AN INVESTIGATION INTO THE EFFECTS AND POSSIBLE MECHANISMS OF ACTION OF CIMETIDINE AND RANITIDINE ON THE SEXUAL BEHAVIOUR OF MALE RATS

BY

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CHAPTER 1: Introduction

The development of H2-receptor antagonists confirmed the physiological role of histamine in gastric acid secretion. More important was the discovery of cimetidine, which revolutionised the medical management of peptic ulcer diseases.

Cimetidine was the first in a new class of drugs, the H2-receptor antagonists, to gain widespread clinical acceptance for the treatment of peptic ulcer diseases. With increased clinical experience, both the number of disorders for which the drug may be used and the range of adverse effects associated with cimetidine therapy have increased. Of particular interest and concern were the emergence of CNS side-effects such as confusion, delirium, drowsiness and restlessness, and side-effects related to sexual dysfunction such as gynaecomastia, loss of libido and impotence. The CNS effects were thought to be due to the interaction of cimetidine with cerebral H2-receptors, whereas the side-effects on sexual dysfunction have been attributed to the antiandrogen activity of the drug. For this reason the development of new H2-receptor antagonists, which do not demonstrate such adverse effects, has become desirable.

Ranitidine, a potent H2-receptor antagonist, was developed by Glaxo-Allenburys and was recently approved for clinical use in several countries. The drug was introduced for

general clinical use in the Republic of South Africa in the mid 1983's. Ranitidine, unlike cimetidine, has not demonstrated antiandrogenic properties.

1.1 The problem

The antiandrogen side-effects of cimetidine noted in clinical usage are well documented. Several case reports of gynaecomastia, loss of libido and impotence were reported in male patients who were on normal or high therapeutic doses of cimetidine (section 2.6.2). Some of these side-effects were particularly distressing. In one study, in which high doses of cimetidine were used for the treatment of gastric hypersecretory states, it was reported that most patients who experienced gynaecomastia were minimally troubled with this side-effect, but almost all who became impotent desired to stop cimetidine and try some other form of treatment, including gastrectomy (1). Despite the appearance of such side-effects in man, no impairment in mating performance was reported by Leslie and Walker (2) in an earlier toxicological study in which male rats were chronically treated with high doses of the drug. However, in this study the various components of sexual behaviour which can be well demonstrated in male rats were not measured.

On the other hand, ranitidine has not demonstrated antiandrogenic properties and has not been reported to impair hypothalamic-pituitary-gonadal function. In

addition, the drug was reported to reverse cimetidine-induced impotence (section 2.3.3). In spite of these properties of ranitidine a few case reports of gynaecomastia and impotence attributed to ranitidine therapy have, nevertheless, appeared in literature (section 2.6.2). The relationship of these side-effects to ranitidine therapy has been questioned by some workers, including the manufacturers (3,4).

A wide range of literature has been published on various pharmacological and clinical aspects of both cimetidine and ranitidine. As far as research concerning the effects of cimetidine and the possible effects of ranitidine on sexual behaviour is concerned, only a few studies on associated aspects of sexual behaviour have been published. The paucity of information on this important aspect of the spectrum of side-effects of the H2-blockers creates an obvious field for research.

1.2 Aim of research

The major purpose of this study was to examine in more detail the effects of cimetidine and ranitidine on sexual behaviour in sexually active adult male rats. In addition, various investigations such as motor activity counts, testosterone levels, cauda epididymal sperm counts and motility, and testes and accessory sex organ weights were included in this study. These additional investigations were done mainly to throw some light on the possible

mechanisms of action of cimetidine and ranitidine on sexual behaviour of male rats.

CHAPTER 2: Histamine H2-receptor antagonists and sexual dysfunction: A literature survey

2.1 Introduction

The discovery of H2-receptor antagonists by Black et al. in 1972 marked the beginning of a new era in the pharmacotherapy of peptic ulcer diseases (5). Cimetidine, the first clinically effective H2-blocker, was introduced in 1976 (6). Ranitidine, a second member of this class of drugs approved for clinical use, was only recently marketed for medical use and has been found to be as effective as cimetidine in the treatment of peptic ulcer diseases (7-11).

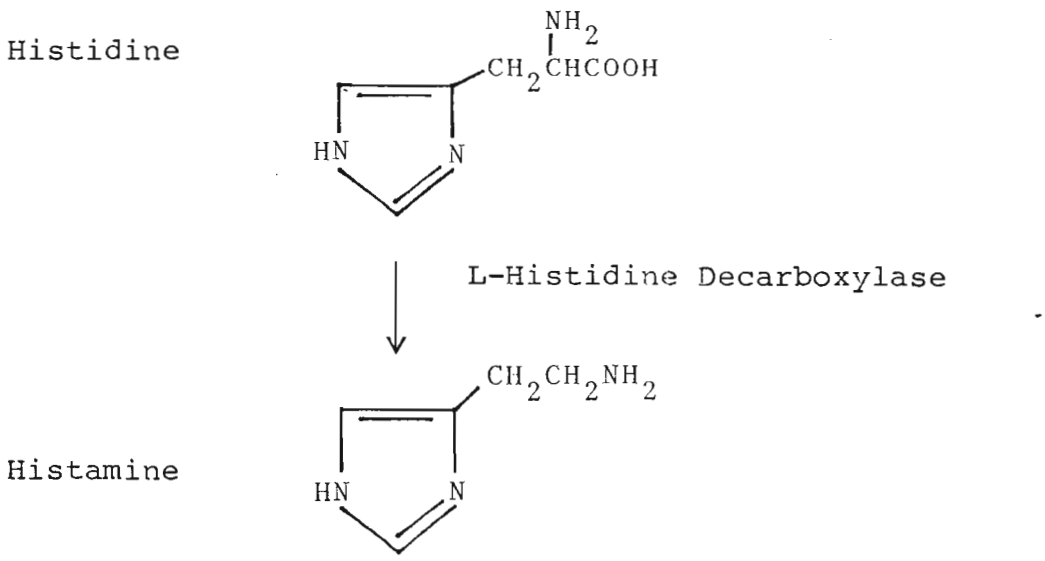
Soon after the introduction of cimetidine some male patients, who were on normal or high doses of cimetidine, developed impotence and/or gynaecomastia. A few unsubstantiated reports have also attributed impotence and gynaecomastia to ranitidine therapy (12,13). This study was initiated to examine the effects of acute and chronic doses of cimetidine and ranitidine on sexual behaviour in adult male rats. Relevant literature, from the early development of interest in the field of histamine research up to the discovery of H2-receptor antagonists, is briefly reviewed in this chapter. The literature on reproductive function, which is closely related to this project, is dealt with in more detail. The scheme of work undertaken in this study is described in chapter 3. The results and

chapter 3. The results and discussions are presented in chapters 4 and 5 respectively.

2.2 Histamine: An overview

The pioneers in this exciting field of research were:- Windaus and Vogt, who were the first to synthesize histamine from imidazoleproprionic acid in 1907; Dale and Laidlaw, who accurately described the pharmacological actions of histamine early in this century; and, Best, Dale, Dudley and Thorpe, who, in 1927 demonstrated beyond doubt that histamine is a natural constituent of the many different tissues of the body (14). Histamine is chemically known as betaimidazolylethylamine and consists of an imidazole ring and an ethylamine side chain. This biogenic amine is formed in the body by the enzymic decarboxylation of the amino acid l-histidine (Figure 1)

Figure 1. Formation of histamine



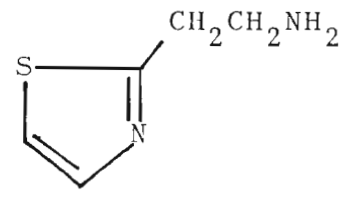
2.2.1 Histamine and its pharmacological effects

The development of specific agonists and antagonists of histamine receptors (Figures 2-5) over the last few decades has aided the investigation of the role of histamine in physiology and pathology.

Histamine and its receptors are widely distributed throughout the body of humans and animals (15,16). Interaction of histamine with these receptors is responsible for the various pharmacological actions of histamine (Tables 1 and 2). It has been established that the pharmacological actions of histamine are mediated through the stimulation of H1- and/or H2-receptors (5,17). However, many histamine-induced pharmacological effects (Table 2) are not completely understood (31). These effects are not blocked by the presently available H1- and/or H2-receptor antagonists. The occurrence of subtypes or possibly a third type of histamine receptor has been hypothesised (18-20).

Figure 2. Some H1-receptor agonists

2-Thiazolyl-ethylamine



2-Methylhistamine

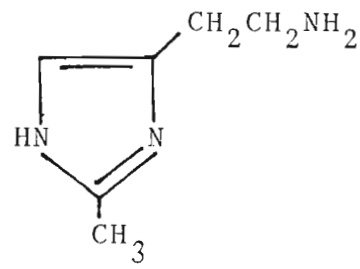
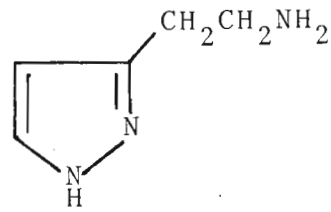
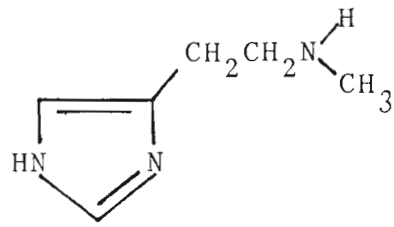


Figure 3. Some well-known H2-receptor agonists

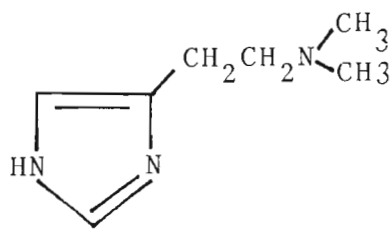
Betazole



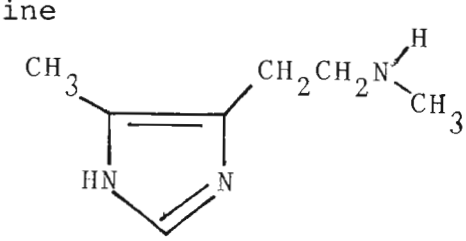
N-methyl Histamine



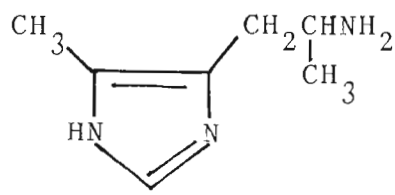
N,N-dimethyl Histamine



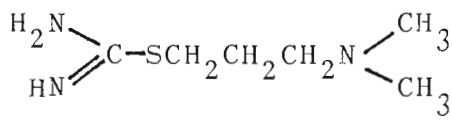
5(4)-methyl-N-methyl Histamine



5(4)-methyl-alpha-methyl Histamine



Dimaprit



Impromidine

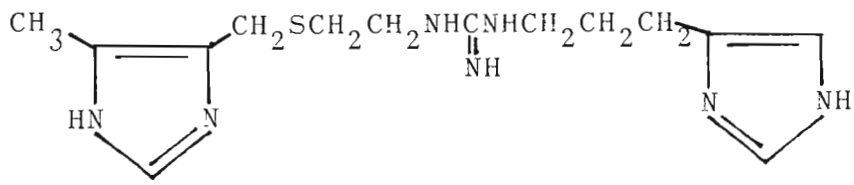


Table 1. Summary of distribution and classification of histamine receptors and pharmacological effects to histamine-stimulation (Modified: Chand N, Eyre P 1975 (16)

Tissue	Species	Receptor	Pharmacological Effect
Blood Vessels			
Pulmonary Artery	Guinea-pig	H1	Vasoconstriction
Pulmonary Vein	Calf	H1	Vasoconstriction
Ear Artery	Rabbit	H1 H2	Vasoconstriction Vasodilatation
Temporal Artery	Man	H2	Vasodilatation
Intracranial Artery (mid cerebral artery)	Cat	H1 H2	Vasoconstriction Vasodilatation
Heart Preparation			
Isolated Heart	Guinea-pig	H2	Positive chronotropic effect
Intact and Isolated Heart	Chicken	H1	Positive chronotropic effect
Intact Heart	Dog	H1 H2	Positive chronotropic effect Negative inotropic effect
Other Tissues			
Uterus	Rat	H2	Relaxation
Gastric Mucosa	Rat, Guinea-pig Man, Dog, Cat	H2	Stimulation of gastric acid secretion
Ileum	Guinea-pig	H1	Contraction
Tracheal Smooth Muscles	Cat	H1,H2	Relaxation

Table 2. Effects of histamine completely or partially unaffected by administration of H1- and H2-receptor blockers (Bertaccini G, Corruzi G 1983 (18))

Effect
Hypothermia in mice
Excitation of hypothalamic cells (rat and cat)
Behavioural changes in the rat (depression)
Excitation of Achatina fulcia neurons
Fast chloride-dependent hyperpolarising response (Aplasia ganglion)
Negative inotropic and chronotropic effect (Isolated rat heart)
Inotropic response (guinea-pig atria)
Relaxation of rabbit trachea
Relaxation of cat bronchi
Contraction of the rat pylorus
Chemoattractant activity of histamine on eosinophils
Inhibitory effect on platelet aggregation
Metabolic changes in chicks (glycogen levels and phosphorylase activity)
Inhibition of electrically-induced twitch responses of guinea-pig ileum
Dipsogenic effect in the rat
Increased formation of fatty acids and PGD2 synthesis in the rat

2.2.2 The H1-receptor and antihistamines

The desire for the development of antihistimine drugs was stimulated by the realization that histamine, which is widely distributed in several tissues and produces a diversity of pharmacological effects, could possibly be associated with certain physiological functions and disease processes. The earlier antihistamine drugs (Figure 4) have recently been referred to as H1-receptor antagonists or merely as H1-blockers (17). This classification differentiates the traditional antihistamines from a new class of antihistamine drugs, the H2-receptor antagonists (5).

The French investigators, Bovet and Staub, were the forerunners in the search for H1-receptor antagonists (21). The effects of phenbenzamine, the first antihistamine drug to undergo clinical trials, were described in 1942. Two years later mepyramine, an analogue of phenbenzamine, was discovered to have a high degree of H1-blocking activity. Mepyramine either reduced or abolished bronchospasms induced by anaphylaxis or by the administration of histamine in guinea-pigs. Clinically, the drug was successfully used in the treatment of urticaria and other conditions such as hay fever and seasonal rhinitis (21,22). Mepyramine, Histalon(R), one of the oldest of the antihistamines, is still commercially available.

Two compounds, diphenhydramine and tripelennamine,

developed in the United States, were introduced for clinical use in 1946 and were found to possess highly effective H1-receptor blocking properties (21). An amazingly large number of antihistamine drugs has since been synthesized (Figure 4).

Pharmacological studies have shown that the conventional antihistamines are not entirely specific in their actions (22,23). In addition to their antihistamine activity, they have numerous other pharmacological actions such as anticholinergic, adrenergic and/or antiadrenergic, and serotonin antagonising effects (24). Although most of the actions of histamine are specifically antagonised by low concentrations of the conventional antihistamines, some effects, such as stimulation of gastric acid secretion (25), increased contractility of the guinea-pig heart (26) and relaxation of the contracted rat uterus (27), are not antagonised by mepyramine and related antihistamines.

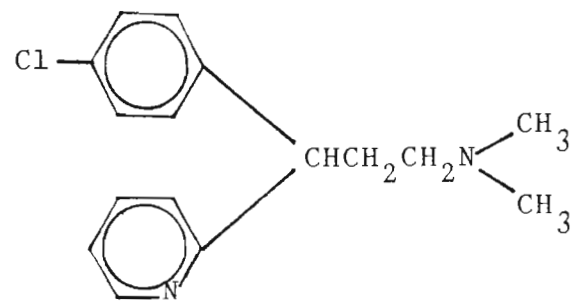
Several workers suggested that these mepyramine resistant actions of histamine might be related to its activity on other histamine receptors (28-31). More evidence for the differentiation of histamine receptors into two classes emerged from investigations on the relative activities of histamine agonists on different tissue systems (32-33). Ash and Schild, in 1966, quantitatively investigated the effects of several histamine analogues and antihistamines in three tissue preparations, namely the perfused rat stomach, the isolated rat uterus, and the isolated

guinea-pig ileum (17). They provided conclusive evidence to support the differentiation of histamine receptors into at least two groups. They suggested the use of the symbol "H1" for those receptors of histamine which are specifically blocked by low concentrations of mepyramine-like antihistamines. The findings of Ash and Schild were later confirmed when Black et al. demonstrated that the relative agonist activity of the 2-methyl derivative of histamine is particularly prominent on the H1-tissue systems while the 4-methyl derivative shows affinity for the non H1-tissue systems (5).

Figure 4. Some well-known H1-receptor antagonists

Alkylamine Derivative:-

Chlorpheniramine



Ethanolamine Derivative:-

Diphenhydramine

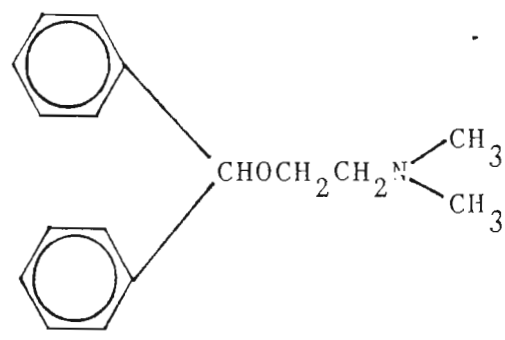
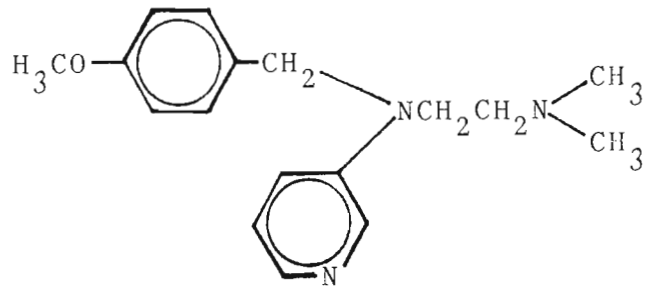


Figure 4.(Continued) Some well-known H1-receptor antagonists

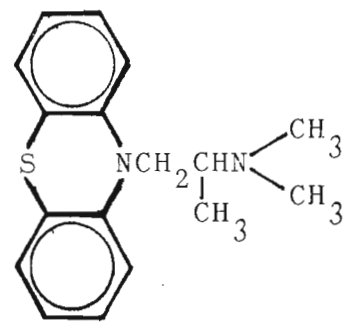
Ethylenediamine Derivative:-

Pyrilamine



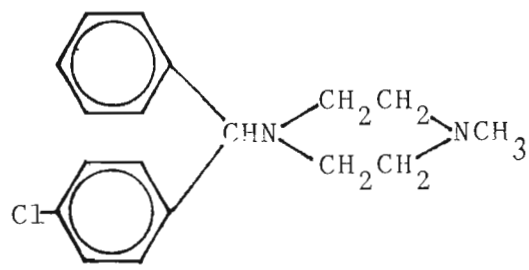
Phenothiazine Derivative:-

Promethazine



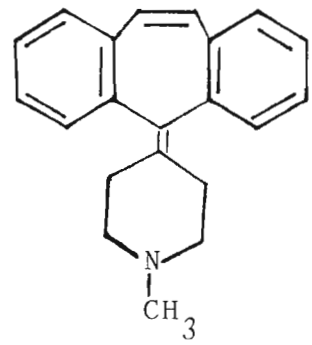
Piperazine Derivative:-

Chlorcyclizine



Other Compounds:-

Cyproheptadine



2.2.3 The H2-receptor and development of H2-receptor antagonists

Up to 1972 all the commercially available antihistamines belonged to the H1-group. The search for antagonists to counteract the mepyramine resistant actions of histamine was initiated in 1964 by Black and his team at the Research Institute of Smith Kline and French Laboratories, Welwyn Garden City, England (5). After the investigation of some 700 compounds they eventually discovered burimamide (Figure 5). Burimamide, the first histamine H2-receptor antagonist, was found to be a specific and competitive blocker of H2-receptors. It effectively antagonised gastric acid secretion evoked by histamine, gastrin or pentagastrin in man (34) and animals (5). However, although the drug was pharmacologically selective it lacked oral effectiveness and was not considered for clinical evaluation.

Metiamide (Figure 5), the successor to burimamide, was orally more active (35). The drug was found to be clinically effective in suppressing gastric acid secretion, relieving duodenal ulcer symptoms and promoting ulcer healing (36). However, it was associated with a low incidence of reversible agranulocytosis (37,38) and subsequently in November 1975 the drug was withdrawn from clinical investigation.

Cimetidine (Figure 5), another imidazole H2-receptor blocker in which the thiourea group of metiamide has been

replaced by a cyanoguanidine group, was synthesized in 1975 (6). Cimetidine became the first H2-receptor blocker to gain widespread clinical acceptance for the treatment of duodenal ulcers and other gastric acid hypersecretory conditions.

All the conventional H2-blockers described so far bear a structural similarity to histamine in possessing an imidazole ring. Until recently the imidazole ring was regarded as an essential feature for H2-receptor site affinity (39). However, ranitidine, a new H2-receptor blocker (Figure 5) developed by Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., differs from the earlier H2-receptor blockers in possessing an alkylated furan ring (40). Studies on isolated tissue preparations have demonstrated that ranitidine is a selective and competitive H2-receptor blocker (40,41). In vivo antisecretory experiments in rats and dogs showed that ranitidine was more potent than cimetidine in antagonising histamine or pentagastrin stimulated gastric acid secretion (40-42). Presently, cimetidine and ranitidine are the only two H2-blockers available for the treatment of peptic ulcers and other related disorders. Their pharmacology is discussed in more detail in section 2.3.

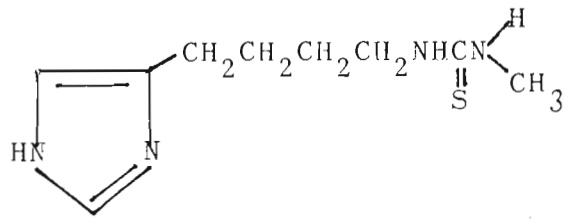
Recently there has been much interest in the search for novel H2-blockers, and a number of pharmaceutical companies are actively engaged in this area of research. Several potent H2-blockers, such as oxmetidine (SK&F92994)

(43), etintidine (BL-5641A-Bristol Lab) (44), famotidine (YM-11170) (45), and SKF 93479 (46) have lately been entered into clinical trials. On the other hand the development of tiotidine (ICI 125.211) (47) was suspended in August 1980 due to its association with a low incidence of gastric tumours in rats treated with high doses of the drug. Likewise, more recently clinical trials with SKF 93479 were halted because of the development of hyperplastic and dysplastic forestomach mucosa in rats treated with massive doses of the drug (1250 times the proposed therapeutic dose) over periods longer than six months (48).

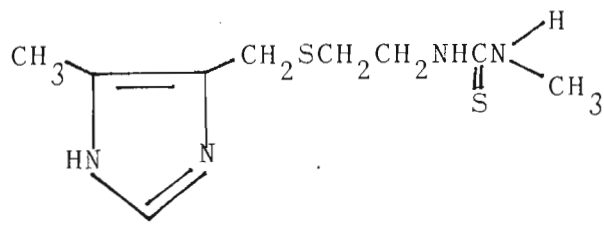
Many other compounds are still in the early stages of development and, so far, data acquired from in vitro or animal studies only have been published. Of interest is a very recent development of a combined H1- and H2-receptor blocker, SK&F 93319 (49). A potential use of SK&F 93319 might be in conditions such as inflammatory skin diseases which may require antagonism of histamine at both H1- and H2-receptors (50).

Figure 5. Some H2-receptor antagonists

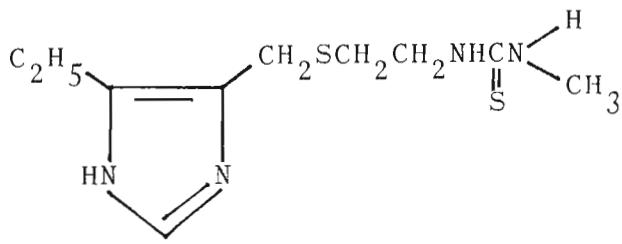
Burimamide



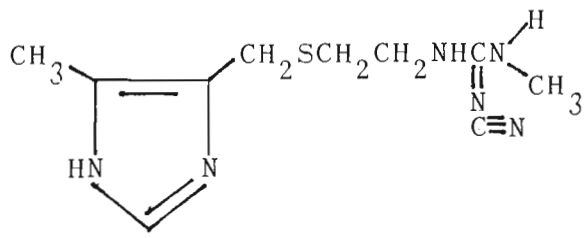
Metiamide



Etiamide

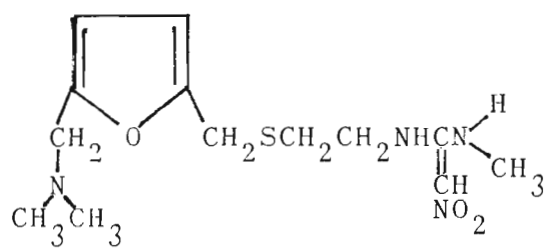


Cimetidine



Ranitidine

(Glaxo AH 19065)



Oxmetidine

(SK&F 92994)

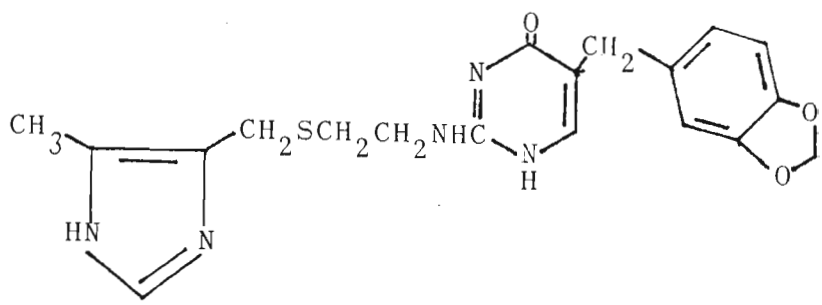
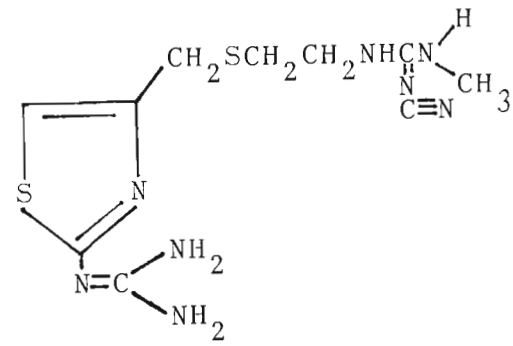


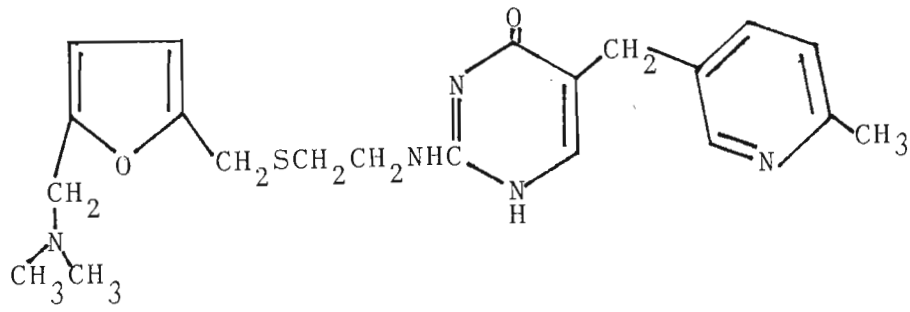
Figure 5.(Continued) Some H2-receptor antagonists

Tiotidine

(ICI 125.211)

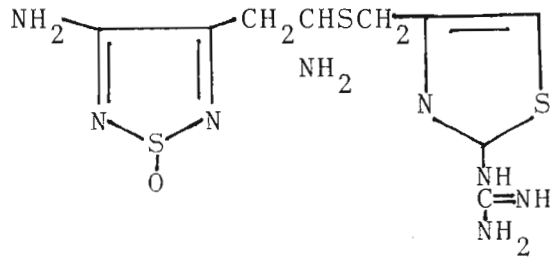


SK&F 93479



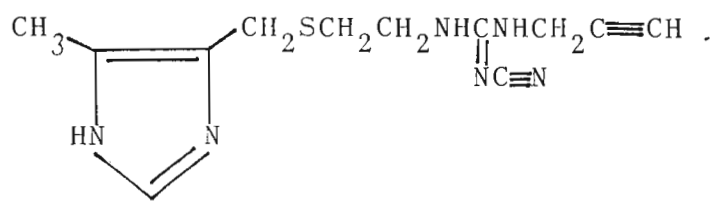
BL-6341A

(Bristol Lab)



Etintidine

(Bristol Lab)



2.3 Pharmacological and other effects of cimetidine and ranitidine.

The most important clinically significant pharmacological effect of H2-receptor blockade is the suppression of gastric acid secretion. This seems to be clinically essential in both relieving ulcer pain and promoting ulcer healing. Cimetidine and ranitidine have the same range of H2-receptor antagonist activity (41). Unlike cimetidine, ranitidine lacks affinity for androgen receptors (21,51) and also does not inhibit the hepatic cytochrome P-450 mixed-function oxidase system to the same extent as cimetidine (52,53). In this section selected aspects of the pharmacology of cimetidine and ranitidine are compared.

