

# Induction of reversible infertility in male rats by oral ornidazole and its effects on sperm motility and epididymal secretions

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Ornidazole (400 mg kg<sup>-1</sup> day<sup>-1</sup>) given by oral gavage rendered male rats infertile by 6.6 ± 0.7 days (mean ± SEM, n = 9, range 3–10) after beginning the treatment and fertility returned within 5–10 days after treatment with ornidazole for 6–7 days. At 200 mg ornidazole kg<sup>-1</sup> day<sup>-1</sup>, fertility was reduced but total infertility was not achieved. No differences were found in the percentage motility of spermatozoa recovered from any region of the epididymides of ornidazole-treated rats compared with controls. However, computer aided sperm analysis revealed significantly lower straight-line and average path velocities in ornidazole-treated animals (400 mg kg<sup>-1</sup> day<sup>-1</sup>) for spermatozoa from the distal regions of the tract than for controls. Curvilinear velocity was significantly lower than that of controls in the distal corpus and cauda regions. The motility characteristics of spermatozoa from animals receiving 200 mg ornidazole kg<sup>-1</sup> day<sup>-1</sup> were lower than, but not significantly different from, motility in controls. There were no differences between the total protein, L-carnitine, glycerophosphocholine or total α-glucosidase content in epididymal homogenates from fertile control and infertile ornidazole-treated animals. Spermatozoa released from the cauda epididymidis of untreated rats into ornidazole solutions displayed no changes in the percentage motility up to 20 mmol l<sup>-1</sup> and were only depressed at 50 mmol l<sup>-1</sup>. All velocities revealed a biphasic response with an initial increase in motility and then inhibition at higher concentrations, but a significant difference from velocities in the absence of ornidazole was evident only for straight line velocity (VSL) at 50 mmol l<sup>-1</sup>. The rapidity of action in inducing infertility is compatible with post-testicular action and an action on the epididymis is suggested by the decline in motility parameters of luminal spermatozoa. The lack of effect on epididymal secretions *in vivo* and on sperm motility *in vitro*, except at very high doses, suggests that there may be a direct action of an ornidazole metabolite on epididymal spermatozoa.

## Introduction

In the search for a reversible antifertility agent that is effective in men, the testicular endocrine approach is most complex but nearest to its goal (Nieschlag *et al.*, 1992), whereas the post-testicular alternative is theoretically simpler but good animal models are not available (Cooper, 1992). Nevertheless, a few compounds are known that act rapidly and reversibly on male fertility (for example chlorohydrins and chlorosugars), most likely through an action on the epididymal spermatozoa. Further knowledge of the action of these drugs in inducing infertility may give clues as to how similar molecules could be designed to achieve similar efficacy with fewer side-effects.

Ornidazole (and the structurally related metronidazole and tinidazole) are effective anti-microbial agents used clinically

for genital tract infections in both men and women. Ornidazole is a 5-nitro-imidazole derivative, which, in contrast to other 5-nitro-imidazoles (Patanelli, 1975), has no effect on the testis at doses that cause infertility (McClain and Downing, 1988b), although extremely high doses do affect spermatogenesis (Linder *et al.*, 1992). In studies to date, infertility appeared in the second week of treatment and McClain and Downing (1988a, b) reported that reversibility was achieved by 6 days.

The aim of this study was (i) to examine more closely under stricter control conditions the onset of infertility in ornidazole-treated animals, (ii) to confirm the rapid reversibility, (iii) to quantify by computer-aided sperm analysis the motility of spermatozoa from all regions of the epididymis, (iv) to measure epididymal secretion products in treated animals in an attempt to pinpoint any epididymal effect and (v) to examine the action of ornidazole on mature spermatozoa *in vitro*. This work forms part of the work submitted for a higher degree by G. Oberländer.

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## Materials and Methods

### Animals and treatments

Male and female rats of the Charles River Sprague Dawley strain (Zentralinstitut für Versuchstierzucht GmbH, Hanover and Charles River Wiga GmbH, Sulzfeld) were kept at about 22°C with a 12 h light:12 h dark cycle (lights on at 07:00 h). They were provided with water and standard rat diet (Altromin GmbH, Lage) *ad libitum*. Surgery was performed in accordance with guidelines of the German code for animal experiments.

### Female rats

Mature female rats weighing 250 g were used. For mating tests, pro-oestrous rats (for those having 4–5 day cycles with 1 day in oestrus) or oestrous rats (for those having 5 day cycles with 2 days in oestrus) were placed in the male's cage overnight.

### Male rats used in mating studies

To standardize mating proficiency and ensure that results were not biased by an improvement in mating competence caused by the serial testing during the course of the study (Schwetz *et al.*, 1980; Zenick and Goeden, 1988), naive mature rats (350–400 g) were allowed to mate four times over a 2–3 week period. For each practice, a receptive female (with ligated oviducts) was introduced into the male's cage (38 cm × 58 cm × 20 cm) in the afternoon and the vaginal smear was examined the next morning for the presence of spermatozoa, which was taken as evidence of successful mating. Only when three practices proved positive was the male recruited into the study.

