EFFECTS OF SYMPATHOMIMETIC DRUGS ON THE CONTRACTILITY OF THE VAS DEFERENS AND ON FERTILITY IN THE MALE RAT AND RABBIT

A THESIS

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This thesis is dedicated to my parents

Mr. M.G. Piyadasa

and

Mrs. Nanda Piyadasa

with respect and gratitude.

SUMMARY

The sympathomimetic drugs noradrenaline, methoxamine, tyramine and norephedrine, caused rhythmic contractions in isolated human vasa deferentia. These contractions were mediated via α-adrenoreceptors. Intravenous administration of some of these drugs into rats and guinea-pigs produced contractions of the vas deferens in vivo but was accompanied by severe cardiovascular side effects. Hence a local method of application, using medical grade silastic in the form of collars or rods was developed. Insertion of these slow-releasing devices around the vas deferens in anaesthetized rats produced rhythmic contractions without serious side effects.

In a fertility screen, these silastic drug mixtures caused a temporary reduction in fertility of male rats and rabbits. The maximal antifertility action occurred in the first week following the insertion of drug-containing collars or rods. At the time of the peak effect on fertility, the numbers of sperm in the ejaculate were reduced to almost zero. In addition the treatment impaired the quality of the ejaculated sperm. Spontaneous restoration of fertility was evident with some of the treatments, but not with others.

It was concluded that the main cause of infertility was the reduction of sperm numbers in the ejaculate resulting from either a block in sperm transport in the vas deferens or by a deficiency in the mechanism of emission. An occlusion of the vas may result from a mechanical block as with methoxamine or from a sustained spasm. A defect in emission may result from depletion of the transmitter, receptor-specific desensitization or by pre-synaptic α-receptor mediated inhibition.

CONTENTS

Communications and papers submitted

Communications

1) Ratnasooriya, W.D., Gilmore, D.P. & Wadsworth, R.M. (1977) An antifertility effect of sympathomimetic drugs on rats. Annual conference of the Society for the Study of Fertility in Dublin, July 1977.

2) Ratnasooriya, W.D., Gilmore, D.P. & Wadsworth, R.M. (1978) The effect of local administration of sympathomimetic drugs to the epididymis on fertility of rats. Annual conference of the Society for the Study of Fertility in Cambridge, July 1978.

Submitted papers

1) The effect of sympathomimetic drugs on contractility of the vas deferens *in vitro* and *in vivo*. J. Reprod. Fert.
2) An antifertility effect of sympathomimetic drugs on male rats when applied locally to the vas deferens. J. Reprod. Fert.
3) The effect of local application of sympathomimetic drugs to the epididymis on fertility of rats. J. Reprod. Fert.
4) The effect of norephedrine on semen quality of rabbits when applied locally to the vas deferens. Fert. Steril.
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6) The short term effect of ligation of the vas deferens on distribution, motility and morphology of spermatozoa in the rat, mouse, guinea-pig, gerbil and hamster. Int. J. Fert.
7) A method of local administration of sympathomimetic amines using silastic implants. J. Pharmacol. Methods.
8) The effect of 6-hydroxydopamine on contractions produced by sympathomimeti amines in the human isolated vas deferens J. Pharm. Pharmac.
9) The effects of Fenclozic acid on fertility of male rats. Contraception.

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INTRODUCTION

Male methods of contraception

Current Methods

Various methods of male contraception at present exist. In coitus interruptus, the penis is withdrawn just before ejaculation occurs, so that the semen does not enter the vagina. However, this method may not be effective as some spermatozoa often escape before full ejaculation, and it can lead to sexual frustration (Rhodes, 1971), as an orgasm may not be reached (particularly by the women).

The condom, another method, has some disadvantages. These are as follows: 1) Its application interrupts courtship behaviour, 2) immediate withdrawal of the penis after ejaculation is necessary to prevent spillage of semen into the vagina, 3) it diminishes sensitivity of intercourse particularly to women (Rhodes,1971) and 4) its use leads to the development of hypersensitive reactions in some persons. Nevertheless, both withdrawal and the condom provide simple, inexpensive, non-clinical methods of contraception which are also free from major side-effects.

A very effective irreversible form of contraception is vasectomy. This involves the removal of a small segment of each vas deferens under local anaesthesia(see Lumbell et al., 1976). Vasectomy does not result in immediate sterility and other contraceptive measures are required until the semen is devoid of spermatozoa (see Hulka et al., 1972). In addition, there are other complications encountered. The most immediate and least serious side-effects are skin discolouration, swelling and pain in up to 50% of men (Wortman, 1975). Development of haematomas in up to 18% of men has also been noted (Lumbell et al., 1976). Distension of the epididymis has also been reported (rabbit: Flickinger, 1975a; Bedford, 1976; rat: Flickinger, 1972; Bedford, 1976; monkey and hamster: Bedford, 1976). Formation of granulomas either in the vas deferens or in the epididymis is also by no means uncommon (rat: Smith, 1962; Bedford, 1976; hamster and monkey: Bedford, 1976; dogs: Vare et al., 1973 and humans: Schmidt, 1975,

Alexander et al., 1977). The presence of granulomas may cause pain (Schmidt, 1975) and can result in recanalization of the vas deferens through the ducts formed within it, leading to failure of the method (Schmidt, 1975). Several workers have reported changes in the testes (rat: Kinson et al., 1975; hamster: Johnson et al., 1975; rabbit: Flickinger, 1975b; Bedford, 1976). However, no major alterations to testicular morphology following vasectomy have been observed by others (rat: Smith, 1962; monkey: : Bedford, 1976; dog: Vare et al., 1973). Immunologically, no serious effect has been reported, although a large proportion of men and animals produce both sperm agglutinating and sperm immobilization antibodies at varying periods following vasectomy (Ansbacher et al., 1975, Alexander, 1975, Alexander et al., 1977). To date no adverse endocrinological consequences have been documented (Bunge, 1972, Johnsonbough et al., 1975).

Methods under investigation

(a) Occlusion of the vas

Occlusion of the vas deferens by injecting sclerolysing agents has been demonstrated (Setty et al., 1972, Coffey et al., 1975). Moreover, Coffey et al. (1975) have shown a decrease in fertility of rats and production of azoospermia in men by this method. The possible limitations of this approach are 1) it appears to be permanent and 2) long term adverse effects have not yet been studied.

The production of either sterility or azoospermia in animals can be achieved by inserting a silastic 'plug' into the vas deferens (Hrdlicka et al., 1967; Hooker et al., 1972). Such an approach also has the difficulty in reversing the induced sterility. Reduction in sperm counts in humans also occurs after blocking the vas deferens with silastic material of different shapes and sizes (Kothari et al., 1967; Brodie, 1975).

The blockage of the vas deferens by tantalum clips results in azoospermia in dogs (Jhaver et al., 1971) and in humans (Gupta et al., 1977). Removal of the clips, however, was not only difficult but left the vas deferens compressed and leaking. With the hope of restoring fertility Free (1975)

blocked spermatozal transport in the vas deferens by means of a mechanical valve. As expected, he obtained sterility with the valve closed and resumption of fertility when the valve reopened. However, the valves are expensive and the insertion must be carried out under general anaesthesia.

(b) Regression of accessory glands

Regression of accessory glands and loss of fertility without a concomitant loss of libido has been induced by administering relatively high doses of the antiandrogen cyproterone acetate to rats or guinea-pigs (Zucker, 1966; Whalen et al., 1969). However, cyproterone acetate in addition to its action on accessory glands causes inhibition of spermatogenesis (see Neumann, 1977).

(c) Disruption of spermatogenesis

(1) Direct suppression of spermatogenesis

Inhibition of spermatogenesis has been induced in cats, dogs, monkeys and humans (Fahim et al., 1977) by applying ultrasound to the testes. Moreover, Fahim et al. (1975) have demonstrated the induction of a reversible sterility in rats by exposing the testes to infra-red microwaves.

Several classes of drugs also inhibit sperm production. The nitrofurans, the thiophenes, the bis-(dichloroacetyl)-diamines and the dinitropyrroles inhibit spermatogenesis, primarily by suppressing maturation of primary spermatocyte stage (see Jackson, 1969; Patanelli, 1975).

Alkylating agents and related compounds like nitrogen mustards, ethyleneimine derivatives and alkane sulphonic esters also affect spermatogenesis by arresting spermatogonial development (see Jackson, 1969; Patanelli, 1975). However, the toxic properties of these drugs preclude their use as contraceptives in the human.

Temporary disruption of spermatogenesis, resulting in infertility without loss of libido, in mice has been recently demonstrated by administering 5-thio-D-glucose (Zysk et al., 1975).

Inhibition of spermatogenesis in a dose dependent manner with the antiandrogen cyproterone acetate has been reported in many species (see

Neumann, 1977) and in man (Koch et al., 1976). In lower doses it arrests the development of spermatids and at higher doses also prevents the division of spermatocytes.

(2) Suppression of gonadotropin release

Reversible suppression of spermatogenesis resulting in azoospermia but with no loss of libido or potency has been reported in man (Heller et al., 1950; Reddy et al., 1972) and in rabbits (Ewing et al., 1973) following daily intramuscular administration of the androgen, testosterone. However, because of its short serum half life, testosterone is not suitable as a contraceptive.

Briggs et al. (1974) claimed that twice-daily oral treatment of a combination of oestrogen and testosterone could cause reversible disruption of spermatogenesis leading to azoospermia in humans . However, a few men complained of loss of libido and potency.

Administration of various concentrations of androgen and progesterone also causeoligospermia or azoospermia in man (Frick et al., 1976), and cessation of the treatment resulted in the reappearance of spermatozoa in the semen. However, loss of libido or development of gynaecomastia was seen in many instances.

The main objections to the use of presently known hormonal therapy are the frequent injections required by their relative short half-life in blood or, if they are orally active, their association with liver toxicity.

(d) Inhibition of epididymal function

Interference with epididymal function has the following advantages over other methods of male contraceptive techniques investigated. Firstly, there is rapid onset and reversibility of action and, secondly, spermatogenesis itself is not affected. Since the functional and structural integrity of the epididymis is androgen-dependent (see Orgebin-Crist et al., 1975) one way to interfere with its activity is by using antiandrogens. Indeed, loss of fertility without changes in libido and accessory gland function has been demonstrated in rats using microquantities of the antiandrogen cyproterone acetate (Prasad et al., 1970; Rajalakshmi et al., 1976).

The other major agent which induces reversible sterility in animals is

α-chlorohydrin (rat: Coppola, 1969; Tsunoda et al., 1976; monkey: Kirton et al., 1970; ram: Brown-Woodman et al., 1974; mouse: Tsunoda et al., 1976). However, recent work suggests that the drug acts directly on the epididymal sperm rather than damaging the epididymal cells (see Glover, 1976). Nevertheless, the drug could also change the composition of the epididymal plasma in a way that has not so far been detected.

Male reproductive tract: structure, function and pharmacology

Anatomy and histology of the vasa efferentia

The vasa efferentia are tiny tubes which connect the extratesticular portion of the rete testis with the initial segment of the epididymis (see Hamilton, 1975). The actual number of tubules present varies with the individual animal. Structurally, each tubule has a central lumen which is surrounded by a layer of columnar epithelial cells resting on a basement membrane. This epithelium consists, primarily, of ciliated and non-ciliated cells (monkey: Ramos et al., 1977; human: Baumgarten et al., 1971; rat: Hamilton, 1975) with occasional macrophages, intra-epithelial lymphocytes and basal cells. The endoplasmic reticulum and the Golgi apparatus are poorly developed in both ciliated and non-ciliated cells, suggesting a low capacity for protein synthesis. On the other hand, the presence of micropinocytotic vesicles, membrane-bound dense bodies and numerous vacuoles of varying size within the non-ciliated cells may perhaps indicate an absorptive function. Large amounts of mitochondria present in the ciliated cells may aid in providing energy for the activity of cilia in the transport of luminal content including the spermatozoa. The epithelial layer is surrounded by (1) a thin layer of circularly arranged contractile cells, (2) connective tissue, (3) blood vessels and (4) unmyelinated nerves. Fluorescence micrographs indicate that these nerves are indeed adrenergic with terminal varicosities which form a loose peritubular plexus in man (Baumgarten et al., 1971) and in the cat (El-Badawi et al., 1962).

Anatomy and histology of the ductus epididymides

The ductus epididymis is an extremely coiled single tube which connects the vasa efferentia with the vas deferens. It is by far the longest part of the male genital duct system; in the human it is about 4-5 metres in length (Dym, 1977). The epididymis is divided topographically into three main portions: the caput, the corpus and the cauda. Microscopically, these parts are further divided into zones and sub-zones (Reid et al., 1957). Glover et al. (1971) divided the epididymis into three segments based on histological and cytological studies: an 'initial' segment, a 'middle' segment or 'intermediate' segment and a 'terminal' segment. These segments do not, however, correspond exactly to the caput, the corpus or the cauda epididymis.

The single epididymal tube contains a central lumen whose diameter varies along the length of the tube (Reid et al., 1957; Martan, 1969; Baumgarten et al., 1971; Hamilton, 1975; Dym, 1977). The lumen is surrounded by a layer of columnar epithelial cells, resting on a basal lamina. There are two main types of epithelial cell: sterociliated or principal cells and non-sterociliated cells. The former is goblet in shape and characterized by the presence of (1) a prominent endoplasmic reticulum and Golgi apparatus, (2) sterocilia and micropinocytotic invaginations in the apical plasmalemma, (3) large numbers of mitochondria and multivesicular bodies including lysosomes. The non-sterociliated cells are pyramidal in shape and contain only typical cellular organelles and inclusions. Electronmicroscopically, these cells have been further subdivided into apical, basal, halo and clear cells, although some workers believe that all four cell types just represent different stages of a halocrine cell-cycle (see Martan, 1969).

The epithelial lining is encircled by a layer of smooth muscle cells. The muscle layer is very thin over most of the length of the tube and up to the mid cauda it is composed of only smooth muscle-like cells (Baumgarten et al., 1971; Dym, 1977). In more distal parts of the cauda, two-layered muscle coat is seen and in the most distal portion this is transformed into a three-layered coat (Baumgarten et al., 1971). Outside the muscle coat is a layer of connective tissue forming the interstitum of the epididymis. Embedded in this layer are

lymphatic vessels, blood vessels and adrenergic varicose nerve terminals forming a peritubular nerve plexus. Marked regional differences occur in the distribution of the nerve plexus. The peritubular nerve fibres are practically absent in the caput and upper corpus regions of the epididymis of the guinea-pig, rabbit and rat and sparse in the monkey and in man (Baumgarten et al., 1971). In the monkey and in man these adrenergic fibres do not penetrate into the muscle layer and generally lie in close association with a blood vessel. In contrast, the adrenergic plexus is well developed in the cauda epididymis, while the fibres penetrate deep into the muscle layers only in the distal cauda epididymis.

Anatomy, histology and pharmacology of the vas deferens

The vas deferens is a tubular organ which connects the terminal portion of the cauda epididymis with the prostatic urethra. In humans it is about 35 cm in length. The average external diameter measures about 2.5 mm and the internal diameter varies from 0.7 to 1.1 mm. The variation in the internal diameter is related to the size of the external genitalia, that is: the lumen of the vas is greater in persons with larger genitalia (Brodie, 1975). Furthermore, in some species including human, the lumen widens near its prostatic end and appears as a spindle shaped enlargement (Sjöstrand, 1965).

The wall of the vas deferens is composed of three well defined layers: the mucosa, the muscularis and the adventitia. The adventitia is the outer-most layer and is composed of blood vessels, small branches of the hypogastric nerve (Popovic et al., 1973) and loose connective tissue (Dym, 1977). The muscularis is made up of smooth muscle cells arranged in bundles. Each cell is about 400μm in length and is surrounded by about twelve neighbouring cells. Each muscle cell is coupled to its neighbours by a flask-shaped protrusion (see Bennett, 1972). Geometrical packing of these bundles gives the characteristic layered appearance to the muscularis. In most mammals a three layered arrangement is seen: a middle circular layer sandwiched between an outer and an inner layer of longitudinally arranged smooth muscle. This arrangement is seen for instance, in man (Baumgarten et al., 1971; Popovic et al., 1973; Mather, 1975) and in the bovine (Mather, 1975) vas

deferens. In the guinea-pig (Gosling et al., 1972) and rabbit (Mather, 1975) only an outer longitudinal and inner circular layer is seen, both being of about equal thickness in the former, and the longitudinal layer being larger in the latter. On the other hand, the canine vas deferens does not show such a discernible pattern of longitudinal and circular layers due to the interweaving of fibres through out the entire length of the ductus deferens (Mather, 1975). Even in a given species the layered arrangement varies from the epididymal to the urethral end (Baumgarten et al., 1971).