2.3.1 Histamine receptor specificity and potency

The actions of histamine on the isolated guinea-pig heart, rat uterine muscle and gastric acid secretion in many species are mediated through the stimulation of H2-receptors. Isolated organ experiments have demonstrated that both cimetidine and ranitidine are competitive blockers of histamine on these H2-tissue systems (40,41). The antagonism was shown to be selective, since neither blockers interfered with beta-adrenergic, H1-histaminergic and muscarinic responses in the guinea-pig atrium, the guinea-pig ileum and the rat uterus. However, the potency of ranitidine on the isolated guinea-pig right atrium and

on the rat uterine horn was shown to be about four and six times greater than that of cimetidine (on a molar basis) respectively. In animals the antisecretory activity of cimetidine and ranitidine has been extensively investigated in the rat (40,41,54) and dog (42,55-58). On a molar basis ranitidine was found to be about four to ten times more potent than cimetidine in inhibiting gastric acid secretion, depending upon the experimental model and the secretory stimulant used.

2.3.2 Inhibition of gastric acid secretion in man

Basal gastric acid secretion in man may be regulated by the three endogenous secretagogues, namely gastrin, acetylcholine and histamine. Several other stimuli such as food, beverages, sham feeding, amino acids and insulin hypoglycaemia, are known to induce gastric acid secretion. Cimetidine and ranitidine effectively inhibit not only basal, but also stimulated gastric acid secretion by blocking parietal cell H2-receptors. Inhibition of gastric acid secretion by the H2-blockers has been extensively investigated in peptic ulcer patients as well as in normal volunteers. Several comparative studies in man have indicated that ranitidine is about four to thirteen times more potent than cimetidine (on a molar basis) in inhibiting basal or stimulated gastric acid secretion (59-62). The increased potency of ranitidine is not significant clinically, since similar ulcer healing rates and relief from pain have been reported with equipotent

doses of both drugs (63-68).

2.3.3 Effects on sex hormones and gonadal function

Several investigators have reported on the effects of cimetidine and ranitidine on male sex hormones. Changes in hormonal levels with cimetidine, but not with ranitidine therapy, have been observed. It has been suggested that the endocrine effects of cimetidine may possibly not be attributed to H2-receptor blockade (69).

The gonadotropic hormones, FSH, LH, and PRL play an important role in the control and regulation of reproductive function (70). Alterations in hormonal mechanisms may contribute to sexual dysfunction. A number of commonly used drugs (Table 3) are known to induce sexual dysfunction (71-74). Cimetidine has been shown to cause significant elevations of PRL levels (75), and reports on impotence associated with this drug were published for the first time in 1979 (76,77).

Table 3. Possible effects of drugs that may induce sexual dysfunction

(Modified: Aldridge SA 1982 (74))

Drug	Loss or Decreased Libido	Impotence (erectile difficulty)	Ejaculatory Difficulty	Hormonal Alteration
Anticholinergics				
Atropine		+		
Benztropine		+		
Propantheline		+		
Scopolamine		+		
Antidepressants				
Amitryptaline	+	+	+	
Doxepin	+		+	
Isocarboxazid		+	+	
Phenelzine		+	+	
Tranylcypromine		+	+	
Antihistamines				
Diphenhydramine	+	+		
Hydroxyzine	+	+		
Antihypertensives				
Clonidine (a)	+	+	+	
Methyldopa (a)	+	+	+	
Phenoxybenzamine			+	
Phentolamine			+	
Prazosin		+		
Propranolol	+	+		
Reserpine	+	+	+	
Spironolactone (a-c)	+	+		+
Thiazides	+	+		
Antipsychotics				
Chlorpromazine	+	+		+
Haloperidol		+	+	+
Thioridazine (b)	+	+	+	+
Narcotics				
Methadone (b)	+	+		+
Morphine sulfate (b)	+	+		+
Sedative-Hypnotics				
Barbiturates	+/(d)			?
Benzodiazepines	+/(d)		?	
Miscellaneous				
Alcohol	+/(d)	+		
Cimetidine (a)	+	+		+
Clofibrate (a)	+	+		
Marijuana (a)	+/(d)	+		?
Oral contraceptives	+			+

(a): May cause gynaecomastia in men or breast enlargement in women.
(b): May cause menstrual irregularities.
(c): May alter vaginal lubrication.
(d): Although increased doses or prolonged use may diminish libido, small doses may have a disinhibiting effect.
+ : Positive effect
? : Uncertain effect

2.3.3.1 Prolactin

Elevated prolactin levels have been observed under experimental conditions in healthy volunteers after the administration of high intravenous bolus doses of cimetidine and ranitidine (75,78-80). The secretion of prolactin stimulated by the H2-receptor blockers has been shown to be dose-dependent (75,80). Evidence for histamine H1- and H2-receptor involvement in regulation of prolactin secretion has been demonstrated (81). The minimum intravenous dose required to induce prolactin release was approximately 65 mg for ranitidine (80) and 100 mg for cimetidine (82). Clinical relevance of this is not yet clear, as reports from short and long term oral use of cimetidine (69,83-87) and ranitidine (48,67,68) have not shown altered plasma prolactin levels.

Nevertheless, in one study significantly increased levels of prolactin were reported with cimetidine, but not with ranitidine therapy (88). In addition, increased prolactin levels were reported in some male patients who developed impotence and/or gynaecomastia (77,89-91) and in female patients with galactorrhoea (90,92) mainly during chronic and high dose cimetidine therapy. In comparison with cimetidine, reports of elevated prolactin levels with ranitidine use have been rare and unsubstantiated. Hyperprolactinaemia with amenorrhoea was reported in one 34 year old patient after one month maintenance therapy with ranitidine (150mg daily) for duodenal ulcer (93). In

a later publication, the same author confirmed, after further investigations on this patient, that the hyperprolactinaemia he had reported previously was not related to ranitidine therapy but to a "probable pituitary microadenoma" (94).

2.3.3.2 Testosterone.

Reports on cimetidine-induced changes in testosterone levels have been contradictory. Two groups of investigators reported normal mean plasma testosterone levels during and after six weeks of cimetidine therapy in males (89,95). Similarly, another group found no alteration in plasma testosterone levels despite development of gynaecomastia in a small number of patients treated with cimetidine for at least three months (96).

Contrary to this, Van Thiel and his group observed a statistically significant ($p<0.05$) increase in basal testosterone levels (21%) after treatment with cimetidine (1200 mg daily for nine weeks) (97). They also reported hypothalamic-pituitary-gonadal dysfunction in a group of seven men treated with cimetidine. Wang et al., in two separate investigations, reported that cimetidine had caused a small but significant elevation of testosterone concentration in duodenal ulcer patients during six months of therapy (98) and ranitidine had shown no effect on testosterone and other gonadotropic hormone levels during twelve months of therapy (88). Furthermore, in one comparative study, a significantly ($p<0.01$) raised basal

level of testosterone in duodenal ulcer patients was reported after four weeks of treatment with cimetidine (800-1000 mg daily) (69). In the same study testosterone levels remained unchanged after treatment with ranitidine (300-320 mg daily). In another comparative investigation, the effects of four weeks of treatment with cimetidine (1 g daily) and ranitidine (300 mg daily) were compared on male sex hormones in duodenal ulcer patients (99). A statistically insignificant increase of 10% in testosterone levels was noted in the cimetidine group, while no increase in testosterone levels was observed in the ranitidine group.

The elevation of basal testosterone levels reported with cimetidine in males has been attributed to the direct antiandrogenic activity of cimetidine, and not to H2-receptor blockade (69,99,87).

Hormonal studies in animals have been rare. However, unlike the reports of elevated plasma testosterone levels observed in man, one study has shown a small but not significant decrease in testosterone levels in the rat (100).

2.3.3.3 Luteinising hormone and follicle stimulating hormone.

In males luteinising hormone (LH) influences the production of testosterone by the interstitial cells of Leydig, while the maturation of the spermatozoa in the

seminiferous tubules is stimulated by follicle stimulating hormone (FSH) and testosterone (70). Reports on changes in basal or stimulated levels of LH and FSH with cimetidine therapy are conflicting (69,97-99). Changes in hormonal levels have not been reported during treatment with ranitidine.

In one of the earlier investigations a reduced responce of LH to luteinising hormone releasing factor after cimetidine therapy (1200 mg daily for nine weeks) was reported (97). Wang et al. noted elevated FSH and unchanged LH levels in duodenal ulcer male patients during six months of cimetidine therapy (98). They also showed that twelve months of treatment with ranitidine (150 mg twice daily for three months and 150 mg nightly for nine months) had no effect on FSH and LH levels (88). In a comparative study Boyd et al. found that neither cimetidine (800-1000 mg daily, thirty-three patients) nor ranitidine (300-320 mg daily, eighteen patients) altered basal levels of LH and FSH in duodenal ulcer patients after four weeks of treatment (69); but in the cimetidine group they observed a significant elevation of basal LH levels after two weeks of treatment, which returned to normal levels on continuation of therapy for a further two weeks. Likewise, in another comparative study no changes were observed in either basal or stimulated levels of LH and FSH in duodenal ulcer patients after four weeks of normal dose therapy with either cimetidine (ten patients) or ranitidine (ten patients) (99).

2.3.3.4 Antiandrogen activity.

Several studies in animals have provided evidence that cimetidine possesses antiandrogenic activity (2,100-102). Similar investigations with ranitidine have shown no evidence of antiandrogenic activity (103).

The gonadotrophins and androgens are responsible for the development and maintenance of the functional and structural integrity of the gonads and the male accessory sex organs. In pharmacological studies, antiandrogen activity can be measured in male rats by determining the weights of the seminal vesicles or the prostates (104). In the rat and dog reduction in gonad weights, atrophy of the prostrate and seminal vesicles have been reported after high doses of chronic treatment with cimetidine (2,100,101). Seminal vesicle weights were found to be significantly reduced in mice treated with high doses of cimetidine (102). Contrary to these reports, Brittain et al. found no changes in prostate and seminal vesicle weights in male rats treated for ten weeks and dogs treated for fifty-two weeks with excessive doses of ranitidine (103).

Further confirmation that cimetidine, and not ranitidine, possesses antiandrogen activity has been obtained from androgen receptor binding studies (51,100,105). Funder and Mercer examined the effect of cimetidine on androgen receptor binding sites in mouse kidney cytoplasmic preparations and demonstrated that cimetidine has affinity

for androgen receptors (51). In another study using the cytosol fraction of the rat ventral prostate, cimetidine was reported to have competitively inhibited dihydrotestosterone from binding to cytoplasmic androgen receptors (100). In addition, the investigators concluded that cimetidine is a nonsteroidal androgen antagonist. In one comparative study the androgen receptor binding affinity of cimetidine and ranitidine was investigated in the mouse kidney cytoplasmic preparation (105). Cimetidine was reported to have competitively displaced 3H-dihydrotestosterone from mouse kidney androgen receptors, while ranitidine showed no effect.

Clinically, the appearance of side-effects such as gynaecomastia, loss of libido and impotence has been attributed to the antiandrogenic activity of cimetidine (1,4,106). Endocrinologically, alteration in levels of the reproductive hormones, testosterone, FSH and LH has been implicated to the antiandrogenic property of cimetidine (97,98). Observations in man with ranitidine therapy for up to twelve months have shown no effect on gonadal function, including sperm counts (88).

2.3.3.5 Effects on sperm count and motility

Reports on the effect of cimetidine therapy on sperm counts in males have been conflicting. Cimetidine was reported to reduce sperm counts in some studies (97,98) but not in another (107). In one study a significant

reduction ($p<0.05$) in sperm counts (without alteration in seminal fluid volumes) was observed in a group of seven men after cimetidine therapy (97). In another group of eleven duodenal ulcer patients treated with cimetidine (1000 mg daily for three months and then 400 mg nightly for another three months) a significantly lower ($p<0.01$) mean sperm count was observed during therapy than after drug withdrawal (98). Sperm motility was not decreased in that study. Peden et al. observed oligospermia in a fifty year old duodenal ulcer patient who complained of impotence after seven months of treatment with cimetidine (1000 mg daily) (77). On the other hand, short term (108) and long term (88) treatment of duodenal ulcer patients with ranitidine showed no significant changes in sperm count and motility.

2.3.3.6 Effects on mating performance of male rats

Leslie and Walker reported on the mating performance and fertility of male rats after oral treatment with high doses of cimetidine (upto 950mg/kg daily for over seventy days) (2). They observed no impairment in sexual performance and fertility although prostate and gonad weights were reduced.

2.4 Pharmacokinetics of cimetidine and ranitidine

The kinetics of cimetidine in the rat, dog and man were reported to be very similar (109). On the basis of these

findings it was proposed that the rat and dog were suitable laboratory animal models for toxicological studies of cimetidine in man. A number of single dose studies in patients and healthy volunteers have shown that ranitidine (110-116) and cimetidine (116-124) are pharmacokinetically similar. Some of the important pharmacokinetic properties of cimetidine and ranitidine are summarised in Table 4.

Table 4. Summary of some important pharmacokinetic properties of oral ranitidine and cimetidine given in single doses to healthy subjects and patients (Brogden RN, et al. 1982 (125))

Drug	Mean peak plasma conc (ng/ml)	Oral bioavail-ability (%)	Half-life (tl/2) (h)	Total plasma clearance (ml/min)	IC50* (ng/ml)	Time >IC50 (h)	Urinary recovery of un-changed drug 0-24h (%)
Ranitidine							
40mg	140-176	39-87	2.1-3.1	568-709	165	2-3	50-70
150mg	480					>8	
Cimetidine							
200mg	700-1500	63-78	1.7-2.1	556-652	780	2.5	48-58

*: Mean plasma concentration resulting in 50% inhibition of stimulated acid secretion after oral administration.

2.4.1 Absorption and peak plasma levels

Cimetidine and ranitidine are rapidly absorbed after oral administration. However, there were wide individual variations reported in attaining peak plasma levels. Griffiths et al. observed average peak blood levels at 90 minutes and 60 minutes after oral dosing with cimetidine 400 mg and 800 mg respectively in two groups of subjects (109). Peak blood levels after oral administration of ranitidine 20, 40, and 80 mg were reported to occur between 60-120 minutes in one study (114) and between 30-90 minutes in another (126). In most subjects a second peak was observed between 1.5 and 4 hours with both drugs when given orally after an overnight fast (122,126-128). Veng Pedersen and Miller investigated this phenomenon and concluded that the secondary peak was possibly due to rapid release of the drug from a drug storage depot such as the hepatic parenchymal tissues (127).

2.4.2 Distribution

Both drugs have been reported to distribute widely throughout the body (129,130). However, ranitidine has been reported to penetrate the blood-brain barrier to a far lesser extent than cimetidine (131). Redolfi reported an apparent volume of distribution of 1.4 - 3 l/kg for cimetidine (132). An apparent volume of distribution of 1.16-1.87 l/kg for ranitidine has been reported (110,133,134).

2.4.3 Elimination and metabolism

The elimination half-life after single intravenous administration of both drugs was reported to be approximately 2 hours (109,110,115,118,133). After oral administration the half-life was between 2.1-3.1 hours for ranitidine (110,115,134) and 2.3-3.8 hours for cimetidine (135). Cimetidine and ranitidine are largely excreted unchanged in the urine (112,118,129,134).

About 10 - 20% of an ingested dose of cimetidine is metabolised in the liver to the sulphoxide, and smaller amounts of desmethyl amide salts have also been detected in the urine (109). At least 3 metabolites of ranitidine have been identified (136). The major metabolite of ranitidine is the n-oxide, and smaller amounts of desmethyl ranitidine and ranitidine-s-oxide have also been detected in the urine. Some of the important pharmacokinetic properties of cimetidine and ranitidine are summarised in Table 4.

2.5 Clinical studies: Comparing therapeutic efficacy of cimetidine and ranitidine in the treatment of peptic ulcer disease

The therapeutic efficacy of ranitidine in the treatment of peptic ulcer diseases has been evaluated in a number of clinical trials. Most studies have compared the rate of ulcer healing after treatment for 4 or 8 weeks with ranitidine 300 mg daily and with cimetidine 1000 mg daily

administered orally. The usual dosagé regimen for the treatment of duodenal and gastric ulcer patients with cimetidine was 200 mg three times a day taken with food and 400 mg at bed time. The dosage regimen for ranitidine was 150 mg taken in the morning and at bed time.

2.5.1 Duodenal ulcer

In several studies ranitidine has been demonstrated to be as effective as cimetidine in the rate of healing of duodenal ulcers. Many trials have shown ulcer healing rates ranging from 69 to 77% in patients treated with ranitidine (300 mg daily) and 60 to 84% in patients teated with cimetidine (1000 mg/daily) over 4 weeks (7-11,67). Ulcer healing rates after 8 weeks of treatment with the usual doses of ranitidine and cimetidine were between 85 and 92% and 88 and 95% respectively (7-10).

However,in one study a very low ulcer healing rate was reported after 4 weeks of treatment with cimetidine (64). In this study the dose of cimetidine (800 mg daily) was below the dosage regimen normally used for the treatment of duodenal ulcer disease.

2.5.2 Gastric ulcer

In one recent well-designed trial, ranitidine (150 mg bid) was compared with cimetidine (200 mg tid and 400 mg at bed time) in the treatment of gastric ulcer patients (137). No

significant difference in the ulcer healing rates between these two drugs was reported. Ulcer healing rates of 58 and 57% after 4 weeks of treatment and 91 and 79% after 8 weeks of treatment with ranitidine and cimetidine respectively were reported.

2.5.3 Zollinger-Ellison syndrome

The Zollinger-Ellison syndrome (ZES) is characterised by marked gastrin production, gastric acid hypersecretion usually above 15 mEq/hour, and peptic ulceration. In a study of 61 patients with ZES, cimetidine therapy in adequate doses was found to be highly effective (138). In most patients gastric hypersecretion was successfully controlled, pain and dyspepsia relieved and ulcers healed after treatment with cimetidine. However, in one study high doses of cimetidine ranging from 1.2 to 10.2 g daily were used for treatment of male ZES patients (1). A high incidence of side-effects related to sexual dysfunction was reported in this study. Furthermore, it was observed that the cimetidine-induced side-effects disappeared when treatment was changed to ranitidine. Similarly, in another study of 8 patients with ZES, in whom cimetidine therapy resulted in tolerance or led to the development of unwanted effects, treatment with ranitidine was reported to be highly successful (139).

2.6 Side-effects: Comparative side-effects of cimetidine and ranitidine, with particular reference to sexual dysfunction

Longterm, widespread usage has shown that cimetidine is a remarkably safe drug with a low incidence of side-effects. Ranitidine is a relatively new drug which has been on clinical trials since 1981. The drug was only recently approved (mid 1983) by the South African Medicines Control Council for general clinical use in the Republic.

2.6.1 General side-effects

Reports from several therapeutic trials comparing ranitidine with cimetidine have shown that side-effects were infrequent with either drug, being up to about 5% and 4% on ranitidine and cimetidine respectively (7-10,67). Generally the nature of the adverse effects reported was similar with both drugs and included trivial and transient side-effects such as diarrhoea, skin rash, headache and dizziness.

A number of CNS effects such as agitation, confusion, delirium, restlessness and hallucinations has been reported to occur particularly in elderly patients with renal and/or hepatic dysfunction (140-143). It has been suggested that the cimetidine-induced CNS effects could be attributed to the blockade of central histamine H2-receptors (142). From the limited information available

it appears that ranitidine does not cause CNS effects because of its lower transfer across the blood-brain barrier (99,131). Furthermore, ranitidine therapy was reported to have reversed cimetidine-induced agitation, confusion and delirium in a 68 year old male with renal failure who was treated for duodenal ulcer (131). Contrary to this, two very recent case reports have appeared in which the occurrence of CNS effects during treatment with ranitidine has been described (144,145). In one report an 86 year old woman was found to have experienced mental confusion, hallucinations and delusions while she was on ranitidine (150 mg twice daily). Subsequently, it was found that cimetidine (1 g daily) was well tolerated by this patient (144). In the other report, a 66 year old woman who had previously experienced confusion with cimetidine therapy (800 mg daily) was treated with ranitidine (300 mg daily) for another episode of recurrent gastric ulceration. Within 2 weeks of commencing therapy symptoms of confusion appeared and persisted even though the dose was reduced to 150 mg daily (145).

Ranitidine, unlike cimetidine, does not significantly inhibit the hepatic cytochrome P450 drug metabolising enzyme system (52,53,146). Accordingly, the pharmacokinetics of drugs such as propranolol (147), warfarin (148), and theophylline (53) were not affected with ranitidine therapy.

2.6.2 Side-effects related to sexual dysfunction.

Many case reports of side-effects such as gynaecomastia, loss of libido and impotence in male patients treated with normal and high therapeutic doses of cimetidine, have appeared in literature. The mechanism of these side-effects has been largely attributed to the antiandrogen activity of cimetidine (4,106).

Wolfe reported decreased libido in a 43 year old male patient during the second week of treatment with cimetidine (300 mg 6 hourly) for the treatment of peptic ulcers (76). His condition soon progressed to complete impotence and on stopping cimetidine normal sexual activity was restored within two weeks. Reproductive hormone levels were not measured.

Another report described three cases of sexual dysfunction in male patients who were treated with cimetidine (1g daily) for duodenal ulcers (77). One man, aged 33 years, complained of loss of libido and thereafter impotence during the first week of treatment. On discontinuing treatment his sexual function returned to normal within four days. The second patient, a 50 year old male, suffered loss of libido which progressed to impotence within three weeks of starting treatment. However, he only complained after seven months of treatment. His impotence had not returned to normal during the eleven months since stopping treatment with cimetidine. Semen analysis showed oligospermia, but examination of a testicular biopsy

specimen showed normal active spermatogenesis in all tubules. The third patient, a 51 year old male, noted loss of libido and subsequently impotence soon after starting a proposed one year course of cimetidine therapy. But his condition was only divulged when he was specifically questioned after eleven months of treatment. It was reported that his sexual activity was not restored after stopping treatment. Testosterone and gonadotropin levels were reported either normal or abnormal in some of these patients and a causal link was not established between cimetidine-induced sexual dysfunction and hormonal levels.

In one longterm study, Jensen et al. treated 22 male patients with gastric hypersecretory states (20 with ZES and 2 with idiopathic gastric hypersecretion) with high doses of cimetidine (1.2 to 10.8 g daily) (1). An unusually high incidence (50%) of antiandrogen side-effects occurring within two to five months of commencing treatment was observed. Impotence, breast tenderness, gynaecomastia or some combinations of these were reported in eleven of the twenty patients. Nine of the eleven patients complained of impotence. It was also reported that when therapy was changed from cimetidine to ranitidine the antiandrogen side-effects disappeared. Similarly, in another study reversal of cimetidine-induced impotence and gynaecomastia were observed in ZES patients who were subsequently treated with ranitidine (139). Furthermore, Jack et al. found no reports of either gynaecomastia or impotence in 300 000 duodenal ulcer and

ZES patients treated with ranitidine (150 to 1200 mg daily) (106).

Although ranitidine has not shown antiandrogenic activity, and was further found to reverse cimetidine-induced impotence, a few case reports of gynaecomastia (12) and impotence (13) associated with ranitidine therapy have, nevertheless, appeared in literature. Tosi and Cagnoli observed right-sided gynaecomastia in a 69 year old male who was on treatment for rheumatoid arthritis and peptic ulcer disease (12). The gynaecomastia appeared after eight days of therapy with ranitidine (150 mg daily) and was reported to have disappeared on stopping treatment and to have reappeared on rechallenge with ranitidine. However, other workers have expressed their doubts that the reported gynaecomastia was related to ranitidine therapy (3,4). Viana reported temporary impotence in a 41 year old man while he was on ranitidine (150 mg twice daily) for the treatment of recurrent duodenal ulcers (13). Smith and Elsdon Dew stated that this patient was entered into one of their clinical trials in Portugal in December 1980 and it was reported to them that the patient experienced loss of libido and not impotence (4). They further pointed out that the patient received a second four-week course of ranitidine without any reports of adverse effects.

2.7 Pharmacology of normal sexual behaviour and drug-effects producing sexual dysfunction

2.7.1 Overview

Sexuality has always been an important aspect of man's life. From early times, man has applied various means and methods to stimulate or revive sexual powers. Some of these included prayer, magic, herbs, oysters, mandrake, ginseng and pornography. One of the earlier references of drug use in human sexuality is found in Macbeth where Shakespeare mentions that alcohol arouses the interest in sexuality but may impair the performance (149).

A large number of drugs used therapeutically are known to affect sexual function adversely. The various types of sexual disorders associated with drug therapy in the male are summarised in Table 5. Sexual drive or libido, with particular emphasis on proposed mechanisms of this sexual dysfunction, is discussed in more detail. It must, however, be noted that many disorders in themselves are associated with sexual dysfunction. Impotence is common even without treatment in hypertensive, diabetic and certain psychiatric patients, for example schizophrenics (151). Furthermore, transient episodes of impotence (usually linked with fatigue, stress, acute illness, alcohol- or drug-ingestion) occur and these have been regarded as normal occurrences (151). The incidence of impotence increases with age. Kinsey et al. quoted a figure of 25% at 65 years and 50% at 75 years, while

occasional cases of impotence were observed below the age of 45 years (152).

Table 5. Types of sexual dysfunction associated with drug therapy in the male (Modified: Stevensen JG, Umstead GS 1984 (153))

DECREASED LIBIDO a decline in the sexual drive or desire; may be either conscious or unconscious
IMPOTENCE inability of the male to achieve or maintain an erection
RETARDED EJACULATION delayed ejaculation or inability to ejaculate
RETROGRADE EJACULATION ejaculation into the urinary bladder due to insufficient tightening of the internal urethral sphincter
GYNAECOMASTIA enlargement and excessive development of the male breast; may be unilateral or bilateral
PRIAPISM persistent abnormal erection of the penis, usually accompanied by pain and tenderness

2.7.2 Effects of drugs on libido

Sexual desire or libido may be influenced by psychological and organic factors (73). Furthermore, the underlying disability, hospitalization and impaired nutrition can be expected to aggravate drug-induced changes in libido.