### Treatment of male rats

Ornidazole (1-[3-chloro-2-hydroxypropyl]-2-methyl-5-nitroimidazole; Sigma Chemie, Deisenhofen) was dispersed in 0.2% carboxymethylcellulose in water (+ two drops Tween 20 per 100 ml suspension) to a concentration of 293 mg ml<sup>-1</sup> (for 400 mg kg<sup>-1</sup> body weight dose) or 147 mg ml<sup>-1</sup> (for 200 mg kg<sup>-1</sup> body weight dose) and homogenized with an Ultra-turrax T 25 (Janke and Kunkel KG, Staufen, Breisgau) at maximum speed for 10 s four to five times in ice-water. The suspension was shaken before administration. The drug was administered by oral gavage at doses of 200 and 400 mg kg<sup>-1</sup> body weight day<sup>-1</sup> in a volume (about 0.6 ml) adjusted according to the body weight obtained immediately before treatment. Control animals received appropriate volumes (0.14 ml per 100 g body weight) of vehicle alone for the same period.

### Mating tests

On scheduled days, one receptive female was introduced to treated or control males. If spermatozoa were seen in the vaginal smears on the following morning, the females were isolated for 11–13 days and then killed by carbon dioxide

asphyxiation and the number of corpora lutea and implantation sites in each uterus was assessed. For each male at each mating, fertility was assessed from the mean number of embryos per corpus luteum (expressed as a percentage calculated for both uterine horns of the female with which he was paired).

### Time course and dose dependence of infertility caused by daily administration of ornidazole

Mating schedules were designed to monitor, as closely as feasible for each rat, the onset and reversal of infertility during the treatment period (with a minimum of 2 days between two consecutive mating tests). After completion of the mating proficiency course, the experiment for each male rat was started when the scheduled mating days would fit the expected cycles of the females. Male rats were assigned at random so that there was always one control rat in the experiment in the same period as other rats in one or more different treatment groups. A pretreatment mating with an intact female was performed on the day before administration of ornidazole (day -1) and ornidazole was administered for 14 days (400 mg kg<sup>-1</sup> day<sup>-1</sup>) or 20 days (200 mg kg<sup>-1</sup> day<sup>-1</sup>). Matings were subsequently scheduled for days 3, 6, 9 and 14 for the 400 mg kg<sup>-1</sup> dose and after days 9, 14, 17 and 20 for the 200 mg kg<sup>-1</sup> dose. Control animals were mated at similar periods.

### Recovery of fertility in animals

A pretreatment mating was performed on the day before ornidazole administration (400 mg kg<sup>-1</sup> day<sup>-1</sup>) (day -1) and ornidazole was fed for 6 days when the male rat mated in the evening of day 6, otherwise for 7 days, after which daily feeding of vehicle alone was continued until day 14. Animals were scheduled to mate on days 3, 6, 9, 12 and 14.

### Epididymal markers

Carnitine, glycerophosphocholine and glucosidase were measured in epididymal homogenates by assays described by Cooper *et al.* (1988) and protein was estimated by a microwell modification of the method using biconchinnic acid (Lane *et al.*, 1986). All markers were measured in one assay for which intra-assay coefficients of variation were 3.1%, 5.5%, 1.2% and 5.3%, respectively.

Approximately 0.6 g tissue from the epididymal cauda was thawed in ice and minced into fine cubes in 0.4 ml ice-cold buffer (Dulbecco's PBS (without bicarbonate) containing Triton X-100 (0.4%, v/v), glycerol (10%, v/v) and phenylmethanesulfonylfluoride (2 mmol l<sup>-1</sup>)) in 1.5 ml tubes. Buffer, 200 µl, was added and stored in ice-water. Tissues were homogenized on ice with an Omni 2000 hand held homogenizer (Süd Laborbedarf GmbH, Gauting) four times at maximum speed (30 000 r.p.m.) for 10 s. Between each homogenization, the tubes were cooled in ice-water for 50 s. Tubes were centrifuged at 20 000 g<sub>max</sub> for 60 min at 8°C (Microcentrifuge 157.MP, Ole Dich Instrument makers, Hvidovre, Denmark) and the

supernatant was cleared by centrifugation through Ultrafree MC Durapore filters (0.65 µm pore size; Millipore, Eschborn) at 2000  $g_{\max}$  and stored at  $-20^{\circ}\text{C}$  before analysis.

#### Motility of spermatozoa recovered from treated rats

Sperm motility was assessed at the end of each treatment, on the day after the last successful mating. Epididymal spermatozoa were collected from five regions of the epididymis of urethane (1.1 g  $\text{kg}^{-1}$  body weight) anaesthetized rats (region 1, mid-caput; 2, proximal corpus; 3, distal corpus; 4, proximal cauda; 5, distal cauda). In each region, a loop of tubule was exposed and cut. The exudated contents were collected onto the tip of a drawn-out glass rod to be dispersed in 100 µl medium. After further dilution of the sperm suspension, movement in 40 µm deep chambers on a heated stage ( $36^{\circ}\text{C}$ ) was recorded using pseudo-dark field optics on videotape and assessed by the Hamilton Thorn computer aided sperm analysis system, as described by Yeung *et al.* (1992). Motility parameters measured included curvilinear velocity (VCL), straight line velocity (VSL), averaged path velocity (VAP), amplitude of lateral displacement (ALH), linearity ( $\text{LIN} = \text{VSL}/\text{VCL} \times 100$ ), straightness ( $\text{STR} = \text{VSL}/\text{VAP} \times 100$ ) and wobble ( $\text{WOB} = \text{VAP}/\text{VCL} \times 100$ ).

