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).

post-ovulation, the majority of fertilized ova in both groups had undergone a single cleavage and no micronuclei were detected in any ova. At 60-h post-ovulation, 87% and 91% of the fertilized ova in the test and control groups, respectively, were in the 5–8 cell stage; at 82 h, all fertilized in the test and control groups were in the morula-blastula stage. About half of the unfertilized ova were fragmented by 60 h post-ovulation.

Individual females: The incidence of mated females with minimally 1 fertilized ova are presented on a weekly basis for all harvesting stages combined (Figure 1B). The percentage of matings were similar for both test and control

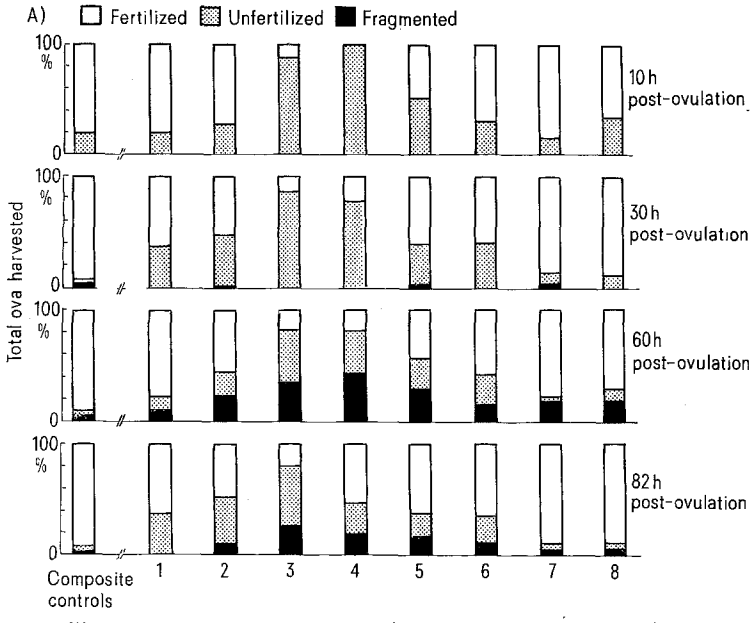
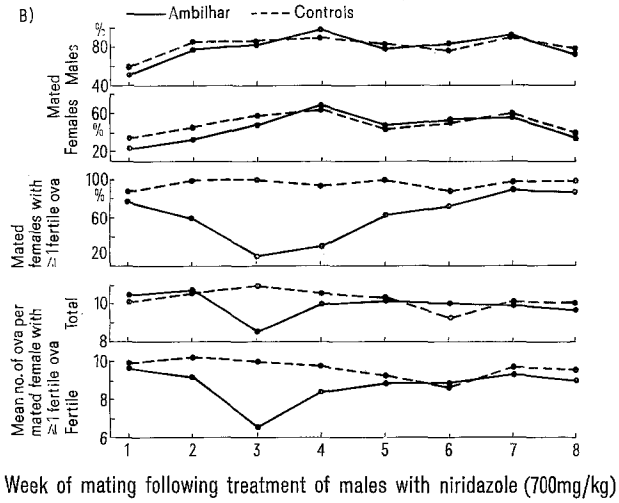


Fig. 1. Incidence of fertilized ova in females (A), and number of ova in females with one or more fertile ova (B) following mating with control and niridazole (Ambilhar)-treated male mice.



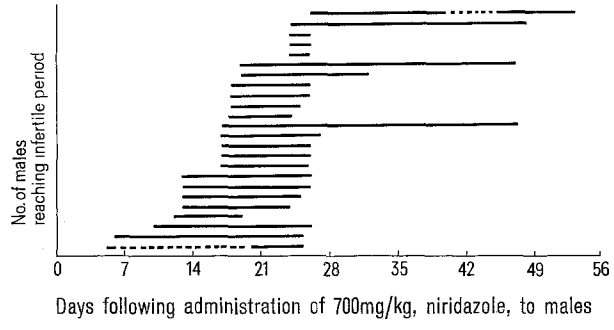


Fig. 2. Consecutive infertility of individual males following niridazole (Ambilhar) treatment. Data arranged in order of day of onset of inertility. ——, infertile period; – – – –, intervening fertile period.

males and females (Figure 1B). However, the incidence of fertile matings, defined as females with vaginal plugs and at least one fertilized ovum, were below control levels at all times and reached the lowest value in the 3rd week; the incidence of fertile matings in test groups was 80% less than in controls. Fertile females in the test group had a reduced number of total ova, both fertile and infertile, in the 3rd week. However, considering only fertilized ova per mating, this reduction below control levels was unequivocal in the 3rd week and persisted until the 5th week. In the 3rd week, the number of fertilized ova in test and control group were 6.6 and 10.0 per female, respectively. Thus, there was a 34% reduction in the number of fertilized ova in the test groups. This effect is less than the 80% difference between test and control groups in the incidence of mated females with at least 1 fertilized ova; the greater effects would have been missed if the vaginal plugs had not been checked.