The mechanisms for drug-induced loss or decrease in libido

have been attributed to central actions (73). Testosterone is considered to play an important role in normal sexual drive. It has been reported that low levels of circulating testosterone, when associated with hyperprolactinaemia, produces loss of libido and impotence (154). Furthermore, it has been found that antiandrogens may cause loss of libido by blocking testosterone receptors and by acting on central sexual behaviour mechanisms (73).

Blockade of central dopamine receptors has also resulted in decreased libido (73). Dopamine acts as an inhibitor of prolactin (PRL) secretion. Drugs such as the antipsychotic agents, for example the phenothiazines, thioxanthines and butyrophenones, may stimulate PRL release by blocking dopamine receptors in the CNS (155). In addition these drugs may have anticholinergic properties which may affect other components of sexual behaviour, for example erection.

The antihypertensive agents are particularly known to decrease sexual desire or impair sexual function. Reserpine has been reported to cause not only decreased libido, but also failure of erection and sometimes ejaculatory dysfunction (73). The mechanism for the decreased libido has been attributed to a depletion of CNS catecholamine levels. The effects of drugs on erectile and ejaculatory function are discussed in section 2.7.3.

To gain a better insight into the mechanism of drug-induced sexual dysfunction it is important to understand the physiology of the normal sexual response.

2.7.3 Physiology of the normal sexual response

Sexual response in humans is extremely complex and may involve the interaction of neurogenic, hormonal, vascular, muscular and psychological mechanisms (156). The effect of drugs on these mechanisms may cause complete impotence or affect some component of sexual behaviour. Since this dissertation is involved with the effect of H2-blockers on male sexual function, the physiology of the normal sexual response of the male, in particular, will be reviewed. An overview of the normal sexual response in the male is presented in Table 6. The normal sexual response in humans may be separated into 4 phases.

Table 6. Overview of the normal sexual response in the male (Modified: Stevensen JG, Umstead GS 1984 (153))

Phase	Effect	Mechanism	Neural Involvement
Arousal	penile erection	stimulation via sacral nerves resulting in vasodilatation of precapillary vessels increased blood flow leads to swelling of vascular erectile tissue	parasympathetic
Plateau	mucous secretion	secretion by Cowper's gland, glands of Littre, and the prostate	parasympathetic
Orgasmic	emission	contraction of smooth muscle of seminal vesicles, vas deferens and ampulla mediated through thoracolumbar region of spinal cord	sympathetic-alpha adrenergic
	relaxation of bladder and contraction of internal urethral sphincter	increase in sympathetic outflow, simultaneous with emission	sympathetic-alpha-adrenergic
	ejaculation	reflex contraction of pelvic and perineal musculature and striated muscle of the penis initiated by semen in posterior urethra	somatic efferents
Refractory	sexual intercourse cannot be repeated		

2.7.3.1 The arousal phase: Erection

The initial effect of sexual stimulation is penile erection, which is produced by increased bloodflow into the erectile tissues, the two corpora cavernosa and the corpus spongiosum. The increased bloodflow results from parasympathetic activity, which causes vasodilatation of penile arteries and occlusion of the penile veins (156). At one time erection was considered to be entirely a cholinergic response (70). It has, however, now been established that the event is also mediated via the autonomic and the somatic nervous system (151). Sympathetic pathways acting on beta-2 and alpha-receptors located in the blood vessels of the corpora cavernosa may also play a part. Detumescence is thought to be brought about by vasoconstriction as a result of sympathetic stimulation of alpha-adrenoceptors.

Erection may be classified as either psychogenic or reflexogenic, depending on the stimulus that evoked the response. Psychogenic erection may result from auditory, olfactory, visual, or imaginary stimuli. The response is mediated by impulses arising from the cerebral cortex and the limbic system to eventually reach both the spinal cord erection centres, the thoracolumbar sympathetic outflow originating around T12 and L1 and the sacral parasympathetic outflow originating around S3, S4 and possibly S2 (73). Reflexogenic erection results from direct sensory stimulation of the penis and is thought to

be mediated through the sacral outflow, which involves parasympathetic, sympathetic and somatic fibres. Somatic sensory impulses, generated by tactile stimulation of the penis or stimuli from the bladder or rectum, travel mainly via the pudendal nerves to the sacral erection center (S2,S4). Efferent parasympathetic impulses arising from the sacral erection center travel via the nervi erigentes (pelvic splanchnic nerve) to the vascular bed of the penis, resulting in vasocongestive response and erection.

The physiological control mechanism for penile erection is dependent on a number of systems and thus erectile dysfunction may result from a variety of psychogenic, neurogenic, vascular and hormonal causes, some of which may be induced by drug therapy. The use of reserpine is associated with mental depression, and loss of libido and impotence have been frequently reported (73,157). Drugs with anticholinergic activity and adrenergic neurone blocking drugs are capable of inducing erectile dysfunction (150). Decreased erection may also arise from excessive sympathetic stimulation, which by increasing muscle blood flow may possibly divert blood away from the penis (73).

2.7.3.2 The plateau phase: Lubrication

During sexual stimulation and with the promotion of erection, secretions from Cowper's glands, glands of Littre' and the prostate flow through the urethra to aid in the lubrication of coitus (153). These secretions,

together with the sperm, constitute semen. However, most of the secretions for lubrication are produced by the female, rather than the male (70). Generally, this phase of the sexual response is more susceptible to adverse drug effects in the female than in the male. Stevenson and Umstead have cited reports of either dimunition or inhibition of vaginal lubrication during treatment with thiazide diuretics and spironolactone (153). The plateau phase is stimulated predominantly through the parasympathetic nervous system (156).

2.7.3.3 The orgasmic phase: Emission and ejaculation

Emission involves the passage of semen into the posterior urethra. At the same time relaxation of the muscle wall of the bladder and constriction of the internal urethral sphincter prevents backflow of semen into the bladder. This response is mediated by sympathetic reflexes originating from the thoracolumbar erection center (T12-L3). Stimulation of alpha-adrenergic receptors results in contraction of the smooth muscles of the prostate, seminal vesicles, vas deferens and the ampulla (153).

Ejaculation is the expulsion of semen from the posterior urethra through the urethral meatus. Clonic contractions of the striated bulbocavernosus and ischiocavernosus muscles result in ejaculation and the response is mediated by somatic efferents in the pudendal nerves (73).
Decreased emission may result from the blockade of

alpha-adrenergic receptors on the vas deferens and epididymus, whilst blockade of these receptors on the internal urethral sphincter may result in incomplete bladder neck closure and retrograde ejaculation (73).

2.7.3.4 The refractory phase

The refractory phase is the period immediately after the orgasmic phase. During this phase sexual intercourse cannot be initiated by the male. However, this phase is not present in the female and is not a well-researched area as regards to human studies and drug effects (73).

2.8 Description of normal sexual behaviour pattern in male rats

The normal sequence of events during copulation in male rats follow a characteristic pattern which consists of mounts (M), intromissions (I), and ejaculations (E). These components of sexual behaviour occur at fairly regular intervals in a group or "series" and are sometimes referred to as a sexual cycle (158). The copulatory cycle is concluded by a distinctive intromission which results in ejaculation. Ejaculation is followed by a period of copulatory inactivity, the refractory period, after which the male attempts to copulate again. The temporal relationship of these components of sexual behaviour is illustrated in Figure 6.

The male rat is capable of completing a number of sexual

cycles before reaching satiety or exhaustion. Miczek and Barry, in their description of the sexual behaviour of rats, mention that the male rat is capable of attaining about 6 to 10 ejaculations with the same female and complete recovery may take up to 10 to 15 days (159). A brief definition and a description of the standard components of sexual behaviour are given below.

2.8.1 Components of sexual behaviour in male rats

2.8.1.1 Mounts

After a short period of courtship involving sniffing of nasal and ano-genital region the male rat approaches the female rat from the back and starts mounting. The male may mount the female several times without vaginal penetration (159). Mount frequency (MF) is the number of mounts that does not result in intromission in a series. Mount latency (ML) is the time from the start of a test to the first mount or intromission.

2.8.1.2 Intromission

A mount with vaginal penetration that is usually followed by a spontaneous dismount with a backward lunge, is referred to as an intromission. Each intromission lasts approximately 0.3 seconds (160,161). During an intromission, initiation of the leg-kick reflex involuntarily throws the male off the female (161) and is often followed by licking of the penis (158(. Intromission

latency (IL) is the time from the start of a test to the first intromission. The interintromission interval is the time between one intromission and the next intromission in a series. Intromission frequency (IF) is the number of intromissions in a series and this usually ranges between 8 to 15 before the male eventually ejaculates (159).

2.8.1.3 Ejaculation

Ejaculation occurs during the final intromission which is considerably longer lasting: from about 1 to 5 seconds (159,161). The leg kicking reflex is inhibited (161), and the rat often dismounts gradually without a backward lunge. Ejaculation latency (EL) is the period beginning from the first intromission of a series to its terminal ejaculation. In satiety tests ejaculation frequency, the number of ejaculations, may also be reported.

2.8.1.4 Refractory period

This is the resting phase or the period of sexual inactivity and is also referred to as the post ejaculatory intromission latency (PEIL). The PEIL is the time between ejaculation and the commencement of the next intromission. The duration of this period is reported to be approximately 4 to 5 minutes (159).

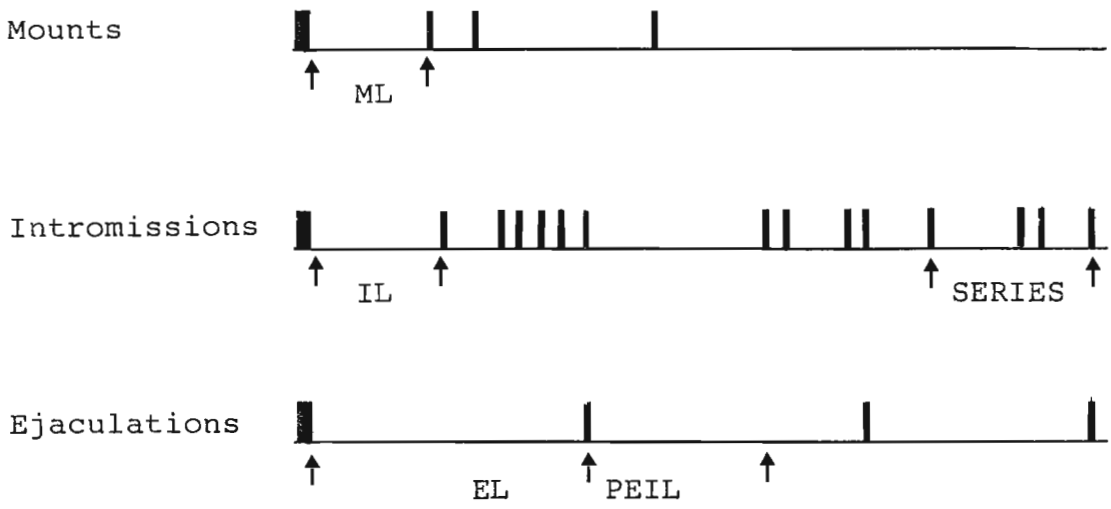


Figure 6. Pattern of copulatory behaviour in normal rats. "▮" indicates commencement of sexual behaviour test. Time moves from the begining of the test at left to right. "|" indicates event mark.

Abbreviations: ML = mount latency; IL = intromission latency; EL = ejaculation latency; PEIL = post ejaculatory intromission latency.

(Modified: Dewsbury DA 1975) (162).

2.9 Effects of drugs on reproductive systems in male rats

The effects of a large number of drugs on sexual behaviour in male rats have been investigated by several workers (158,163). Decreases and increases in frequencies and latencies of the various measures of copulatory behaviour in the male rat have been interpreted as stimulatory (facilitatory) and retardatory (inhibitory) respectively (162).

Bignami investigated the effects of d-amphetamine, LSD-25, strychnine and various anticholinergic agents on mating behaviour in male rats (163). He observed that d-amphetamine and LSD-25 showed facilitative effects at low doses and inhibitory effects at higher doses. Soulairac and Soulairac further investigated the effects of d-amphetamine on sexual behaviour in male rats (158). They found that when the same dose of d-amphetamine which stimulated sexual behaviour was administered daily, sexual behaviour diminished very markedly between the 4th and 5th days and in certain rats disappeared entirely.

2.9.1 Effects on sexual behaviour, reproductive organs and sex hormones

In a study by Leslie and Walker, rats treated with high doses of cimetidine (up to 950 mg/kg/day orally for at least 70 days) showed reductions in gonad, prostate and seminal vesicle weights, but no impairment in mating performance (2). However, the study by Leslie and Walker

did not include direct observations on mating behaviour. A similar study with ranitidine has not demonstrated deleterious effects on reproductive organs (103). In another study, Sodersten et al. treated male rats with flutamide (50 mg/kg/day for 30 days) and found marked reduction in prostate and seminal vesicle weights, but no changes were observed in sexual behaviour components (164). Flutamide, like cimetidine, is a non-steroidal antiandrogen. Sodersten et al. have suggested that the lack of inhibitory effects of antiandrogens on sexual behaviour might possibly be related to the difficulty of antagonising the maintenance of sexual behaviour in experienced rats. The studies by Leslie and Walker, and by Sodersten et al. did not report on serum testosterone and gonadotrophin levels.

Winters et al. treated male rats with much lower doses of cimetidine (50 mg/kg/day for 7 days) and despite marked reductions in prostate and seminal vesicle weights, they found no significant changes in the size of the testes and in the reproductive hormone levels LH, FSH and testosterone (100). They did not conduct mating behaviour tests. However, antiandrogens may alter gonadotrophin and testosterone levels by their influence on the hypothalamic-pituitary-gonadal axis as well as on peripheral androgen target tissues. It has been suggested that flutamide and cyproterone elevate serum LH levels in male rats possibly by blocking the negative feedback control of LH secretion by androgens (100). Contrary to

this, medrogestone and danazol were reported to suppress plasma gonadotrophins by possibly acting as impeded androgens (100).

Any agent that affects the hypothalamic-pituitary-gonadal-axis in addition to disruption of sexual behaviour, may also affect spermatogenesis. The maintenance of normal spermatogenesis was believed to depend on the continued stimulation of pituitary gonadotrophins, since arrest of spermatogenesis was observed after hypophysectomy in rats (165). FSH acts synergistically with LH to stimulate the Leydig cells to produce testosterone (165). It has been subsequently shown that both testosterone and dihydrotestosterone may maintain spermatogenesis in adult hypophysectomized rats (166).

2.10 Mechanisms influencing sexual behaviour in male rats

Recent studies have shown that neurotransmitters and hormones may play a role in the control of sexual behaviour (158,162,165,167). Monoaminergic control of sexual behaviour has been demonstrated by drugs that deplete or increase brain monoamine levels (158,162,171). Depletion of serotonin by p-chlorophenylalanine (158,162,169) and of all monoamines by reserpine and tetrabenazine (162), was reported to facilitate copulatory behaviour in the male rat. On the other hand, elevation in brain monoamine levels by the monoamine oxidase inhibitors iproniazid, nialiamide, and pargyline was reported to

produce retardation of copulatory behaviour (162).

2.10.1 Dopaminergic mechanisms

Dopaminergic involvement on sexual behaviour has been demonstrated with apomorphine and L-DOPA; facilitation of male rat sexual behaviour by stimulation of central dopamine receptors has been reported with these compounds (170,171). Furthermore, it was shown that the facilitatory effects of apomorphine were antagonised by pimozide, a dopamine receptor antagonist (171). Haloperidol and the phenothiazines are potent blockers of dopamine receptors and these drugs have been reported to suppress sexual behaviour in both animals and man (172).

2.10.2 Cholinergic mechanisms

Cholinergic stimulation by low doses of nicotine (tartrate), showed significant facilitation of sexual behaviour (158,162). Anticholinergic agents such as atropine, methylatropine and scopolamine have been reported to inhibit sexual behaviour (163). Scopolamine was found to be the most active (about 50 times more effective than methylatropine) in inhibiting sexual behaviour in the male rat (163). The low sensitivity of methylatropine, a quaternary anticholinergic agent, may be explained by the fact that the quaternary compounds do not penetrate the blood-brain barrier easily (173).

2.10.3 Serotonergic mechanisms

Stimulation of sexual behaviour in male rats has been reported with treatments which deplete brain serotonin levels. Facilitation of sexual behaviour in male rats has been observed after treatment with parachlorophenylalanine, 5,6-dihydroxytryptamine and tryptophan-free diets (168). On the other hand, suppression of sexual behaviour has been observed with drugs that elevate brain serotonin levels. Monoamine oxidase inhibitors such as pargyline, phenelzine and iproniazid inhibit copulatory behaviour in male rats (168). The suppression of sexual behaviour observed with pargyline was reported to coincide with maximal accumulation of brain serotonin concentration (168).

2.10.4 Adrenergic mechanisms

The possibility of a balance between alpha- and beta-adrenoceptor activity in the control of sexual behaviour has been suggested. Evidence for facilitation of sexual behaviour has been provided with stimulation of beta-receptors, while stimulation of alpha-receptors has suggested inhibitory effects (158).

Epinephrine (50 ug/kg) was reported to stimulate sexual behaviour; the number of ejaculations was increased from an average of 3.1 to 5.0 without any change in the refractory period. At the higher dose (100 ug/kg) sexual activity was suppressed completely and the animal usually

fell asleep in the experimental cage (158). Norepinephrine (50 to 100 ug/kg i.p.) completely inhibited sexual behaviour in about 10 to 15 minutes and the effect was reported to last several hours. Moreover, the alpha-adrenergic blockers, dihydroergotamine (0.6 mg/kg i.p.) and dibenamine (0.3 mg/kg i.p.) given 15 minutes before the test, stimulated sexual behaviour by increasing the number of ejaculations, while the beta-blocker, propranolol (0.3 mg/kg i.p.), produced minor stimulatory changes which were not statistically significant (158).

2.10.5 Hormonal mechanisms

Evidence of stimulatory actions of androgens in both neural and target tissues has been demonstrated in castrated animals (167). It has been reported that castration reduces blood testosterone and elevates the pituitary gonadotrophin levels, LH and FSH, in animals (165). Exogenous androgen therapy has resulted in restoration, not only of sexual behaviour, but also of normal suppression of gonadotrophin secretion and maintenance and growth of sex accessory tissues (167).

Before 1968 it was generally believed that testosterone was the major hormone responsible for androgen effects (167). However, it has now been established that dihydrotestosterone (a metabolite of testosterone) is a more potent androgen than testosterone. Furthermore, it has been suggested that testosterone can be metabolically

converted intracellularly to dihydrotestosterone in both neural and peripheral tissues (167).

It has been established that substances with dopaminergic activity inhibit prolactin secretion while substances with dopamine blocking actions elevate prolactin secretion (174). It is believed, firstly, that prolactin diminishes the responsiveness of the male gonads to LH and thus exerts an inhibitory effect on plasma testosterone levels (74) and, secondly, it is suspected that prolactin may inhibit sexual behaviour by antagonising the peripheral actions of testosterone (153).

2.10.6 Influence of locomotor activity

Most studies of copulatory behaviour have not included measures of non-copulatory behaviour, for example locomotor activity. Nevertheless, Sachs & Barfield (175), in an extensive review on the functional analysis of male rat copulatory behaviour, have cited one paper by Malmnas (1973) in which it is mentioned that facilitation or depression of sexual behaviour in animals is not necessarily correlated with locomotor activity. They observed that para-chlorophenylanine sharply depressed locomotor activity and sharply potentiated copulation. Other reports have indicated that drugs may affect locomotion and copulation in the same direction. For example, 5-hydroxytryptamine increases brain serotonin levels leading to decreases in both sexual and motor

activity (172). Similarly, haloperidol has been reported to block aggressive and violent activity in animals and man and also to suppress sexual activity (172).

CHAPTER 3: The influence of cimetidine and ranitidine on sexual behaviour and gonadal function in male rats: Experimental design, materials and procedures

3.1 Introduction

The rat is one of the most widely used laboratory animals for the study of drug-effects on sexual behaviour. The mating behaviour of male rats has been extensively investigated (158-162,176). Early researchers described and analysed animal copulatory behaviour from observations of untamed animals in their natural habitat. However, with recent methodological advances, techniques have been developed for the study of animal copulatory behaviour under controlled conditions in the laboratory (159).

This chapter deals with the design of the experiments and a description is given of the various procedures employed in this study.

3.2 Experimental design

Controlled studies to examine the effects of single doses and subchronic treatment with cimetidine, ranitidine and placebo on sexual behaviour patterns and gonadal function, were undertaken. The subjects of the study were intact adult male albino rats especially selected for their sexual vigour. Ovariectomised, responsive female rats were the sexual stimulus. Motor activity counts were recorded

immediately before conducting the sexual behaviour tests in both the acute and subchronic-dose studies. The experimental designs of both the single-dose and the subchronic-dose behavioural studies are depicted in Schemes 1 and 2 respectively.

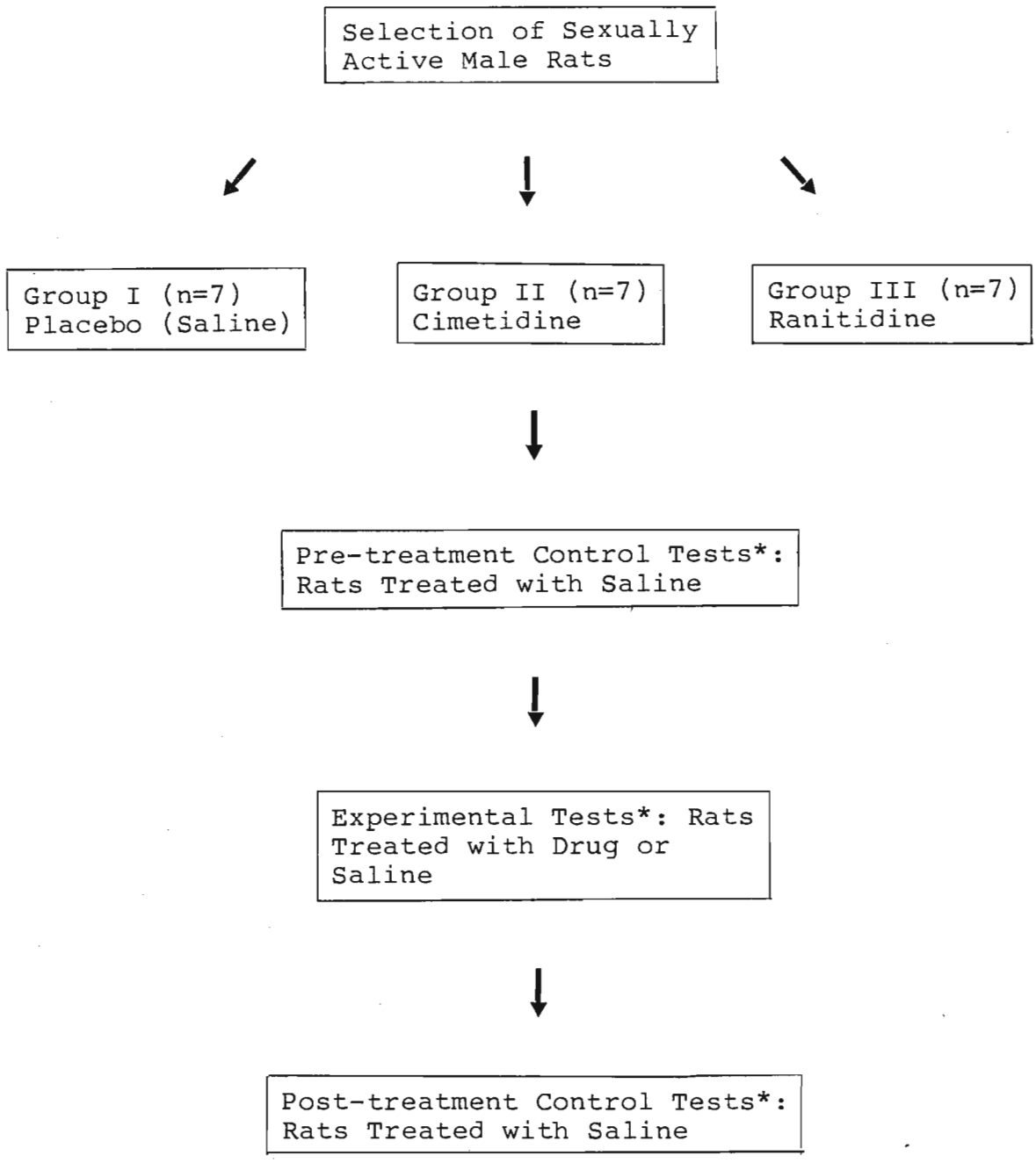
3.2.1 Single-dose design

In designing the single-dose study, pre- and post-treatment behavioural tests were done in all groups of subjects. This design enabled each animal to serve as his own control. The inclusion of a separate group of placebo-treated rats permitted comparison of the performance of independant groups of animals, and also served to indicate the consistency of sexual behaviour in the strain of rats used in this study. The main consideration in designing this experiment was to observe whether the H2-blockers may affect sexual behaviour either by some direct or indirect action. It has been suggested that maximal behaviour effect should be correlated with maximal pharmacological effect of a drug (177). Therefore, on the strength of pharmacokinetic data (section 2.4), the animals in this experiment were observed in sexual behaviour tests 2 hours after dosing; it was presupposed that the drugs would exert their maximal pharmacological effect at about this time.

3.2.2 Subchronic-treatment design

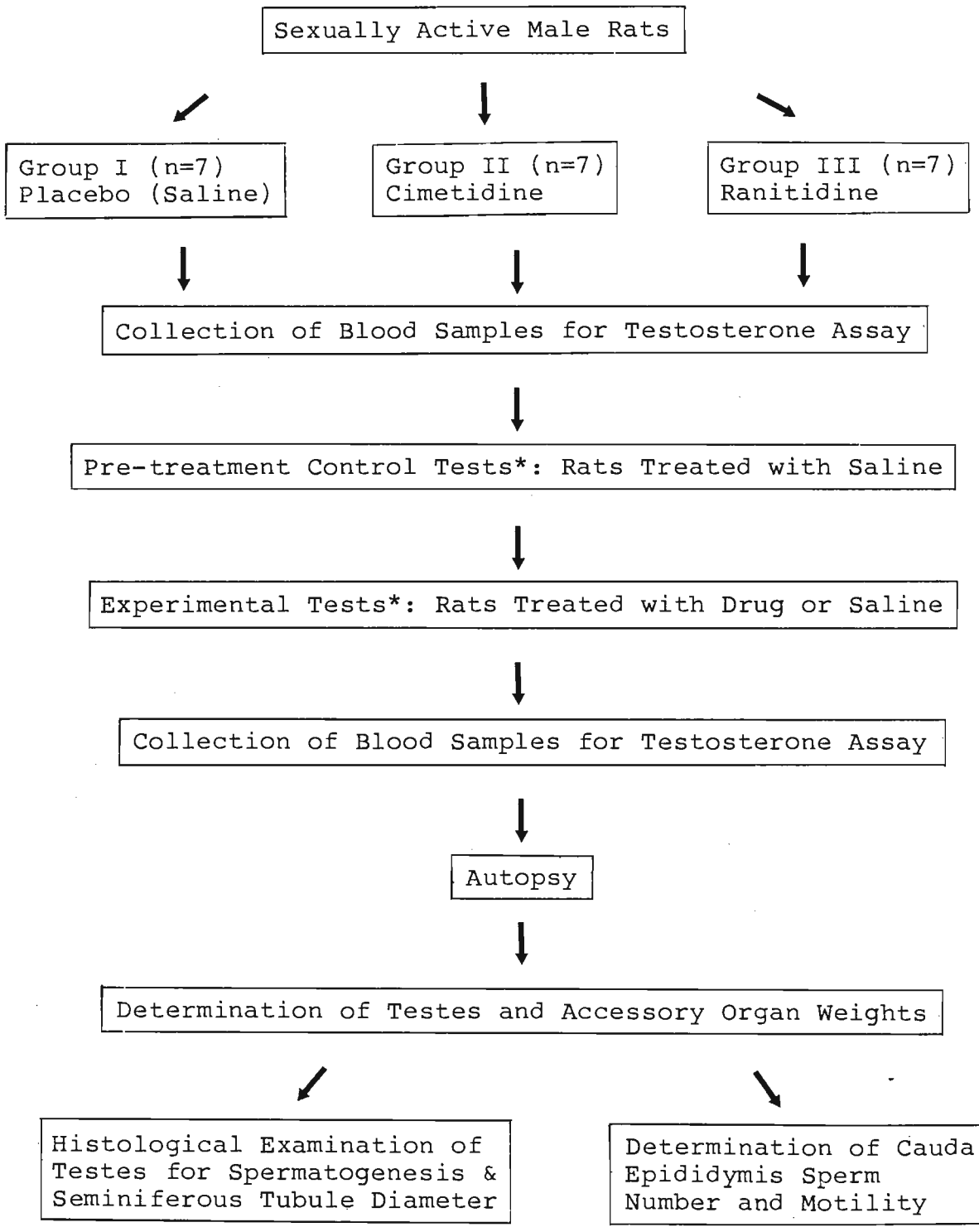
The main emphasis of this design was to detect whether changes in copulatory behaviour may result secondary to possible alterations in testosterone levels or structural impairment to the testes and accessory sex organs. To eliminate any possible direct effect of the drugs on sexual behaviour, the animals were tested four to seven hours after the last treatment. Basal testosterone levels were estimated on blood samples taken before and after the subchronic treatment. Other investigations included an assessment of the effect of cimetidine and ranitidine on testes, prostate and seminal vesicle weights after subchronic treatment. Sperms from the cauda epididymus were enumerated and examined for motility. In addition, the testes were examined histologically and the seminiferous tubule diameter was also measured.