#### Haematocrit

Duplicate estimates of the haematocrit were obtained (using Hettich haematocrit centrifuge, Lattingen) from mixed arterial and venous blood from the tip of the tail of rats anaesthetised with urethane.

#### Organ weights

After assessment of sperm motility, the liver, kidneys, seminal vesicles with coagulating glands (emptied of contents), ventral prostate, epididymides and testes were blotted and weighed. Epididymides were frozen at  $-20^{\circ}\text{C}$ .

#### Incubation of rat epididymal spermatozoa with ornidazole in vitro

Several short segments of the dissected tubule from the cauda epididymidis were prepared from rats anaesthetized with urethane by sealing both ends with 8/0 suture thread (Weiss & Sons, London) and incubating in medium H (Yeung *et al.*, 1992) at  $34^{\circ}\text{C}$  in 5%  $\text{CO}_2$ . Spermatozoa were released from these sacs, as described by Yeung *et al.* (1992), into medium H containing ornidazole at various concentrations. Medium H was modified by a reduction of 2.5 to 25 mmol  $\text{NaCl l}^{-1}$  to redress the difference in osmolality. The solutions of 50 mmol  $\text{l}^{-1}$  were solubilized by vigorously mixing for up to 1.5 h at room temperature until the solution was clear (Eppendorf shaker 5432) and then diluted and stored at  $4^{\circ}\text{C}$  overnight. Motility was assessed immediately after sperm release as described by Yeung *et al.* (1992).

#### Statistical analysis

Differences in fertility between control and ornidazole-fed rats at different times were compared by analysis of variance

followed by Duncan's multiple range test. Differences in sperm motility in different regions of the epididymis and with different doses of ornidazole *in vitro*, as well as differences in body and organ weights of different groups, were compared by analysis of variance and Tukey's multiple range test. Correlation between individual fertility and sperm motility was statistically tested by linear regression using the computer software Statgraphics (version 2.6; STSC Inc., Rockville, MD).

## Results

#### General health of the treated animals

The body weights of the rats did not increase during treatment in either the control or ornidazole-treated groups (Table 1). Some ornidazole-treated animals appeared inactive in their cages up to a few hours after feeding but could mate with sexually receptive females. Only one rat was rejected from recruitment to the study for failing to meet the criteria relating to mating proficiency and one rat was killed before the end of the study owing to respiratory difficulties during treatment.

Haematocrits of fertile control animals (mean  $\pm$  SEM  $47.6 \pm 1.6$ ,  $n = 5$ ) and infertile rats receiving ornidazole continuously ( $48.6 \pm 1.2$ ,  $n = 6$ ) were similar.

#### Organ and body weights

There were no major changes in organ weights during treatment with ornidazole (Table 1). The slight but statistically significant decrease in epididymal weight was seen only in the reversibility group in which less ornidazole was given than in the continuous administration group which displayed no such decrease. When expressed in terms of body weight, the decrease in relative epididymal weight was no longer significant, although renal weights expressed per unit body weight were higher in groups treated with 200 and 400 mg ornidazole  $\text{kg}^{-1} \text{day}^{-1}$  compared with the control group.

#### Fertility of male rats

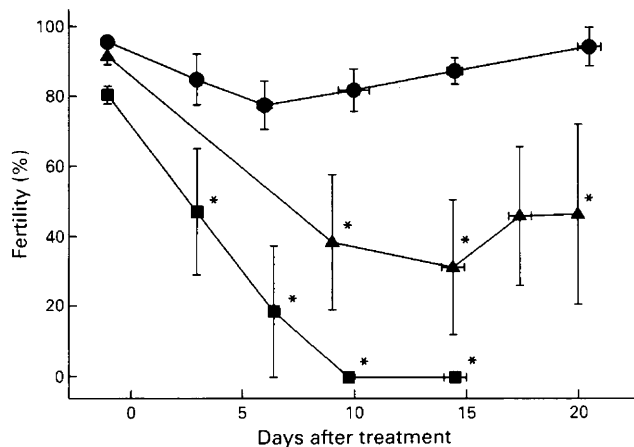
The fertility of the pretreatment matings was not different between the control and ornidazole-treated groups of animals. Despite the mating practice, control animals displayed a temporary slight decrease in their fertility at about day 6 but then recovered to prestudy levels (Fig. 1). Individual fertility never fell below 60% in this group. All females became pregnant in the control group and the number of implantations was high (Table 2). In marked contrast, four of five animals treated with 400 mg ornidazole  $\text{kg}^{-1} \text{day}^{-1}$  showed a decline in fertility after 3 days. Two of the rats produced only one fetus in the females. All five treated rats eventually became infertile: three on day 6, one on day 7 and one on day 10. Females were never pregnant at further matings (Table 2). Significant differences in fertility between these animals and the controls at the same time point and with the pretreatment values were found on days 3, 6, 10 and 14 (Fig. 1).