The mucosa surrounds the lumen and is undulated and irregular in appearance. It is composed of pseudostratified epithelial cells (Popovic et al., 1973; Hamilton, 1975; Hoffer, 1976) arranged in two layers (a) small basal cuboidal cells resting on the basement membrane and (b) a luminal layer of columnar cells. The cuboidal cells possess a prominent nucleus but have a poorly developed endoplasmic reticulum and Golgi apparatus (Hamilton, 1975; Hoffer, 1976). At least two different types of cells have been reported to be present in the columnar layer: the principal cell and the pencil cell. The latter type is simply a dead or dying cell (Hoffer, 1976). The former type is endowed with (1) sterocilia at the apical surface (2) endocytotic invaginations in the plasmalemma between sterocilia and (3) well developed endoplasmic reticulum and Golgi. It would seem that the principal cells have a high degree of metabolic activity. A third cell type is unique to humans and monkeys (Hoffer, 1976), and is known as the mitochondrion rich cell. It is basically similar to the principal cell but contains an extremely large number of mitochondria in the cytoplasm and a poorly developed endoplasmic reticulum and Golgi apparatus. Lymphocytes are also reported to be present, occasionally scattered amongst epithelial cells (Popovic et al., 1973; Hamilton, 1975; Hoffer, 1976).

(a) Innervation

1. Cholinergic

In most mammals the vas deferens seems to be the most densely innervated smooth muscle organ in the body (Sjöstrand, 1965). It has apparently a dual innervation, consisting of a predominate adrenergic and a supplementary

cholinergic supply. The main evidence for the existence of a cholinergic innervation are: (1) The observation that a spontaneous release of acetylcholine occurs in desheathed isolated rat vasa deferentia. Field stimulation also caused a release of acetylcholine in a dose-dependent manner (Knoll et al., 1972), (2) Atropine sensitive spontaneous junctional potentials have been demonstrated in the vasa deferentia of guinea-pigs (Inomata et al., 1971), (3) Histochemical studies have revealed the presence of acetylcholine esterase positive nerve fibres in the muscularis of the vasa deferentia of the mouse (Jones et al., 1975), the cat (Jacobowitz et al., 1965), the guinea-pig (Jacobowitz et al., 1965; Robinson, 1969; Gosling et al., 1972; Al-Zuhair et al., 1976) or the human (Shirae et al., 1973). Moreover, a patch of acetylcholine esterase activity has been recorded on the muscle membranes adjacent to acetylcholine esterase positive axons when they approached within 1100 Å of the smooth muscles (Robinson, 1969), (4) In electron micrographs intra-axonal large agranular vesicles (450-600 Å), believed to contain acetylcholine, have been observed in the vas deferens of the rat (Farrell, 1968), the guinea-pig (Gosling et al., 1972), the mouse (Jones et al., 1975) and in man (Baumgarten et al., 1971). Neither reserpinization (Farrell, 1968) nor sympathectomy (Jones et al., 1975) reduced the number of agranular vesicles seen in the rat or the mouse.

2. Adrenergic

There is morphological, histological, physiological and pharmacological evidence for the existence of an extremely dense adrenergic innervation of the vasa. In mammals the vas deferens receives sympathetic fibres via the hypogastric nerves, which emanate from the inferior mesenteric ganglion or ganglia (see Sjöstrand, 1965). However, in contrast to the general pattern of adrenergic innervation in other organs, the final pathway to the effector organ originates from peripherally located ganglion cells. Thus there is no decrease in noradrenaline content after hypogastric denervation (rat: Owman et al., 1972; guinea-pig: Owman et al., 1972; monkey and rabbit: Sjöstrand, 1965). These "short adrenergic neurones" (Sjöstrand, 1965) differ in several physiological and pharmacological properties from the conventional adrenergic

fibres:- e.g. (1) high doses of reserpine are required to deplete the noradrenaline stores in the vas deferens (Ambache et al., 1971) (2) there is resistance against the neurotoxic effects of 6-hydroxydopamine in the mouse (Jenkins et al., 1977), (3) there is difficulty in depleting the endogenous store of noradrenaline in the vas deferens (Swedin, 1971) by repeated nerve stimulation.

A very dense adrenergic plexus has also been demonstrated in the musculature of vasa deferentia of a number of species, using Falck and Hillarp's histological technique (rat: Norberg et al., 1967; Owman et al., 1972; Anton et al., 1977; guinea-pig: Jacobowitz et al., 1965; Owmen et al., 1972; cat: Jacobowitz et al., 1965; human: Baumgarten et al., 1971). Consistent with this finding, an exceptionally high concentration of noradrenaline is present in the vas deferens of the hedgehog, monkey, rabbit, rat, mouse, guinea-pig, dog, fox, cat, bull (Sjöstrand, 1965) and man (Baumgarten, 1971). Moreover, noradrenaline appears in the bathing fluid after field stimulation of isolated vasa deferentia of the mouse (Farnebo et al., 1971) and the rat (Langer, 1970). Studies using the electron microscope have also indicated the presence of intra-axonal small granular vesicles (about 400 A) thought to contain noradrenaline, in the ductus deferens of the rat (Farrell, 1968) the guinea-pig (Al-Zuhair et al., 1976) and man (Baumgarten et al., 1971). In the rat, pretreatment with reserpine caused a marked reduction in the number of granular vesicles in the nerve varicosities (Farrell, 1968).

There is considerable but not overwhelming pharmacological evidence that noradrenaline is the transmitter of the motor nerves. After pretreatment with 6-hydroxydopamine (which causes necrosis specifically of the adrenergic neurones), the motor responses of isolated vasa deferentia were abolished in the guinea pig (Wadsworth, 1973) and in the mouse (Jones et al., 1975) and the fluorescent nerve terminals could no longer be demonstrated by the method of Falck and Hillarp. A tenfold increase in sensitivity in the mouse and moderate increase in sensitivity in the guinea-pig vasa, to exogenous noradrenaline, were also reported by the same investigators. Similarly, a supersensitivity to exogenous noradrenaline has been found after surgical

denervation of the ductus deferens of the rat and guinea-pig (Birmingham, 1970). Furthermore, tyramine (a drug which causes the release of endogenous noradrenaline from nerve terminals) did not produce contractions after denervation. Cocaine, a drug which inhibits neuronal uptake of noradrenaline, produces supersensitivity to exogenously added noradrenaline in the vas deferens of the mouse without any enhancement in the potency of methoxamine , which is not taken up by the adrenergic neurones (Buckner et al., 1975; Pennefather, 1976). Another uptake blocking drug, desipramine, was also found to potentiate the contractions resulting from nerve stimulation without any change in the output of noradrenaline in the mouse isolated vas deferens preparations (Farnebo et al., 1971). Guanethidine, a drug which prevents the release of noradrenaline from adrenergic neurones, also significantly reduced the nerve-mediated contractions both in vitro (rat: Anton et al., 1977; guinea-pig: Furness, 1974; mouse: Jones et al., 1975) and in vivo (Anton et al., 1977).

Using electrophysiological techniques in the guinea-pig vas deferens it has been found that spontaneous junctional potentials reduced both in amplitude and in frequency after reserpinization (Burnstock et al., 1962). These spontaneous junctional potentials are depressed or sometimes even abolished after chronic administration of bretylium, another drug which prevents the release of noradrenaline (Nakanishi et al., 1970).

In spite of such a large body of evidence to suggest that the main motor innervation in the vas deferens is adrenergic, there are results which cannot be completely explained on this theory, and some workers believe that an as yet unidentified substance is the transmitter. The main lines of evidence are as follows:- (1) Field stimulation of the isolated rat or guinea-pig vas deferens evokes contractions which are not blocked by α-adrenoreceptor blockers, although they do prevent the contractions produced by exogenously added noradrenaline or tyramine (Ambache et al., 1971, 1972). Furthermore, chronic administration of α-blockers also failed to prevent nerve-mediated contractions of the vas deferens of guinea-pig in vivo (Nakanishi

et al., 1970) (2) Very low concentrations of noradrenaline inhibit the twitch response to a single pulse field stimulation of isolated mouse vas deferentia (Jenkinset al., 1977) or the guinea-pig (Ambache et al., 1971) (3) Relatively weak contractions are produced in the vas deferens by exogenous noradrenaline compared with those to nerve-stimulated contractions (Ambache et al., 1971; Jenkinset al., 1977) (4) Pretreatment with (-) β -hydroxyphenethyl guanidine (a more potent agent than guanethidine) did not impair motor transmission in the vas deferens of guinea-pig (Henderson et al., 1976). Yet the treated animals showed a total lack of catecholamine fluorescence.

Explanations have been advanced to account for these observations on the 'noradrenaline theory'. The ineffectiveness of α -receptor antagonists in reducing nerve-mediated contractions of the vas deferens may be due to the presence of a diffusion barrier which prevents the penetration of drugs to the neuroeffector junction. Indeed, Furness et al. (1972) have demonstrated the existence of a 'seal' formed at the orifice of the groove of smooth muscle by the confluence of basement membrane associated with the nerves and muscles. This complex arrangement of nerve and muscle cell may arise during post-natal development (Furness et al., 1970), as Swedin (1971) has demonstrated a complete block of nerve-mediated contraction with neonatal rats. Secondly, during nerve stimulation the large amounts of noradrenaline released (Langer, 1970; Farnebo et al., 1971) into the narrow sub-synaptic gap may compete for the receptors. Thirdly, it is also possible that noradrenaline uptake blocking action of α-adrenoreceptor antagonists prevents them from displaying antagonistic action. Fourthly, α-adrenoreceptor antagonists contribute to their own ineffectiveness by increasing the amount of noradrenaline released during nerve stimulation.

The failure of exogenous noradrenaline to produce a contraction of similar magnitude to that of electrical stimulation may also be a consequence of the diffusion barrier. The observation that noradrenaline inhibits the contractions produced by nerve stimulation could be explained by receptor specific desensitization as seen in guinea-pig vas deferens (Wadsworth, 1974).

Hence, there is evidence both for and against the idea that noradrenaline

is the motor transmitter. To some extent it may be possible to reconcile these opposing theories as there is evidence of both adrenergic and non-adrenergic components of the motor response.

3. Evidence for dual motor innervation

Swedin (1971) and Birmingham et al. (1971), however, demonstrated that in the rat and guinea-pig the mechanical response of the vas deferens to motor nerve stimulation is complex, consisting of two phases, an initial rapid 'twitch' response and slower better-maintained 'secondary response' which is a sustained a rise in tension. Subsequently, this complex response of the vas deferens to motor nerve stimulation was confirmed in the rat, guinea-pig, rabbit and mouse (Duncan et al., 1976; Anton et al., 1977) but not in man (Anton et al., 1977). If the vasa deferentia were bisected, then the responses in the two halves were different, with the 'secondary' component dominating in the epididymal half in the rat (Duncan et al., 1976; Anton et al., 1977), the guinea-pig, the rabbit or the mouse (Anton et al., 1977). In contrast, in the prostatic half, the 'twitch' component dominated. Also, in spite of low noradrenaline content (McGrath et al. cited by Anton et al., 1977) and a smaller total muscle mass, the epididymal half produced a greater tension than the prostatic half when electrically stimulated (Pennefather et al., 1974; Duncan et al., 1976; Anton et al., 1977) or when exogenous noradrenaline or tyramine was added (Pennefather et al., 1974). Yet these variations in the response of the two halves cannot be due to differences in the circular and longitudinal muscle layers present in the two regions, since in pithed rats the vas deferens perfusion pressure response to field stimulation is also biphasic and of about equal magnitude (Anton et al., 1977) as the muscle response when recorded isometrically or isotonically.

Pharmacological studies indicate that guanethidine (Swedin, 1971; Anton et al., 1977) and hexamethonium (Anton et al., 1977) in higher doses (5mg/kg) depress both components of the response. Reserpine and lysergic acid diethylamide (LSD) preferentially inhibit the initial twitch component of the response (Gillespie et al., 1975; Anton et al., 1977). Phentolamine and lower doses of hexamethonium (1mg/kg) preferentially abolished the secondary component

(Duncan et al., 1976; Anton et al., 1977). In addition, modifications in the bath temperature and stimulatory parameters also influenced the two components differentially (Birmingham et al., 1971). From these results it seems that the vas deferens is innervated by two sets of adrenergic motor fibres with different pharmacological properties and distribution.

4. Modulation of transmitter release

Euler et al. (1975) proposed that potassium (K^+) released from sympathetic nerves during stimulation acts as the transmitter in the vas deferens of the guinea-pig. However, Stjärne (1976) suggested that potassium released from adrenergic neurones in the course of impulse conduction may act as a modulator rather than acting as the transmitter itself, since higher concentrations of potassium lowered the output of noradrenaline in the guinea-pig vas deferens with a simultaneous rise in the twitch contraction.

Similarly, it has been suggested that endogenous and locally formed prostaglandins of the E series (PGE) may act as modulators in adrenergic neurone transmission in the vas deferens. This suggestion is based on the observation that (1) prostaglandin E's inhibit both mechanical response (Taylor et al., 1972; Hedqvist, 1973) and the noradrenaline output (Fredholm&Hedqvist,1973; Hedqvist,197. during nerve stimulation in the vas deferens; (2) indomethacin, a drug which inhibits prostaglandin synthesis increases the release of noradrenaline from electrically stimulated guinea-pig vas deferens (Fredholm et al., 1973).

In complete contrast, based on pharmacological studies on the rat vas deferens Simon et al. (1976) suggested that dopamine is the physiologically functional neurohumoral transmitter in the vas deferens which, when released, stimulates dopamine receptors. Nevertheless, this suggestion seems unlikely because dopamine has failed to evoke contractions in the vasa deferentia of rat, mouse or guinea-pig (Stone, 1977).

Maturation and transport of spermatozoa in the male reproductive tract

Maturation of Spermatozoa

Studies upon both eutherian and metatherian mammals have demonstrated

that the spermatozoa leaving the testis undergo physiological, morphological and biochemical changes as they transit the epididymis. These transformations are collectively described as maturation or 'ripening' of the spermatozoa.

(a) Physiological changes

1) Fertilizing capacity

Testicular spermatozoa are infertile. That is they are incapable of penetrating an ovum and producing viable offspring (rabbit: Cooper et al., 1975, 1977; bull: Amann et al., 1974). The cumulative weight of evidence suggests that the epididymis is the region within which the spermatozoa acquire their fertilizing power (rabbit: Orgebin-Crist, 1967; Cooper et al., 1977; bull: Amann et al., 1974; hamster: Cummins, 1976). In the epididymis, most of the spermatozoa achieve the fertilizing potential in the distal corpus, as in the rabbit (Bedford, 1966; Orgebin-Crist, 1967; Orgebin-Crist et al., 1977) or in the proximal cauda as in the hamster (Cummins, 1976).

The fertilizing power, once acquired, does not last indefinitely. Its maximum duration is about 25 days in the mouse and hamster (Lubicz-Nawrocki et al., 1973) and 49 days in the rabbit (Tesh et al., 1969).

The development and the maintenance of the fertilizing ability of the spermatozoa is androgen dependent (rat: Vreeburg et al., 1976; hamster: Lubicz-Nawrocki, 1976). Nevertheless, there is still no direct evidence for the existence of an androgen dependent epididymal factor(s) capable of inducing fertilizing power of immature spermatozoa.

2) Sperm motility

Spermatozoa either within the seminiferous tubules or immediately upon leaving the testis are immotile (Mann, 1970) or show only weak vibratile motions (see Orgebin-Crist et al., 1975). The extent and vigour of motility then starts to increase as the spermatozoa pass the flexure of the caput epididymis. The spermatozoa released from the caput epididymis in many laboratory rodents (Gaddum, 1968; Wyker et al., 1977) exhibit a circular motion which changes to unidirectional progressive movement by the time they reach the cauda epididymis. In contrast, however, human sperm does not

show such a change (Bedford et al., 1973) during epididymal passage but exhibits a range of activity throughout the epididymis. The motility once achieved, lasts for 42-60 days (Tesh et al., 1969; Orgebin-Crist et al., 1976).