Scheme 1. Schematic representation of experimental design on acute dose sexual behaviour studies in male rats



*Tests which included measurements of motor activity and sexual behaviour observations were done on every third day. The treatments administered are shown in appendix A, Table A1.

Scheme 2. Schematic representation of experimental design on subchronic dose sexual behaviour studies and investigations related to reproductive function in male rats



*: Tests which included measurements of motor activity and sexual behaviour observations, were conducted on every third day. The treatments administered are shown in appendix B, Table B1.

3.3 Materials

3.3.1 Subjects

The subjects of the study were intact, adult male albino rats, a commercial breed originally derived from the Wistar strain, obtained from a local supplier, The Natal Institute of Immunology. The weights of the animals averaged 350 g. These animals were selected from a larger population on the basis of their performance in pre-experimental sexual behaviour tests. Animals that consistently showed a high level of sexual activity - that is, commenced copulation within 0.25 minutes on introduction to a receptive female - were included as experimental subjects.

3.3.2 Stimulus Female Rats: Ovariectomy

The study of male rat sexual behaviour requires responsive female stimulus rats. The sex hormones in female animals show considerable variation and fluctuate according to the oestrus cycle (159). To achieve uniform responsiveness ovariectomised females were brought into artificial heat by injecting sex hormones before the test.

Mature female rats, weighing about 200g each, were ovariectomised via dorsolateral incisions. Surgery was performed using aseptic technique while the rats were under ether anaesthesia. The ovaries, fallopian tubes and approximately 5mm of the uterine horns were removed. The

procedure for removal of the right ovary is illustrated in Figures 7 to 10. The procedure for removal of the left ovary is similar; after incision, the skin is slid towards the left and the left ovary is finally removed. Three to four weeks after ovariectomy the females were ready for use in the sexual behaviour tests.

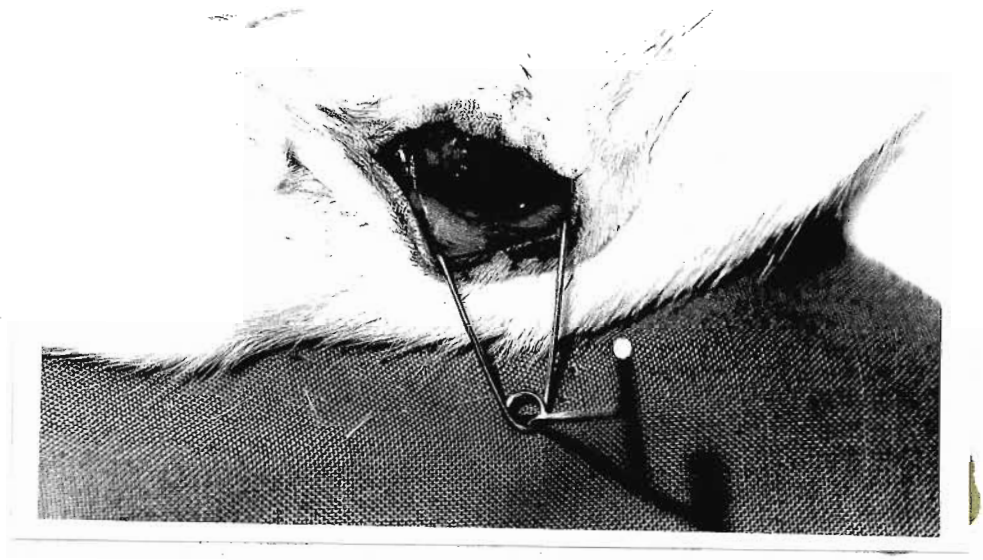


Figure 7. Photograph showing the incised skin on the mid-dorsal surface of the female rat. The skin is pulled towards the right side of the animal with the use of a hook and the peritoneum is exposed.

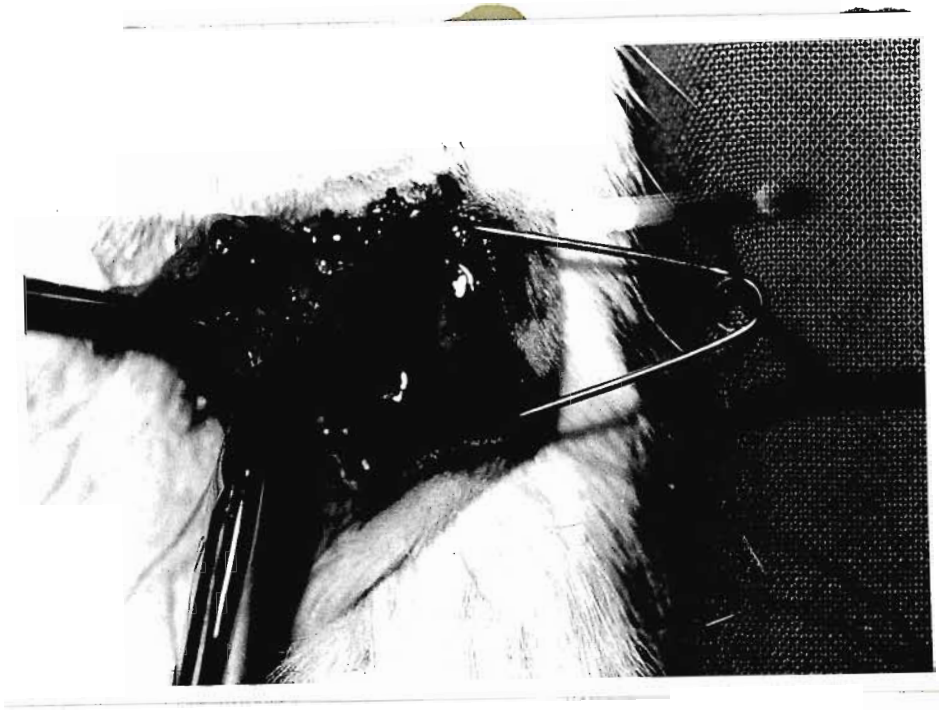


Figure 8. Incision of the peritoneum and removal of ovary.

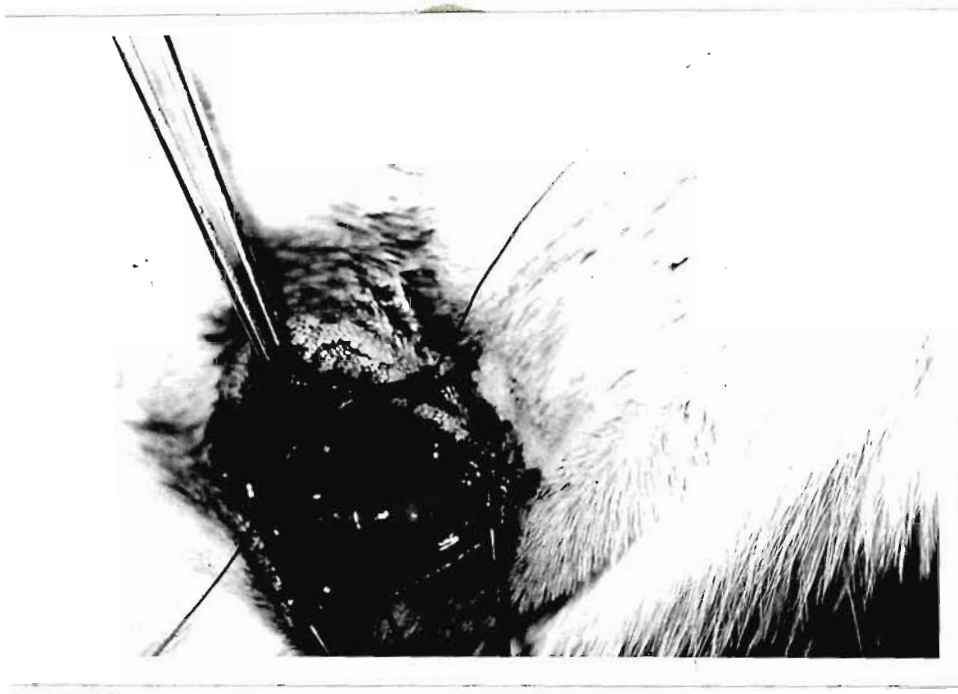


Figure 9. Suture of peritoneum after ovariectomy.

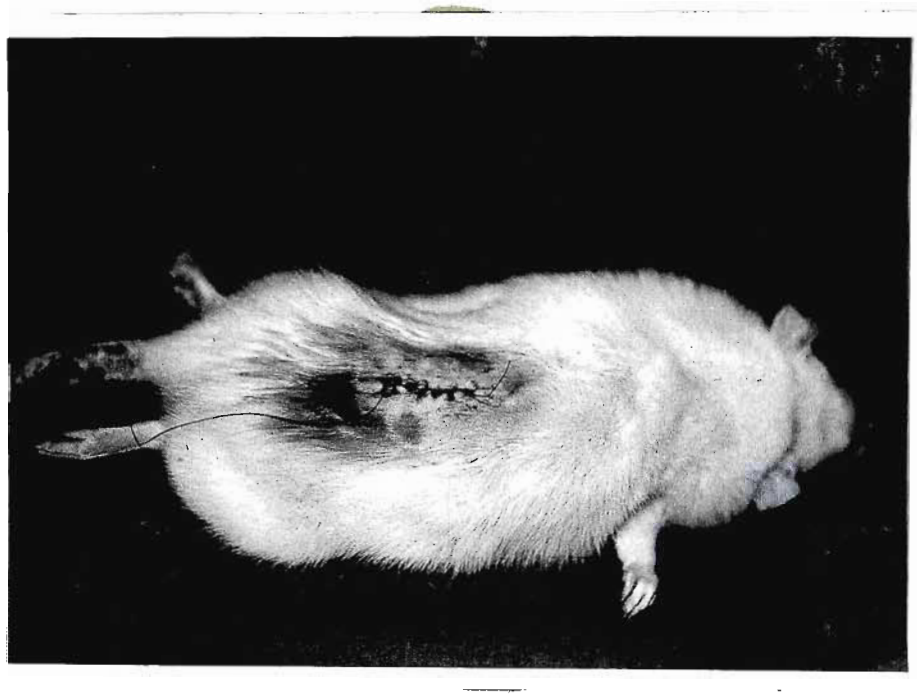


Figure 10. Suture of skin terminates surgical procedure.

3.3.2.1 Induction of oestrus

The sex hormones oestradiol benzoate (doses ranging from 0.02 to 0.1 mg/rat) and progesterone (doses ranging from 0.5 to 1.0 mg/rat) have been used to induce oestrus (159-162). The time intervals between administration of these hormones and the commencement of mating tests have also varied amongst different workers. Oestradiol benzoate has been administered from 48 to 72 hours, and progesterone from 3 to 10 hours before testing. Despite such variations in dosage and time intervals before testing, the main criteria appears to be in the careful selection of receptive female rats.

In this study female rats were made sexually receptive by injecting sub-cutaneously, 0.1mg oestradiol benzoate (Sigma Chemical Co.), in 0.1ml sweet oil 54 hours prior to testing and 0.75mg progesterone (Sigma Chemical Co.), in 0.1ml sweet oil 4 to 6 hours before testing.

3.3.2.2 Selection of receptive female rats

Female rats that were sexually receptive were used as sexual partners. Each female rat was tested in advance with a non-experimental, sexually active male and was selected only if she made a lordosis response and allowed mounting with intromission within 0.25 minutes, without displaying aversive or aggressive behaviour towards the male.

3.3.3 Housing of animals

The subjects were housed separately from the females and were kept five or six to a cage in a darkened room with forced ventilation. Free access to food (Epol mice cubes) and water was possible at all times except during behavioural tests. The ambient room-temperature was maintained between 19 to 23 degrees centigrade. Reversed lighting conditions were regulated by an automatic time-switch. White fluorescent ceiling lights were on from 22h00 to 10h00. Rats are nocturnal animals and it has been suggested that their sexual behaviour is more sensitive during the dark portion of the regulated cycle (162). Animals were allowed two to five weeks to become accustomed to laboratory conditions before testing.

3.3.4 Behavioural testing apparatus

The equipment, illustrated in Figures 11, 12 and 13 was used in the behavioural studies.

3.3.4.1 Animal activity monitor

The "Opto-Varimex-3" control unit (Columbus Instruments, Columbus, Ohio), (Figure 10), was used for measuring motor activity counts. The monitor consisted of a control unit, in the centre of which was placed a standard perspex animal cage (interior dimensions 390mm x 390mm x 290mm) with a lid.

The animal cage used in this study was modified by

inverting another identical cage over the standard cage (Figure 11). This modification allowed the rats "unrestricted" movement within the cage. In preliminary studies it was observed that some animals persistently pushed against the lid of the cage in an attempt to escape, thus possibly producing erroneous motor activity counts.

Horizontal activity - that is the amount of movement of the animal over the surface of the floor - was recorded by the interruption of infrared beams that passed from one wall to the opposite wall at a height of about 35 mm from the bottom of the cage. Vertical movements, consisting of rears and jumps, were monitored by two infrared vertical sensors, an emitter and a detector, hung at a height of 125mm from the bottom of the cage on opposite walls. Separate electronic counters located on the front panel of the "Opto-varimex" displayed the horizontal and vertical animal activity counts.

3.3.4.2 Sexual behaviour observation cage

The observation cage (Figure 12) was a circular box with a wooden base 450 mm in diameter, surrounded by a clear perspex wall 300 mm high. The floor was covered with fresh sawdust on each test day.

3.3.4.3 Recorder

A single channel JJ Instruments CR650S recorder (Figure 13) with a time and event marker was used for recording the various components of sexual behaviour.

3.3.5 Drugs investigated

The drugs investigated were cimetidine (Smith Kline and French Ltd), and ranitidine hydrochloride (Glaxo Group Research Ltd). The injections, 200mg cimetidine/2ml (Tagamet(R)) and 50mg ranitidine (as hydrochloride)/5ml (Zantac(R)) were used.

Figure 11. Animal activity monitor: "Opto-Varimex-3"

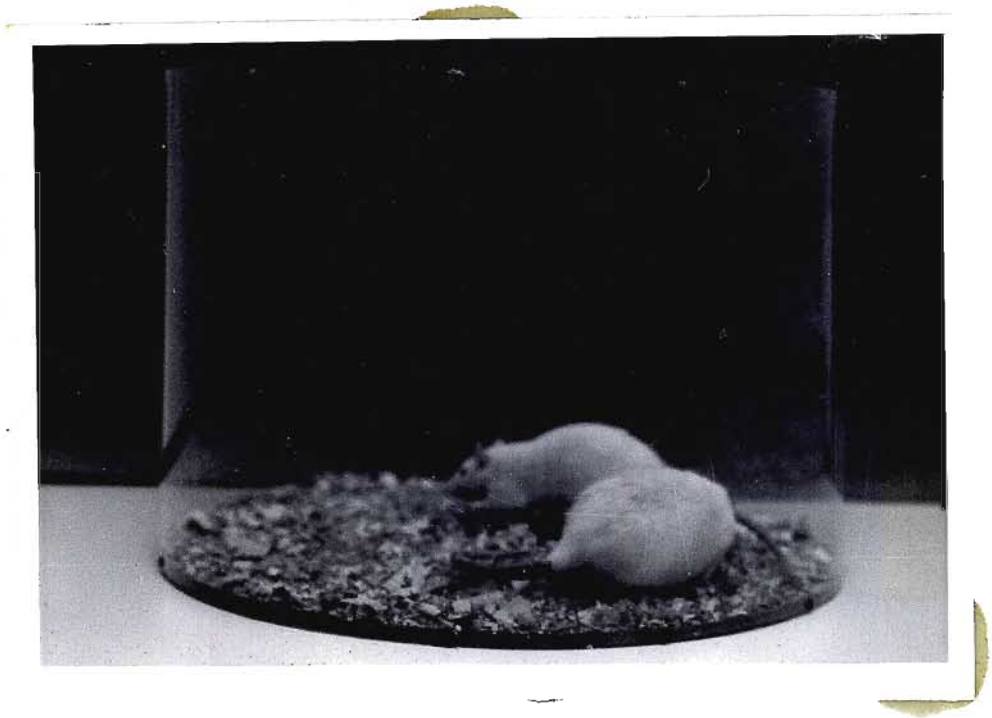


Figure 12. Sexual behaviour observation cage.

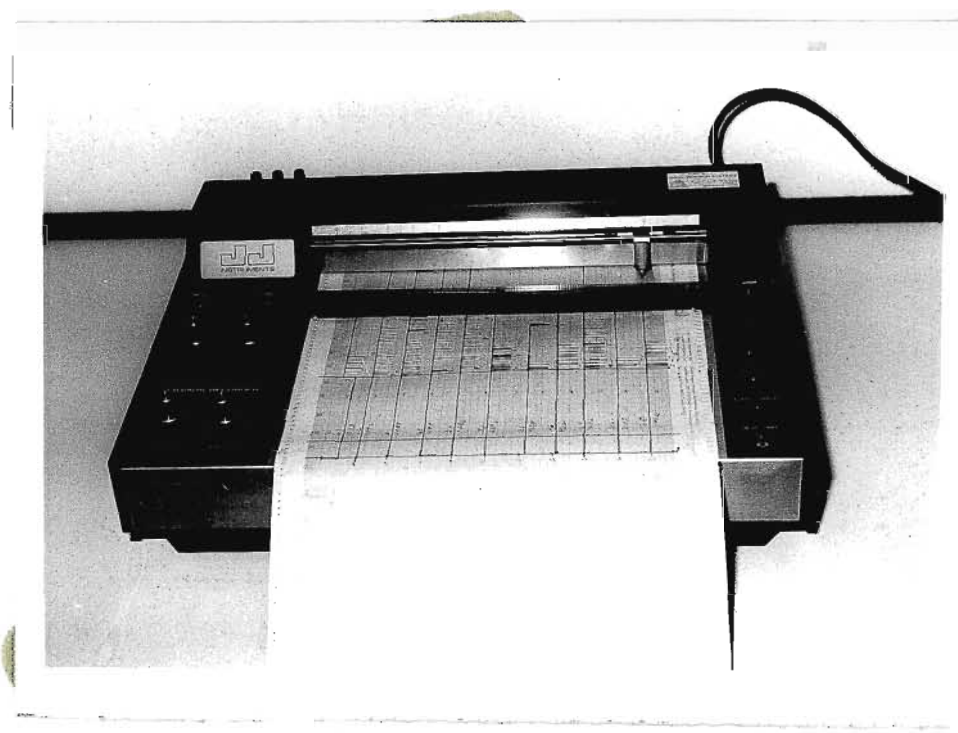


Figure 13. Recorder: "JJ Instruments CR650S Recorder", showing a recording of motor activity and sexual behaviour components.

3.4 General procedures

Tests for locomotor activity and sexual behaviour were conducted in a dimly lit room. A 15 watt bedside lamp placed approximately 4 meters away from the test apparatus provided sufficient lighting for accurate observations. To avoid possible influence on the order of testing effects, the different treatment groups were equally distributed over the entire testing period. In order to minimise circadian effects on behaviour, tests were always commenced during the beginning of the dark cycle and were done during the same time of the day for each test session. Two days intervened between tests.

3.4.1 Measurement of motor activity

Animal movements were measured by an "Opto-Varimex-3" activity monitor. Motor activity consisting, firstly, of animal movements on the surface of the floor of the activity cage were recorded as horizontal activity counts; and secondly, animal movements comprising mainly of rears and on rare occasions, a few jumps performed by some rats, were recorded as vertical activity counts. The number of interruptions of infra-red light beams was recorded as activity counts on digital counters. In order for the rats to adapt to the motor activity counting apparatus, they were kept for a one minute period, in the counting cages before the horizontal and vertical activity counters were reset. Rats were placed in the centre of the cage and counts were recorded for a period of 4 minutes.

Rearing responses were defined by the number of times the rat stood on its hind limbs with its head raised at least 125 mm above the floor of the cage. Jumps above this height were included in the rearing counts.

3.4.2 Sexual behaviour tests

Mating behaviour was observed and recorded after measuring the motor activity counts. The standard measures of sexual behaviour (section 2.8) as described by Bignami (163) and Ahlenius et al., (169) were recorded.

The male rats were removed from the motor activity cage and placed in the centre of the sexual behaviour cage. After a two minute period of adaptation to the observation cage, a receptive female rat was introduced as far away from the male as possible; preferably outside his line of vision. Observations were made about 1 meter away from the sexual behaviour cage. The presentation of the female rat to the observation cage coincided with the activation of the paper speed switch on the recorder. The paper speed was set at 20 mm/minute. Sexual behavioural components consisting of mounts, intromissions and ejaculations were identified by observation and recorded manually by operating a time and event marker on the recorder (Figure 13). Sessions lasted until the first intromission of the second series, at the end of which the rats were removed to their respective home cages. A different female rat was used with each male partner.

3.4.2.1 Measures of sexual behaviour

The following response frequencies and latencies (min) were obtained by analysing the sexual behavioural components which were recorded during the mating tests:-

i. Mount Frequency (MF): The number of mounts with pelvic thrust but without vaginal penetration before ejaculation.

ii. Intromission Frequency (IF): The number of mounts with vaginal penetration before ejaculation.

iii. Mount Latency (ML): Time between presentation of the female and the first mount, either with or without intromission.

iv. Intromission Latency (IL): Time between presentation of the female and the first intromission.

v. Ejaculation Latency (EL): Period between the first intromission and ejaculation.

vi. Post Ejaculatory Intromission Latency (PEIL): Interval between ejaculation and the first intromission of the next series of copulations.

3.5 Procedure for single-dose behavioural studies

3.5.1 Subjects

The subjects of this study were 21 sexually active male rats selected as described in section 3.3.1. They were randomly divided into three groups (n=7, for each group)

and numbered on their tails with indelible ink from 1 to 21. Group I was the placebo group while groups II and III were the drug-treatment groups.

3.5.2 Schedule of drug treatment

The drug and placebo dosages used are indicated in appendix A, Table 1. Multiples of equipotent doses of cimetidine and ranitidine (in terms of treating Zollinger-Ellison syndrome patients) were used. Based on dosage information from Drugdex (R), a drug information retrieval system (178), the maximum starting dose of cimetidine was calculated to be 8.57 mg/kg (single dose/weight of average man: 600mg/70kg = 8.57mg/kg) and for ranitidine 2.142 mg/kg (150mg/70kg = 2.142mg/kg). All treatments were administered intraperitoneally 2 hours before testing began. For the pre-treatment control tests the three groups of animals were injected with saline. After the base-line sessions rats in group I continued to receive saline injections. The volumes of saline administered in each test session were adjusted to approximate the volumes of drug solutions administered in the drug-treatment groups. Groups II and III received single doses of cimetidine and ranitidine respectively. After treatment the rats were returned to their respective home cages until tests commenced.

3.5.3 Measurement of motor activity and sexual behavioural components

Two hours after treatment motor activity counts were recorded and immediately thereafter the animals were observed in mating behaviour tests. All tests commenced at 10h30 on each test day. The first and the last motor activity and mating behaviour tests of the series were control tests. With the exception of the aforementioned, the experimental procedures were as described in section 3.4, under general procedures.

3.6 Procedure for subchronic-dose behavioural study

3.6.1 Subjects

The animals used in this study were the same animals which were used in the single-dose behavioural studies. A rest period of 6 weeks was allowed to dissipate any drug effects before the animals were used. During this period the rats were deprived of sexual contact and apart from routine husbandry they remained undisturbed. After the rest period the rats were pooled and regrouped by random selection into 3 groups, (n=7 for each group); group I being the control group and groups II and III being the cimetidine- and ranitidine-treated groups respectively. The rats were numbered with indelible ink from 1 to 21. Individual rats belonging to a particular group are identified in appendix B, Table B1.

3.6.2 Schedule of drug treatment

The drug and placebo preparations used are shown in Table B1, appendix B. Drug dosages were calculated on the same basis as for the single dose study, but treatments were administered daily in 3 equal doses at 8 hourly intervals by the intraperitoneal route. The first dose was administered at 14h00. For the initial base-line motor activity and sexual behavioural measurements all three groups of animals were injected with saline. After the base-line sessions rats in group I continued to receive saline injections; the volumes of solution administered in subsequent test sessions were adjusted to approximate the volumes of drug solution administered in the drug-treatment groups. Groups II and III were treated with cimetidine and ranitidine respectively. Dosages were doubled after every 2 test sessions. After dosing the animals were returned to their respective home cages until tests began.

3.6.3 Measurement of motor activity and sexual behavioural components

Four to seven hours after dosing, motor activity counts were recorded and the animals were then observed in sexual behaviour tests. The first motor activity and mating behaviour test of the series was a control test. Tests were done between 10h30 and 14h00. With the exception of the aforegoing, the experimental procedures for these tests were as described in section 3.4, under general procedures.

3.7 Procedures for additional investigations related to gonadal function

3.7.1 Radioimmunoassay of serum testosterone

Radioimmunoassay provides one of the most sensitive techniques in the quantitative analysis of serum testosterone. The procedure employed in this study was based on the competitive binding principles of radioimmunoassay as developed by Yallow and Berson (179).

3.7.1.1 Collection of blood samples

Blood samples were collected between 09h00 and 12h00 by the tail vein route before the commencement of the subchronic-dose behavioural tests and by cardiac puncture one day after the termination of the behavioural tests. Blood (0.5 to 1.0 ml) was collected in plain tubes from both control and treatment groups, allowed to clot at room temperature for about 30 minutes, and centrifuged for 10 minutes. Serum was withdrawn and kept at -20 degrees centigrade until assayed for testosterone levels.

3.7.1.2 Assay procedure

Assays of testosterone were done on duplicate samples of serum using the immunochem method (180). All samples from the pre- and post-chronic-dose behavioural study, together with 6 testosterone standards (0ng/ml; 0.2ng/ml; 0.6ng/ml; 2.0ng/ml; 6.0ng/ml and 20.0ng/ml) and control serum, were analysed in a single assay.