**Table 1.** Organ weights of ornidazole- and vehicle-treated rats

	Body weight (g)			Organ weight (g)					
	Begin	End	Gain	Testis	Epididymis	SV + CG	Ventral prostate	Kidney	Liver
Control ( <i>n</i> = 6)									
Mean	406.67	395.50	- 11.17	3.67	1.22	0.82	0.48	2.70	13.77
SEM	10.06	26.43	17.75	0.08	0.02	0.09	0.07	0.18	1.19
Ornidazole (200 mg kg <sup>-1</sup> day <sup>-1</sup> for 20 days) ( <i>n</i> = 5)									
Mean	434.00	403.20	- 30.80	3.43	1.10	0.86	0.49	3.11	13.84
SEM	10.65	13.51	14.14	0.10	0.04	0.05	0.06	0.13	0.61
Ornidazole (400 mg kg <sup>-1</sup> day <sup>-1</sup> for 14 days) ( <i>n</i> = 5)									
Mean	424.00	400.40	- 23.60	3.69	1.18	0.77	0.39	3.23	15.12
SEM	8.28	8.53	2.93	0.05	0.05	0.07	0.12	0.12	0.57
Recovery ( <i>n</i> = 5)									
Mean	402.00	380.80	- 21.20	3.49	1.06*	0.84	0.63	2.77	13.78
SEM	10.79	21.29	13.76	0.07	0.03	0.08	0.13	0.13	1.17

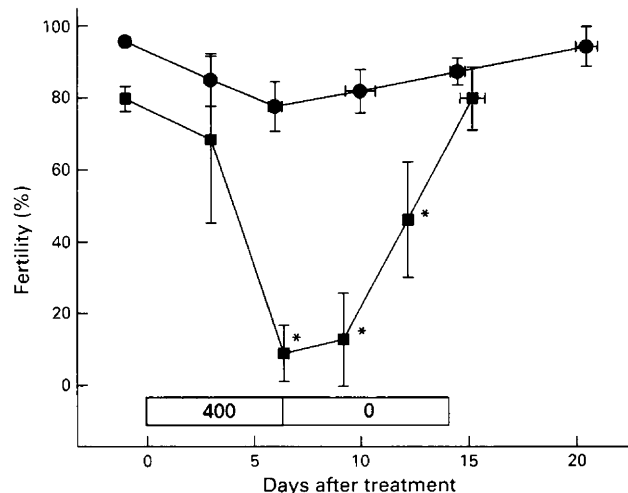
SV + CG: seminal vesicle with coagulating gland.

\*Significantly different from control ( $P < 0.05$ ).



**Fig. 1.** Fertility (expressed as  $100 \times$  number of implants per number of corpora lutea) of male rats (mean  $\pm$  SEM,  $n = 4-6$ ) on various days (mean  $\pm$  SEM) before and after daily administration of vehicle (●) or ornidazole (▲: 200 mg kg<sup>-1</sup> day<sup>-1</sup>; ■: 400 mg kg<sup>-1</sup> day<sup>-1</sup>). \*Significantly different ( $P < 0.05$ ) from the control group at the same time point as well as from their own pretreatment fertility value (see also Table 2).

At a dose of 200 mg kg<sup>-1</sup> day<sup>-1</sup>, fertility was influenced by ornidazole to markedly different extents. Most of the females mated to this group became pregnant, although there was a decline in the number of implantations to about half that in the control group (Table 2). Only three males became totally infertile (two on day 14, one on day 16) and two of these regained some fertility in subsequent mating tests. The other rat, infertile on day 14, was fertile when tested on days 16 and 20. The lowest fertility rates expressed by the other rats during the 21 day treatment were 25% and 71%. Such wide variations gave rise to large standard errors, yet the fertility rates were significantly lower than those of the control group and the pretreatment controls on days 9, 14 and 20 (Fig. 1).



**Fig. 2.** Fertility (expressed as  $100 \times$  number of implants per number of corpora lutea) of male rats (mean  $\pm$  SEM,  $n = 4-6$ ) on various days (mean  $\pm$  SEM) before and during administration of 400 mg ornidazole kg<sup>-1</sup> day<sup>-1</sup> (■) for 6 days (open bar '400') followed by vehicle alone (open bar '0') or vehicle for the entire period (●). \*Significantly different ( $P < 0.05$ ) from the control group at the same time point as well as from their own pretreatment fertility value (see also Table 2).