(b) Morphological changes

Morphological alterations associated with sperm maturation are:-

i) A reduction in the dimensions of the acrosome occurs in rabbits (Bedford, 1963), monkeys (Flechon et al., 1975) and elephants (Jones et al., 1974). However, no changes in the acrosome morphology have been seen in rats, mice (Nicander, cited by Bedford, 1975) or humans (Bedford et al., 1973).

ii) The cytoplasmic droplet migrates away from the neck of the spermatozoa (rabbit: Bedford, 1963; monkey: Flechon et al., 1975; elephant: Glover, 1973; Jones et al., 1974; hyrax: Glover, 1973). The cytoplasmic droplet is generally absent in the ejaculated spermatozoa. The disappearance of the cytoplasmic droplet is, however, a variable event in man (Bedford et al., 1973) and a cytoplasmic cuff is frequently present at the neck in a proportion of human spermatozoa in the cauda epididymis and the ejaculate.

iii) The net negative charge on the plasma membrane increases (Cooper et al., 1971; Bedford et al., 1973).

iv) Disulphide links are established in certain proteins within the nucleus (Calvin et al., 1971; Bedford et al., 1973) and in the outer membrane of the mitochondria (Bedford et al., 1973).

v) A reduction in size (about 10%) of the spermatozoa (Brotherton, 1976).

(c) Biochemical changes

Alterations in the content of lipids (Terner et al., 1975) and sialoproteins (Arova et al., 1975) have been demonstrated during epididymal transport. There is also a decrease in alkaline phosphotase activity and an increase in lipase activity (Temer et al., 1975).

Transport of spermatozoa in the male genital tract

Sperms are produced in the seminiferous tubules of the testes. It is

a continuous process in non-seasonal breeding, scrotal mammals and possibly also in some testicond mammals (elephant, hedgehog and most marine mammals) from puberty to old age. But in seasonal breeders, spermatogenesis ceases completely in the non-breeding season (Skinner, 1971).

It has been suggested that spermatozoa once formed are released from the seminiferous epithelium by dissolution of the tubulobulbar complexes, the sperms and sertoli cells (Russel et al., 1976). In addition, actin or actin-like filaments found the sertoli cells (Toyama, 1976) may possibly aid the release of spermatozoa into the lumen of the seminiferous tubule. These testicular spermatozoa are either immotile (Mann, 1970) or exhibit only weak oscillatory movements without any forward progression in the rat (Wyker et al., 1977) and rabbit (Cooper et al., 1975; Orgebin-Crist et al., 1975). Moreover, Johnson et al. (1975, 1976) demonstrated that the mean pressure in the caput epididymis is greater than that of the seminiferous tubule and the mean pressure in the distal cauda epididymis is greater than that of the proximal cauda epididymis in the golden hamster and guinea-pig. Therefore, their transport most logically depends on some propelling force(s) and indeed, numerous mechanisms have been postulated.

The undulating movements of the seminiferous tubules of mature dogs and rats observed by Roosen-Runge (1958) in vitro may be involved in the propulsion of the non-mobile sperm to the epididymis. Similar contractions of the seminiferous tubules moving in the direction of rete testis of rats has also been described (Clermont, 1958). Moreover, the movement of spermatozoa may be assisted by the contraction of myoid elements lying around the seminiferous tubules of the larger mammals including man (Bustos-Obregon, 1976; Hermo et al., 1977). Furthermore, contractions of smooth muscle cells in the rete (Bustos-Obregon et al., 1976), by changing its shape and the rhythmic contractions of the vasa efferentia (Risley, 1958) may also be involved in sperm transport.

The periodic spontaneous contractions shown in the testicular capsules of the rabbit both in vitro and in vivo (Davis et al., 1971; Hargrove et al., 1973; 1976) and in the human in vitro (Firlit et al., 1975) represent another

dominant force capable of transporting non-motile spermatozoa from the seminiferous tubule to the caput epididymis. Furthermore, the testicular capsule is also equipped with smooth muscle cells which could be responsible for such contractions. (Firlit et al., 1975) In addition, there have been suggestions that the testes are capable of undergoing spontaneous contractions as a whole organ (Wojcik et al., 1966, cited by Firlit et al., 1975), which would cause a localized increase in pressure, capable of moving immotile sperms and fluid into the caput epididymis.

Finally, the ciliary currents of the rete epithelium (Reid et al., 1957) and ductuli efferents (El-Badawi et al., 1976) are also offered as a possibility for transport of non-motile psermatozoa. However, Leeson (1962) by electron microscope studies concluded the number of cilia in the rete testes were insufficient to bring about sperm transport.

Once the spermatozoa reach the caput epididymis, transport from here through the corpus may result from tubular contractions and/or the resting hydrostatic pressure gradient. Glover (1976) suggested that the upper part of the epididymis is spontaneously contracting in a peristaltic manner. Indeed, rhythmic contractions of the epididymis are reported both in vitro and in vivo (Hib, 1976, 1977). Furthermore, in the corpus and the caput region of the rat epididymis localized contractions of a pendular and peristaltic type are seen in vivo (Risley, 1963, cited by Bedford, 1975).

The motility of the spermatozoa increases as they pass through the epididymis (Bedford, 1966; Igboeli et al., 1968; Gaddum, 1968), but recent micropuncture studies have failed to show any progressive movement in vivo (Wyker et al., 1977). Hence whether motility of sperms per se contributes to their transport through the epididymis is doubtful.

Transit time of spermatozoa through the caput and corpus epididymes is surprisingly uniform (between 2 and 6 days) among the species studied so far (see Amann et al., 1976). Moreover, transit time of sperms in these parts of the epididymis is not altered by the frequency of ejaculation, although transit time in the cauda epididymis is shorter in sexually active animals (Marton et al., 1963; Amann et al., 1974).

Movement through the cauda epididymis against the resting pressure gradient must result from the intermittent peristalsis in sexually-rested animals. The continuous flow of spermatozoa seen after cannulation of the vas deferens of sexually abstinent animals (Holtz et al., 1974), and the presence of sperms in appreciable numbers in the vasa deferentia (Lambiase et al., 1969; Jones et al., 1974; Richardson et al., 1976) indicates there is a continuous flow of sperms from the epididymis. Similarly, spermatozoa are transported through the cauda epididymis by a sudden powerful contraction of its smooth muscle during ejaculation (Potts, 1957; Cross et al., 1958; Knight, 1974).

Within the vas deferens spermatozoa may perhaps be transported towards the urethral end, during periods of sexual rest by spontaneous contractions. In fact, such contractions are seen in human vasa obtained under general anaesthesia (Ventura et al., 1973) and from cadavers (Martins, 1940). Contractions of vasa deferentia have also been demonstrated in vivo (rabbit: Melin, 1970; Bruschini et al., 1977; dog: Kimura et al., 1975; Bruschini et al., 1977). In contrast, human vasa deferentia obtained under local and spinal anaesthetic are quiescent (MacLeod et al., 1973. Hepperlen et al., 1976). The persistant accumulation of spermatozoa in the vas deferens of rats (Evans et al., 1972) suggests the existence of a mechanism involving adrenergic nerves, responsible for constant transportation of sperms along the vas deferens towards the ampullary region where storage could take place.

On the other hand, at ejaculation the vasa deferentia and cauda epididymis contract almost simultaneously, transporting spermatozoa stored within them to the pelvic urethra (Potts, 1956; Cross et al., 1958; Knight, 1974). The number of spermatozoa expelled by such orgasmic contraction depends on the frequency of ejaculation and on the species. However, there appears to be no agreement upon the nature of the contraction of the vas deferens during ejaculation. Vanwelkenhuyzen (1966) proposed that at ejaculation the contraction of the vas deferens starts at the urethral end and spreads towards the epididymal end. This would induce a rapid rise in pressure

inside the vas deferens. Since the contents of the vas deferens cannot enter the epididymis, the sperm flow occurs towards the posterior urethra. This process, according to Vanwelkenhuyzen (1966) occurs only once during ejaculation and the vas deferens does not undergo a series of contractions in transporting the spermatozoa. However, whether a contraction starting at urethral end would be able to create a momentum in the opposite direction seems to be doubtful.

Alternatively, Kimura et al. (1975) have demonstrated contractile movement en masse during stimulation of hypogastric nerves of the dog. Similarly a single contraction has been demonstrated by Knight (1974) in rams during copulation. These results would indicate a single reflex contraction per ejaculate. Furthermore, X-ray cinematographic studies using radio-opaque materials in man (Mitsuya et al., 1960) also suggest that the vas deferens empties itself in a single reflex. Nevertheless, this observation does not necessarily permit the elimination of other possible mechanisms.

Finally, Ventura et al. (1973) have suggested a co-ordinated series of contractions of the vas deferens and cauda epididymis which propel the sperm towards the urethra during ejaculation, based on the fact that sympathomimetic drugs cause rhythmic contractions of the vasa deferentia. Batra (1974) also suggests that a peristaltic pumping mechanism for sperm transportation is also mathematically feasible. Nevertheless, the exact nature of the contraction of vas during copulation is still not known.

Fate of unejaculated spermatozoa

In non-seasonally breeding mammals spermatogenesis continues throughout most the adult life. Yet there is no clear indication of the fate of unejaculated spermatozoa in sexual continence.

Continuous dissolution of sperm has been reported (Simeone et al., 1931) in the epididymis and proximal vas deferens of guinea-pigs. Recently, Cooper et al. (1977) using electron microscopic techniques also observed degeneration of spermatozoa in the terminal epididymis and proximal vas deferens of adult rats, mice, hamsters and guinea-pigs. However, the 'keratinoid' nature of the sperm (Calvin et al., 1971) renders them very resistant to disruption.

Reabsorption of spermatozoa in the excurrent ducts has been reported in rabbits (Glover, 1961; Swanson et al., 1968; Holtz et al., 1972) and occasionally in man (Phadke, 1964) and the bull (Nicander, 1965). Ultrastructural evidence for sperm resorption is reported in the domestic fowl (Tingari et al., 1972). Lubicz-Nawrocki (1974) showed that sperm removal process takes place in the cauda epididymis and depends on the circulating androgen level. On the other hand, Glover (1961) suggested resorption as a method of disposal of abnormal sperm in rabbits, bulls, goats, dogs and cats. But the site of absorption was not specified. Roussel et al. (1967) suggested removal of abnormal sperm is effected largely, if not wholly, by macrophages primarily in the cauda and corpus epididymides of bulls, rabbits and monkeys. In vitro phagocytosis of mouse sperm by macrophages has been documented (Ball et al., 1977). In addition, the possible involvement of lymphocytes in sperm resorption of rats and monkeys have been suggested (Dym et al., 1975). Yet there has been no substantial evidence either from electron microscopy or from light microscopy to support this contention.

The nocturnal emission and masturbation in man speculated as a mechanism to ensure minimum incidence of teratozoospermia and avoidance of hyperspermia (Levin, 1975) is perhaps another possible fate of spermatozoa. Spontaneous seminal discharge is also experienced by the rat (Orbach, 1961; Marten et al., 1963; Kihlstrom, 1966; Ågmo, 1976), mice (Lakomaa et al., 1972) and guinea-pigs (Marten, 1968; Lakomaa et al.,1972). Masturbation has also been reported in wild apes, monkey and deer (Cohen, 1977).

In contrast, Lino et al. (1967) indicated spermaturia as the major mechanism for disposal of surplus sperm (88%) in sexually rested rams. Spermatozoa were also found in the urine of rats, (Fernandez-Collazo et al., 1971; Vreeburg et al., 1974), man (Richardson et al., 1977) and in some marsupials (Rodger et al., 1976).

SECTION 1.

SYMPATHOMIMETIC DRUGS ON THE MOTILITY OF THE VAS AND EPIDIDYMIS IN VITRO.

METHODS

Specimens of human vas deferens (10-35 mm long) were obtained from patients undergoing elective vasectomy under local anaesthesia. The operations were done between 16.00 and 18.00 hours. The tissues removed were immediately placed in iced Krebs-Henseleit solution and stored at 4°C until use the following morning.

Wistar rats (350 - 400 g) were anaesthetised with ether and the terminal 2 - 3 cm of the cauda epididymis dissected free. A thread was tied around each end of the straight tubular section so obtained. One preparation could be thus obtained from each epididymis.

Organ bath procedure

The tissues were suspended in 7.5 ml baths under a resting tension of 1.0 g (vasa) or 0.5 g (epididymal tubule). Contractions were isometrically recorded with Grass FT03C or Dynamometer UF1 strain gauges using a Grass 7B or Washington 400 MD2 recorder. The physiological salt solution used for transportation, storage and during the experimental investigation had the following composition (mM): Na^+, 143; K^+, 5.8; Ca^{++}, 2.6; Mg^{++}, 1.2; Cl^-, 128; $H_2PO_4^-$, 1.2; HCO_3^- 25, $SO_4^=$, 1.2 and glucose 11.1. It was gassed with 95% O_2 and 5% CO_2 and maintained at 36-38°C throughout the experiment.

Some vasa deferentia were electrically stimulated through platinum ring electrodes using a Grass SK4 stimulator, at a frequency of 3 - 10 Hz, with impulses of 0.5 ms duration and 40 - 70 volts.

Drugs were either added cumulatively, increasing the concentration every 8 minutes, or sequentially, when each dose was washed out once a stable response had been established. Potassium contractions were obtained by the addition of 2 M KCl (to give a final potassium concentration of 150 mM). In the experiments with 6-hydroxydopamine, 1000 µg/ml of the drug was added simultaneously with

PLAN OF STUDY

Long-term objectives

In family planning, individual choice plays an important role in the method of contraception employed. Hence it is desirable to have a variety of methods available for use by both partners. However, at present no pharmacological method is available for the safe and reversible control of male fertility. Therefore one reason for undertaking this study was to investigate the possibility of reducing the fertilizing potential of the male by using sympathomimetic drugs to alter the environment in the ductular system through which spermatozoa must pass before ejaculation.

Design

1) To investigate whether sympathomimetic drugs could cause rhythmic contractions in isolated vasa deferentia. If such contractions were initiated, the mechanism underlying their induction would be analysed.
2) To find the optimal conditions for the initiation of rhythmic contractions in the vas deferens of anaesthetized animals.
3) To discover whether sympathomimetic drugs reduce the fertility of male rats and rabbits.
4) If an antifertility effect was observed, to investigate the mechanism of action of drugs by
   (a) studying the sperm distribution in various regions of the male reproductive tract,
   (b) assessing the numbers and quality of ejaculated sperm,
   (c) investigating the histology of the testes and the genital ducts.

50 µg/ml of tyramine or norephedrine and washed out after 15 - 20 minutes. The procedure was repeated until the tyramine or norephedrine response was completely abolished.

The following drugs were used, and concentrations are expressed as µg/ml of the salt methoxamine hydrochloride (Borroughs Wellcome): (-)-noradrenaline hydrochloride, tyramine hydrochloride, (-)-norephedrine hydrochloride, 6-hydroxydopamine HBr (Sigma) and phentolamine mesylate (Ciba).

Prior to the administration of drugs the tissues were incubated for 0.5 - 1 hour (cauda epididymis) or 3 - 5 hours (vas deferens) in order to equilibrate to the experimental conditions.

RESULTS

Human vas deferens

20 out of 44 vasa investigated showed spontaneous regular contractions during the equilibration period. The time between mounting the tissue in the bath to the start of spontaneous motility varied from 2 to 15 minutes. These contractions lasted 10 - 15 minutes after which the muscles became quiescent. The amplitude of these contractions varied from 0.5 - 1.0 g and the frequency was 2 - 3 contractions per minute. Repeated washings enhanced the disappearance of spontaneous contractions.

Potassium response

Addition of potassium produced a sustained contraction (0.25 - 2.5 g) which was maintained until washing. In a few preparations repeated phasic contractions were superimposed on it.