Test samples, standards, control serum, anti-testosterone coated tubes and testosterone-125I were allowed to reach room temperature before use. Coated tubes were marked to identify standards, controls and test specimens. Ten microlitres each of standard, control, and test serum were pipetted into coated tubes. To each tube 1.0 ml testosterone-125I was added, vortex-mixed and incubated at 37 degrees centigrade for 120 minutes. The contents of each tube was emptied into an appropriate radioactive waste container and then drained onto a paper towel. All tubes were counted for one minute in a "Berthold Multicrystal Gamma Counter LB2100". Data from the gamma counter was transmitted to a "Berthold Printer, Model 43" and used in plotting the standard curve (Appendix D, Figure D1) and for the determination of the levels of testosterone in the samples.

3.7.1.3 Calculations

i. Average count per standard sample: Since the tests were done in duplicate the average count for each standard sample was determined.

ii. %B/Bo for standard samples: %B/Bo was calculated by expressing the average counts for each non-zero standard as a percentage of the zero standard.

iii. Standard curve: A standard curve of %B/Bo against standard concentrations was plotted (Appendix D, Figure D1).

iv. %B/Bo for test samples: The %B/Bo was determined for each test sample and the testosterone concentration was read from the standard curve (Appendix D, Table D1). Testosterone concentrations were expressed in ng/ml.

3.7.2 Determination of testes and accessory sex organ weights

The animals were autopsied after light etherization and decapitation on the day following the last behavioural test. The testes, prostate and seminal vesicles of each animal were removed, trimmed of any extraneous tissue and weighed.

3.7.3 Sperm analysis

3.7.3.1 Determination of sperm motility

The right cauda epididymis was removed and sperm squeezed out of it and evenly distributed with a pasteur pipette in a petri dish containing 5 ml of phosphate buffered saline. A drop of the suspension was placed on a microscope slide and sperms were counted in 5 random fields at a magnification of 40x. Motile and non-motile sperms were counted and the number of motile sperms was expressed as a percentage of the total number of sperms. Sperm motility was ascertained immediately after death.

3.7.3.2 Determination of sperm numbers

The original sperm suspension was diluted 1 in 10 in phosphate buffered saline containing a few drops of formalin. Sperms were counted on a modified Neubauer haemocytometer.

3.7.4 Preparation of testis for histological examination

Testes were fixed in Bouin-Hollande fixative for 48 hours. The tissues were embedded in paraffin wax and sectioned at 8 microns. Sections were stained with haematoxylin and eosin for histological evaluation of qualitative spermatogenesis and determination of the average seminiferous tubule diameter. A microscope micrometer was used to measure the diameter of seminiferous tubules (Figure 14).

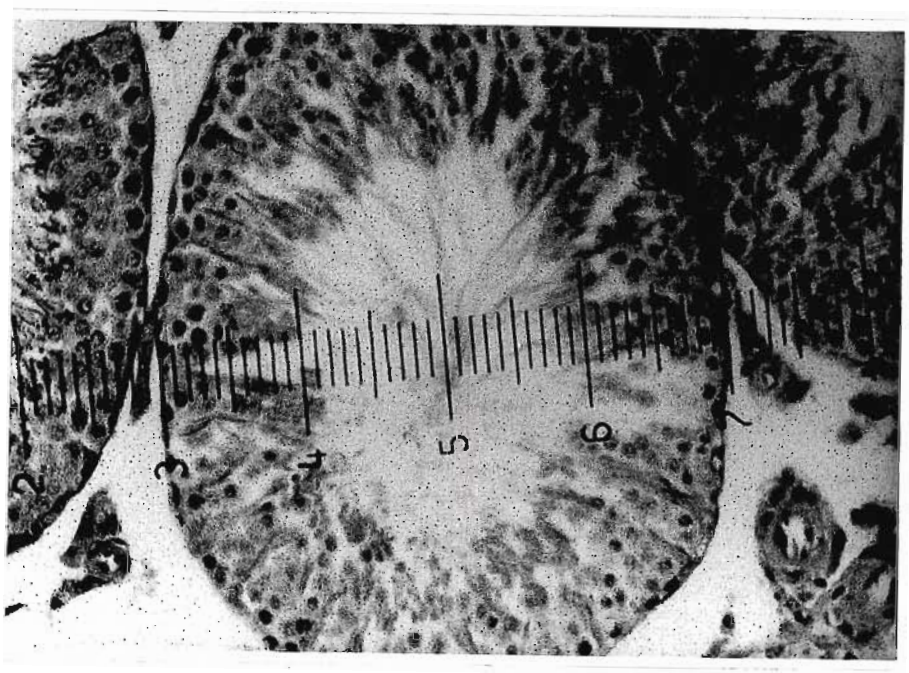


Figure 14. Photomicrograph of a cross section of a testis showing micrometer grid readings across a seminiferous tubule (H and E Stain, x250).

CHAPTER 4: Results

In all the behavioural experiments described, repeated observations were made on the same animals in control and treatment tests. The Wilcoxon matched-pairs signed ranks test was employed for statistical analysis of significance. For the studies on additional investigations the Mann-Whitney U test was used for comparisons between groups. All decisions were based on one-tailed probabilities.

4.1 Single-dose behavioural studies

Detailed data on mating performances and locomotor activity of individual male rats after treatment with single doses of cimetidine, ranitidine and placebo are tabulated in appendix A. Sexual behavioural components, consisting of mount latency (ML), intromission latency (IL), mount frequency (MF), intromission frequency (IF), ejaculation latency (EL) and post ejaculatory intromission latency (PEIL) are presented in Tables A1 to A6. Tables A7 and A8 contain data on indices of locomotor activity, namely horizontal and vertical activity counts.

4.1.1 Effects on components of sexual behaviour

The results (group medians) for the effects of cimetidine, ranitidine and placebo on the various components of sexual behaviour, ML, IL, MF, IF, EL, PEIL are summarised in Table 7.

Table 7 Effects of various intraperitoneal doses of cimetidine, ranitidine and placebo on sexual behaviour in male rats

Drug Treatment	Sexual behaviour components*					
	MF	IF	ML	IL	EL	PEIL
			Group I: Placebo			
(mls saline/rat)						
0.50	1	8	0.20	0.25	3.35	3.95
0.50	3	9	0.25	0.25	2.65	4.30
0.80	2	8	0.20	0.20	2.45	4.05
1.25	2	9	0.10	0.20	1.60	4.00
1.60	0	8	0.15	0.15	1.70	3.90
2.00	2	7	0.10	0.15	2.60	4.35
2.50	3	8	0.05	0.20	3.05	4.30
2.50	1	8	0.15	0.20	1.85	4.40
			Group II: Cimetidine			
Pr-TCT 0.50**	2	8	0.15	0.20	3.40	4.65
Cimetidine (mg/kg)						
8.57	2	8	0.20	0.25	1.95	5.15
85.70	2	8	0.15	0.20	2.35	5.20
128.60	1	6	0.10	0.20	1.10$	4.60
171.40	1	6	0.15	0.20	1.65	4.85
214.40	1	6	0.10	0.20	2.15	5.25
257.10	1	6	0.20	0.20	2.30	5.75$$
Po-TCT 2.5**	1	6	0.15	0.20	1.20	4.55
			Group III: Ranitidine			
Pr-TCT 0.5**	1	7	0.15	0.20	2.40	5.10
Ranitidine (mg/kg)						
2.143	1	8	0.15	0.20	2.45	4.55
21.430	1	7	0.02	0.25	2.15	4.15
32.150	1	7	0.15	0.15	2.40	4.60
42.860	0	7	0.10	0.10	1.05	4.40
53.580	1	6	0.15	0.15	2.05	4.75
64.290	1	5	0.20	0.25	1.85	4.50
Po-TCT 2.5**	1	7	0.15	0.20	2.6	3.95

* : Each value is the median of 7 observations. Latencies in minutes.
**: Denotes volume(mls) of saline administered/rat.
Abbreviations used: MF= mount frequency; IF= intromission frequency; ML= mount latency; IL= intromission latency; EL= ejaculation latency; PEIL= post ejaculatory intromission latency; Pr-TCT= pre-treatment control test; Po-TCT= post treatment control test.
$: Significantly reduced from pre-treatment control test ($p<0.01$).
$$:Significantly increased from pre-treatment control test ($p<0.05$).

The results show that throughout the dosage range, cimetidine and ranitidine showed no effect on ML, IL, MF, and IF.

The EL after treatment with cimetidine at the 128.6 mg/kg dose was significantly reduced ($p<0.01$) when compared to the pre-treatment control tests. With increasing doses of cimetidine a slight but progressive increase in the EL was noted and on challenge with saline, the EL decreased markedly (in 5 of 7 rats). The reduction in the EL after rechallenge with saline was not statistically significant.

The median PEIL, after treatment with cimetidine, was elevated after most of the doses employed, with the exception of the 128.6 mg/kg dose after which the PEIL reverted to almost normal levels. However, progressive increase in the PEIL was observed from 171.4 mg/kg up to 257.1 mg/kg; the increase being significant ($p< 0.05$) at the 257.1 mg/kg dose level when compared to the pre-treatment control levels. After treatment with ranitidine and placebo the PEIL remained fairly consistent throughout the dosage ranges employed.

The sexual behaviour of rat No.9, cimetidine group (Appendix A, Table A5.) deserves particular reference. The EL of this animal was markedly reduced at the 128.6 mg/kg dose (2.05 minutes) and dramatically increased with increasing doses of cimetidine; at the highest dose of cimetidine studied, 257.1 mg/kg, the EL increased almost five fold (11.65 minutes) and the PEIL also increased (9.8

minutes). Furthermore, on withdrawal of cimetidine and challenge with saline, the EL and the PEIL were markedly reduced (2.60 and 6.10 minutes respectively); These levels were below the pre-treatment control levels. After treatment with ranitidine the EL remained fairly consistent throughout the dosage range used.

4.1.2 Effects on locomotor activity

The results on locomotor activity consisting of horizontal and vertical activities are summarised in Table 8. Both the horizontal and vertical activities were significantly ($p < 0.02$) reduced after treatment with cimetidine, 214.4 and 257.1 mg/kg. Locomotor activity in rat No.9 was markedly reduced, almost 50%, at the high dosage of cimetidine.

Equipotent doses of ranitidine, 53.58 and 64.29 mg/kg, also reduced locomotor activity, but to a lesser extent than cimetidine. At the 53.58 mg/kg dose only the vertical activity was significantly depressed ($p<0.025$), whereas at the higher dose, 64.29 mg/kg, both the horizontal and vertical activities were markedly depressed ($p<0.05$).

Table 8 Motor activity in male rats prior to sexual behaviour observations after treatment with cimetidine, ranitidine and placebo

Drug	Motor activity (counts/min)*	
Treatment	HAC	VAC
	Group I: Placebo	
(mls saline/rat I.P.)		
0.50	452 +/- 38	11 +/- 1.6
0.50	455 +/- 76	14 +/- 2.3
0.80	507 +/- 29	23 +/- 1.9
1.25	453 +/- 37	19 +/- 2.3
1.60	426 +/- 62	19 +/- 2.8
2.00	468 +/- 50	18 +/- 3.4
2.50	470 +/- 65	21 +/- 2.7
2.50	485 +/- 35	21 +/- 2.1
	Group II: Cimetidine	
Pr-TCT		
0.50**	357 +/- 29	10 +/- 1.1
Cimetidine (mg/kg I.P.)		
8.57	440 +/- 55	15 +/- 1.8
85.70	470 +/- 63	20 +/- 2.9
128.60	393 +/- 39	17 +/- 2.9
171.40	400 +/- 73	17 +/- 2.8
214.40	313 +/- 84$	9 +/- 2.1$
257.10	272 +/- 96$	10 +/- 3.0$
Po-TCT		
2.5**	467 +/- 81	19 +/- 3.5
	Group III: Ranitidine	
Pr-TCT		
0.5**	533 +/- 67	19 +/- 2.0
Ranitidine (mg/kg I.P.)		
2.143	488 +/- 76	20 +/- 3.3
21.430	505 +/- 68	17 +/- 2.8
32.150	503 +/- 51	22 +/- 3.1
42.860	538 +/- 69	21 +/- 3.1
53.580	464 +/- 71	16 +/- 3.4$$
64.290	447 +/- 56$$$	16 +/- 3.3$$
Po-TCT		
2.5**	529 +/- 89	23 +/-4.6

Abbreviations used: HAC= horizontal activity counts; VAC= vertical activity counts; Pr-TCT= pre-treatment control tests; Po-TCT= post treatment control test.
*: Each value is the mean +/- SEM of 7 measurements.
**:Denotes volume(mls) of saline administered/rat.
$: Significantly lower than post-treatment control test, ($p<0.01$).
$$:Significantly lower than post-treatment control test, ($p<0.025$).
$$$:Significantly lower than post-treatment control test, ($p<0.05$).

4.2 Subchronic-dose behavioural studies

Detailed data on mating performances and locomotor activity of individual male rats during subchronic treatment with increasing, graded doses of cimetidine, ranitidine and placebo are tabulated in appendix B. Sexual behaviour components, consisting of mount latency (ML), intromission latency (IL), mount frequency (MF), intromission frequency (IF), ejaculation latency (EL) and post ejaculatory intromission latency (PEIL) are presented in Tables B1 to B6. Tables B7 and B8 contain data on indices of locomotor activity, namely horizontal and vertical activity counts.

4.2.1 Effects on components of sexual behaviour

The results (group medians) for the effects of cimetidine, ranitidine and placebo on the various components of sexual behaviour, ML, IL, MF, IF, EL, PEIL are summarised in Table 9. The results show that throughout the treatment period with cimetidine, ranitidine and placebo the sexual behaviour pattern remained fairly consistent. No significant changes were observed in any of the sexual behavioural components when compared to pre-treatment control tests.

4.2.2 Effects on locomotor activity

The results on locomotor activity are summarised in Table 10. No significant changes in either the horizontal or vertical activities were recorded during treatment with cimetidine, ranitidine or placebo.

Table 9. Sexual behaviour components in male rats during subchronic treatment with cimetidine, ranitidine and placebo

Drug Treatment	Sexual behaviour components* MF	IF	ML	IL	EL	PEIL
			Group I: Placebo			
(mls saline/-rat/day)						
0.75**	1	6	0.17	0.20	1.43	3.45
0.75**	1	5	0.60	0.60	2.55	3.95
0.75	2	7	0.10	0.15	2.25	3.95
0.75	1	8	0.10	0.10	1.92	3.98
1.50	1	6	0.15	0.15	1.15	3.80
1.50	1	6	0.10	0.10	1.05	3.65
3.00	1	6	0.10	0.10	1.40	3.45
3.00	1	8	0.10	0.10	2.05	4.30
6.00	1	7	0.20	0.20	1.95	4.40
6.00	2	6	0.25	0.25	1.25	4.05
			Group II: Cimetidine			
Pr-TCT						
0.75**	2	7	0.10	0.20	1.70	3.90
0.75**	0	8	0.15	0.15	2.40	4.40
Cimetidine (mg/kg/day)						
85.70	2	7	0.10	0.15	2.75	4.60
85.70	2	7	0.15	0.20	2.05	4.30
171.40	3	9	0.10	0.15	2.65	4.10
171.40	2	9	0.10	0.10	2.80	4.20
342.80	2	7	0.10	0.10	1.95	4.15
342.80	2	8	0.10	0.15	2.25	4.45
685.60	1	8	0.10	0.10	1.75	4.10
685.6	1	8	0.15	0.25	1.90	3.90
			Group III: Ranitidine			
Pr-TCT						
0.75**	2	8	0.20	0.15	1.75	4.40
0.75**	2	6	0.15	0.15	2.15	4.60
Ranitidine (mg/kg/day)						
21.4	4	7	0.15	0.20	4.60	4.35
21.4	2	7	0.15	0.15	2.40	4.60
42.8	1	7	0.15	0.15	2.10	4.15
42.8	2	8	0.10	0.10	2.60	4.80
85.6	1	7	0.10	0.10	2.25	4.00
85.6	1	7	0.15	0.15	3.10	4.55
171.2	1	8	0.15	0.15	1.85	4.50
171.2	2	8	0.10	0 10	2.50	4.40

* : Each value is the median of at least 6 observations. Treatments were administered intraperitoneally in divided doses at 8 hourly intervals. Latencies in minutes.

**: Denotes volume(mls) of saline administered/rat in pre-experimental control tests.

Abbreviations used: MF= mount frequency; IF= intromission frequency; ML= mount latency; IL= intromission latency; EL= ejaculation latency; PEIL= post-ejaculatory intromission latency

Table 10. Motor activity counts in male rats measured prior to sexual behaviour observations during subchronic treatment with cimetidine, ranitidine and placebo

Drug	Motor Activity (counts/min)*	
Treatment	HAC	VAC
	Group I: Placebo	
(mls saline/-rat/day)		
0.75**	416 +/- 46	16 +/- 3.0
0.75**	376 +/- 28	15 +/- 2.4
0.75	336 +/- 31	16 +/- 3.0
0.75	365 +/- 33	20 +/- 4.4
1.50	407 +/- 20	17 +/- 2.3
1.50	401 +/- 33	16 +/- 2.6
3.00	421 +/- 34	20 +/- 1.0
3.00	390 +/- 45	14 +/- 2.5
6.00	396 +/- 45	18 +/- 2.0
6.00	420 +/- 40	18 +/- 2.9
	Group II: Cimetidine	
Pr-TCT		
0.75**	490 +/- 59	20 +/- 1.9
0.75**	461 +/- 57	17 +/- 1.4
Cimetidine (mg/kg)		
85.70	440 +/- 51	20 +/- 2.4
85.70	447 +/- 39	19 +/- 1.7
171.40	533 +/- 63	19 +/- 1.8
171.40	543 +/- 52	21 +/- 2.0
342.80	577 +/- 74	25 +/- 1.7
342.80	506 +/- 47	17 +/- 1.8
685.60	481 +/- 58	18 +/- 2.3
685.60	420 +/- 55	13 +/- 2.3
	Group III: Ranitidine	
Pr-TCT		
0.75**	548 +/- 47	21 +/- 3.4
0.75**	513 +/- 43	21 +/- 3.9
Ranitidine (mg/kg)		
21.40	488 +/- 39	20 +/- 2.5
21.40	530 +/- 57	22 +/- 2.9
42.80	537 +/- 42	23 +/- 3.5
42.80	529 +/- 64	22 +/- 3.9
85.60	541 +/- 44	25 +/- 3.7
85.60	506 +/- 54	18 +/- 2.4
171.20	535 +/- 53	24 +/- 2.9
171.20	487 +/- 58	22 +/- 3.5

*: Each value is the mean +/- SEM of 7 measurements. Treatments were administered intraperitoneally in divided doses at 8 hourly intervals.

**:Denotes volume(mls) of saline administered in pre-experimental control tests.

Abbreviations: HAC= horizontal activity counts; VAC=vertical activity counts; Pr-TCT= pre-treatment control tests.

4.3 Additional investigations related to gonadal function

4.3.1 Effects of cimetidine and ranitidine on serum testosterone levels

Detailed data on basal serum testosterone levels in samples taken before and after subchronic treatment with cimetidine and ranitidine are presented in appendix C, Table C1.

The results, depicted in Figure 15 and Table 11, show that the mean post-treatment serum testosterone levels rose in the placebo and ranitidine groups, but were depressed in the cimetidine group when compared to pre-treatment testosterone levels. These changes in testosterone levels were not statistically significant. However, inter-group comparison between cimetidine and placebo, revealed that the post-treatment testosterone level in the cimetidine group was significantly lower ($p<0.05$) than in the placebo group.

The serum testosterone levels (Appendix C, Table C1.) rose in 5 animals in the control group, 4 in the ranitidine group and in 2 in the cimetidine group.

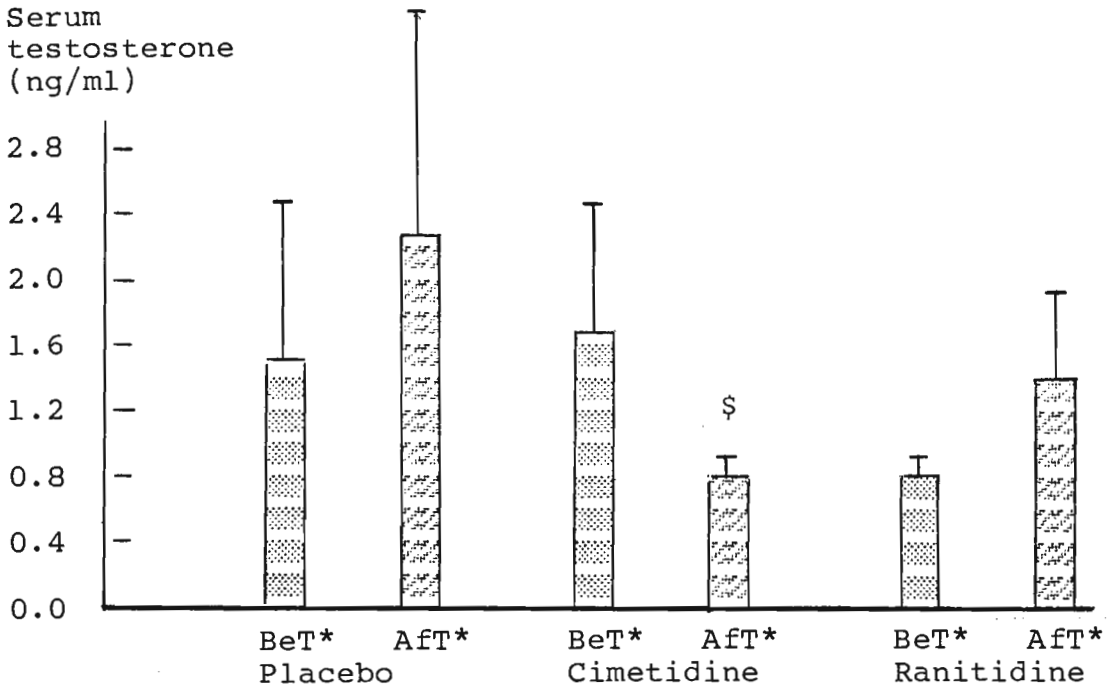


Figure 15. Effect of cimetidine and ranitidine on serum testosterone levels in sexually potent male rats before and after subchronic treatment.

$: Significantly lower than post-treatment placebo group, ($p<0.05$).

*: Treatments were administered as shown in appendix B, Table B1.

Abbreviations: BeT= before treatment; AfT= after treatment.

Table 11. Serum testosterone levels in rats before and after subchronic treatment with cimetidine, ranitidine and placebo

Group I: Placebo (Saline)

Rat No.	Testosterone (ng/ml)	
	Before treatment*	After treatment*
2	1.007	2.470
5	1.458	4.168
8	0.575	0.825
11	0.825	1.736
14	1.124	4.060
17	3.764	2.483
20	1.589	0.522
Mean	1.477	2.323
SEM	0.987	1.325

Group II: Cimetidine

Rat No.	Testosterone (ng/ml)	
	Before treatment*	After treatment*
3	6.445	0.314
6	2.002	1.137
9	0.610	0.765
12	1.250	1.126
15	0.572	1.128
18	0.331	0.357
21	0.513	0.900
Mean	1.675	0.818$
SEM	0.820	0.140

Group III: Ranitidine

Rat No.	Testosterone (ng/ml)	
	Before treatment*	After treatment*
1	0.484	0.714
4	1.021	0.572
7	1.171	0.531
10	1.161	0.402
13	0.945	3.222
16	0.532	1.092
19	0.415	3.267
Mean	0.818	1.400
SEM	0.120	0.480

*: Treatments were administered as indicated in appendix B, Table B1.

$: Significantly lower when compared to after-treatment placebo group, ($p<0.05$).

4.3.2 Effects of cimetidine and ranitidine on testes and accessory sex organ weights

Detailed data on testes, prostate and seminal vesicle weights after subchronic treatment with cimetidine, ranitidine and placebo are presented in appendix C, Table C2.

The results, summarised in Table 12, are presented as the means and standard error of the mean of the actual organ weights. The results show that the testes ($p < 0.025$), prostate ($p<0.05$) and seminal vesicle ($p<0.001$) weights of the cimetidine group were significantly lower than those of the control group.

No changes in organ weights were observed in the ranitidine group.

Table 12. Effects of cimetidine and ranitidine on weights of testes, prostate, and seminal vesicles

	Organ weights (g) mean +/- SEM.		
Organ	Cont	Cimet	Ranit
Testis R.	1.61 0.04	1.47$ 0.03	1.61 0.07
Testis L.	1.63 0.03	1.54$ 0.08	1.59 0.07
Prostate	0.88 0.09	0.69$$ 0.07	0.74 0.06
Seminal vesicle	1.63 0.11	1.04$$$ 0.03	1.76 0.08

Treatments were administered as shown in appendix B, Table B1.

Abbreviations: Cont = control; Cimet = cimetidine;

Ranit = ranitidine.

$: Significantly different from control group, ($p<0.025$).

$$: Significantly different from control group, ($p<0.05$).

$$$: Significantly different from control group, ($p<0,001$).

4.3.3 Effects of cimetidine and ranitidine on epididymal sperm and the seminiferous tubule

Detailed results on sperm motility, sperm counts and seminiferous tubule diameter for individual rats are given in appendix C, Tables C3, C4, and C5 respectively. The results are summarised in Table 13.

4.3.3.1 Effects on sperm motility and sperm numbers

The results (Appendix C, Table C4) on sperm counts showed a marked inter-individual variation. Although the mean sperm count in the cimetidine group was markedly low, this was not statistically different from the sperm concentration of the control group. Sperm motility in the cimetidine and ranitidine groups showed no significant changes when compared with the control group.

4.3.3.2 Effects on seminiferous tubule: Diameter and qualitative spermatogenesis

Histological examination of haematoxylin and eosin stained sections of the testes showed no gross changes in the seminiferous tubules. However, the diameters were slightly reduced in the cimetidine (statistically significant, $p < 0.05$) and ranitidine (statistically not significant) groups (Table 13). Normal seminiferous tubules from one of the control animals is illustrated in Figure 16.

Apparantly normal spermatogenesis was observed in all treatment groups (Figures 17, 18, 19).

Table 13. Effects of cimetidine and ranitidine on epididymal sperm and the seminiferous tubule

Parameter	Control	Cimetidine	Ranitidine
Sperm number (million/cauda)	96.5 +/- 7.6	75.9 +/- 16.5	111.1 +/- 10.6
Sperm motility (%)	59.9 +/- 4.7	66.9 +/- 7.3	64.3 +/- 6.0
Sem Tub Dia (micrometer)	247 +/- 6.6	232 +/- 3.9$	235 +/- 2.3

$: Significantly lower than control group ($p<0.05$).

Abbreviation: Sem Tub Dia = seminiferous tubule diameter.