### Reversibility of infertility

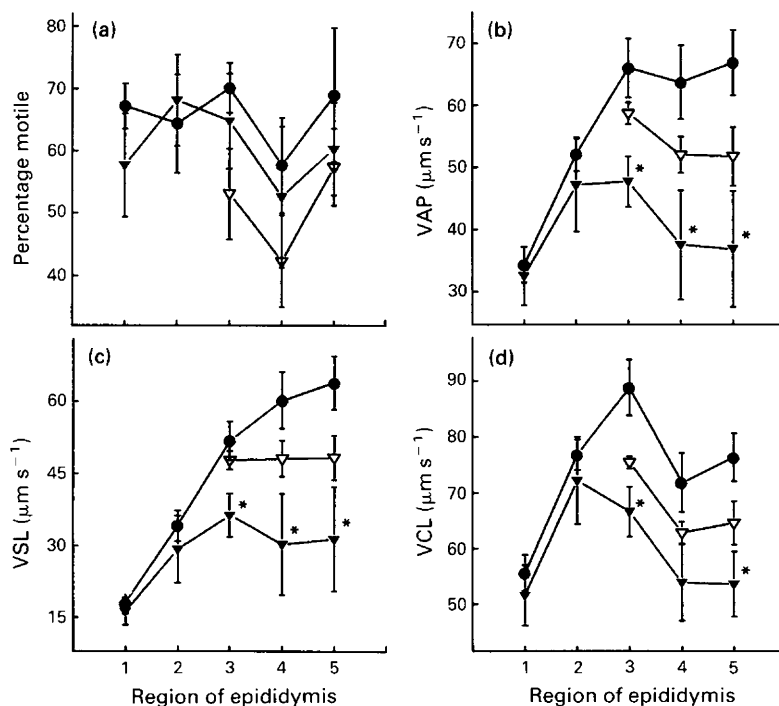
Five males fed ornidazole at 400 mg kg<sup>-1</sup> day<sup>-1</sup> for 6 or 7 days followed by vehicle ingestion for the next 8 days showed an abrupt decline in fertility during the course of treatment followed by a recovery (Fig. 2 and Table 2). Four rats became totally infertile, three during treatment (one rat on day 3 and two rats on day 6) and one on day 10 when the drug was no longer being administered. One rat did not become infertile, but showed a decrease in fertility (lowest 40% on day 7). During the recovery phase when vehicle alone was fed, fertility

Table 2. Reproductive ability of male rats during and after feeding with ornidazole or vehicle

	Sequential mating					
	1	2	3	4	5	6
Control (vehicle) ( <i>n</i> = 6)						
Days after treatment:	-1 ± 0	3 ± 0	6 ± 0.3 (5-7)	10 ± 0.7 (9-12)	14.5 ± 0.3 (14-16)	20.5 ± 0.5 (20-21)
Pregnant/mated (%)	6/6 (100)	4/4 (100)	5/5 (100)	4/4 (100)	6/6 (100)	2/2 (100)
Implantations:	15.3 ± 0.8	13.0 ± 1.2	13.4 ± 0.7	15.0 ± 0.9	14.3 ± 0.8	16.0 ± 0
Ornidazole (200 mg kg <sup>-1</sup> day <sup>-1</sup> ) ( <i>n</i> = 5)						
Days after treatment:	-1 ± 0	9 ± 0	14.4 ± 0.5 (13-16)	17.4 ± 0.5 (16-19)	20 ± 0	
Pregnant/mated (%)	5/5 (100)	4/4 (100)	2/5 (40)	4/5 (80)	3/4 (75)	
Implantations:	15.4 ± 0.9	7.3 ± 3.3**	5.8 ± 3.6**	7.8 ± 3.5*	6.5 ± 3.5**	
Ornidazole (400 mg kg <sup>-1</sup> day <sup>-1</sup> ) ( <i>n</i> = 5)						
Days after treatment:	-1 ± 0	3 ± 0	6.4 ± 0.2 (6-7)	9.8 ± 0.3 (9-10)	14.5 ± 0.5 (14-16)	
Pregnant/mated (%)	5/5 (100)	5/5 (100)	4/5 (80)	0/4 (0)	0/4 (0)	
Implantations:	15 ± 0.4	9.2 ± 3.4*	3.2 ± 3.2**	0 ± 0**	0 ± 0**	
Ornidazole (400 mg kg <sup>-1</sup> day <sup>-1</sup> until after the third mating) ( <i>n</i> = 5)						
Days after treatment:	-1 ± 0	3 ± 0	6.4 ± 0.2 (6-7)	9.2 ± 0.2 (9-10)	12.2 ± 0.2 (12-13)	15.2 ± 0.6 (14-17)
Pregnant/mated (%)	5/5 (100)	3/4 (75)	2/5 (40)	1/5 (20)	4/5 (80)	5/5 (100)
Implantations:	14.6 ± 0.5	10.8 ± 3.6	1.4 ± 1.2**	2.2 ± 2.2**	8.0 ± 2.7**	12.8 ± 1.0

Values are means ± SEM. Range in parentheses.

\*Significantly different from day - 1 of the same treatment group (own control) (*P* < 0.05).\*\*Significantly different from the control group on the same day after treatment (*P* < 0.05).



**Fig. 3.** (a) Percentage motility and (b) averaged path velocity (VAP), (c) straight line velocity (VSL), (d) curvilinear velocity (VCL) (mean  $\pm$  SEM,  $n = 5$ ) of spermatozoa obtained from various regions of the epididymis (region 1: mid-caput; region 2: proximal corpus; region 3: distal corpus; region 4: proximal cauda and region 5: distal cauda) 14 days after daily treatment with vehicle alone ( $\bullet$ ), or ornidazole at 200 mg  $\text{kg}^{-1}$   $\text{day}^{-1}$  ( $\nabla$ ) or 400 mg  $\text{kg}^{-1}$   $\text{day}^{-1}$  ( $\blacktriangledown$ ). \*Significantly different ( $P < 0.05$ ) from the control group at the same region.

returned to all animals, in three animals reaching 100% (on days 12, 14 and 17) and in others reaching 75% and 56% on days 15 and 16, respectively. Significant differences from the control group and pretreatment values were found on days 6, 9 and 12 (Fig. 2).