Noradrenaline, methoxamine, tyramine and norephedrine

All the agonist drugs investigated elicited rhythmic contractions. Four patterns of response could normally be distinguished (Fig. 1). Each of these was occasionally observed with any of the drugs at all effective concentrations. However, any one preparation usually exhibited one or, at the most, two types of activity.

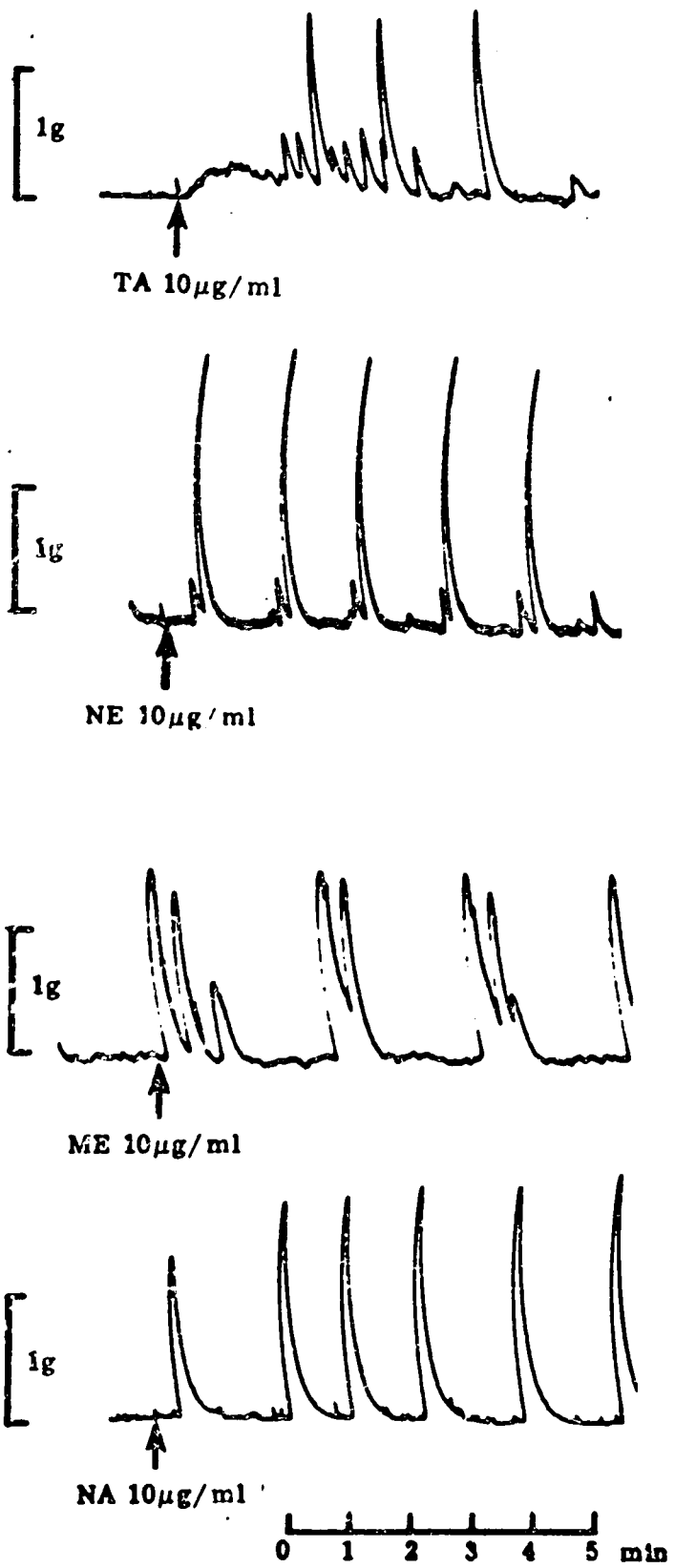


Fig. 1

Examples of contractions produced by sympathomimetic drugs in isolated human vasa deferentia to show 4 types of responses commonly observed. The drugs used were tyramine (TA), norephedrine (NE), methoxamine (ME) and noradrenaline (NA), although all of them produced on different occasions responses of each of the type shown. The arrows indicate the addition of 10 μg/ml of the drugs into the bath and left in contact of the tissue*. The top tracing shows an initial tonic contraction followed by phasic contractions. The second trace shows single spikes with small contractions preceeding them. The third shows phasic contractions in groups of 2 or 3 of approximately equal size. The bottom tracing shows phasic contractions in single spikes.

* for the remainder of the period shown.

1) An initial tonic contraction (with or without superimposed phasic contractions) followed by phasic contractions.
2) Single spikes with small contractions preceding or following them.
3) Phasic contractions in groups of 2 or 3 of roughly equal size.
4) Phasic contractions in single spikes.

Figure 2 shows the dose response curve to noradrenaline (13 specimens) when the drug was added sequentially. A lag of 0.2 to 9 minutes was observed between addition of each dose and the appearance of the response. With each dose the phasic contractions grew gradually larger, reaching a maximum after 0.5 to 8 minutes. The contractions could be abolished by repeated washings.

The cumulative concentration-response curve for methoxamine is depicted in Figure 3. From this it can be inferred that the maximum responses were not evoked by the highest concentration studied. The lag period with the first dose was short (0.5 - 1.0 minute). In contrast to noradrenaline, the maximum response with methoxamine was achieved in less than 0.5 minute after the first contraction. Once repeated phasic contractions had developed, they could not be abolished by repeatedly washing out the bath. The persistence of action of the drug may result from its slow rate of inactivation (Iversen, 1967).

The cumulative dose relationship for tyramine (8 vasa) is shown in Figure 4. Tyramine-induced contractions were similar to those induced by noradrenaline. Tyramine was, however, less potent than noradrenaline. Furthermore, a pronounced lag was observed prior to the initial response. This lag was presumably due to the drug's indirect mode of action.

Figure 5 (cumulative) and Figure 6 (sequential) show the concentration response relationship for norephedrine (3 vasa each). The effect produced by norephedrine was very similar to that brought about by tyramine, suggesting a similar mode of action.

Phentolamine

Phentolamine (2 µg/ml) abolished the contractions produced by noradrenaline, methoxamine and tyramine within 2 minutes. Phentolamine in this concentration is a selective α-adrenoreceptor antagonist (Wohl et al., 1967).

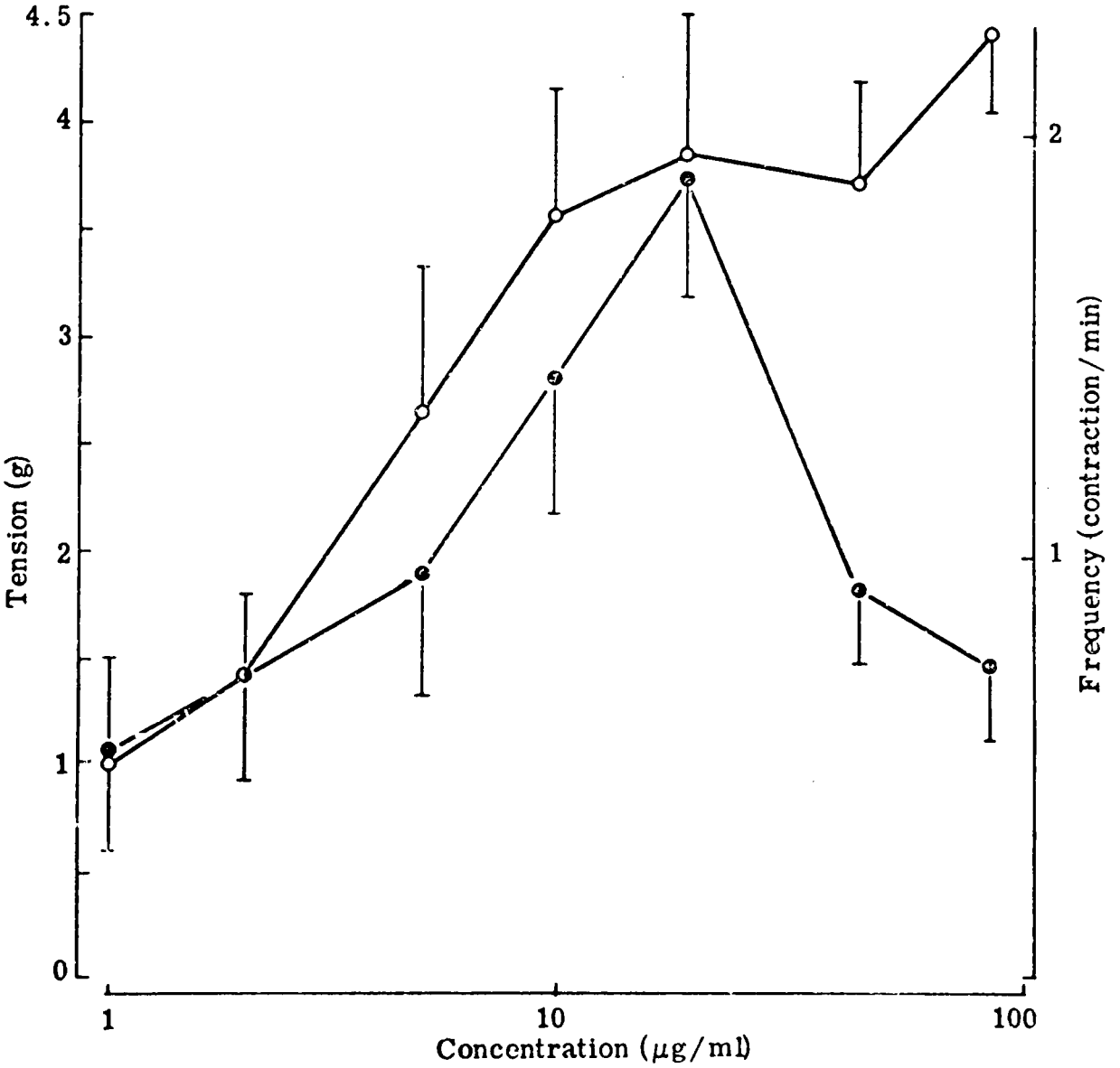


Fig. 2

Sequential dose response curve showing the effect of noradrenaline on the frequency (●) or peak tension (o) of isolated human vasa deferentia. Each point is the mean of 13 observations. Vertical bars represent s.e.mean.

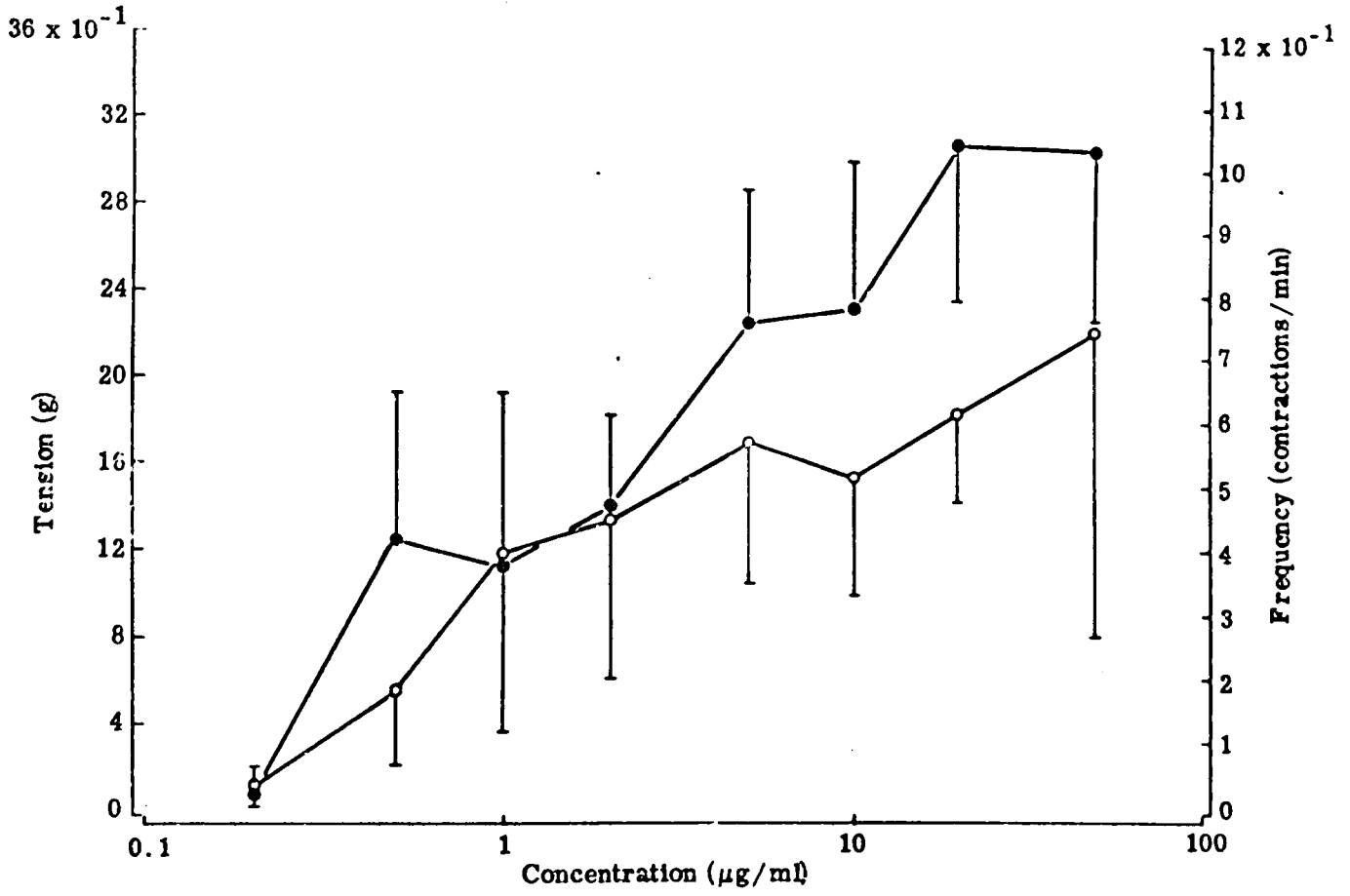


Fig. 3

Cumulative dose response curve showing the effect of methoxamine on the frequency (●) or peak tension (o) of contractions of isolated vasa deferentia. Each point is the mean of 6 observations. Vertical bars represent the s.e.mean

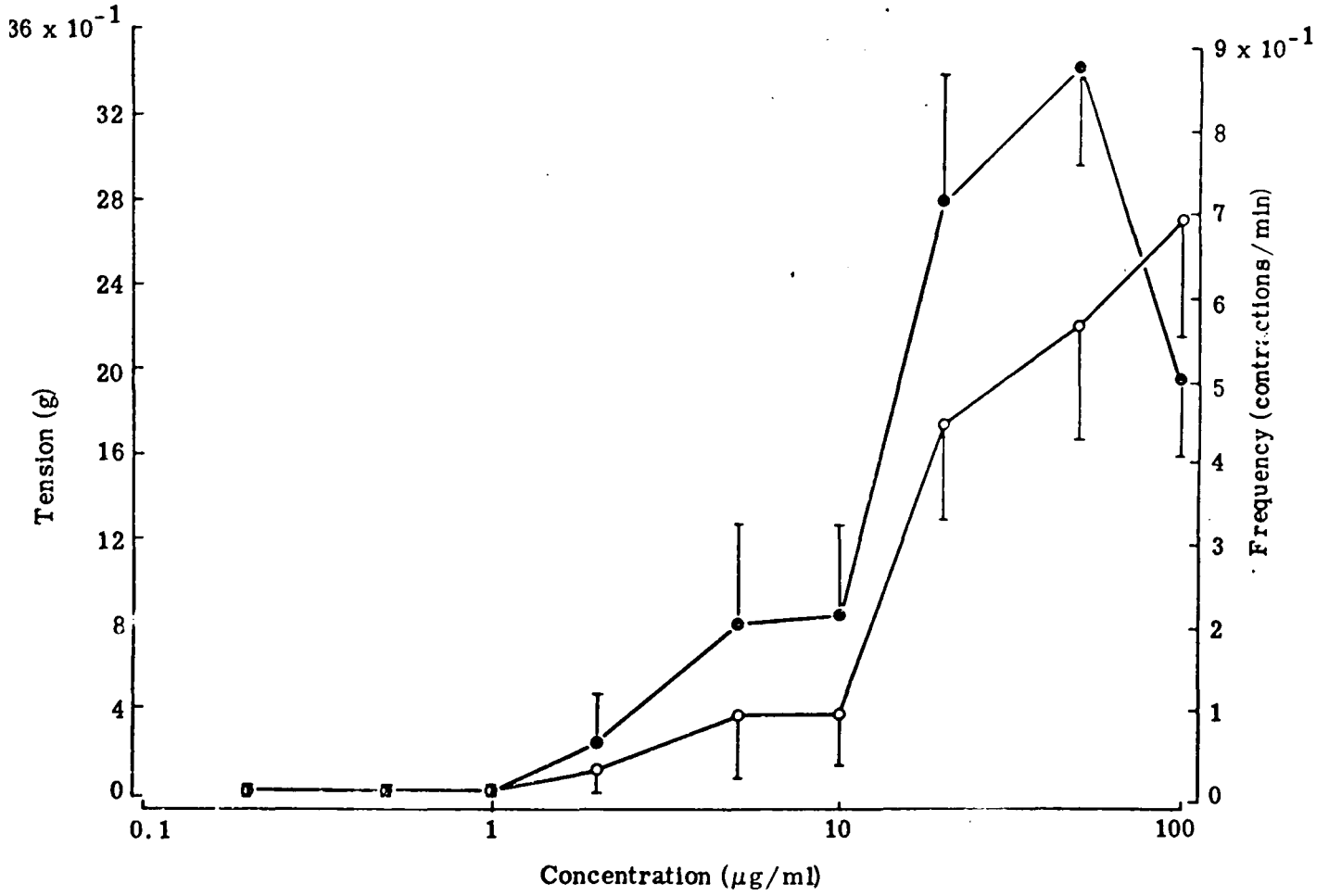


Fig. 4

Cumulative dose response curve showing the effect of tyramine on the frequency (●) or peak tension (o) of contractions of isolated human vasa deferentia. Each point is the mean of 8 observations. Vertical bars represent the s.e.mean.

