.30 ± 1.05

[a] $p < 0.001$ (group 1 differs significantly from group 2; t-test).

[3] B. G. HENRIKSSON and T. JÄRBE, Psychon. Sci. *27*, 25 (1972).
[4] J. MASUR, R. M. W. MÄRTZ and E. A. CARLINI, Psychopharmacologia *19*, 388 (1971).
[5] P.-O. SJÖDÉN, T. U. C. JÄRBE and B. G. HENRIKSSON, Pharmac. Biochem. Behav., in press (1973).
[6] S. SIEGEL, *Non-Parametric Statistics for the Behavioral Sciences* (McGraw-Hill, New-York, Toronto, London 1956) p. 75.
[7] R. K. KUBENA and H. BARRY, III, Nature, Lond. *235*, 397 (1972).

Parenthetically it seems that the present method could become a suitable tool for studying tolerance development, since it is not open to criticism based on state-dependency which many other procedures are[8,9].

[8] The help from Drs. I. Dureman, J. O. Johansson, G. Krook and Mr. G. Ohlin and G. Ågren is acknowledged.
[9] This research was supported by the Swedish Council for Social Science Research No. 181/72 P.

Résumé. La tolérance de l'effet du 1-Δ^9-tétrahydrocannabinol (Δ^9-THC) a été examinée par une méthode nouvelle. Les résultats obtenus permettent une interprétation de tolérance pharmacologique pour Δ^9-THC.

T. U. C. Järbe and B. G. Henriksson

University of Uppsala, Department of Psychology, Slottsgränd 3, S-752 20 Uppsala (Sweden),
29 March 1973.

Reduced Fertility in Male Mice Following Treatment with Niridazole

Niridazole is toxic to the gonads of schistosomes; low doses inhibit spermatogenesis and higher doses induce testicular necrosis[1]. Reversible inhibition of spermatogenesis has also been induced in rats and mice following oral administration of niridazole[2]. More recently, niridazole was shown to induce temporary sterility in male mice, but no preimplantation losses or early fetal deaths in the dominant lethal assay[3]. We report here quantitative data on fertilization and early embryonic development in mice following paternal treatment with niridazole and subsequent mating over the duration of the spermatogenic cycle.

Random-bred ICR-Ha Swiss male mice, 8–10 weeks old, were given a single i.p. injection of 700 mg/kg (LD_{25}) of niridazole 1-(5-nitro-2-thiazolyl)-2-imidazolidinone (Ambilhar®, Ciba Pharmaceuticals), in fine suspension in 0.1 ml of tricaprylin; controls were concurrently injected with solvent alone. Each test and control male was then individually caged for 1 week with 3 untreated virgin 8–10-week-old females. Females were replaced weekly and consecutively with fresh animals for a total of 8 weeks. 2 replicate tests were performed with groups of 21 and 33 males; groups of 10 and 20 males, respectively, served as concurrent controls. Females were examined daily for vaginal plugs and ova from all females with vaginal plugs were harvested at approximately 10, 30, 60, and 80 h[2] post-ovulation. Ova were stained, examined by phase-contrast microscopy, and evaluated as described previously[4,5].

The two replicate test groups yielded a total of 582 mated females out of 1,296 exposed (45%), as determined by the presence of vaginal plugs; correspondingly, 324 females mated out of 720 exposed (45%) in the controls. Thus, there was no difference in the frequency of mating between the test and control males. The total ova harvested were 5,449 from 582 females (mean 9.36) in the test group and 3,310 from 324 females (mean 10.22) in the controls. This discrepancy was attributed to the difficulty of detecting unfertilized ova as the embryonic development progresses, and was particularly noticeable in the 3rd and 4th mating week when infertility was high (Table). The following data are based on all ova harvested from all females.

Individual ova: The incidence of unfertilized ova in the test group was highest in the third and fourth week after niridazole treatment of the males; fertility returned to control levels by week 7 (Figure 1A). At 10 h post-ovulation, the overall percentage of unfertilized ova in controls was 21%; contrastingly, the mean incidence of unfertilized ova in test groups in weeks 3 and 4 was 88% and 100%, respectively.

In the fertilized ova no differences were detected between rates of cleavage and no malformations in zygotes harvested for test and control groups. At 10 h post-ovulation, pronuclei were present in 67% and 64% of the ova in test and control groups, respectively; the incidence of polyspermy was less than 1% in both groups. By 30 h

Incidence of females with vaginal plugs and number of ova harvested subsequent to mating with niridazole-treated male mice

Week	Group		Time post-ovulation (h)				
			10	30	60	82	Total
1	Test	A	11	13	15	5	44
		B	112	126	152	58	448
		C	10.2	9.7	10.1	11.6	10.1
	Control	A	11	6	9	6	32
		B	103	64	84	62	313
		C	9.4	10.7	9.3	10.3	9.7
2	Test	A	4	18	12	20	54
		B	44	179	133	203	559
		C	11.0	9.9	11.1	10.2	10.3
	Control	A	4	11	9	16	40
		B	38	134	102	151	425
		C	9.5	12.2	11.3	9.4	10.5
3	Test	A	15	20	17	21	73
		B	141	191	129	177	638
		C	9.4	9.6	7.6	8.4	8.7
	Control	A	11	10	12	12	45
		B	118	117	132	126	493
		C	10.7	11.7	11.0	10.5	10.9
4	Test	A	19	40	13	24	96
		B	208	385	116	234	943
		C	11.0	9.6	9.0	9.8	9.9
	Control	A	14	18	10	7	49
		B	154	191	87	77	509
		C	11.0	10.6	8.7	11.0	10.4

A, No. of females with vaginal plugs; B, No. of ova harvested; C, Average ova per female.

[1] H. P. Striebel and F. Kradolfer, Acta trop. Suppl. *9*, 54 (1966).
[2] C. R. Lambert, V. S. P. Sinari and J. Tripod, Acta trop. *22*, 155 (1965).
[3] S. S. Epstein, E. Arnold, J. Andrea, W. Bass and Y. Bishop, J. Toxic. appl. Pharmac. *23*, 288 (1972).
[4] S. S. Epstein, S. R. Joshi, E. Arnold, E. C. Page and Y. Bishop, Nature *225*, 1260 (1970).
[5] S. R. Joshi, E. C. Page, E. Arnold, Y. Bishop and S. S. Epstein, Genetics *65*, 483 (1970).