#### *Motility parameters of spermatozoa recovered from male rats administered ornidazole*

No differences were found in either the percentage motility (Fig. 3) or ALH (Fig. 4) of spermatozoa recovered on the morning after the last experimental mating from any region of the ornidazole-treated epididymides compared with controls. However, significantly lower velocities (both VSL and VAP) were recorded in ornidazole-treated animals (400 mg  $\text{kg}^{-1}$   $\text{day}^{-1}$ ) for spermatozoa from regions 3, 4 and 5. VCL was significantly lower than that of controls in regions 3 and 5 of ornidazole-treated animals (Fig. 3). The derived parameters STR and WOB were significantly lower in spermatozoa from ornidazole-treated rats in regions 4 and 5, but the difference in LIN was statistically significant only in region 4. As a group, the motility characteristics of spermatozoa from animals receiving 200 mg ornidazole  $\text{kg}^{-1}$   $\text{day}^{-1}$  were lower than, but not significantly different from, sperm motility of controls.

Data from individual rats in control and both ornidazole-treated groups, in which some animals were fertile and others

infertile, revealed that there were no significant correlations between any motility parameter and fertility.

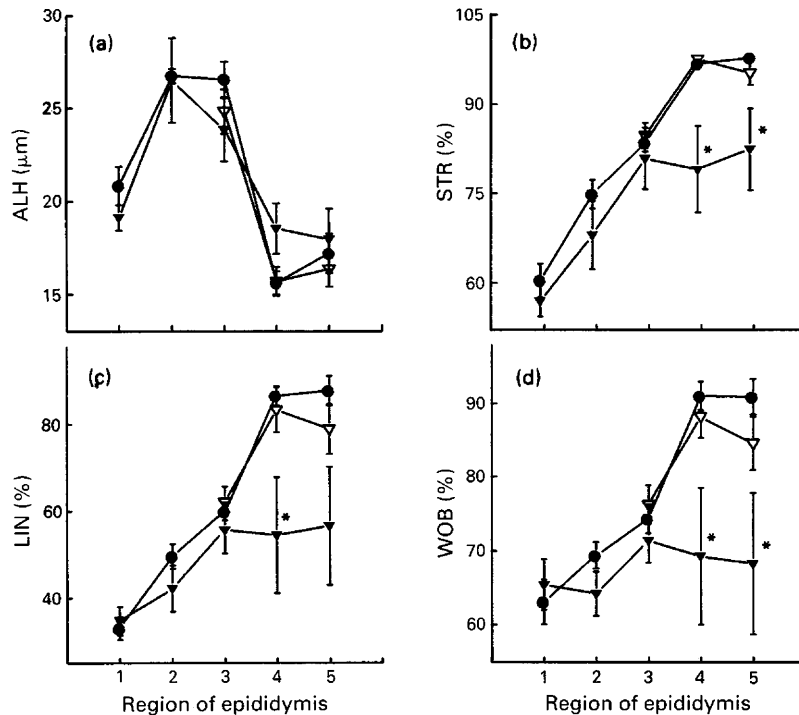
#### *Effect of 400 mg ornidazole $\text{kg}^{-1}$ $\text{day}^{-1}$ for 14 days on epididymal secretions*

There were no differences in the total protein, L-carnitine, glycerophosphocholine or total glucosidase content in epididymal homogenates between fertile control and infertile ornidazole-treated animals (Table 3).

#### *Effect of incubation with ornidazole in vitro on motility of mature spermatozoa*

Upon release of spermatozoa into ornidazole solutions, no changes in ALH were found at any concentration and the percentage of motile cells was depressed only at a concentration of 50 mmol  $\text{l}^{-1}$  (Fig. 5). A dose-dependent decline in STR was noted which was significantly lower than the control at 20 mmol ornidazole  $\text{l}^{-1}$ . All velocities revealed a biphasic response with initial increases followed by inhibition at higher concentrations, but a statistically significant difference from velocities obtained in the absence of drug was evident only for VSL for 50 mmol ornidazole  $\text{l}^{-1}$ .

Spermatozoa were incubated with 10 mmol ornidazole  $\text{l}^{-1}$  for 2 h, but no inhibitory effects on motility were observed (data not shown).



**Fig. 4.** Motility parameters (a) amplitude of lateral displacement (ALH), (b) straightness (STR), (c) linearity (LIN), (d) wobble (WOB) (mean  $\pm$  SEM,  $n = 5$ ) of spermatozoa obtained from various regions of the epididymis (region 1: mid-caput; region 2: proximal corpus; region 3: distal corpus; region 4: proximal cauda and region 5: distal cauda) 14 days after daily treatment with vehicle alone ( $\bullet$ ), or ornidazole at  $200 \text{ mg kg}^{-1} \text{ day}^{-1}$  ( $\nabla$ ) or  $400 \text{ mg kg}^{-1} \text{ day}^{-1}$  ( $\blacktriangledown$ ). \*Significantly different ( $P < 0.05$ ) from the control group at the same region.