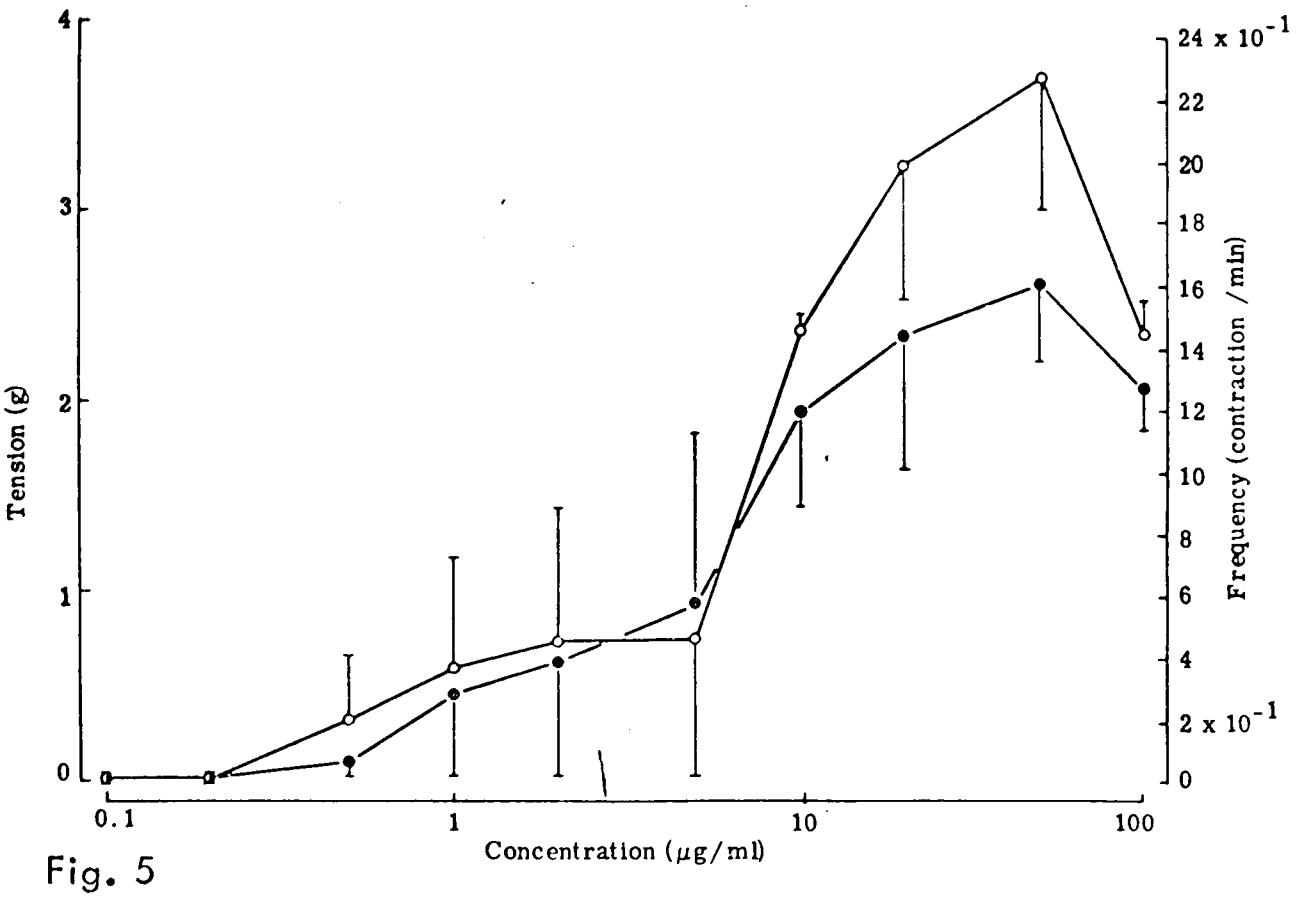


Fig. 5

Cumulative dose response curve showing the effect of norephedrine on the frequency (●) or peak tension (o) of contractions of isolated human vasa deferentia. Each point is the mean of 3 observations. Vertical bars represent s.e.mean.

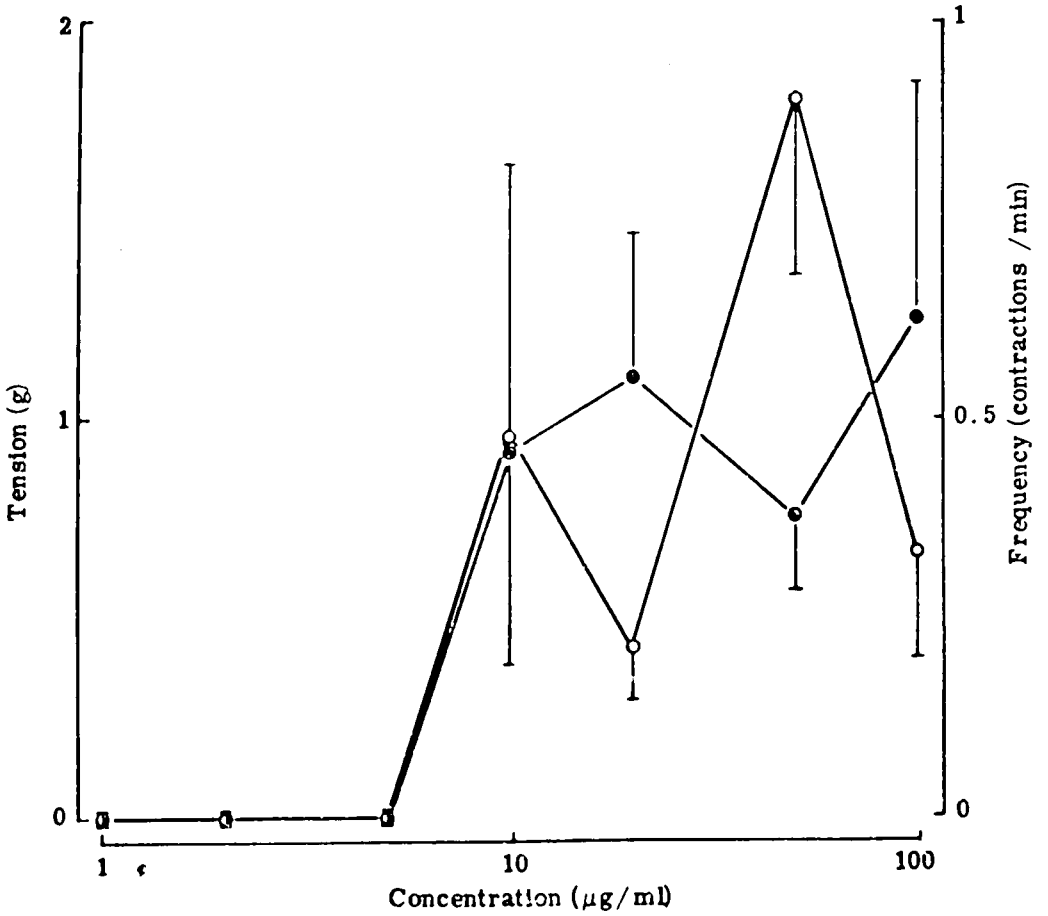


Fig. 6

Sequential dose response curve showing the effect of norephedrine on the frequency (●) or peak tension (o) of contractions of isolated human vasa deferentia. Each point is the mean of 3 observations. Vertical bars represent s.e.mean.

6-hydroxydopamine

Contractions elicited by either tyramine, norephedrine or electrical stimulation were completely abolished in 10 vasa out of 11 studied. In contrast, little or no effect on methoxamine or potassium-induced contractions was produced in the same experiments (Table 1). An augmentation of tyramine or norephedrine induced contractions was usually seen during the first 1 -3 treatments with 6-hydroxydopamine. The time required for the complete abolition of contractions was about $1\frac{1}{2}$ to 2 hours. The abolition of the responses was not due to trachyphyllaxis.

Rat isolated terminal cauda epididymis

Spontaneous contractions were observed in 2 out of 3 tubules. Repeated washings failed to abolish these contractions. This contrasts with the effect seen in the human vas deferens. The methoxamine-induced contractions occurred (Fig.7b) in a dose dependent manner as shown in Figure 7a.

DISCUSSION

Human vas deferens

Ventura et al. (1973), McLeod et al. (1973) and Hepperlen et al. (1976) reported no spontaneous motility of human vasa obtained under local anaesthesia. In contrast to their observations, about half of the vasa deferentia in the current study exhibited spontaneous contractions for a short period. The development of these spontaneous contractions may be perhaps due to changes in the ionic balance as normal cell metabolism is restored, or to liberation of spasmogenic substances as a result of cold storage. Alternatively, the divergent results of Ventura et al. (1973), McLeod et al. (1973) and Hepperlen et al. (1976) might have been caused by (1) differences in the composition of the physiological solutions used, (2) methods of recording the contractions or (3) the age and the health of the patients.

Several authors have demonstrated that rhythmic contractions can be induced with noradrenaline in the human vasa deferentia (Martins et al., 1940; Birmingham, 1968; Ventura et al., 1973; McLeod et al., 1973; Hepperlen et al.,

Table 1. Effect of 6-hydroxydopamine on the contractility of the isolated human vasa deferentia. The first part shows the control responses obtained with 150 mM potassium, 5 - 50 µg/ml methoxamine or by electrical stimulation of the postganglionic neurones via ring electrodes. In the second part is indicated the number of doses (100 µg/ml) of 6-hydroxydopamine (repeated at 15 - 20 min intervals) required to abolish contractions induced either by norephedrine or by tyramine. The third part shows the magnitude of responses obtained with the original doses of potassium or methoxamine following 6-hydroxydopamine treatment.

Before 6-hydroxydopamine					6-hydroxydopamine treatment		After 6-hydroxydopamine			
Length of vas (mm)	Potassium response Amplitude (g)	Methoxamine response Amplitude (g)	Methoxamine response Frequency contraction/min	Electrical Amplitude (g)	Indirectly acting drug used	No.of 6-hydroxy-dopamine treatments	Potassium response Amplitude (g)	Methoxamine response Amplitude (g)	Methoxamine response Frequency contraction/min	Dose of methoxamine x 10^{-6}
20	0.25				Norephedrine	8		0.25	0.528	10
22	0.75	5	0.48		Norephedrine	8		4.0	1.66	10
18	1.0				Norephedrine	7		4.2	1.5	10
10	0.25				Norephedrine	7		0.5	0.736	10
16	1.0				Tyramine	5	2.0			
11	0.40				Tyramine	6	2.0			
20	1.0			6.25	Tyramine	7		4.0	0.369	5
15	0.8				Tyramine	7	0.75	0.75	0.21	5
30	2.5				Tyramine	8*	1.0			
15		1.0	0.24		Tyramine	6	0.5	1.5	0.20	50
30	1.5				Tyramine	7	0.75	1.0	0.40	50

* 90% abolished

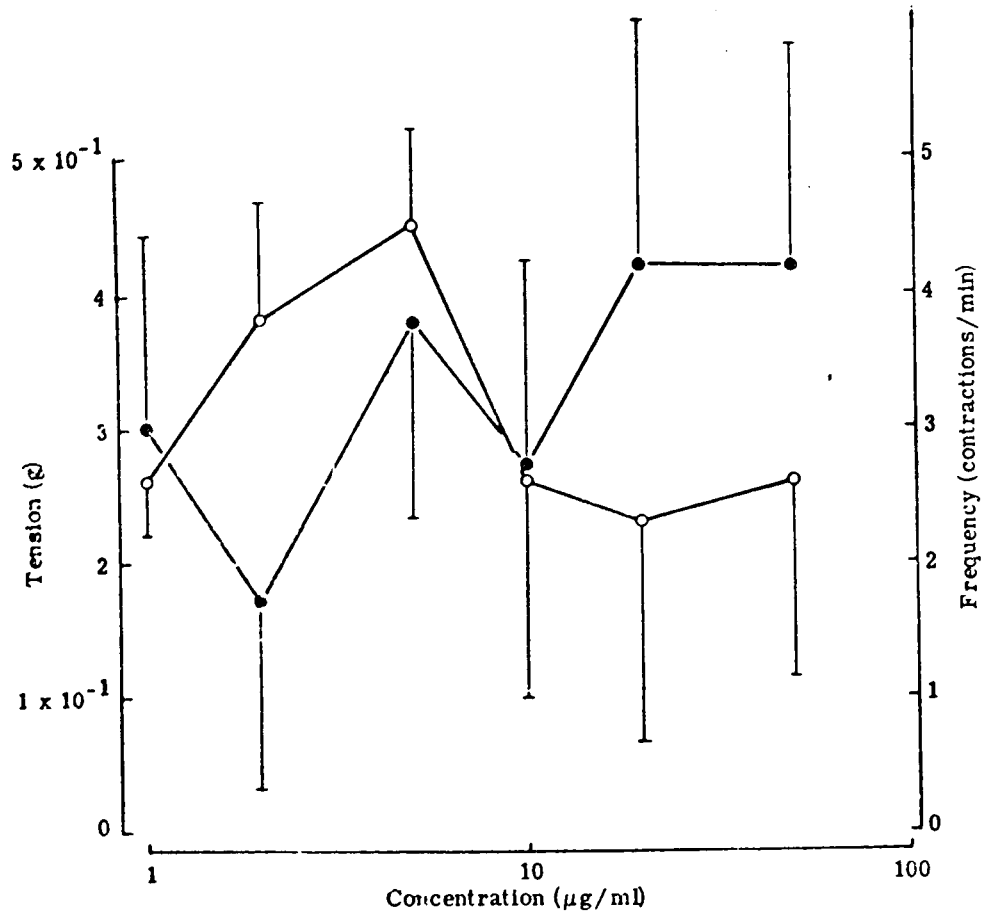


Fig. 7a

Cumulative dose response curve showing the effect of methoxamine on the frequency (e) or peak tension (o) of contractions of isolated rat terminal cauda epididymal tubules. Each point is the mean of 3 observations. Vertical bars represent s.e.mean

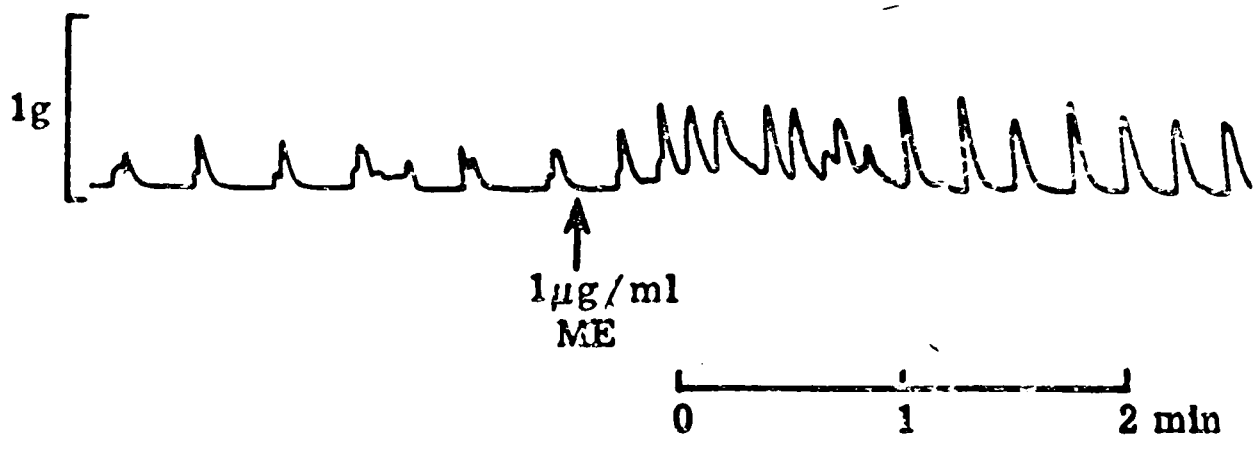


Fig. 7b

Effect of methoxamine on the contractility of the isolated rat terminal cauda epididymal tubule. The spontaneous contractions are shown on the left hand side of the arrow. The arrow indicates addition of 1 µg/ml methoxamine to the bath. Methoxamine augmented the frequency and amplitude of the contractions.