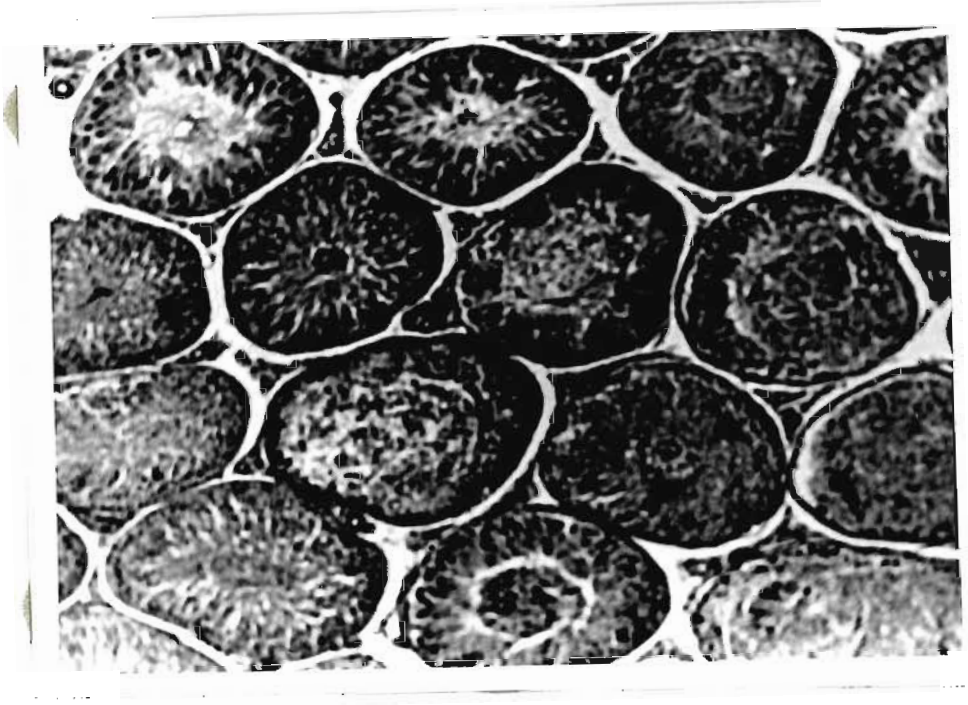


Figure 16. Photomicrograph of testis section from a control rat showing normal seminiferous tubules (H & E Stain, x100).

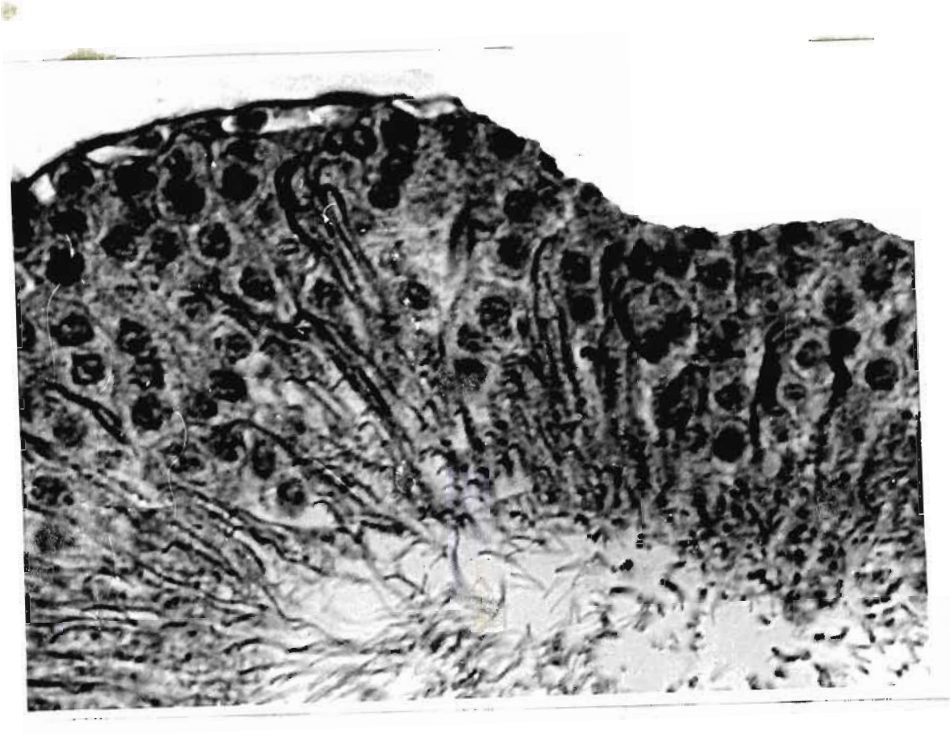


Figure 17. Photomicrograph of part of seminiferous tubule (rat No.2) showing normal spermatogenesis after treatment with saline (H & E Stain, x640).

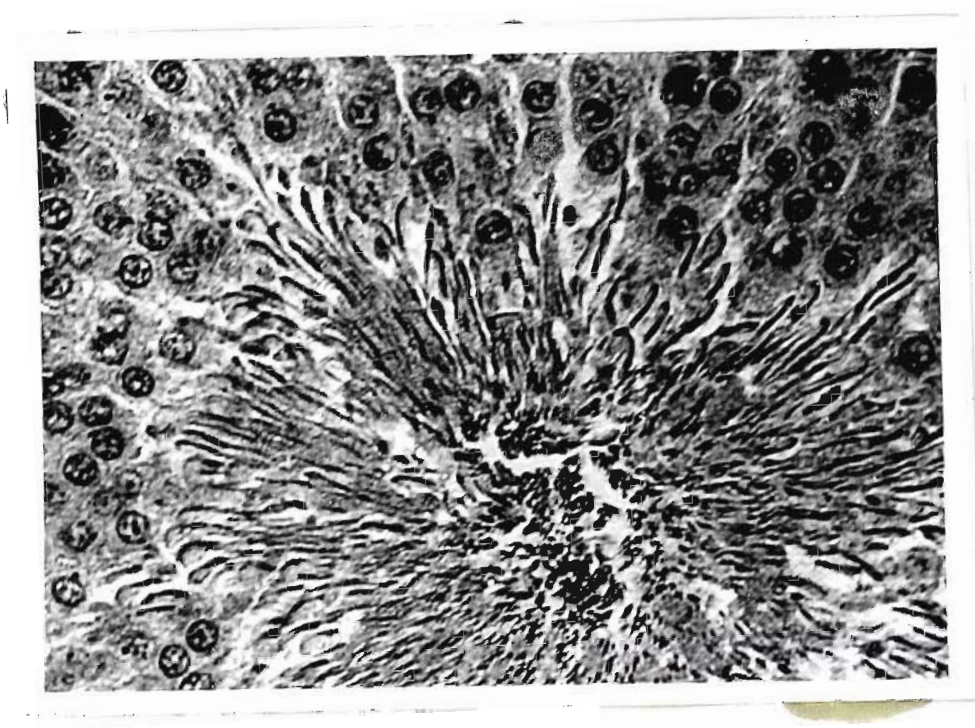


Figure 18. Photomicrograph of part of seminiferous tubule (rat No.6). Spermatogenesis appears to be normal after treatment with cimetidine (H & E Stain x640).

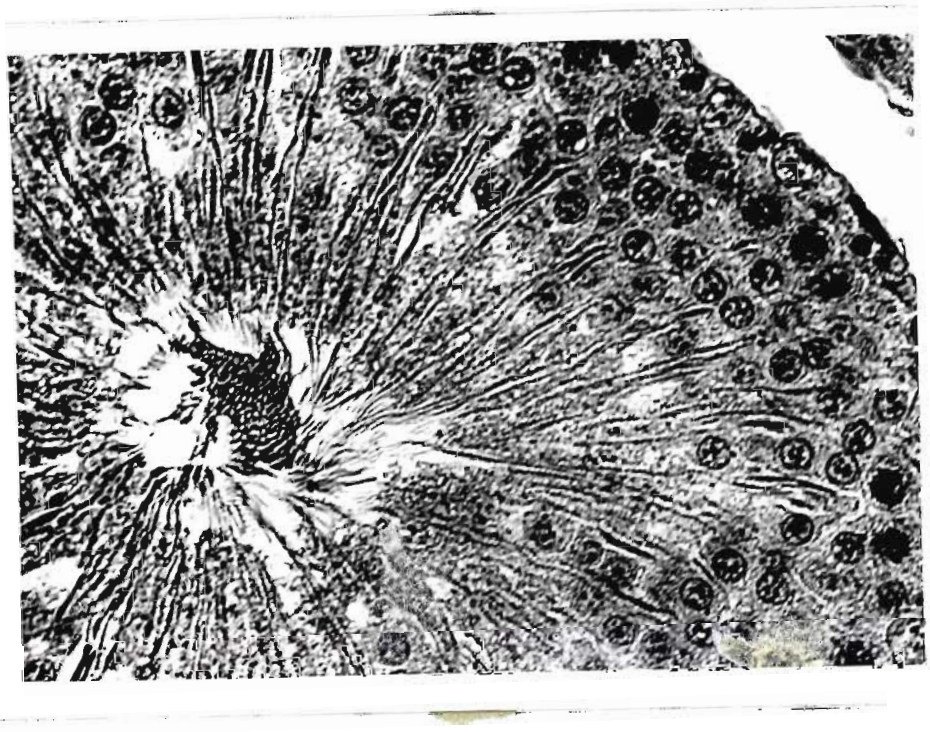


Figure 19. Photomicrograph of part of seminiferous tubule (rat No.1). Spermatogenesis appears to be normal after treatment with ranitidine (H & E Stain, x640).

CHAPTER 5: Discussion and conclusions

5.1 Discussion

5.1.1 Single-dose behavioural study

The results obtained in this study showed that cimetidine produced marked and statistically significant alterations in sexual behaviour at different dosage levels (Table 7). Ranitidine, tested in parallel with cimetidine showed no effect on sexual behaviour. These experimental findings show that cimetidine and ranitidine exert similar effects in the male rat as in the human male. Several reports of sexual dysfunction have appeared with cimetidine therapy (1,76,77,139), whereas ranitidine has been reported to be free from side-effects related to sexual dysfunction (3,4,106) or has been found to reverse cimetidine-induced impotence (1,139).

A stimulatory and an inhibitory response was observed at the lower and the higher dosage levels of cimetidine, respectively. A marked reduction in the ejaculatory latency together with a slight lowering of the post ejaculatory intromission latency (refractory period) was observed at the lower dose, 128.6 mg/kg. At the higher dose, 257.1 mg/kg, a significant increase in the refractory period together with a slight increase in the ejaculation latency was observed. The sensitivity to cimetidine varied from one animal to another; rats No.9

and 12 (Appendix A, Tables A1 TO A8) merit special discussion in this regard.

Rat No.9 was particularly susceptible to both the stimulatory and the inhibitory effects of cimetidine on sexual behaviour. Facilitation of sexual behaviour in this animal was strikingly demonstrated by a marked reduction in the ejaculation latency (from 5.10 to 2.05 minutes) after the 128.6mg/kg dose of cimetidine. When compared with pre-treatment control levels, retardation of sexual behaviour after the high dose of cimetidine, 257.1 mg/kg, was accompanied by marked increases in the mount frequency (from 3 to 6), intromission latency (from 0.35 to 0.80 minutes), ejaculation latency (from 5.10 to 11.65 minutes) and the refractory period (from 6.5 to 11.65 minutes). The inhibitory effect of cimetidine in this rat was accompanied by marked depression in locomotor activity. Furthermore, on rechallenge with saline (post-treatment control tests) all these parameters reverted to equivalent or below control levels, suggesting a return to normal copulatory behaviour. Similar observations have been made in peptic ulcer patients by Wolfe (76) and Peden et al. (77).

In contrast to rat No.9, rat No.12 was remarkably resistant to the facilitative and inhibitory effects of cimetidine on sexual behaviour. Throughout the dosage range this animal showed no appreciable alteration in sexual behaviour components. Furthermore, unlike the

appearance of reduced motor activity in most of the rats in this group, locomotor activity in this animal was not affected, in spite of the very high doses of cimetidine administered. The lack of effects on sexual behaviour and motor activity in this animal could probably be attributed to a very effective blood-brain barrier.

Similar to the findings on cimetidine in this study, Bignami found that male rats treated with d-amphetamine and LSD-25 displayed facilitative effects at low doses and inhibitory effects at higher doses (163). Further investigations revealed that when the same dose of amphetamine that stimulated sexual behaviour was administered daily to male rats, sexual behaviour diminished very markedly by the 4th day, and in certain rats disappeared entirely (159). Thus, it would be of interest to know whether similar treatment of male rats with cimetidine would show similar effects on sexual behaviour. The loss of libido and impotence reported by Wolfe (76) and Peden et al. (77) were associated with low-dose cimetidine therapy (1000 to 1200 mg/day) and, furthermore, these side-effects occurred during the first or second week of treatment.

Recent studies have established that several neurotransmitter systems and hormones may influence sexual behaviour. The question now arises as to whether the effects of cimetidine on sexual behaviour are hormonally mediated or whether they result from an interaction with

some neurotransmitter system responsible for the control of sexual behaviour.

Cimetidine was reported to produce elevated prolactin levels in some male patients who developed impotence and/or gynaecomastia (77,89-90) and in female patients with galactorrhoea (90,92). It has been established that substances with dopaminergic activity inhibit prolactin secretion while substances with dopamine blocking actions elevate prolactin secretion (174). It may be possible that the inhibition in sexual behaviour observed with high doses of cimetidine could result from a blockade of dopamine receptors; however, this cannot be regarded as conclusive until further investigations have been done. Furthermore, the cimetidine-induced inhibitory effects on sexual behaviour were accompanied by a significant reduction in motor activity. Similar findings have been reported with haloperidol (a dopamine receptor antagonist) in man and animals (172).

It is not clear whether the effect of cimetidine on sexual behaviour can be attributed to the reduced motor activity. It has been reported that facilitation or depression of copulatory behaviour in animals may not necessarily be related to changes in locomotor activity (175). However, it is of interest to note that haloperidol was reported to suppress aggressive and violent activities in animals and man and was also found to suppress sexual activity in both species (172). The findings of this experiment thus

provide a strong suggestion for a possible dopaminergic involvement in the cimetidine-induced suppression of sexual behaviour in the male rat.

Finally, the data in this experiment suggest that the effects of cimetidine on sexual behaviour do not appear to be related to H2-receptor blockade as equipotent doses of ranitidine showed no effect on sexual behaviour. It seems that the effects of cimetidine on sexual behaviour are possibly related to some independent mechanisms responsible for the control of sexual behaviour, as discussed earlier.

5.1.2 Subchronic-dose behavioural study and additional investigations related to gonadal function

In the subchronic-dose experiments no changes in sexual behaviour components were observed during the treatment period (24 days) with high doses of cimetidine, ranitidine and placebo. These experiments were designed to determine whether changes in copulatory behaviour would result from possible endocrine or antiandrogenic effects which may arise from subchronic treatment with high doses of cimetidine and ranitidine. In this study significant reductions in seminiferous tubule diameter, testosterone levels, and weights of testes, prostates and seminal vesicles were observed in the cimetidine group at autopsy. Furthermore, cauda epididymal sperm counts in the cimetidine group were lower than control values, although these reductions were not statistically significant. All

these parameters were not affected in the ranitidine group. Similar studies with ranitidine have not demonstrated antiandrogenic properties (103).

Reports on the effects of cimetidine and ranitidine on the seminiferous tubule and on epididymal sperm are apparantly lacking. However, Scott (personal communication) has suggested that the reductions in the seminiferous tubule diameter, although statistically significant, are too small to be regarded as pathologically significant (181). The effects of cimetidine on sex organ weights are in complete agreement with those of Leslie and Walker (2) and in partial agreement with the findings of Winters et al. (100). Winters et al. found no changes in the size of the testes; a possible explanation could be the lower dosage and the shorter treatment period employed (50 mg/kg/day for 7 days). Leslie and Walker too, did not observe impairment in mating performance in rats treated with high doses of cimetidine, despite reductions in gonad, prostate and seminal vesicle weights. However, the study of Leslie and Walker did not include direct observations on mating behaviour. In another study, Sodersten et al. treated male rats with flutamide (50 mg/kg/day for 30 days) and found marked reduction in prostate and seminal vesicle weights but no changes were observed in sexual behavioural components (164). Flutamide, like cimetidine, is a non-steroidal antiandrogen. The studies of Leslie and Walker (2) and Sodersten et al. (164) have not reported on serum testosterone levels. However, Winters et al. treated

male rats with much lower doses of cimetidine (50 mg/kg/day for 7 days) and in addition to significant reductions in prostate and seminal vesicle weights their results showed slight but not statistically significant decreases in plasma testosterone levels (100).

It is of interest to note that the quality of sexual performance remained unchanged in the cimetidine-treated animals despite marked reductions in serum testosterone levels and weights of testes and accessory sex organs. Similar findings have been reported with flutamide and Sodersten el al. have suggested that the lack of inhibitory effects of antiandrogens on sexual behaviour might possibly be related to the difficulty of antagonising the maintenance of sexual behaviour in experienced rats (164).

5.2 Conclusions.

The results of this study have demonstrated for the first time that cimetidine disrupts sexual behaviour in the male rat.

On final analysis, after taking into consideration the findings of the single dose studies, the subchronic-dose studies and the additional investigations related to gonadal function, the following conclusions may be drawn:

i. Cimetidine stimulates sexual behaviour in low doses and inhibits sexual behaviour in high doses in the male rat.

ii. Ranitidine has no effect on sexual behaviour in male rats.

iii. The stimulatory effect of cimetidine on sexual behaviour was not accompanied by changes in motor activity.

iv. The inhibitory effect of cimetidine on sexual behaviour was correlated with a significant depression in motor activity.

v. The effects of cimetidine on sexual behaviour appear to be mediated by some direct or indirect action on some neurotransmitter system responsible for the control of sexual behaviour,.

vi. Both the stimulatory and inhibitory effects of cimetidine on sexual behaviour in the male rat appear to be related to some non-specific action of cimetidine, and not to H2-receptor blockade, as equipotent doses of ranitidine showed no effect on sexual behaviour.

vii. Sexual behaviour in experienced male rats is not impaired secondary to cimetidine-induced antiandrogenic effects.

viii. The stimulatory effect of cimetidine probably has a peripheral locus of action and the inhibitory effect is possibly related to a central mechanism.

SUMMARY

The development of a new class of antihistamines, the H2-receptor antagonists, introduced a new era in the treatment of peptic ulcer diseases. Cimetidine, the first clinically effective H2-blocker, was introduced in 1976. Recently ranitidine, a second member approved for clinical use, has been found to be as effective as cimetidine in the management of peptic ulcer diseases. Soon after the introduction of cimetidine several reports of loss of libido, impotence and gynaecomastia were described in male patients who were on normal or high therapeutic doses of cimetidine. A few unsubstantiated reports of loss of libido and gynaecomastia attributed to ranitidine therapy have also appeared in literature.

This study was undertaken to examine in detail the effects of acute and subchronic treatment with cimetidine and ranitidine on mating behaviour in sexually active male rats. Motor activity counts were recorded immediately before sexual behaviour observations. The animals were tested on every third day and observations were terminated after the first intromission of the next series of copulations. In the single dose study, mating behaviour tests were commenced 2 hours after treatment; mating tests during the subchronic dose studies were done 4 to 7 hours after the 6h00 dose. The following measures were used in the analysis of data: mount latency, intromission latency,

mount frequency, intromission frequency, ejaculation latency, and the postejaculatory intromission latency. At the termination of the subchronic dose studies blood samples were collected by cardiac puncture and the animals were subsequently autopsied. Cauda epididymal sperm counts and motility were determined, testes and accessory sex organs were weighed, and one testis was processed for histological examination.

Cimetidine in the low dose, 128.6 mg/kg, significantly shortened the ejaculatory latency and to a lesser extent the postejaculatory intromission latency. At the higher dose, 257.1 mg/kg, cimetidine markedly prolonged the postejaculatory intromission latency and to a lesser extent increased the ejaculation latency. The inhibitory effect of cimetidine on copulatory behaviour at the higher dose level was accompanied by significant depression in motor activity.

At the conclusion of the subchronic dose studies marked reductions in serum testosterone levels and decreased testes and accessory organ weights were observed in the cimetidine group. No significant changes in sperm counts were observed, although the sperm counts in the cimetidine group were lower than the control values. Histological examination of testes showed apparently normal spermatogenesis in all three treatment groups.

However, in spite of the reduced testosterone levels and decreased testes and accessory sex organ weights in the

cimetidine group, no impairment in mating behaviour was observed.

In both the acute and the subchronic dose studies, similar to placebo, treatment with ranitidine showed no effect on mating behaviour.

On final analysis of the results it is concluded that cimetidine, and not ranitidine, disrupts sexual behaviour in male rats. Furthermore, it is concluded that the effect of cimetidine on sexual behaviour is not related to H2-receptor blockade as equipotent doses of ranitidine did not produce similar effects. The mechanism of cimetidine-induced impairment of sexual performance in the male rat may possibly be attributed to some non-specific, direct or indirect action of cimetidine on some neurotransmitter system responsible for the control of sexual behaviour. It is further suggested that the effect may possibly be mediated by a blockade of central dopamine receptors. However, it must be stressed that further experimentation is necessary to elucidate the mechanism of action of cimetidine on sexual behaviour.

APPENDICES

APPENDIX A: Sexual behaviour and motor activity in male rats after single doses of cimetidine, ranitidine and placebo.

Table A1. Mount frequency in rats after single doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat I.P.)							
No.	0.5	0.5	0.8	1.25	1.6	2.0	2.5	2.5
2	1	3	1	1	0	2	2	0
5	5	0	2	3	3	4	4	7
8	0	0	1	0	0	0	0	0
11	1	3	3	1	0	4	3	0
14	0	6	2	5	0	3	6	1
17	4	3	2	2	2	2	3	1
20	1	2	1	3	1	1	1	3
Mdn	1	3	2	2	0	2	3	1

Group II: Cimetidine

Rat	Cimetidine (mg/kg I.P.)							
No.	0.5*	8.57	85.7	128.6	171.4	214.4	257.1	2.5**
3	6	3	3	4	1	1	0	2
6	1	1	2	1	0	1	1	0
9	3	13	2	2	4	3	6	1
12	2	0	2	0	5	2	1	2
15	2	3	2	0	1	2	5	2
18	2	2	0	3	0	1	2	0
21	9	0	1	0	0	1	0	1
Mdn	2	2	2	1	1	1	1	1

Group III: Ranitidine

Rat	Ranitidine (mg/kg I.P.)							
No.	0.5*	2.143	21.43	32.15	42.86	53.58	64.29	2.5**
1	2	1	1	1	4	2	1	3
4	1	2	4	1	0	1	1	1
7	0	1	3	3	4	1	2	5
10	1	3	1	1	0	1	0	1
13	0	1	1	0	0	2	0	0
16	2	1	0	2	0	0	1	1
19	2	1	0	0	0	3	1	3
Mdn	1	1	1	1	0	1	1	1

*: Denotes volume (mls) of saline administered/rat in pre-treatment control tests.
**: Denotes volume (mls) of saline administered/rat in post-treatment control tests.
Mdn:Median of 7 observations. Tests were done on every 3rd day, 2 hours after treatment.

Table A2. Intromission frequency in rats after single doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat I.P.)							
No.	0.5	0.5	0.8	1.25	1.6	2.0	2.5	2.5
2	5	7	8	8	5	6	8	8
5	10	10	9	14	14	10	12	15
8	8	6	6	6	7	5	6	7
11	8	10	10	9	8	10	11	12
14	8	9	9	9	9	7	8	7
17	7	12	7	7	8	12	13	8
20	5	5	8	9	4	5	6	7
Mdn	8	9	8	9	8	7	8	8

Group II: Cimetidine

Rat	Cimetidine (mg/kg I.P.)							
No.	0.5*	8.57	85.7	128.6	171.4	214.4	257.1	2.5**
3	8	9	6	7	6	4	6	6
6	8	4	5	4	4	4	5	5
9	8	11	5	14	15	9	9	8
12	6	5	7	6	6	6	6	6
15	8	8	9	6	7	9	7	5
18	13	8	9	8	6	4	9	6
21	27	8	8	4	6	10	6	5
Mdn	8	8	8	6	6	6	6	6

Group III: Ranitidine

Rat	Ranitidine (mg/kg I.P.)							
No.	0.5*	2.143	21.43	32.15	42.86	53.58	64.29	2.5**
1	7	5	6	7	8	6	4	7
4	5	7	8	6	6	6	4	6
7	9	8	7	8	10	8	9	10
10	8	9	6	5	8	6	5	6
13	4	9	6	5	5	3	5	4
16	6	6	8	8	6	8	8	8
19	13	10	7	9	7	14	10	9
Mdn	7	8	7	7	7	6	5	7

*: Denotes volume (mls) of saline administered/rat in pre-treatment control tests.
**: Denotes volume (mls) of saline administered/rat in post-treatment control tests.
Mdn:Median of 7 observations. Tests were done on every 3rd day, 2 hours after treatment.

Table A3. Mount latency (min) in rats after single doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat No.	Saline (mls/rat I.P.)							
	0.5	0.5	0.8	1.25	1.6	2.0	2.5	2.5
2	0.05	0.15	0.20	0.05	0.15	0.05	0.05	0.20
5	0.10	0.60	0.10	0.10	0.10	0.10	0.05	0.25
8	0.25	0.40	0.45	0.65	2.10	0.60	0.45	0.25
11	0.20	0.20	0.05	0.10	0.05	0.10	0.10	0.05
14	0.30	1.60	0.20	0.10	0.10	0.15	0.05	0.15
17	0.05	0.15	0.15	0.05	0.15	0.05	0.05	0.10
20	0.35	0.25	0.30	0.15	0.15	0.05	0.20	0.10
Mdn	0.20	0.25	0.20	0.10	0.15	0.10	0.05	0.15

Group II: Cimetidine

Rat No.	Cimetidine (mg/kg I.P.)							
	0.5*	8.57	85.7	128.6	171.4	214.4	257.1	2.5**
3	0.25	0.35	0.15	0.10	0.20	0.20	0.10	0.30
6	0.15	0.20	0.10	0.10	0.10	0.10	0.95	0.20
9	0.25	2.25	0.25	0.25	0.70	0.25	0.35	0.25
12	0.10	0.60	0.15	0.10	0.20	0.25	0.05	0.10
15	0.20	0.15	0.20	0.25	0.15	0.05	0.35	0.15
18	0.15	0.20	0.10	0.10	0.15	0.10	0.20	0.10
21	0.15	0.05	0.10	0.25	0.05	0.05	0.10	0.10
Mdn	0.15	0.20	0.15	0.10	0.15	0.10	0.20	0.15

Group III: Ranitidine

Rat No.	Ranitidine (mg/kg I.P.)							
	0.5*	2.143	21.43	32.15	42.86	53.58	64.29	2.5**
1	0.10	0.10	0.20	0.15	0.20	0.30	0.20	0.10
4	0.15	0.20	0.25	0.10	0.05	0.10	0.20	0.20
7	0.05	0.15	0.70	0.15	0.10	0.15	0.50	0.20
10	0.30	0.15	0.10	0.10	0.10	0.05	0.10	0.20
13	0.50	0.15	0.05	0.15	0.10	0.20	0.30	0.05
16	0.20	0.25	0.25	0.15	0.05	0.15	0.05	0.15
19	0.10	0.20	0.20	0.25	0.20	0.15	0.10	0.10
Mdn	0.15	0.15	0.20	0.15	0.10	0.15	0.20	0.15

*: Denotes volume (mls) of saline administered/rat in pre-treatment control tests.
**: Denotes volume (mls) of saline administered/rat in post-treatment control tests.
Mdn:median of 7 observations. Tests were done on every 3rd day, 2 hours after treatment.