**Table 3.** Epididymal secretions in homogenates of the cauda epididymidis from fertile control and infertile ornidazole-treated rats

Secretions	Controls ( $n = 6$ )	Treated ( $n = 5$ )
Protein ( $\text{mg g}^{-1}$ tissue)	$36.77 \pm 0.75$	$34.71 \pm 0.68$
$\alpha$ -Glucosidase ( $\text{mU g}^{-1}$ tissue)	$98.13 \pm 2.45$	$92.03 \pm 1.99$
( $\text{mU mg}^{-1}$ protein)	$2.67 \pm 0.04$	$2.65 \pm 0.07$
L-Carnitine ( $\mu\text{mol g}^{-1}$ tissue)	$14.40 \pm 0.90$	$12.14 \pm 1.25$
( $\text{nmol mg}^{-1}$ protein)	$394 \pm 31$	$348 \pm 30$
GPC ( $\mu\text{mol g}^{-1}$ tissue)	$6.86 \pm 1.02^a$	$6.23 \pm 1.55$
( $\text{nmol mg}^{-1}$ protein)	$191 \pm 32$	$177 \pm 40$

Animals were fed vehicle alone (controls) or ornidazole at  $400 \text{ mg kg}^{-1} \text{ day}^{-1}$  (treated) for 14 days. Values are means  $\pm$  SEM. <sup>a</sup> $n = 4$ . No significant differences were found between tissues from control and treated rats. GPC: glycerophosphocholine.

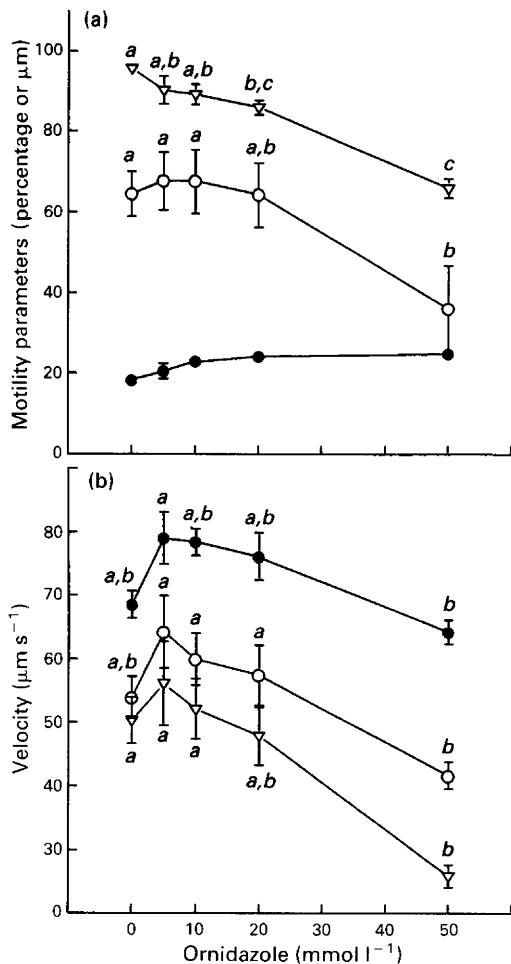
## Discussion

The loss of weight of the controls as well as of ornidazole-fed animals suggests that the effect was due to stress caused by

handling or to mating activities rather than a selective effect of ornidazole. A decline in body weight of ornidazole-fed animals was noted by McClain and Downing (1988a) but that study entailed no handling as ornidazole was administered in the food. The similarity in haematocrit values of control and treated animals, which remained in the normal range (Mitraka and Raunsley, 1977), suggests that there was no marked change in composition of blood.

With the frequent scheduling of mating tests on stringently recruited and trained animals in this study, a more rapid decline in male rat fertility against stable control values was detected (3 days) than in the earlier study; all animals became infertile within 10 days, the earliest by day 3 and most by day 6. Such an observation precludes an action on the testis, a view supported by the unchanged weight of the testis itself and the androgen-dependent accessory organs in the infertile animals. A rapid recovery in fertility after drug withdrawal was suggested by McClain and Downing (1988a) from studies in which the groups of rats continuously fed  $400 \text{ mg kg}^{-1} \text{ day}^{-1}$  for 64 days still achieved 62% pregnancies. In this study reversibility, following a near total inhibition, was convincingly demonstrated.