1976; Anton et al., 1977)or tyramine (Birmingham, 1968). The present study, in addition to confirming the above responses, also shows dose-dependent response to methoxamine and norephedrine. Abolition of these contractions with phentolamine indicates that the effect was mediated via α-receptors. The complete inhibition of tyramine and norephedrine-induced responses by 6-hydroxydopamine was evident in the current study. On the other hand, there was little or no change in response to a directly acting agonist or to depolarization. Since 6-hydroxydopamine causes necrosis of adrenergic nerves specifically (see Rotman et al., 1976) the above observations indicate that tyramine or norephedrine produced their actions in the vas indirectly, by releasing the endogenous transmitter from the adrenergic varicosities. However, confirmation of this hypothesized mechanism requires further studies in fluorescence microscopy (see Wadsworth, 1973).

Lastly, the results show that 6-hydroxydopamine could be used in vitro to cause selective sympathetic degeneration within an hour or so without using high concentrations of ascorbic acid which itself causes some damage.

Rat isolated terminal cauda epididymis

Spontaneous contractions of cauda epididymides have been demonstrated in humans (Martins et al., 1940), guinea-pigs (Da Silva et al., 1975) and in rats (Hib, 1976). In agreement with their observations, similar contractions were seen in this study, in the absence of drugs. Inability to abolish these contractions with repeated washings perhaps indicates a myogenic activity in the smooth muscle. Abolition of the methoxamine-induced contractions with phentolamine indicates that the responses are mediated through α-receptors as in the human vas deferens.

SECTION 2.

SYMPATHOMIMETIC DRUGS AND MOTILITY OF THE VAS IN VIVO

METHODS

In this study 24 male rats (250 - 350 g) and 10 male guinea-pigs (350 - 500 g) were used. The animals were anaesthetised with urethane (0.6 ml of 25% w/v solution/100 g of animal) given intra-peritoneally, and then placed on a heated operating table in the supine position with their limbs and head secured by adhesive tape. A 2 - 3 cm midline incision was made in the neck and the right carotid artery and jugular vein were exposed. Both of these vessels were cannulated with polythene catheters (internal diameter 0.76 mm and external diameter 1.2 mm, Portex). The trachea was usually cannulated in an attempt to facilitate respiration. Intravenous injections or infusions were made through the catheter placed in the jugular vein. The arterial pressure was monitored continuously throughout the experimental period from the carotid artery using a pressure transducer (Siatham P23V), and a Grass 7B pen recorder. The pressure transducer was calibrated against a mercury manometer.

One of the vasa deferentia was exposed by a longitudinal incision made from the scrotum to the pubic region. A 5/0 thread was sewn through the connective tissue surrounding the epididymal end of the vas and connected to an isometric transducer (Grass FT03C) via a spring with a compliance of 1g/cm. The contractions were therefore recorded semi-isotonically. The minimum contraction that could be detected with the recorder sensitivity that was used was 0.05 g (equivalent to 0.5 mm movement). Some vasa deferentia were electrically stimulated (50 Hz, 40 - 70 volts, pulse width = 0.5 msec) through surface electrodes using a Grass SK4 stimulator.

In two rats, the skin surrounding the exposed vas was pinned in such a way that a pool could be created in which to immerse the vas. The pool contained 1 ml of a 1 mg/ml solution of the drug under test. Throughout the experimental period, the exposed organs of the body were covered with pads of cotton wool soaked in warm saline.

RESULTS

Vas deferens

Spontaneous contractions

No spontaneous activity was observed in any rat or guinea-pig vas deferens under the experimental conditions of this study. The period of observation in different experiments was 3 - 6 hours (Table 2).

Electrical stimulation

Single contractions ranging from 0.5 g to 3.5 g (and 5 - 35 mm shortening) were exhibited as shown in Table 2. Three guinea-pig vasa, however, gave a double response upon stimulation.

Intravenous administration of drugs

a) Injection

Only 3 out of the 10 guinea-pig vasa deferentia responded to intravenous drug administration (methoxamine hydrochloride or oxymetazoline hydrochloride) and even then very high doses (1600 - 3000 µg) were required (Table 2). Single contractions ranging from 0.1 to 0.6 g were produced. The same drugs in lower doses (100 - 800 µg) failed to produce contractions in rat vasa deferentia (11 preparations).

b) Infusion

When similarly high doses of methoxamine hydrochloride were administered as an infusion, no contractions were produced (Table 2).

Local application of drugs

a) Pool

Contractions ranging from 0.2 to 0.4 g were produced at a frequency/min of 1.5 - 1.86. These responses lasted about 30 - 45 minutes.

b) Collars

Contractions up to 14 days after the insertion of collars were seen in all animals tested (Fig. 8). On the other hand control collars did not produce any contractions.

Table 2. Effects of intravenous and local administration of sympathomimetic drugs on the contractility of the vas deferens and on the blood pressure of anaesthetised guinea pigs (a) or rats (b). Intravenous administrations were made either by injection or by infusion into the jugular vein. The local application was made by pinning the skin surrounding the vas in such a way that a drug pool could be created in which the vas was immersed.

(a)
guinea pig

Animal number	Drug	Method of administration	Dose (µg/kg) or µg/kg/min	Control measurements			Peak drug effects			
				Arterial pressure systolic/diastolic mm Hg	Contraction of vas		Arterial pressure systolic/diastolic mm Hg		Contraction of vas	
					Electrically induced (g)	Spontaneous (g)	Lowest dose	Highest dose	Tension (g)	Frequency (contr./min)
4	ME	Injection	200-400	75/60	1.0	absent	100/90	120/110	absent	absent
2	ME	Injection	200-800	90/52	-	absent	120/80	140/100	absent	absent
3	ME	Injection	400-800	72/60	1.5	absent	128/88	120/100	0.5	0.33
1	ME	Injection	400-2000	60/35	-	absent	121/78	104/78	0.6	0.4
7	OM	Injection	200-800	80/40	-	absent	100/80	72/24	absent	absent
8	OM	Injection	200-800	150/100	3.5	absent	180/140	190/160	absent	absent
5	OM	Injection	200-1000	152/120	3.0	absent	192/160	200/160	absent	absent
6	OM	Injection	400-3000	56/43	1.6	absent	130/86	65/43	0.6	0.58
9	ME	Infusion	28.57/min	-	3.0	absent	-	-	absent	absent
10	ME	Infusion	57.14/min	-	2.0	absent	-	-	absent	absent

ME = methoxamine hydrochloride
OM = oxymetazoline hydrochloride

Animal number	Drug	Method of administration	Control measurements				Peak drug effects			
			Dose (µg/kg) or µg/kg/min	Arterial pressure systolic/diastolic mm Hg	Contraction of vas		Arterial pressure systolic/diastolic mm Hg		Contraction of vas	
					Electrically induced (g)	Spontaneous (g)	Lowest dose	Highest dose	Tension (g)	Frequency (contr./min)
108	ME	Injection	100-500	128/60	0.5	absent	140/80	190/100	absent	absent
109	ME	Injection	100-800	-	-	absent	-	-	absent	absent
107	ME	Injection	200	130/70	-	absent	200/152	-	absent	absent
105	ME	Injection	200-400	144/72	0.5	absent	200/140	200/180	absent	absent
106	ME	Injection	200-400	140/60	-	absent	180/120	200/148	absent	absent
110	ME	Injection	200-1200	72/40	-	absent	110/60	190/120	absent	absent
100	OM	Injection	200-400	90/40	-	absent	110/50	120/50	absent	absent
102	OM	Injection	200-400	80*	1.2	absent	120*	160*	absent	absent
103	OM	Injection	200-400	100/86	-	absent	100/86	168/140	absent	absent
104	OM	Injection	200-400	60/52	-	absent	120/112	128/120	absent	absent
101	OM	Injection	200-800	110*	-	absent	140*	120*	absent	absent
111	OM	Pool	-	-	-	absent	-	-	0.2	1.5
112	OM	Pool	-	-	-	absent	-	-	0.4	1.86

* The mean pressure is given where the recording was damped due to clotting in the cannula.

ME = methoxamine hydrochloride

OM = oxymetazoline hydrochloride

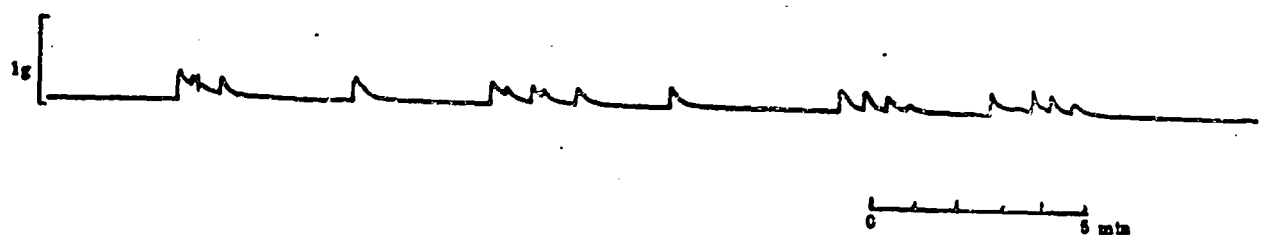


Fig. 8

Effect of local application of tyramine on the contractility of the rat vas deferens. A collar containing 50% tyramine was inserted around each vas deferens. 14 days following the insertion, the animal was anaesthetised and the contractions of the vas were recorded semi-isotonically in situ.

Blood pressure responses

A transient rise in blood pressure was seen immediately after each drug administration (Table 2). In some animals the greater rise was in response to the lower dose of the drug.

It was not possible to obtain control values of arterial pressure in those animals with collars. However, comparison of post-operative blood pressure (Table 3) with the control values of a different group (Table 2) would suggest that tyramine or amphetamine hydrochloride collars had raised blood pressure. Experiments with conscious rats (see p. 78) confirmed this observation.

DISCUSSION

These experiments demonstrate that the vasa deferentia of anaesthetised rats and guinea-pigs do not exhibit spontaneous contractions or at least, if present, they must be extremely small (< 0.05 g). In contrast, spontaneous rhythmic contractions of the vas *in vivo* have been observed in rabbits (Melin, 1970; Bruschini *et al.*, 1977) and dogs (Kimura *et al.*, 1975; Bruschini *et al.*, 1977). These differences may be perhaps due to 1) species variation or 2) differences in the recording systems employed, as the above workers have monitored intra-luminal pressure changes and not changes in longitudinal tension. In fact, no spontaneous contractions have been reported in the guinea-pig vas deferens *in vivo*, when longitudinal tension was recorded (Sannomiya *et al.*, 1977).

Contractions of the vas were produced by intravenous administration of sympathomimetic drugs, given in high doses, but severe cardiovascular effects were also produced. In contrast, a selective effect on the vas was obtained when the drugs were applied locally in the form of a pool. This suggested that it would be effective if the drugs were delivered locally by a slow-releasing formulation, made from a drug/silastic mixture, as has been described for several other classes of drug (Robert *et al.*, 1970; Prasad *et al.*, 1970; Kimball *et al.*, 1978). It was encouraging to find that such a drug/silastic collar did indeed produce rhythmic contractions over a long period of time with no greater effects on the blood pressure.

Table 3 Effect of collars containing tyramine or amphetamine on the blood pressure and contractions of the vas deferens of anaesthetised rats at various times after insertion.

Animal number	Type of collar	Time after insertion of collars	Arterial pressure systolic / diastolic (mm Hg)	Contraction of vas Amplitude (g)	Frequency (min)
151	50% AM	45 min	120/80	0.05 - 0.08	0.43
152	50% AM	45 min	168/120	0.24 - 0.28	0.27
155	50% TA	30 min	180/120	0.08 - 0.24	0.24
157	50% TA	30 min	152/100	0.16 - 0.4	0.15
154	50% TA	45 min	100/60	0.05 - 0.08	0.07
153	50% TA	45 min	160/100	0.24 - 0.48	0.37
158	50% TA	45 min	156/112	0.16 - 0.24	0.19
156	50% TA	1 day	-	0.16 - 0.24	0.10
159	50% TA	14 days	156/116	0.08 - 0.16	0.20

AM = amphetamine hydrochloride; TA = tyramine hydrochloride.

SECTION 3.

LOCAL APPLICATION OF SYMPATHOMIMETIC DRUGS TO THE EPIDIDYMIS

METHODS

Animals

Adult Sprague-Dawley or Wistar rats (males weighing 300 - 350 g and females weighing 200 - 250 g) were used in this study. The animals were housed under constant environmental conditions with free access to food (Diet 41B, Millers) and water.

Construction of rods

Rods were made by mixing an appropriate weight of the powdered sympathomimetic drug with a known volume of fluid polysiloxane polymer (silastic 383 Medical Grade Elastomer, Dow Corning Corp., Maryland 48650, U.S.A.) in a mortar for about 5 minutes. A drop of the hardener (Stannous octoate) was then added and the resulting paste mixed for another 1 - 2 minutes. This was then transferred into a length of vinyl tubing and left to set (about 15 minutes). The rods so formed were removed from the tubing and cut into lengths, 15 - 20 mm long by 3.5 mm diameter, weighing approximately 200 mg each. Control rods consisting entirely of silastic were constructed in a similar fashion.

Construction of cones

Hollow cones fitting loosely around the cauda epididymis were made from silastic is a similar manner to that described above, except that a suppository mould was used. Control cones were inserted into 3 rats, one around each cauda epididymis. All 3 animals were fertile 3 days later, but were infertile on days 7 and 14. On day 15 it was found that bilateral granulomas had developed in the cauda epididymis and this presumably caused the observed sterility. Hence this method of local administration to the epididymis was discontinued.

Insertion of rods

A group of 27 rats were operated upon under light ether anaesthesia using aseptic precautions. A 2 - 3 cm incision was made in the midline of the scrotum, with the animals lying in supine position. A small incision (about 5 mm) was then made in the tunica vaginalis of each side and one rod containing 25% methoxamine hydrochloride (3 rats), 50% methoxamine hydrochloride (7 rats) or 50% tyramine hydrochloride (2 rats) was inserted adjacent to each epididymis. In an attempt to increase the dose administered, two rods containing either 50% norephedrine hydrochloride (3 rats) or 50% tyramine hydrochloride (3 rats) were applied adjacent to each epididymis in another set of rats. The incision in the tunica vaginalis was sutured with 5/0 silk suture and sprayed with an antibiotic mixture which contains bacitracin, neomycin and polymyxin (Polybactrin, Calmic Ltd.). The incision in the scrotal sac was then closed with 12 mm Michel wound clips. Control animals (3 rats) had inserted a single drug-free rod, adjacent to each epididymis. The whole operative procedure took about 5 minutes.