Table A4. Intromission latency (min) in rats after single doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat No.	Saline (mls/rat I.P.)							
	0.5	0.5	0.8	1.25	1.6	2.0	2.5	2.5
2	0.10	0.15	0.20	0.35	0.15	0.15	0.05	0.20
5	0.20	0.60	0.15	0.10	0.15	0.10	0.10	0.45
8	0.25	0.40	0.45	0.65	2.10	0.60	0.60	0.25
11	0.25	0.25	0.15	0.20	0.05	0.15	0.25	0.05
14	0.30	1.60	0.20	0.15	0.10	0.20	0.20	0.20
17	0.10	0.15	0.40	0.05	0.20	0.05	0.05	0.10
20	0.35	0.25	0.30	0.20	0.20	0.05	0.20	0.10
Mdn	0.25	0.25	0.20	0.20	0.15	0.15	0.20	0.20

Group II: Cimetidine

Rat No.	Cimetidine (mg/kg I.P.)							
	0.5*	8.57	85.7	128.6	171.4	214.4	257.1	2.5**
3	0.80	0.40	0.30	0.20	0.20	0.20	0.10	0.30
6	0.20	0.25	0.20	0.15	0.10	0.40	1.05	0.20
9	0.35	2.75	0.30	0.35	0.95	0.25	0.80	0.25
12	0.15	0.60	0.15	0.10	0.20	0.30	0.20	0.15
15	0.55	0.20	0.20	0.25	0.20	0.10	0.35	0.20
18	0.15	0.25	0.10	0.15	0.15	0.10	0.20	0.10
21	0.20	0.05	0.10	0.25	0.05	0.05	0.10	0.10
Mdn	0.20	0.25	0.20	0.20	0.20	0.20	0.20	0.20

Group III: Ranitidine

Rat No.	Ranitidine (mg/kg I.P.)							
	0.5*	2.143	21.43	32.15	42.86	53.58	64.29	2.5**
1	0.10	0.10	0.30	0.20	0.25	0.30	0.45	0.35
4	0.20	0.25	0.35	0.10	0.05	0.15	0.25	0.25
7	0.05	0.30	0.75	0.15	0.10	0.25	0.50	0.25
10	0.30	0.15	0.20	0.15	0.10	0.10	0.10	0.20
13	0.50	0.20	0.15	0.15	0.10	0.25	0.30	0.05
16	0.20	0.25	0.25	0.20	0.05	0.15	0.05	0.15
19	0.10	0.20	0.20	0.25	0.20	0.15	0.10	0.10
Mdn	0.20	0.20	0.25	0.15	0.10	0.15	0.25	0.20

*: Denotes volume (mls) of saline administered/rat in pre-treatment control tests.
**: Denotes volume (mls) of saline administered/rat in post-treatment control tests.
Mdn:Median of 7 observations. Tests were done on every 3rd day, 2 hours after treatment.

Table A5. Ejaculation latency (min) in rats after single doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat No.	Saline (mls/rat I.P.)							
	0.5	0.5	0.8	1.25	1.6	2.0	2.5	2.5
2	0.80	2.10	1.75	1.45	0.75	1.35	1.60	1.30
5	4.65	3.45	3.90	5.60	4.10	2.60	3.80	6.40
8	3.45	2.10	2.45	1.50	1.70	1.65	1.70	1.00
11	3.35	3.85	2.95	2.65	2.40	4.20	3.10	4.05
14	4.65	6.70	3.15	3.50	2.85	4.20	3.80	3.85
17	1.35	2.65	2.40	1.55	1.70	2.70	3.05	1.85
20	0.45	0.70	0.70	1.60	0.35	0.55	0.95	1.20
Mdn	3.35	2.65	2.45	1.60	1.70	2.60	3.05	1.85

Group II: Cimetidine

Rat No.	Cimetidine (mg/kg I.P.)							
	0.5*	8.57	85.7	128.6	171.4	214.4	257.1	2.5**
3	4.75	3.95	2.40	3.80	2.35	3.20	3.35	3.40
6	2.20	0.55	1.50	0.80	0.35	1.05	1.95	1.20
9	5.10	8.20	5.00	2.05	7.00	9.20	11.65	2.60
12	3.40	1.95	2.95	1.40	1.65	1.95	2.00	2.60
15	1.75	1.00	2.10	1.10	1.70	2.20	2.30	1.00
18	2.25	1.05	2.20	1.00	1.10	0.60	2.80	0.65
21	7.05	2.25	2.35	0.45	0.95	2.15	0.80	0.70
Mdn	2.25	1.95	2.35	1.10	1.65	2.15	2.30	1.20

Group III: Ranitidine

Rat No.	Ranitidine (mg/kg I.P.)							
	0.5*	2.143	21.43	32.15	42.86	53.58	64.29	2.5**
1	4.10	1.50	1.65	2.80	2.40	2.40	1.85	2.65
4	2.20	2.50	4.20	2.40	1.60	2.05	1.65	2.80
7	2.40	3.30	5.20	3.00	3.10	3.75	4.00	4.70
10	2.35	2.25	2.27	0.95	1.05	1.60	0.70	1.70
13	0.35	1.35	0.75	0.40	0.75	0.70	0.70	0.35
16	4.85	3.15	2.15	4.00	1.05	1.90	3.20	3.65
19	3.05	2.45	0.85	2.35	1.10	2.75	2.05	1.45
Mdn	2.40	2.45	2.15	2.40	1.05	2.05	1.85	2.65

*: Denotes volume (mls) of saline administered/rat in pre-treatment control tests.
**: Denotes volume (mls) of saline administered/rat in post-treatment control tests.
Mdn: Median of 7 observations. Tests were done on every 3rd day, 2 hours after treatment.

Table A6. Post ejaculatory intromission latency (min) in rats after single doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat I.P.)							
No.	0.5	0.5	0.8	1.25	1.6	2.0	2.5	2.5
2	4.80	4.30	3.70	4.60	4.30	4.60	4.65	4.40
5	3.85	4.60	3.70	4.00	5.05	4.20	4.70	4.75
8	4.00	4.10	4.10	3.90	3.90	3.70	3.85	3.10
11	3.80	3.70	3.80	4.15	3.50	4.45	4.30	4.45
14	5.80	6.55	4.85	5.95	5.30	6.05	6.00	5.65
17	3.95	4.20	4.05	3.75	3.30	4.35	4.20	3.95
20	4.25	5.65	5.50	3.65	3.75	3.85	4.00	4.20
Mdn	3.95	4.30	4.05	4.00	3.90	4.35	4.30	4.40

Group II: Cimetidine

Rat	Cimetidine (mg/kg I.P.)							
No.	0.5*	8.57	85.7	128.6	171.4	214.4	257.1	2.5**
3	5.02	4.75	4.35	4.60	3.85	4.55	5.90	4.55
6	4.65	5.15	5.20	5.40	4.85	6.30	5.75	4.65
9	6.50	5.25	6.05	6.20	6.40	5.75	9.80	6.10
12	6.00	5.40	5.35	5.15	5.50	5.40	5.80	5.55
15	4.05	5.15	4.45	4.10	4.90	5.25	5.65	4.05
18	3.75	4.30	5.50	4.35	4.50	4.35	4.65	4.25
21	4.40	3.25	4.15	4.20	4.05	4.40	4.10	3.45
Mdn	4.65	5.15	5.20	4.60	4.85	5.25	5.75	4.55

Group III: Ranitidine

Rat	Ranitidine (mg/kg I.P.)							
No.	0.5*	2.143	21.43	32.15	42.86	53.58	64.29	2.5**
1	5.65	4.00	4.15	4.95	4.40	4.80	4.50	3.50
4	4.60	5.65	4.65	4.60	4.40	4.75	4.90	4.80
7	5.05	4.55	5.10	4.75	5.70	5.15	5.55	5.95
10	5.10	3.70	3.60	3.10	3.95	3.30	3.80	3.80
13	3.40	3.35	3.85	3.35	3.30	4.45	3.45	3.15
16	5.85	5.30	5.55	5.40	5.30	5.45	5.30	5.90
19	4.30	4.60	4.00	4.10	4.15	4.30	4.40	3.95
Mdn	5.10	4.55	4.15	4.60	4.40	4.75	4.50	3.95

*: Denotes volume (mls) of saline administered/rat in pre-treatment control tests.
**: Denotes volume (mls) of saline administered/rat in post-treatment control tests.
Mdn: Median of 7 observations. Tests were done on every 3rd day, 2 hours after treatment.

Table A7. Horizontal activity (counts/min) in rats after single doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat I.P.)							
No.	0.5	0.5	0.8	1.25	1.6	2.0	2.5	2.5
2	524	245	600	403	202	522	469	437
5	469	596	472	531	509	486	506	500
8	317	267	429	299	308	303	213	358
11	474	694	618	552	648	633	776	620
14	584	700	515	551	545	610	506	588
17	482	335	420	446	273	307	343	451
20	316	345	495	386	497	417	479	439
MEAN	452	455	507	453	426	468	470	485
SEM	38	76	29	37	62	50	65	35

Group II: Cimetidine

Rat	Cimetidine (mg/kg I.P.)							
No.	0.5*	8.57	85.7	128.6	171.4	214.4	257.1	2.5**
3	321	388	324	455	296	178	24	290
6	324	432	486	372	485	221	339	537
9	477	495	549	448	275	357	149	467
12	348	667	632	551	802	747	801	906
15	253	184	341	271	255	84	142	273
18	443	494	263	388	390	194	198	355
21	332	421	698	266	298	413	251	440
MEAN	357	440	470	393	400	313	272	467
SEM	29	55	63	39	73	84	96	81

Group III: Ranitidine

Rat	Ranitidine (mg/kg I.P.)							
No.	0.5*	2.143	21.43	32.15	42.86	53.58	64.29	2.5**
1	541	246	304	441	281	205	305	307
4	259	670	600	557	571	566	532	558
7	467	392	287	429	487	457	433	446
10	380	387	549	504	458	476	362	354
13	661	325	393	347	429	260	336	340
16	650	613	654	773	810	525	423	886
19	773	783	749	473	727	758	740	811
MEAN	533	488	505	503	538	464	447	529
SEM	67	76	68	51	69	71	56	89

*: Denotes volume (mls) of saline administered/rat in pre-treatment control tests.

**: Denotes volume (mls) of saline administered/rat in post-treatment control tests. Tests were done on every 3rd day, 2 hours after treatment.

Table A8. Vertical activity (counts/min) in rats after single doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat I.P.)							
No.	0.5	0.5	0.8	1.25	1.6	2.0	2.5	2.5
2	17	8	19	10	16	19	13	19
5	11	20	31	22	29	20	24	24
8	5	10	17	15	15	10	13	21
11	9	20	26	18	23	15	28	25
14	15	20	26	30	27	36	31	30
17	13	14	20	20	8	16	19	15
20	8	6	19	19	16	10	17	15
MEAN	11	14	23	19	19	18	21	21
SEM	1.6	2.3	1.9	2.3	2.8	3.4	2.7	2.1

Group II: Cimetidine

Rat	Cimetidine (mg/kg I.P.)							
No.	0.5*	8.57	85.7	128.6	171.4	214.4	257.1	2.5**
3	8	11	17	9	8	4	0	7
6	9	14	26	17	18	8	12	25
9	15	22	22	16	23	10	6	19
12	11	15	26	31	28	17	25	34
15	8	9	6	8	8	0	4	11
18	13	21	15	20	19	10	12	24
21	8	16	26	20	13	11	11	14
MEAN	10	15	20	17	17	9	10	19
SEM	1.1	1.8	2.9	2.9	2.8	2.1	3.0	3.5

Group III: Ranitidine

Rat	Ranitidine (mg/kg I.P.)							
No.	0.5*	2.143	21.43	32.15	42.86	53.58	64.29	2.5**
1	4	5	11	12	12	3	5	4
4	11	27	19	34	24	24	18	33
7	17	31	17	32	23	19	21	25
10	6	20	30	25	26	14	16	26
13	7	20	9	15	18	7	19	21
16	8	12	10	18	13	17	5	12
19	17	22	20	20	36	28	29	40
MEAN	10	20	17	22	22	16	16	23
SEM	2.0	3.3	2.8	3.2	3.1	3.4	3.3	4.6

*: Denotes volume (mls) of saline administered/rat in pre-treatment control tests.

**: Denotes volume (mls) of saline administered/rat in post-treatment control tests. Tests were done on every 3rd day, 2 hours after treatment.

APPENDICES

APPENDIX B: Sexual behaviour and motor activity in male rats during subchronic treatment with cimetidine, ranitidine and placebo.

Table B1. Mount frequency in rats during subchronic treatment with increasing doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat/day I.P.)*									
No.	0.75	0.75	0.75	0.75	1.50	1.50	3.00	3.00	6.00	6.00
2	0	0	2	1	0	0	1	1	0	2
5	0	1	3	3	5	2	1	1	0	3
8	1	1	2	0	0	0	0	2	1	1
11	1	1	1	1	2	2	1	1	0	1
14	1	0	2	3	0	3	0	6	4	3
17	6	1	0	1	1	1	3	0	2	1
20	**	0	1	**	0	1	1	0	0	1
Mdn	1	1	2	1	0	1	1	1	0	1

Group II: Cimetidine

Rat	Cimetidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	85.7	85.7	171.4	171.4	342.8	342.8	685.6	685.6
3	1	2	2	5	0	1	2	3	0	2
6	2	2	6	5	5	7	1	2	1	2
9	3	0	1	0	13	1	0	2	2	1
12	0	0	4	2	0	3	4	2	1	2
15	0	0	2	0	3	2	2	0	0	1
18	2	2	2	5	1	1	1	1	0	0
21	2	0	3	0	3	2	7	1	2	1
Mdn	2	0	2	2	3	2	2	2	1	1

Group III: Ranitidine

Rat	Ranitidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	21.4	21.4	42.8	42.8	85.6	85.6	171.2	171.2
1	5	2	4	4	1	4	1	4	9	2
4	0	0	3	0	0	2	1	0	0	1
7	3	2	9	3	2	3	2	1	2	2
10	1	3	5	4	3	2	8	3	6	5
13	0	0	1	1	0	1	0	1	0	1
16	3	0	4	1	3	0	3	1	0	3
19	2	2	2	2	0	2	1	3	1	2
Mdn	2	2	4	2	1	2	1	1	1	2

*: All treatments were administered in divided doses at 8 hourly intervals. Tests were done on every 3rd day, 4 to 7 hours after treatment.
**: Rat failed to copulate within 15 minutes.
$: Denotes volume (mls) of saline administered/rat in pre-treatment control tests.

Table B2. Intromission frequency in rats during subchronic treatment with increasing doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat/day I.P.)*									
No.	0.75	0.75	0.75	0.75	1.50	1.50	3.00	3.00	6.00	6.00
2	4	4	5	4	3	4	8	8	9	5
5	6	6	7	8	7	5	7	10	7	8
8	6	4	5	11	7	4	7	7	8	7
11	5	6	6	6	5	6	5	4	4	4
14	8	4	9	15	4	9	6	18	12	6
17	8	7	8	7	6	7	6	8	7	6
20	**	6	7	**	7	6	6	5	7	5
Mdn	6	5	7	8	6	6	6	8	7	6

Group II: Cimetidine

Rat	Cimetidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	85.7	85.7	171.4	171.4	342.8	342.8	685.6	685.6
3	5	9	5	11	7	5	7	8	4	8
6	8	10	12	13	13	11	5	10	12	9
9	7	8	5	6	7	6	6	6	7	8
12	7	7	8	9	11	12	7	10	8	8
15	7	8	10	7	9	9	6	7	8	7
18	10	8	7	7	4	5	8	6	4	5
21	7	8	5	5	12	10	13	10	10	9
Mdn	7	8	7	7	9	9	7	8	8	8

Group III: Ranitidine

Rat	Ranitidine (mg/kg/day I.P.)*									
No	0.75$	0.75$	21.4	21.4	42.8	42.8	85.6	85.6	171.2	171.2
1	9	9	7	10	7	8	7	10	10	8
4	6	5	8	6	6	7	5	7	4	7
7	11	12	14	17	10	9	8	13	8	7
10	7	8	7	7	10	9	11	7	11	10
13	4	6	4	3	4	3	5	5	4	4
16	8	6	12	10	10	4	10	5	9	8
19	8	6	6	6	7	9	6	11	6	9
Mdn	8	6	7	7	7	8	7	7	8	8

*: All treatments were administered in divided doses at 8 hourly intervals. Tests were done on every 3rd day, 4 to 7 hours after treatment.
**:Rat failed to copulate within 15 minutes.
$: Denotes volume (mls) saline administered/rat in pre-treatment control tests.

Table B3. Mount latency (min) in rats during subchronic treatment with increasing doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat/day I.P.)*									
No.	0.75	0.75	0.75	0.75	1.50	1.50	3.00	3.00	6.00	6.00
2	0.05	0.05	0.10	0.10	0.10	0.10	0.10	0.10	0.20	0.25
5	0.20	0.60	0.15	0.15	0.20	0.10	0.15	0.30	0.05	0.40
8	0.15	0.05	0.05	0.10	0.10	0.05	0.05	0.10	0.10	0.05
11	0.02	0.65	0.10	0.20	0.15	0.05	0.05	0.10	0.20	0.10
14	0.10	0.15	0.10	0.05	0.10	0.10	0.10	0.05	0.10	0.05
17	0.25	1.00	0.10	0.10	0.20	0.30	0.05	0.15	0.25	0.35
20	**	4.80	0.25	**	1.00	0.70	6.20	0.25	0.65	0.25
Mdn	0.17	0.60	0.10	0.10	0.15	0.10	0.10	0.10	0.20	0.25

Group II: Cimetidine

Rat	Cimetidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	85.7	85.7	171.4	171.4	342.8	342.8	685.6	685.6
3	0.55	0.75	0.15	0.30	0.25	0.10	0.05	0.10	0.15	0.25
6	0.10	0.15	0.15	0.15	0.05	0.10	0.10	0.10	0.10	0.05
9	0.05	0.10	0.10	0.10	0.10	0.05	0.10	0.35	0.10	0.15
12	0.15	0.25	0.15	0.15	0.15	0.10	0.20	0.10	0.10	0.25
15	0.10	0.15	0.05	0.25	0.15	0.10	0.05	0.05	0.05	0.15
18	0.05	0.15	0.10	0.10	0.05	0.10	0.05	0.15	0.10	0.25
21	0.10	0.05	0.10	0.15	0.10	0.05	0.10	0.10	0.05	0.05
Mdn	0.10	0.15	0.10	0.15	0.10	0.10	0.10	0.10	0.10	0.15

Group III: Ranitidine

Rat	Ranitidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	21.4	21.4	42.8	42.8	85.6	85.6	171.2	171.2
1	0.50	0.20	0.10	0.15	0.15	0.40	0.10	0.15	0.10	0.15
4	0.30	0.05	0.25	0.15	0.10	0.10	0.10	0.70	0.15	0.10
7	0.10	0.10	0.10	0.15	0.05	0.05	0.20	0.15	0.10	0.15
10	0.20	0.20	0.15	0.15	0.05	0.20	0.30	0.20	0.20	0.05
13	0.20	0.15	0.20	0.15	0.20	0.05	0.10	0.20	0.15	0.10
16	0.15	0.05	0.10	0.10	0.15	0.05	0.20	0.10	0.10	0.05
19	0.05	0.20	0.20	0.30	0.20	0.10	0.10	0.15	0.15	0.10
Mdn	0.20	0.15	0.15	0.15	0.15	0.10	0.10	0.15	0.15	0.10

*: All treatments were administered in divided doses at 8 hourly intervals.
**:Rat failed to copulate within 15 minutes.
$: Denotes volume (mls) of saline administered in pre-experimental control tests.

Table B4. Intromission latency (min) in rats during subchronic treatment with increasing doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat/day I.P.)*									
No.	0.75	0.75	0.75	0.75	1.50	1.50	3.00	3.00	6.00	6.00
2	0.05	0.05	0.10	0.10	0.10	0.10	0.10	0.10	0.20	0.25
5	0.20	0.60	0.30	0.15	0.30	0.10	0.15	0.30	0.05	0.40
8	0.20	0.05	0.15	0.10	0.10	0.05	0.05	0.10	0.10	0.10
11	0.20	0.65	0.10	0.20	0.15	0.10	0.05	0.10	0.20	0.15
14	0.10	0.15	0.15	0.05	0.10	0.10	0.10	0.05	0.15	0.05
17	0.30	1.00	0.10	0.10	0.25	0.30	0.10	0.10	0.25	0.30
20	**	4.80	0.25	**	1.00	0.70	6.20	0.25	0.65	0.25
Mdn	0.20	0.60	0.15	0.10	0.15	0.10	0.10	0.10	0.20	0.25

Group II: Cimetidine

Rat	Cimetidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	85.7	85.7	171.4	171.4	342.8	342.8	685.6	685.6
3	0.55	0.75	0.20	0.30	0.25	0.15	0.05	0.20	0.15	0.25
6	0.15	0.15	0.15	0.15	0.25	0.10	0.15	0.10	0.10	0.10
9	0.20	0.10	0.15	0.10	0.45	0.05	0.10	0.35	0.15	0.25
12	0.15	0.25	0.20	0.55	0.15	0.15	0.25	0.10	0.15	0.25
15	0.10	0.15	0.05	0.25	0.20	0.10	0.10	0.05	0.05	0.15
18	0.10	0.20	0.20	0.25	0.10	0.10	0.05	0.15	0.10	0.25
21	0.35	0.05	0.10	0.15	0.15	0.05	0.20	0.15	0.05	0.05
Mdn	0.20	0.15	0.15	0.20	0.15	0.10	0.10	0.15	0.10	0.25

Group III: Ranitidine

Rat	Ranitidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	21.4	21.4	42.8	42.8	85.6	85.6	171.2	171.2
1	0.50	0.25	0.25	0.20	0.15	0.40	0.10	0.20	0.10	0.15
4	0.30	0.05	0.25	0.15	0.10	0.10	0.10	0.70	0.15	0.10
7	0.15	0.10	0.20	0.15	0.15	0.05	0.20	0.15	0.15	0.20
10	0.25	0.20	0.20	0.30	0.10	0.20	0.30	0.25	0.30	0.10
13	0.20	0.15	0.25	0.15	0.25	0.05	0.10	0.30	0.15	0.15
16	0.15	0.05	0.10	0.10	0.20	0.05	0.25	0.15	0.10	0.05
19	0.15	0.30	0.20	0.30	0.20	0.10	0.15	0.15	0.15	0.10
Mdn	0.15	0.15	0.20	0.15	0.15	0.10	0.10	0.15	0.15	0.10

*: All treatments were administered in divided doses at 8 hourly intervals.
**:Rat failed to copulate within 15 minutes.
$: Denotes volume (mls) of saline administered in pre-experimental

Table B5. Ejaculation latency (min) in rats during subchronic treatment with increasing doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat/day I.P.)*									
No.	0.75	0.75	0.75	0.75	1.5	1.5	3.0	3.0	6.0	6.0
2	0.35	0.35	1.00	0.85	0.80	0.45	1.20	1.95	1.95	1.00
5	1.90	3.25	2.60	2.25	2.40	0.80	1.40	3.25	1.20	3.50
8	1.50	0.70	1.00	1.60	1.00	0.35	0.85	2.00	1.25	1.25
11	1.10	3.10	2.90	1.15	1.15	1.05	1.05	0.80	0.50	0.65
14	1.35	0.55	2.25	2.95	0.45	2.25	2.10	5.50	2.55	1.05
17	3.25	3.45	1.55	2.85	1.50	2.05	2.20	2.60	2.05	1.50
20	**	2.55	2.25	**	1.20	1.45	2.90	1.14	4.70	1.25
Mdn	1.43	2.55	2.25	1.92	1.15	1.05	1.40	2.05	1.95	1.25

Group II: Cimetidine

Rat	Cimetidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	85.7	85.7	171.4	171.4	342.8	342.8	685.6	685.6
3	0.65	6.55	1.20	3.00	1.95	0.80	1.00	2.85	0.95	1.75
6	1.70	3.25	3.90	4.15	2.60	3.45	1.20	2.75	2.20	1.80
9	2.60	3.05	4.85	1.70	7.15	1.85	1.90	2.15	2.00	2.60
12	0.95	1.10	2.75	2.01	2.65	3.40	1.95	2.25	2.00	1.30
15	1.25	2.40	3.40	2.05	2.90	3.85	2.30	2.30	1.75	2.30
18	3.30	2.90	2.10	3.95	2.20	1.10	2.60	1.45	0.90	1.95
21	2.00	1.20	0.95	0.60	3.00	2.80	3.30	1.90	1.35	1.90
Mdn	1.70	2.40	2.75	2.05	2.65	2.80	1.95	2.25	1.75	1.90

Group III: Ranitidine

Rat	Ranitidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	21.4	21.4	42.8	42.8	85.6	85.6	171.2	171.2
1	5.70	5.10	4.95	7.35	4.20	4.75	3.25	6.60	5.50	5.85
4	3.50	1.65	4.60	2.40	0.80	1.90	0.80	2.80	1.50	2.50
7	2.90	3.10	5.78	6.40	2.60	3.35	2.25	5.20	3.60	4.10
10	1.75	2.55	6.60	5.95	4.10	3.60	5.20	5.45	4.30	5.05
13	0.45	0.90	0.45	0.40	0.55	0.90	0.50	0.85	1.00	1.05
16	1.60	0.80	3.95	1.95	2.10	0.70	2.60	1.25	1.85	1.45
19	1.65	2.15	1.8	2.00	1.50	2.60	1.90	3.10	1.45	2.00
Mdn	1.75	2.15	4.60	2.40	2.10	2.60	2.25	3.10	1.85	2.50

*: All treatments were administered in divided doses at 8 hourly intervals.
**:Rat failed to copulate within 15 minutes.
$: Denotes volume (mls) of saline administered in pre-experimental control tests.

Table B6. Post ejaculatory intromission latency (min) in rats during subchronic treatment with increasing doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat/day I.P.)*									
No.	0.75	0.75	0.75	0.75	1.5	1.5	3.0	3.0	6.0	6.0
2	2.75	3.05	3.95	3.55	3.40	3.60	3.35	4.20	4.00	0.60
5	3.30	3.95	4.35	3.90	4.30	3.55	3.40	4.05	3.25	3.60
8	4.20	4.00	4.40	4.00	3.75	3.80	3.75	4.55	3.80	4.05
11	3.35	3.55	3.60	3.95	3.80	3.50	3.50	3.65	3.40	3.60
14	3.85	4.10	3.85	4.60	4.50	3.50	4.45	4.65	4.40	4.30
17	3.60	4.60	4.15	4.40	4.40	4.30	3.45	5.30	4.40	4.30
20	**	3.95	3.25	**	3.40	4.10	4.05	4.30	4.55	4.50
Mdn	3.45	3.95	3.95	3.98	3.80	3.65	3.45	4.30	4.00	4.05

Group II: Cimetidine

Rat	Cimetidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	85.7	85.7	171.4	171.4	342.8	342.8	685.6	685.6
3	3.90	5.60	4.75	5.05	4.75	3.95	4.20	4.55	4.75	5.30
6	2.70	4.00	4.60	4.30	4.10	4.20	3.85	4.45	3.35	3.25
9	5.40	5.20	7.10	5.30	5.70	6.00	5.85	5.50	5.30	5.35
12	3.35	4.40	3.75	3.95	4.20	4.25	4.15	3.15	4.10	3.70
15	3.10	3.50	3.60	4.05	3.70	3.60	3.65	3.95	3.75	3.90
18	4.85	5.20	4.90	6.00	5.15	4.90	5.75	5.65	5.00	4.75
21	4.30	4.05	4.10	3.80	4.00	3.65	3.65	3.65	3.55	3.60
Mdn	3.90	4.40	4.60	4.30	4.10	4.20	4.15	4.45	4.10	3.90

Group III: Ranitidine

Rat	Ranitidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	21.4	21.4	42.8	42.8	85.6	85.6	171.2	171.2
1	5.50	6.45	6.35	7.20	6.10	6.65	5.50	6.75	6.00	5.95
4	4.65	4.00	4.75	4.60	4.30	4.20	4.25	5.00	4.60	4.40
7	4.30	4.60	4.15	4.75	3.85	4.25	4.00	4.20	4.50	5.45
10	5.40	6.00	6.30	5.50	4.85	5.80	5.20	6.90	5.60	4.10
13	4.40	4.75	4.10	3.95	3.80	4.80	3.70	4.55	4.35	4.45
16	4.00	1.30	4.35	4.55	4.15	3.45	1.45	3.65	4.25	3.45
19	3.40	3.15	3.45	3.60	3.60	4.90	3.55	3.65	3.40	3.75
Mdn	4.40	4.60	4.35	4.60	4.15	4.80	4.00	4.55	4.50	4.40

*: All treatments were administered in divided doses at 8 hourly intervals.
**:Rat failed to copulate within 15 minutes.
$: Denotes volume (mls) of saline administered in pre-experimental control tests.