The decline in straight-line velocity of spermatozoa obtained from the cauda epididymidis of rats fed  $400 \text{ mg kg}^{-1} \text{ day}^{-1}$  (for 64 days in their study; McClain and Downing, 1988b) was confirmed in this study where a



**Fig. 5.** Motility parameters (mean  $\pm$  SEM,  $n = 5$ ) of spermatozoa obtained from incubated distal cauda epididymal sacs and released immediately into different concentrations of ornidazole *in vitro* (a) straightness (STR) (%) ( $\nabla$ ), percentage motile ( $\circ$ ), amplitude of lateral displacement (ALH) ( $\mu\text{m}$ ) ( $\bullet$ ), (b) curvilinear velocity (VCL) ( $\bullet$ ), averaged path velocity (VAP) ( $\circ$ ), straight line velocity (VSL) ( $\nabla$ ). Values with the same superscript are not significantly different from each other; lack of superscripts indicates that no significant differences between treatments were found.

much shorter exposure to ornidazole was used. The observations were extended by measurement of other parameters of spermatozoa from other regions of the epididymis. A significant reduction in velocities of spermatozoa when taken from the epididymides of rats treated with 400 mg ornidazole  $\text{kg}^{-1} \text{day}^{-1}$  was noticeable only in regions 3, 4 and 5 (distal corpus, proximal and distal cauda) but not more proximally. Normally, an increase in sperm velocities occurs along the epididymis (Yeung *et al.*, 1992) as in the control studies here. Lower velocities were also accompanied by a reduction in the derived values STR and WOB. These two kinematic parameters represent the closeness of the smoothed path from a straight line and closeness of the actual track from the averaged path, respectively. This indicates that sperm tracks were more erratic and less forward directed than those in control spermatozoa, as well as slower. The motility thus appears to be

'arrested' at a state found in immature cells. However, the reduced motility may not be the underlying cause of infertility since there was no statistical correlation between any sperm motility parameter in the distal cauda epididymidis on the day after mating and pregnancies induced from that mating.

As major epididymal secretions, L-carnitine, glycerophosphocholine and glucosidase were measured to determine whether epididymal epithelial functions (synthesis, secretion and transport) were disrupted in the infertile animals. However, the finding that they were not lower than those of control tissues suggests that they were not responsible for the reduced motility or fertility. If other important epididymal secretions are not reduced, an alternative mechanism is that ornidazole interacts directly with spermatozoa.

Incubation *in vitro* revealed that ornidazole itself had an effect on sperm motility, similar to that found *in vivo*, but only at extremely high concentrations, which are unlikely to be present within epididymal tissue of the treated animals. There are no data on circulating concentrations of ornidazole in treated rats, but men given a lower dose (about  $11 \text{ mg kg}^{-1}$ ) have concentrations of ornidazole in blood of only  $20 \mu\text{mol l}^{-1}$  at 24 h after a single ingestion (Schwarz and Jeunet, 1976). It is possible that a metabolite of ornidazole is responsible for the decline of motility, but neither the distribution nor metabolism of the drug in the infertile animals is yet known. The changes in motility suggest that either the less mature, proximally situated, spermatozoa are unresponsive to ornidazole arriving from the peripheral circulation, or that ornidazole accumulates in higher concentrations distally within the epididymis or spermatozoa. There is some evidence from man that 5-nitroimidazoles do accumulate in accessory gland secretions, as a related compound, metronidazole, is rapidly excreted in human semen (Eliasson and Dornbusch, 1980). This compound also induces infertility in male rats, but requires more than 4 weeks to be effective, suggesting a slow action on the testis (McClain *et al.*, 1989).

A final comment concerns the similarity of the side chain at position 1 of the imidazole ring of ornidazole to the known antifertility agents *epi*- and  $\alpha$ -chlorohydrin. This arises from the synthesis of substituted nitroimidazoles from *epi*-chlorohydrin (Hoffer and Grunberg, 1974). It has therefore to be considered (i) whether there were impurities in the ornidazole used, (ii) whether *epi*-chlorohydrin is liberated from ornidazole *in vivo* and (iii) if the attached side chain can act in a manner similar to that of the chlorohydrins.

From the evidence below we consider it unlikely that the infertility brought about by ornidazole stems from contamination by, or conversion to, chlorohydrins. Concerning (i), the purity of the ornidazole used was 99.0% (by t.l.c. analysis), but if ornidazole were completely inactive, a contamination of over 6% by weight of *epi*-chlorohydrin would be required to bring about the fertility rates approaching those found in this study (Toth *et al.*, 1991). Concerning (ii), the metabolites of ornidazole identified in man are hydroxy derivatives of ring and side-chain methyl groups, with no evidence for cleavage of an intact side chain (Richle *et al.*, 1978). Consideration of (iii) requires a short discussion of the action of the chlorohydrins. To exert its antifertility action *epi*-chlorohydrin is thought to be converted *in vivo* to  $\alpha$ -chlorohydrin (Toth *et al.*, 1989), which acts to decrease the supply of energy to epididymal



spermatozoa by interfering with energy metabolism (Ford and Rees, 1990). Reductions in VCL, VSL, ALH and LIN were also observed after treatment with *epi*-chlorohydrin (Toth *et al.*, 1991). However, both chlorohydrins also induce epididymal spermatozoa (Ford, 1982; Toth *et al.*, 1989) which were not found in this study or that by McClain and Downing (1988a, b).

In conclusion, this study has shown that ornidazole induces a rapid and reversible infertility in male rats, by an action not involving major epididymal secretions. Only extremely high concentrations of the drug modify sperm motility *in vitro*, so that indirect effects of ornidazole or a metabolite on spermatozoa are postulated.

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