Fertility tests

The libido and fertility of the treated and the control males were tested from day 3 after the operation (counting the day of operation as day 0) at varying time intervals until about the 51st day. This was done by separately pairing each male overnight with a mature pro-oestrus female rat which had been cycling regularly. Vaginal smears were examined on the morning following pairing to check for the presence of spermatozoa. The presence or absence of copulatory plugs was noted. Females showing no signs of having mated were examined again on days 4, 5, 8 and 9. Since the oestrus cycle of rat is 4 days (Lane-Petter, 1972), if they have not mated then they would be either in pro-oestrus or oestrus on these days. On the other hand, if mating had taken place they would be in continuous dioestrus (pregnancy or pseudo-pregnancy). Females were either killed or laparotomized 10-12 days post-

coitum. The ovaries were examined for the presence of corpora lutea and the number of implantation sites was recorded. If the females were consistently in dioestrus following mating and functional corpora lutea were found to be present at laparotomy, it was assumed that mating had in fact taken place. In a few occasions, the females were allowed to litter and the young examined for any gross abnormalities.

Sperm counts in the male tract

In 6 rats a rod containing 50% methoxamine hydrochloride was placed adjacent to the right epididymis as described earlier. Within the left tunica vaginalis of each animal a control rod was inserted. On day 3 after the operation the animals were anaesthetized with ether. The excurrent duct from each side was then removed and cleared of adhering tissue and the animals then killed. The vas deferens was removed from the cauda epididymis and the length of the whole epididymis measured. The caput was then separated by dividing the epididymis at the thinnest region towards the tail region of the epididymis. The remaining part was placed on 2.5 mm squared graph paper and divided into two equal lengths which were designated corpus and cauda. All the segments including the vas deferens were separately weighed before being placed in 3.5 mm petri dishes. In an attempt to expose the contents of the vas deferens, a longitudinal cut was made along its length before dividing it into 8 segments of approximately equal size. Each portion of the epididymis was also divided into 8 approximately equal sized pieces. The tissue was transferred to a glass homogenizer and the petri dishes thoroughly washed with saline.

A concentrated suspension of spermatozoa was prepared from each segment of the epididymis and the entire vas deferens by gently mascerating the tissue with about 3 ml saline, using a Griffiths tube ground glass homogenizer (Baird and Tatlock). Following suitable dilutions, the number of spermatozoa in each region of the duct was determined using the improved Neubauerhaemocytometer. The counts were made in duplicate and the average of the two determinations was used. The results were expressed as sperm content ($\times 10^6$).

Sperm counts in the female tract

The method was a modification of that described by Matthews et al. (1977). 3 male rats were separately paired overnight with one mature proestrus female rat. Vaginal smears were taken on the following morning to check the presence of spermatozoa. The females were then anaesthetized with ether and the peritoneal cavity opened with a longitudinal incision in the abdomen. Uterine horns were exposed and freed from adhering tissue. Artery forceps were clamped on each fallopian tube and on the lower end of the vagina. The pubic symphyses were divided and the entire reproductive tract, including vagina, cervix, uterus and ovaries was removed (with the 3 arterial forceps in place), and transferred to a 10 cm petri dish containing saline at 37°C. A drop of the uterine contents was sucked into a syringe using an 18G needle and transferred to a glass slide and the motility of any spermatozoa present was assessed. The artery forceps were removed and the entire tract was cut open longitudinally from the vagina to the fallopian tubes. The mucosa was scraped to remove any spermatozoa embedded in the wall and the tract then flushed with saline. This saline was then transferred to a measuring cylinder and after suitable dilution used for sperm counts as described above. Counts were made of repeated ejaculations of the same individual male rat, with not less than 3 days rest between consecutive matings. At least two control counts were made for each rat, after which one rod containing 50% methoxamine hydrochloride was inserted adjacent to each epididymis as described above. Further counts were made on days 3 and 7 after the operation.

Histology

Following the last mating, the 3 male rats used to estimate sperm counts in the female tract were anaesthetized with ether and parts of the testis and excurrent duct (cauda, corpus, caput epididymides and the vas deferens) were removed. The tissue was fixed in Bouin's fluid for subsequent histological examination. Sections were cut at 7 μm and then stained with haematoxylin and eosin before microscopic examination at 100x and 400x magnification.

Motility of the epididymal spermatozoa

Immediately prior to fixation of the cauda epididymis for histological purposes, spermatozoa were extracted from a portion of it into saline. The motility of the spermatozoa was scored on a subjective scale of 0 - 5 (the former showing absence of motility and the latter showing the greatest motility ever observed).

Sperm counts in the urinary bladder

A single rod containing 50% methoxamine hydrochloride was placed adjacent to each epididymis in 4 rats as described earlier. On day 4 after operation, the animals were paired individually with a pro-oestrus female at 17.00 - 18.00 hours. The paired animals were observed until mating occurred. Immediately following copulation the males were sacrificed by ether overdose and the urinary bladder was exposed. The entire contents of the urinary bladder were removed using a 1 ml syringe. A drop of the urine was transferred to a microscope slide and examined for any spermatozoa. A vaginal smear was also taken from the female to check for the presence of spermatozoa.

Rate of release of methoxamine hydrochloride from the rods

The amount of methoxamine released from the rods containing 50% methoxamine hydrochloride was calculated by subtracting the final from the initial weight after the rods had been dried at 60 - 70°C until a constant weight was reached (about 3 days).

Statistics

The significance of differences between the treated and the control groups were assessed using Mann-Whitney or Wilcoxon rank-sum non-parametric tests (Seigel, 1956), taking $p < 0.05$ as significant.

RESULTS

Fertility tests

The fertility of rats into which drug-free and drug-containing rods had been placed is shown in Table 4 and Figures 9 and 10.

a) Control rats

All 3 controls were fertile as evidenced by the normal numbers of implantations produced in the females with which they were mated.

b) 50% methoxamine (1 rod per epididymis)

Of the 7 rats in this group, 1 died two days after the operation. Of the remainder, none were fertile at the first mating but subsequently a progressive return to fertility was seen in all 6 males. However, up to day 28, there was a significant reduction in the average number of implants recorded in the mated females compared to those produced by the control group (combined matings days 3, 7, 14 and 21: $p < 0.00006$). No abnormalities were detected amongst the litters born from the last two matings (Table 4).

c) 25% methoxamine (1 rod per epididymis)

With the exception of one mating on day 3, all 3 males in this group were little affected by the treatment, although the average number of implants was less than in the controls. However, when all the mating results up to 21 days were pooled, a marginal reduction in fertility was seen compared with the pooled results from the control group ($p < 0.02$).

d) 50% tyramine (1 or 2 rods per epididymis) and 50% norephedrine (2 rods/epididymis)

No significant reduction in fertility was seen in any of these groups. In addition, no gross abnormalities were encountered in the litters resulting from the female rats mated to those males in which tyramine rods had been placed.

Table 4. The effect of local application of sympathomimetic drugs to the epididymis on the fertility of male rats. Each male was tested individually with a different female at various times after insertion of the rods. The table shows the number of implantation sites resulting from each mating.

Animal Number	Number of implantations												
	Days after operation												
	3	4	7	11	14	21	23	28	31	34	44	51	65
Control rods													
56		14	15	14			14		10			14	
57		10	13	14			18		14			17	
90		14	11	8			16		10			12	
50% ME													
1	0		4		9	11		7		10*			12*
2	0		16		10	11		8		16*			14*
3	0		4		0	5		7		12*			10*
4	0		0		0	0		11		14*			14*
5	0		0		6	6		12		10*			--
6	0		0		0	4		10		11*			-
25% ME													
7	0		12		9	12							
8	10		15		5	11							
9	12		7		6	10							
50% TA													
58		13	14	0			11		12				17
59		0	11	0			15		11				16
2 x 50% TA													
70	15		10		16	0					9*		
74	16		14		15	7					3*		
75	11		14		15	16					15*		
2 x 50% NE													
66	14		12		0	12							
67	8		10		6	3							
68	14		16		8	0+							

ME - methoxamine hydrochloride

TA - tyramine hydrochloride

NE - norephedrine hydrochloride

* Females allowed to litter

\+ Adhesions in ovary.

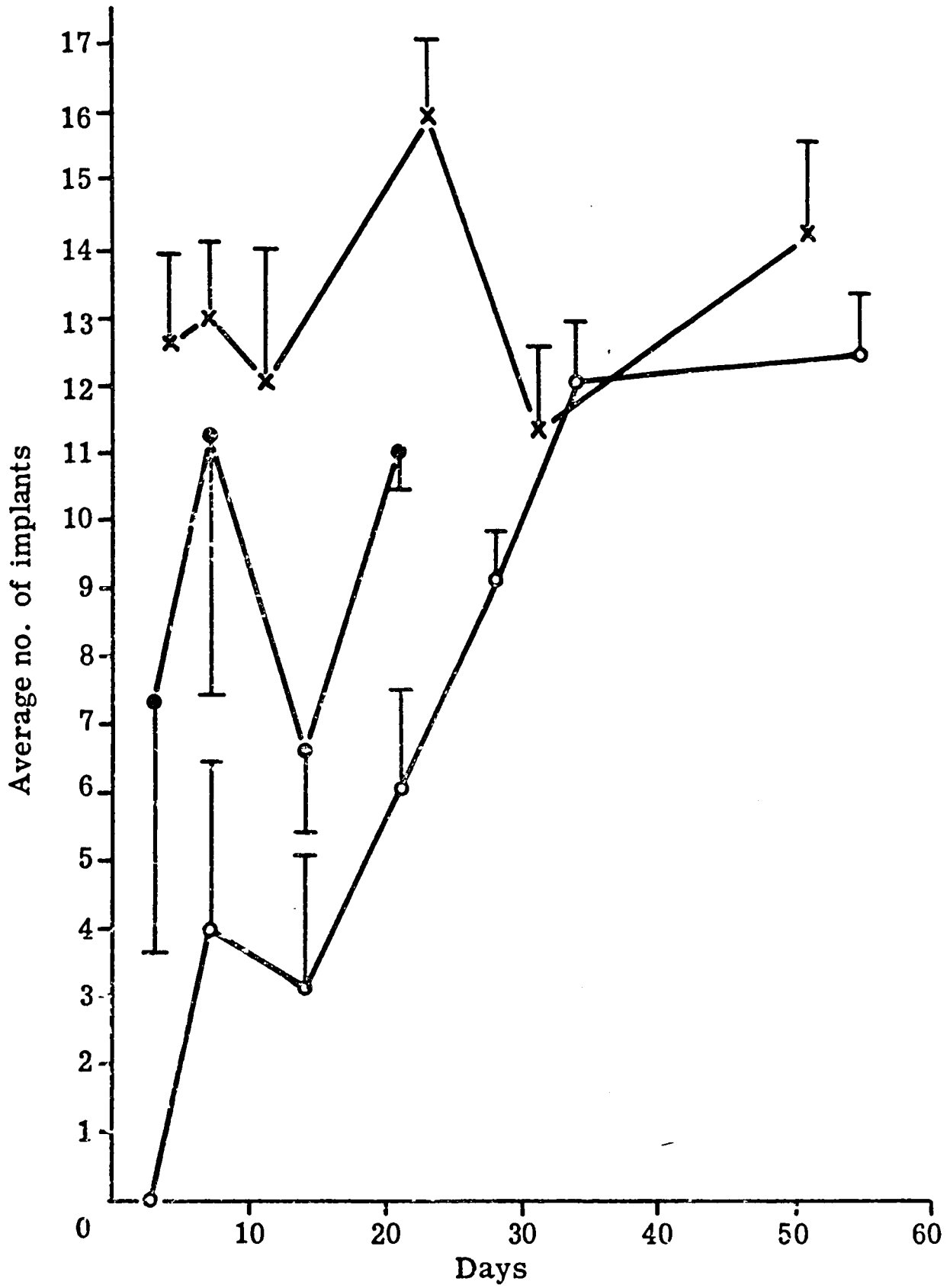


Fig. 9

The effect of rods containing 25% or 50% methoxamine or drug-free rods placed adjacent to the epididymis on the fertility of male rats. Males were paired individually with a different female at various times from day 3 after operation. Each point is the mean of the following numbers of results: 25% methoxamine rods (●) n = 3, 50% methoxamine rods (o) n = 6, control rods (x) n = 3. The standard errors are represented by the vertical bars.

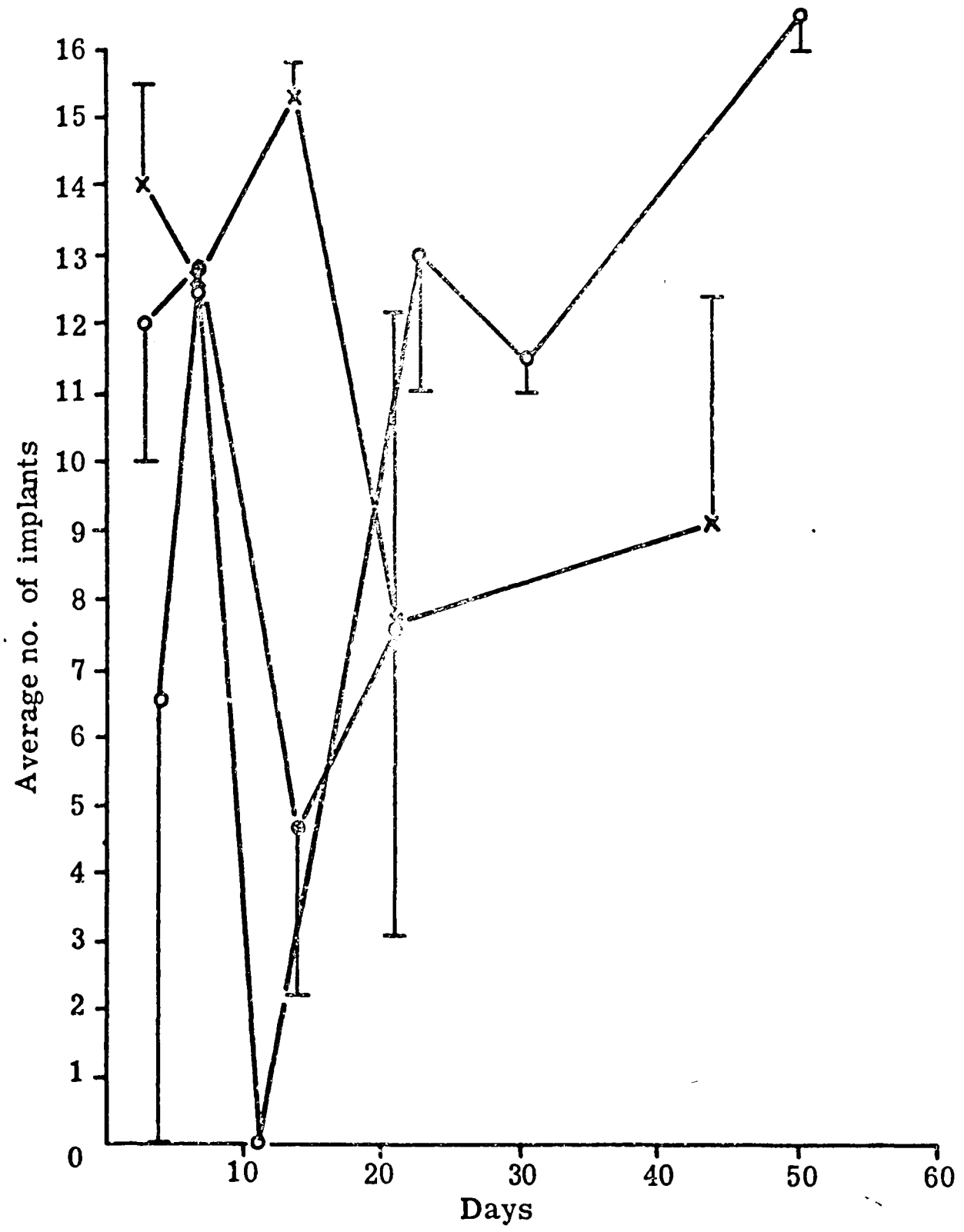


Fig. 10

The effect of norephedrine and tyramine applied locally to the epididymis on the fertility of male rats. Males were paired individually with a different female at various times from day 3 after operation. Each point is the mean of the following numbers of results: 50% tyramine rods (o) n = 2, 2 x 50% tyramine rods (x) n = 3, 2 x 50% norephedrine rods (•) n = 3. The standard erros are represented by the vertical bars.