Table B7. Horizontal activity (count/min) in rats during subchronic treatment with increasing doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat/day I.P.)*									
No	0.75	0.75	0.75	0.75	1.50	1.50	3.00	3.00	6.00	6.00
2	389	315	210	331	400	327	478	326	307	356
5	542	444	349	464	399	479	500	507	358	390
8	555	431	332	339	453	300	467	329	449	573
11	284	446	410	268	436	425	439	332	475	361
14	346	421	368	415	461	410	307	375	427	399
17	516	373	436	476	396	534	472	597	546	561
20	270	240	250	260	304	335	281	262	185	298
Mean	416	376	336	365	407	401	421	390	396	420
SEM	46	28	31	33	20	33	34	45	45	40

Group II: Cimetidine

Rat	Cimetidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	85.7	85.7	171.4	171.4	342.8	342.8	685.6	685.6
3	372	360	267	373	485	427	392	358	252	205
6	398	390	470	420	532	459	459	432	352	419
9	782	735	665	586	782	807	969	750	619	587
12	345	361	280	423	314	425	438	434	423	318
15	607	561	460	566	685	553	638	538	613	508
18	510	521	464	463	572	510	622	521	447	583
21	417	315	473	297	364	622	523	508	663	321
Mean	490	461	440	447	533	543	577	506	481	420
SEM	59	57	51	39	63	52	74	47	58	55

Group III: Ranitidine

Rat	Ranitidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	21.4	21.4	42.8	42.8	85.6	85.6	171.2	171.2
1	574	537	590	755	574	808	716	548	658	586
4	653	644	423	541	759	671	628	755	645	624
7	476	418	439	459	442	443	480	283	553	482
10	459	451	480	438	463	469	488	493	539	472
13	445	385	459	461	522	387	485	492	413	311
16	771	668	663	712	554	587	617	552	652	669
19	459	464	364	341	443	335	371	419	284	268
Mean	548	513	488	530	537	529	541	506	535	487
SEM	47	43	39	57	42	64	44	54	53	58

*: All treatments were administered in divided doses at 8 hourly intervals.
$: Denotes volume (mls) of saline administered in pre-experimental control tests.

Table B8. Vertical activity (count/min) in rats during subchronic treatment with increasing doses of cimetidine, ranitidine and placebo

Group I: Placebo

Rat	Saline (mls/rat/day I.P.)*									
No.	0.75	0.75	0.75	0.75	1.5	1.5	3.0	3.0	6.0	6.0
2	7	8	1	7	10	8	22	4	13	11
5	29	23	22	24	25	21	23	15	27	29
8	13	14	18	14	17	13	19	13	15	20
11	14	12	10	42	10	7	17	8	16	8
14	18	16	15	23	24	18	22	14	14	12
17	15	8	24	21	14	26	20	25	23	22
20	15	8	22	11	19	18	16	16	15	23
Mean	16	15	16	20	17	16	20	14	18	18
SEM	3.0	2.4	3.0	4.4	2.3	2.6	1.0	2.5	2.0	2.8

Group II: Cimetidine

Rat	Cimetidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	85.7	85.7	171.4	171.4	342.8	342.8	685.6	685.6
3	14	10	11	20	19	19	17	9	5	3
6	17	15	15	25	24	22	28	19	15	13
9	27	20	21	11	23	26	28	14	20	22
12	16	16	20	16	10	12	24	21	21	12
15	25	18	15	21	23	28	31	13	18	16
18	19	19	28	21	20	19	23	18	12	9
21	23	21	27	21	17	23	25	22	33	16
Mean	20	17	20	19	19	21	25	17	18	13
SEM	1.9	1.4	2.4	1.7	1.8	2.0	1.7	1.8	2.3	2.3

Group III: Ranitidine

Rat	Ranitidine (mg/kg/day I.P.)*									
No.	0.75$	0.75$	21.4	21.4	42.8	42.8	85.6	85.6	171.2	171.2
1	7	6	9	13	8	10	11	8	13	15
4	23	30	20	21	31	28	24	20	37	25
7	30	15	29	25	21	22	33	12	28	33
10	24	19	23	27	26	27	32	24	25	19
13	14	22	16	22	27	15	21	23	24	13
16	31	37	25	34	35	40	38	24	26	36
19	15	15	17	13	16	15	16	18	18	16
Mean	21	21	20	22	23	22	25	18	24	22
SEM	3.4	3.9	2.5	2.9	3.5	3.9	3.7	2.4	2.9	3.5

*: All treatments were administered in divided doses at 8 hourly intervals.
$: Denotes volume (mls) of saline administered in pre-experimental control tests.

APPENDICES

APPENDIX C: Results of additional investigations related to gonadal function.

Table C1. Data on serum testosterone levels in rats subchronically treated* with cimetidine and ranitidine

Group I: Placebo (Saline)

	Testosterone (ng/ml)					
Rat	Before treatment			After treatment		
No.	Assay 1	Assay 2	Av T1	Assay 1	Assay 2	Av T1
2	1.198	0.814	1.007	2.376	2.564	2.470
5	1.197	1.420	1.458	4.217	4.119	4.168
8	0.594	0.557	0.575	0.900	0.750	0.825
11	1.017	0.633	0.825	1.848	1.625	1.736
14	1.332	0.916	1.124	4.000	4.119	4.060
17	3.885	3.643	3.764	2.476	2.490	2.483
20	1.892	1.286	1.589	0.516	0.528	0.522
Mean			1.477			2.323
SEM			0.987			1.325

Group II: Cimetidine

	Testosterone (ng/ml)					
Rat	Before treatment			After treatment		
No.	Assay 1	Assay 2	Av T1	Assay 1	Assay 2	Av T1
3	6.426	6.464	6.445	0.307	0.321	0.314
6	2.102	1.903	2.002	1.144	1.130	1.137
9	0.716	0.504	0.610	0.696	0.834	0.765
12	1.301	1.198	1.250	1.234	1.017	1.126
15	0.583	0.560	0.572	1.177	1.079	1.128
18	0.355	0.307	0.331	0.353	0.361	0.357
21	0.498	0.528	0.513	1.017	0.782	0.900
Mean			1.675			0.818
SEM			0.820			0.140

Group III: Ranitidine

	Testosterone (ng/ml)					
Rat	Before treatment			After treatment		
No.	Assay 1	Assay 2	Av T1	Assay 1	Assay 2	Av T1
1	0.498	0.470	0.484	0.712	0.716	0.714
4	1.029	1.011	1.021	0.547	0.597	0.572
7	1.079	1.263	1.171	0.492	0.570	0.531
10	1.066	1.256	1.161	0.428	0.376	0.402
13	0.854	1.035	0.945	3.222	3.222	3.222
16	0.467	0.597	0.532	1.137	1.048	1.092
19	0.430	0.399	0.415	2.934	3.601	3.267
Mean			0.818			1.400
SEM			0.120			0.480

Av T1: Average testosterone level (ng/ml).
*: Treatments were administered as indicated in appendix B, Table B1.

Table C2. Body weights, and organ weights (testes, prostates and seminal vesicles) of rats subchronically treated* with cimetidine, ranitidine and placebo

Group I: Placebo (Saline)

Rat	Body weight (g)		Organ weights (g)			
No.	BeT	AfT	R T	L T	Pro	SV
2	388	375	1.7	1.7	0.9	1.8
5	330	340	1.7	1.7	0.7	1.7
8	320	328	1.6	1.6	0.9	1.5
11	374	384	1.7	1.7	1.0	2.0
14	293	290	1.6	1.6	0.6	1.2
17	367	375	1.4	1.5	1.2	1.6
20	354	378	1.6	1.5	1.0	2.0
Mean	346.6	352.9	1.61	1.63	0.88	1.63
SEM	12.7	13.2	0.04	0.03	0.09	0.11

Group II: Cimetidine

Rat	Body weight (g)		Organ weights (g)			
No.	BeT	AfT	R T	L T	Pro	SV
3	336	320	1.6	1.6	0.8	1.0
6	305	291	1.4	1.4	0.6	1.0
9	385	358	1.4	1.4	0.6	1.1
12	327	323	1.5	1.5	0.5	1.0
15	310	305	1.5	1.6	0.9	1.2
18	423	384	1.5	1.4	0.9	1.0
21	273	255	1.4	1.4	0.5	1.0
Mean	337	319	1.47	1.47	0.69	1.04
SEM	19.3	16.1	0.03	0.03	0.07	0.03

Group III: Ranitidine

Rat	Body weight (g)		Organ weights (g)			
No.	BeT	AfT	R T	L T	Pro	SV
1	381	377	1.7	1.6	0.5	1.6
4	400	407	1.7	1.7	0.8	2.0
7	366	374	1.8	1.7	0.6	1.9
10	303	294	1.3	1.3	0.9	1.9
13	413	413	1.4	1.4	0.8	1.8
16	297	298	1.6	1.6	0.7	1.7
19	359	349	1.8	1.8	0.9	1.4
Mean	359.9	358.9	1.61	1.59	0.74	1.76
SEM	16.98	18.14	0.07	0.07	0.06	0.08

*: Treatments were administered as shown in appendix B, Table B1.

Abbreviations used: BeT= before treatment; AfT= after treatment; R T= right testis; L T= left testis; Pro= prostate; SV= seminal vesicles.

Table C3. Effects of subchronic treatment* with cimetidine and ranitidine on sperm motility

Group I: Placebo (Saline)

Rat	Sperm motility scores													
No.	M	N	M	N	M	N	M	N	M	N	TM	TN	M+N	%M
2	12	12	3	8	35	15	15	8	15	10	80	53	133	60
5	10	1	20	6	16	5	16	12	19	14	81	38	119	68
8	18	10	22	10	14	11	10	17	13	23	77	71	148	52
11	7	9	17	6	5	7	3	14	6	12	38	48	86	44
14	9	6	7	10	9	6	7	15	11	16	43	53	96	45
17	20	3	25	5	12	12	30	8	15	8	102	36	138	74
20	9	4	6	1	15	7	30	9	54	15	114	36	150	76
Mean														59.9
SEM														4.7

Group II: Cimetidine

Rat	Sperm motility scores													
No.	M	N	M	N	M	N	M	N	M	N	TM	TN	M+N	%M
3	9	1	9	1	10	0	10	0	15	0	53	2	55	96
6	7	10	12	13	8	16	10	15	12	8	49	62	111	44
9	45	6	50	12	45	15	50	6	50	8	240	47	287	84
12	45	8	40	6	100	25	35	20	100	20	320	79	399	80
15	3	5	3	8	5	10	10	4	8	4	29	31	60	48
18	35	18	18	6	30	16	18	6	20	7	121	53	174	70
21	4	3	5	3	3	2	7	0	9	5	28	13	61	46
Mean														66.9
SEM														7.3

Group III: Ranitidine

Rat	Sperm motility scores													
No.	M	N	M	N	M	N	M	N	M	N	TM	TN	M+N	%M
1**														
4**														
7**														
10	2	2	2	1	1	1	2	0	1	4	8	8	16	50
13	30	5	10	3	4	8	3	10	4	15	51	41	92	55
16	15	3	9	2	12	6	12	8	25	6	73	25	98	74
19	65	15	45	12	28	14	45	9	85	25	268	75	343	78
Mean														64.3
SEM														6.0

*: Treatments were administered as indicated in appendix B, Table B1.
**: Not determined, due to death of animals.

Abbreviations used: M= motile; N= non-motile; TM= total number of motile sperms; TN= total number of non-motile sperms; M+N= total number of sperms counted; %M= percent motile.

Table C4. Effects of subchronic treatment* with cimetidine, ranitidine and placebo on cauda epididymal sperm numbers

Group I: Placebo (Saline)

Rat	Sperm numbers**		
No.	Count 1	Count 2	Mean
2	85	115	100
5	92.5	97.5	95
8	115	125	110
11	77.5	55	65
14	110	70	90
17	125	112.5	118.8
20	70	127.5	98.8
Mean			96.8
SEM			6.4

Group II: Cimetidine

Rat	Sperm numbers**		
No.	Count 1	Count 2	Mean
3	170	117.5	143.8
6	20	22.5	21.3
9	35	60	47.5
12	85	65	75
15	102.5	90	96.3
18	90	130	110
21	35	40	37.5
Mean			75.9
SEM			16.5

Group III: Ranitidine

Rat	Sperm numbers**		
No.	Count 1	Count 2	Mean
1	70	95	82.5
4	120	110	115
7	112.5	105	107.5
10	117.5	80	97.5
13	160	147.5	152.5
16	160	125	142.5
19	65	95	80
Mean			111.1
SEM			10.6

*: Treatments were administered as indicated in appendix B, Table B1.
**:in million/cauda

Table C5. Seminiferous tubule diameter in rats after subchronic treatment* with cimetidine, ranitidine and placebo: Micrometer readings and calculations

Group I: Placebo (Saline)

Seminiferous tubule diameter													
Rat No. 2		5		8		11		14		17		20	
Micrometer readings													
50	49	43	43	44	41	36	40	39	33	42	37	45	35
48	46	40	47	60	43	40	39	35	31	46	46	50	21
40	54	41	39	59	36	37	41	33	30	40	43	40	29
38	53	44	37	41	37	37	39	33	37	38	39	48	44
40	55	45	32	45	40	44	34	38	36	39	47	47	45
48	47	42	37	45	40	39	38	40	32	37	52	40	39
40	50	49	37	41	47	37	39	35	43	38	47	38	47
48	42	37	37	45	45	35	40	39	39	40	41	35	44
44	45	44	38	47	36	36	30	45	32	42	33	40	42
46	50	39	42	52	41	40	38	49	34	45	34	36	48
49	53	55	38	42	44	36	38	44	38	21	38	39	49
49	48	48	40	39	38	31	35	43	37	45	38	30	41
49	45	43	45	46	43	39	37	35	34	41	42	38	41
52	52	34	40	46	35	40	36	53	33	35	47	28	40
48	51	42	39	41	35	40	37	39	36	33	36	35	40
42	47	40	34	40	35	40	36	34	36	40	39	40	37
44	40	37	41	46	46	39	37	33	40	42	44	40	43
43	41	30	41	49	48	40	44	33	40	38	44	38	49
48	39	60	45	46	49	40	39	40	38	55	43	41	44
49	41	45	41	42	46	43	36	36	35	48	37	45	40
40	46	42	49	43	45	33	36	36	34	46	31	26	47
40	38	39	47	37	40	40	37	36	38	44	43	48	38
47	43	41	46	35	40	37	37	36	33	37	43	43	47
45	42	42	43	46	45	39	41	38	33	42	43	45	45
41	45	40	52	44	45	38	38	41	38	41	40	48	48
Sum of 50 readings													
1128+1163 =2291		1062+1030 =2092		1121+1050 =2171		993+ 942 =1935		963+890 =1853		1015+1027 =2042		1003+1043 =2046	
Average reading													
45.82		41.84		43.42		38.7		37.06		40.84		40.92	
Conversion to mm (average reading x .006)													
0.275		0.251		0.261		0.232		0.222		0.245		0.246	
Conversion to micrometers (mm x 1000)													
275		251		261		232		222		245		246	
Group mean +/- SEM: 247.4 +/- 6.6													

Group II: Cimetidine .....continued

Table C5. (continued)

Group II: Cimetidine

Seminiferous tubule diameter													
Rat No. 3		6		9		12		15		18		21	
Micrometer readings													
32	34	38	37	40	50	35	35	37	45	45	31	48	33
37	31	39	46	45	36	38	41	38	45	42	31	44	39
39	41	39	39	38	38	35	35	40	51	43	35	40	49
33	32	40	44	36	33	35	35	37	48	40	35	48	38
34	33	43	36	35	39	36	31	37	41	40	34	43	37
36	48	47	38	30	50	33	38	40	46	49	30	53	35
48	44	42	43	37	50	31	36	38	44	40	33	49	39
43	30	44	43	37	43	33	37	36	42	45	30	55	40
43	34	45	43	41	52	38	41	35	35	43	40	56	36
40	45	34	38	40	36	35	48	40	39	45	37	36	31
44	42	39	39	49	43	37	44	39	38	41	35	36	35
39	32	38	42	46	40	33	43	35	40	37	32	41	38
43	35	39	45	45	32	35	42	36	44	35	37	45	33
43	40	39	40	46	38	30	45	36	39	37	40	36	37
55	50	39	44	43	36	36	47	37	40	34	36	39	40
57	37	34	42	42	39	35	47	42	38	31	36	40	40
30	28	45	30	40	38	34	41	49	35	32	44	40	38
37	31	46	40	38	41	30	40	33	39	34	40	44	37
35	35	44	31	40	41	37	35	37	39	25	32	41	40
40	35	45	36	34	40	37	44	49	38	29	35	30	40
36	35	44	41	39	37	36	42	36	39	31	31	31	41
38	34	42	41	38	41	34	31	37	36	35	30	35	39
31	40	41	34	42	34	33	39	43	40	35	32	32	40
28	28	42	36	39	41	32	36	44	38	34	38	38	39
34	33	44	36	45	38	37	38	43	35	30	38	33	41
Sum of 50 readings													
975+ 906 =1881		1032+ 984 =1964		1005+1006 =2038		865+ 991 =1856		974+1014 =1988		932+ 872 =1804		1033+ 955 =1988	
Average of 50 readings													
37.62		39.28		40.76		37.12		39.76		36.08		39.76	
Conversion to mm (average x .006)													
0.226		0.236		0.245		0.223		0.239		0.217		0.239	
Conversion to micrometers (mm x 1000)													
226		236		245		223		239		217		239	

Group mean +/- SEM: 232.1 +/- 3.9

Group III: Ranitidine.....continued

Table C5. (continued)

Group III: Ranitidine

Seminiferous tubule diameter													
Rat No. 1		4		7		10		13		16		19	
Micrometer readings													
40	37	48	50	43	38	43	38	38	31	46	36	41	40
41	35	45	48	43	42	44	44	42	32	41	42	47	40
41	40	46	38	38	34	49	38	41	34	47	29	47	35
40	37	38	36	40	40	42	36	40	40	47	28	48	42
39	40	31	39	36	42	41	35	39	39	51	28	43	33
40	39	45	41	35	38	41	41	42	37	46	32	44	35
38	39	39	41	34	38	35	36	41	36	53	30	46	39
39	36	45	36	37	37	39	39	40	37	53	29	39	40
36	38	41	34	40	36	45	38	40	40	45	28	39	33
39	35	38	37	33	39	41	37	41	37	47	31	47	34
36	37	41	26	37	39	50	38	36	40	45	31	41	37
41	38	43	43	36	45	36	40	42	37	49	30	45	42
40	38	43	35	42	33	41	40	46	41	50	36	43	43
40	37	39	38	43	33	37	37	40	36	50	31	40	43
44	40	42	39	42	50	39	32	41	36	50	28	41	47
38	39	46	38	37	44	35	31	38	42	44	38	40	47
37	37	43	38	37	38	42	29	43	50	44	34	39	41
43	40	46	43	38	34	45	31	39	44	38	33	40	39
42	40	37	47	45	40	37	30	37	44	33	38	40	45
35	37	46	46	44	40	34	33	39	38	48	37	41	40
36	37	43	29	47	46	32	38	34	39	38	33	41	36
37	39	36	43	33	39	39	39	37	35	39	35	45	34
40	35	34	37	36	40	32	37	35	40	36	32	44	38
37	37	43	36	37	35	44	35	37	40	36	40	43	33
43	38	48	32	35	41	35	37	38	39	38	40	36	37
Sum of 50 readings													
982+ 945 =1927		1046+ 978 =2024		968+ 981 =1949		988+ 909 =1897		986+ 964 =1950		1114+ 829 =1943		1060 973 =2033	
Average of 50 reading													
38.54		40.48		38.98		37.94		39.00		38.86		40.66	
Conversion to mm (average x .006)													
0.231		0.243		0.234		0.228		0.234		0.233		0.244	
Conversion to micrometers (mm x 1000)													
231		243		234		228		234		233		244	
Group mean +/- SEM: 235.3 +/- 2.3													

*: Treatments were administered as shown in appendix B, Table B1.

APPENDICES

APPENDIX D: Data on serum testosterone and standard curve.

Table D1. Data on serum testosterone concentration for test samples

Rat No.	cpm-1	cpm-1	%c.v.	%B/Bo	%B/Bo	conc.	conc. avg	conc.
Before treatment								
1	13374.5	13480.3	0.56	85.38	86.07	0.498	0.470	0.484
2	11472.3	12393.0	5.45	72.90	78.94	1.198	0.814	1.007
3	6907.9	6897.1	0.11	42.95	42.88	6.426	6.464	6.445
4	11846.8	11892.5	0.27	75.36	75.65	1.029	1.011	1.021
5	10883.0	11019.4	0.88	69.03	69.93	1.497	1.420	1.458
6	9957.9	10228.0	1.89	62.96	64.73	2.102	1.903	2.002
7	11734.8	11339.0	2.43	74.62	72.02	1.079	1.263	1.171
8	13056.1	13176.0	0.65	83.29	84.07	0.594	0.557	0.575
9	12675.8	13358.1	3.71	80.79	85.27	0.716	0.504	0.610
10	11762.1	11342.3	2.57	74.80	72.04	1.066	1.256	1.161
11	11877.5	12930.2	6.00	75.55	82.46	1.017	0.633	0.825
12	11255.1	11458.8	1.27	71.47	72.81	1.301	1.198	1.250
13	12282.3	11831.1	2.65	78.21	75.25	0.854	1.035	0.945
14	11202.0	12122.0	5.57	71.13	77.16	1.332	0.916	1.124
15	13086.2	13168.1	0.44	83.48	84.02	0.583	0.560	0.572
16	13486.6	13039.2	2.38	86.11	83.18	0.467	0.597	0.532
17	8242.7	8413.3	1.45	51.71	52.83	3.885	3.643	3.764
18	13925.4	14128.0	1.02	88.99	90.32	0.355	0.307	0.331
19	13628.6	13746.1	0.61	87.04	87.81	0.430	0.399	0.415
20	10243.4	11285.0	6.83	64.84	71.67	1.892	1.286	1.589
21	13376.7	13272.8	0.55	85.39	84.71	0.498	0.528	0.513
After treatment								
1	12688.1	12671.7	0.09	80.88	80.77	0.712	0.716	0.714
2	9606.8	9399.6	1.54	60.66	59.30	2.376	2.564	2.470
3	14124.1	14059.8	0.32	90.29	89.87	0.307	0.321	0.314
4	13207.1	13044.6	0.88	84.28	83.21	0.547	0.597	0.572
5	8023.8	8078.0	0.48	50.27	50.63	4.217	4.119	4.168
6	11588.2	11606.4	0.11	73.66	73.78	1.144	1.130	1.137
7	13393.1	13130.6	1.40	85.50	83.78	0.492	0.570	0.531
8	12165.1	12566.3	2.29	77.44	80.07	0.900	0.750	0.825
9	12735.4	12343.6	2.21	81.18	78.61	0.696	0.834	0.765
10	13633.5	13838.1	1.05	87.07	88.42	0.428	0.376	0.402
11	10316.3	10664.0	2.34	65.31	67.59	1.848	1.625	1.736
12	11395.9	11866.2	2.86	72.39	75.48	1.234	1.017	1.126
13	8755.1	8758.9	0.03	55.07	55.10	3.222	3.222	3.222
14	8167.6	8082.8	0.74	51.21	50.66	4.000	4.119	4.060
15	11505.4	11735.6	1.40	73.11	74.63	1.177	1.079	1.128
16	11594.9	11803.4	1.26	73.70	75.07	1.137	1.048	1.092
17	9504.5	9489.0	0.11	59.99	59.89	2.476	2.490	2.483
18	13928.9	13898.0	0.16	89.02	88.81	0.353	0.361	0.357
19	9025.8	8446.5	4.69	56.85	53.05	2.934	3.601	3.267
20	13311.8	13271.4	0.21	84.96	84.70	0.516	0.528	0.522
21	11878.4	12476.1	3.47	75.56	79.48	1.017	0.782	0.900

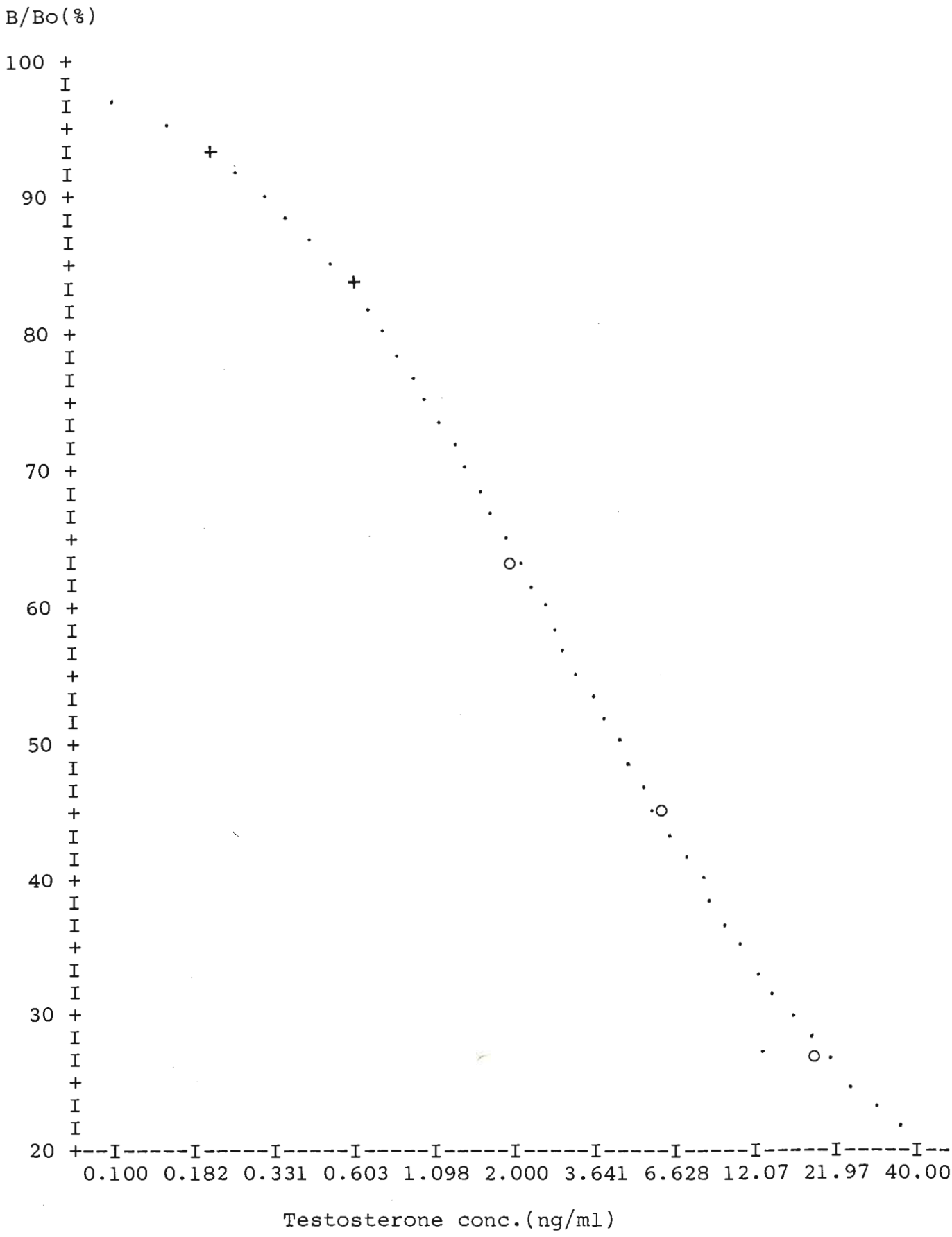


Figure F1. Standard curve for testosterone (% B/Bo vs. concentration).

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