Individual males: Data for 33 test and 20 control individual males in 1 replicate were analyzed. The fertility of the males did not seem to be directly related to the total number of matings; the overall percentage of fertile matings in the test and controls was 66.7% and 95.3%, respectively. Some treated males had periods of fertile matings followed by periods of no mating or only infertile matings, with subsequent return to fertile mating. Thus, it was possible to determine the periods of infertility induced by niridazole (Figure 2); these periods may be underestimates, as consecutive periods of failure to mate were excluded. In most males, the period of infertility was continuous. In the treated group, 2 out 33 were consistently fertile, and 6 showed no consistent periods of infertility. Of the 24 males with infertile periods, 22 were infertile between day 21 and 25, thus confirming observations in individual females (vide supra) that these effects were maximal on the 25th day following the treatment. In all of these 24 males, infertility commenced between days 17 and 19; mean and S.D. were 17.4 ± 4.9. The mean and S.D. of the duration of the infertile period in these males, including the 2 males which remained fertile, was 11.1 ± 7.87 days; median 10 days. Infertile matings in controls were few and scattered with no consecutive periods of infertility.

The only manifest effects of treatment of male mice was reduction in their fertilization rates, based on scoring of non-penetrated ova in females with vaginal plugs. This was evident from calculations based on individual ova, individual females with vaginal plugs, or individual males. Fertility began to decline on the 12th day following drug treatment, decreased progressively till the 25th day, and then gradually recovered until it was normal by the 39th day; 22 out of 24 males were infertile between days 21 and 25.

The spermatogenic cycle in the mouse, from spermatogonia to sperms in ejaculate, takes 42 days and is comprised of: spermatogonial mitosis, 6 days; spermatocytes, 14 days; spermatids, 9 days; testicular sperms, 5.5 days; epididymal sperms, 7.5 days. Meiosis occurs at the end of 3rd week[6]. Thus, niridazole primarily affects spermatids and meiotic division of spermatocytes, resulting in reduced fertility. These results are consistent with the findings in the dominant lethal assay in mice, where reduced incidence of pregnancies and the number of total implants were observed in females mated with males which had been treated 3 to 4 weeks previously with niridazole[3]. Infertility induced by niridazole is in marked contrast with the biphasic infertility, both pre-meiotic and post-meiotic, induced by mutagenic alkylating agents, such as TEPA. Infertility induced by TEPA, as evidenced by pre-implantation losses, is due to the effects on both pre- and post-meiotic stages of spermatogenesis. The mechanism involved, however, is different for those two stages. With post-meiotic drug-exposed sperms, fertilization is normal but subsequent development of the zygote is retarded and malformed embryos fail to implant. Contrastingly, with pre-meiotic effects infertility is characterized by failure of fertilization possibly due to aspermia[5,7,8].

Data reported here indicate that sterility induced by niridazole is due to inhibition of fertilization and not due to the induction of dominant lethal mutagenic effects. Reduction in total number of implants in the dominant lethal assay cannot then be necessarily equated with pre-implantation losses and mutagenic effects[3,5].

Zusammenfassung. Nach einmaliger i.p.-Injektion von 700 mg/kg Niridazol wurden männliche Mäuse wiederholt mit unbehandelten Weibchen wöchentlich über 2 Monate hinweg gepaart und die Ova gesammelt. Es konnte eine starke Herabsetzung der Befruchtungsraten 3–4 Wochen nach Paarung festgestellt werden, was die Annahme einer Wirkung auf die meiotische Teilung der Spermatozyten und Spermatiden nahelegt. Nach der 7. Woche trat eine Normalisierung der Fruchtbarkeitsrate ein.

S. R. Joshi, Y. Bishop and S. S. Epstein[9,10]

National Cancer Institute, National Insitutes of Health, Bethesda, Maryland; Children's Cancer Research Foundation, Boston, Massachusetts; and Case Western Reserve University Medical School, Dept. of Pharmacology, Cleveland, (Ohio, 44106, USA), 9 April 1973.

[6] A. J. Bateman, Heredity *12*, 213 (1958).
[7] S. S. Epstein, W. Bass, F. Arnold, Y. Bishop, S. R. Joshi and I. D. Adler, Toxic. app. Pharmac. *19*, 134 (1971).
[8] S. S. Epstein, E. Arnold, K. Steinberg, D. Mackintosh and H. Shafner, Toxic. appl. Pharmac. *17*, 23 (1970).
[9] This work was supported by NIH Grants No. C-6516 and No. FR 05526 and by the Environmental Protection Agency Contract No. CPA-70-17.
[10] Reprint requests to Dr. S. S. Epstein, Director Environmental Health Programs, Case Western Reserve University Medical School, Cleveland, Ohio 44106, USA.

β-Sympatholytics as Non-Specific Inhibitors of Serum Cholin-Esterase

The therapeutic use of β-sympatholytics is not limited to their specific antiadrenergic effects. Also their non-specific actions have aroused clinical interest. It is therefore desirable to gather more information on the non-specific effects of this group of drugs. It was the aim of this work to investigate the inhibiting effects and the type of inhibition of 11-β-sympatholytics on the serum cholinesterase as a model of an isolated enzyme, and the correlation between the inhibitory potency of the drugs and their hydrophobic properties.

Materials and methods. The octanol-buffer partition coefficients (P) of the β-sympatholytics were determined