Libido

It was clear that none of the treatments reduced libido although methoxamine produced infertility. The mating response of the treated males was as vigorous as in control animals, with the usual nudging and sniffing of the females and typical mounting behaviour. Confirmation of mating was obtained in all cases by the presence of sperm in a vaginal smear and/or by consistent dioestrus and the presence of corpora lutea in the ovary at laparotomy.

Histological observations

Slides were made of tissues taken from 3 rats, killed 8 days after implantation of one 50% methoxamine rod adjacent to each epididymis. In the testes no disturbance was seen in the spermatogenic process as such. All the stages from spermatogonia to spermatozoa were observed. However, many of the seminiferous tubules contained large numbers of rounded cells, presumably spermatids or spermatocytes sloughed into the lumen. Very few tubules showed severe damage.

The epithelium of the epididymides were normal except in one male whose corpus epididymis showed disintegration. This was presumably due to some local action of the rod since the cauda and the caput were normal in this individual. Numerous spermatozoa and increased numbers of rounded cells (perhaps spermatids) were seen in the lumen of the epididymides along their length.

No change either in the wall or in the epithelial lining was seen in the vasa deferentia of any of the treated animals. However, in addition to spermatozoa, the lumen also contained varying numbers of spermatids.

Motility of the epididymal spermatozoa

The motility score of the spermatozoa extracted into saline from the cauda epididymis, 8 days after implantation of 50% methoxamine rods, was 0 in contrast to control animals which had a motility score of 5. In addition, large numbers of spermatozoa in the treated animals were decapitated.

Sperm counts in the male tract

In 6 rats the distribution and the general appearance of spermatozoa were investigated, 3 days following the insertion of a control rod and 50% methoxamine containing rod as described earlier. The most consistent effects seen were:-
(1) a reduction in sperm numbers in the cauda and the whole epididymis and
(2) a varying proportion of the sperm in the cauda epididymis and vas deferens were decapitated (Table 5).

In the whole epididymis there was 39 $\pm$ 5% reduction ($p < 0.05$) and in the cauda epididymis 46 $\pm$ 9% reduction ($p < 0.05$). There was no significant change in the mean corpus epididymis count (fall of 4 $\pm$ 23%). In 5 out of 6 animals the number of sperm in the caput was reduced (mean change 33 $\pm$ 15%) but did not achieve statistical significance. One of the treated animals also showed approximately 25% decapitation of spermatozoa in the caput epididymis. The mean total number of sperm in the vas deferens of the treated side showed an increase of 53 $\pm$ 22% but there was a considerable individual variation and this was not significant. A granuloma was observed in one of the rats, in the vas deferens of the treated side.

Sperm counts in the female tract

The ejaculated sperm numbers in the 3 rats investigated ranged from 1 - 100 million before the insertion of the 50% methoxamine rods (Table 6). 3 - 7 days following the treatment the sperms in the ejaculate was reduced either to very low numbers or to zero.

Sperm counts in the urinary bladder

No spermatozoa were seen in the urine in any of the 4 treated males. However, a few spermatozoa were seen in the vaginal smears in two of the mated females. Copulatory plugs devoid of sperm were seen in the other two females.

Table 5

Effect of methoxamine on the sperm distribution in the genital tract of male rats. A 50% methoxamine rod was inserted adjacent to the right epididymis. A drug-free rod was inserted adjacent to the left epididymis. sperm numbers in different regions of the right side compared with that of the left. Each result is the mean $\pm$ s.e.m. from 6 rats.

	Total sperm numbers (millions)	
	control (left vas deferens)	50% methoxamine (right vas deferens)
caput epididymis	132 $\pm$ 6	88 $\pm$ 19
corpus epididymis	21 $\pm$ 4	19 $\pm$ 4
cauda epididymis	175 $\pm$ 9	$^{+}$93 $\pm$ 13*
whole epididymis	328 $\pm$ 9	200 $\pm$ 18*
vas deferens	35 $\pm$ 20	$^{++}$48 $\pm$ 22

* significantly different from control at $p < 0.05$ (Wilcoxon test)

$^{+}$ approximately 95% of sperm decapitated

$^{++}$ approximately 92% of sperm decapitated.

Table 6

Effect of 50% methoxamine rods on ejaculated sperm count of rats. Male rats were paired individually and the total sperm numbers in the mated females was estimated on the following day. Control sperm counts on different occasions are shown in the first column. Sperm numbers on different occasions after insertion of rods are shown in the third column.

	Total sperm counts in the uterus	
Animal number	6-13 days before operation	3-7 days after operation
1	105×10^6, 25×10^6	5×10^4, $< 2.5 \times 10^4$ *
2	2.5×10^6, 40×10^6	0, 0, 0, $< 2.5 \times 10^4$ *
3	15×10^6, 1×10^6	2.5×10^4, $< 2.5 \times 10^4$ *

* If spermatozoa were found in the vaginal smear, but none counted in the Improved Neubaeur haemocytometer, it is recorded as $< 2.5 \times 10^4$ as this value represents the minimum number that could be counted.

DISCUSSION

The main result showed that local administration of a directly acting sympathomimetic drug, methoxamine, adjacent to the epididymis, caused a dramatic reduction in fertility in male rats. The peak effect on fertility was seen at the first mating on day 3 following insertion of the drug-containing rods, when all the females paired with treated males failed to become pregnant. Over the next 3 weeks there was a gradual restoration of fertility which was probably due to exhaustion of the drug as indicated by the reduction in weights of implants (see Table 7). A lower dose of methoxamine appeared to reduce fertility slightly. The indirectly acting sympathomimetics, tyramine (Ambache et al., 1972; Wadsworth, 1973) and norephedrine (see p. 31) whose effect is brought about by the release of endogenous noradrenaline from adrenergic neurones, failed to produce any antifertility effect when administered in a similar manner. The muscular coat of the caput and the proximal corpus epididymis of the rat is virtually free of adrenergic neurones in contrast to the cauda and vas deferens which receive a dense adrenergic innervation (El Badawi et al., 1962; Norberg et al., 1966). If the sympathomimetic drugs are acting to induce sterility by an action on smooth muscle in the epididymis it is to be expected that the indirectly acting sympathomimetics would be ineffective.

The total number of spermatozoa in the uteri of rats was counted on the morning after mating and this was used as a measure of the numbers of spermatozoa ejaculated by individual males. In control animals the number of spermatozoa ejaculated was in the range of 1 - 100 million, but there was found to be a considerable individual variation in agreement with the results of Matthews et al. (1977) and there was also a marked variation in the same individual on different occasions. 3 - 7 days after application of methoxamine-containing rods, the number of spermatozoa in the ejaculate was reduced either to very low numbers or to zero. This is presumably the main reason why the treated males were infertile, though in addition there was a loss of motility and decapitation which would by itself be sufficient to produce infertility.

Table 7

Loss of weight of 50% methoxamine rods after insertion adjacent to the epididymis of rats. The table shows the initial weight and the final weight after drying. The difference is presumed to indicate the quantity of drug released.

Number of rods inserted	Initial wt. (mg)	Number of days after insertion	Final wt. (mg)	Difference in wt. (mg)
2	432	8	222	210
2	425	8	215	210
1	175	3	144	31
1	187	3	150	37
1	186	3	170	16

Table 8

Effect of 50% methoxamine rods inserted adjacent to the right epididymis on the transit time of spermatozoa through the epididymis as estimated by the method of Amann et al. (1976) compared to that of left epididymis which acted as control (drug-free rod). Each result is the mean from 6 rats.

	Transit time (days)	
	Control	Treated
caput epididymis	3.06	2.05
corpus epididymis	0.49	0.45
cauda epididymis	4.06	2.15
whole epididymis	7.53	4.65

After treatment with 50% methoxamine rods, there was a reduced, but still considerable number of spermatozoa in the cauda (about 90 million). Since the ejaculate was virtually free of spermatozoa, it seems likely that the drug in some way prevented spermatozoa from entering it. This might have resulted from diminished contractions of the vas deferens and cauda epididymis at copulation. Such a diminished contraction could perhaps result from stimulation of presynaptic α-receptors (Farnebo et al., 1971) which is probably produced by this method of local administration. Another mechanism for the absence of sperms would be if sperms were retrogradely ejaculated into the urinary bladder. But this is ruled out by the observation that no sperms were detected in the urine after copulation.

The reduction in spermatozoal populations in the cauda and the total epididymis may have been caused by contraction of the epididymal smooth muscle. Sympathomimetic drugs have been shown to induce rhythmic contractions in the cauda epididymis (Da Silva et al., 1975; Hib, 1976, see p. 31). Such contractions in the epididymis produced by methoxamine could decrease the sperm numbers in the cauda if there was a continuous expulsion of spermatozoa into the vas deferens. Indeed, there was a slight, but non-significant, increase in the mean sperm numbers in the vas deferens. Spermatozoa have been detected in the urine of animals including rats (Fernández-Collazo et al., 1971; Vreeburg et al., 1974). Since the increase in sperm numbers in the vas was not significant, we presume that the expelled sperm from the cauda were eventually voided in the urine or spontaneously ejaculated (Orbach, 1961; Kihlström, 1966; Åmgo, 1976). Further experiments are necessary to see if the urine or spontaneous ejaculates contain sufficient spermatozoa to account for the reduction in the spermatozoal population in the cauda and the vas deferens caused by methoxamine.

The lack of motility and decapitation of sperm in the cauda and vas deferens but not in the upper regions of the epididymis, suggests a local action on the cauda epididymis. Lack of motility might occur if there were enhanced transport of spermatozoa from the testis to the cauda epididymis so that sufficient time is not allowed for the spermatozoa to mature before reaching the cauda epidi-

dymis. Using the formula described by Amann et al. (1976) an enhanced transport of spermatozoa is seen on the treated side (see Table 8). However, the calculations are based on the assumption that production of spermatozoa in the testis is unaffected by the treatment. An alternative explanation for immotility could be a direct toxic effect of the drug or any changes in the epididymal environment that might have been caused by the drug. The decapitation might also have resulted from similar effects. Alternatively, the effect on motility and decapitation might have resulted from a decrease in blood supply to the epididymis, since methoxamine is known to be a potent vasoconstrictor (Hoffbrand et al., 1973).

Contractions in the testicular capsule are reported with sympathomimetic drugs (Davis et al., 1971; Hargrove et al., 1973, 1976; Firlit et al., 1975). Too severe contractions in the testicular capsule caused by methoxamine could possibly cause the sloughing of the immature forms in the testis as observed in the treated animals. Alternatively, it is possible that the vasoconstrictor actions of methoxamine may have produced a disturbance of the function of the pampiniform plexus which might have resulted in changes in temperature regulation or blood flow to the testis. Marked changes in blood flow or blood temperature to the gonads could result in exfoliation of immature cell forms since the rat's reproductive tract is known to be particularly vulnerable to stress (Ericsson, 1975). Alternatively, the sloughing of the spermatids could have been caused by a direct effect of methoxamine on immature germinal cells. However, the exfoliation of germinal cells is unlikely to be the cause of the antifertility effect, at least in the early stages because the onset was so rapid (3 days) and also because considerable numbers of sperm could still be seen in histological sections and on maceration of the tract.

There was no decrease in libido as judged by courting behaviour and the undiminished ability of the male to mate. This suggests that the treatment has not brought about any significant change in the blood testosterone levels. The rapid development of the antifertility effect (3 days) suggests that it could not have been produced by inhibition of spermatogenesis.

The results of this study indicate that rods containing 50% methoxamine can cause reversible sterility in male rats without loss of libido. The primary cause of infertility is the reduction of the spermatozoa in the ejaculate. In addition, the treatment causes decapitation and immotility of spermatozoa which would reduce the fertilizing potential of any spermatozoa which do enter the ejaculate. It is suggested that the main cause of this effect is contraction of smooth muscle in the epididymis, combined with an effect on the ejaculatory process.

SECTION 4.

LOCAL APPLICATION OF SYMPATHOMIMETIC DRUGS TO THE VAS DEFERENS OF RATS

METHODS

Animals

Adult Sprague-Dawley rats (males weighing 300 - 350 g and females weighing 200 - 250 g) were used in this study. The animals were housed under constant environmental conditions with free access to food (Diet 41B, Millers) and water.

Construction of collars

An appropriate weight of the powdered sympathomimetic drug was mixed together with a known volume of polysiloxane polymer (Silastic 382 Medical Grade Elastomer, Dow Corning Corp., Maryland 48640, U.S.A.) in a mortar for about 5 minutes. A drop of the hardener (Stannous octoate) was then added and further mixing carried out for another 1 - 2 minutes. The paste thus formed was immediately transferred into a mould constructed from the inner barrel of a 2 ml disposable polycarbonate syringe. A knitting needle (diameter 2.8 mm) was pushed through the centre of the mixture, which was then allowed to set (about 15 minutes). The needle was later withdrawn and the hardened drug-containing silastic tube removed by crushing the barrel of the syringe. A further layer of the polymer, mixed with its catalyst, was applied to the outer surface of the tube (since preliminary experiments had suggested that such an application reduced systemic absorption of drugs). The resulting cylinder was finally cut into 2 or 3 collars, each about 15 mm long by 8 mm diameter with a 2.8 mm central hole and weighing about 250 mg. A longitudinal cut was then made in the wall of the collar to enable its placement around the vas deferens. Control collars consisting entirely of silastic were prepared in a similar manner.

Insertion of collars around the vas deferens

A group of 24 male rats was operated upon under ether anaesthsia using aseptic precautions. With the animals lying in supine posture, a 2 - 3 cm midline

incision was made in the lower abdomen. Both vasa deferentia were exposed at the prostatic end by pulling on the epididymal fat pad with a pair of forceps. Care was taken not to withdraw the testes into the abdomen. A collar containing 25% methoxamine hydrochloride (5 rats), 50% tyramine hydrochloride (6 rats), 75% tyramine hydrochloride (7 rats), 50% norephedrine hydrochloride (6 rats) or drug-free (5 rats) was then placed around each vas deferens before returning them and the fat pads to their natural positions in the abdominal cavity. The incision in the abdominal wall was closed with a 2/0 silk suture and sprayed with an antibiotic mixture which contains bacitracin, neomycin and polymyxin (Polybactrin-Calmic Ltd.). The incision in the skin was closed with 12 mm Michel wound clips. The wound was sprayed with a transparent plastic dressing (Nobecutane, B.D.H. Ltd.) and the animals allowed to regain consciousness.

In another group of 3 rats, a collar containing 50% tyramine was inserted around just one vas.

In the Results section, the day of operation is always designated day 0, and the date of later procedures (e.g. mating) is defined with respect to this.

Insertion of collars into the abdominal cavity

Two collars containing 50% norephedrine hydrochloride (2 rats), 50% tyramine hydrochloride (4 rats) or 75% tyramine hydrochloride (1 rat) were placed in the abdominal cavity, near the pelvic viscera using similar surgical techniques to those described above. The collars were anchored to the inner surface of the abdominal wall by means of a 2/0 silk suture in 2 of the 5 rats in the 50% tyramine group.

Removal of collars from the vas deferens

Collars containing 50% tyramine hydrochloride (7 rats) and 50% norephedrine (10 rats) were implanted around each vas deferens in the manner previously described. The animals were mated subsequently on days 3 and 7 after surgery. On day 10 following the first surgery, a second operation was carried out on these rats. Both vasa deferentia were exposed and the connective tissue sheath, which had now usually grown around the collars, cut open and the collars

removed. Care was taken not to damage the vas deferens. In instances where there was doubt about damage caused to either one or both vasa deferentia by removal of the collars, the animals were deleted from the study. Fertility tests with these animals were again resumed from day 7 following the second surgery.

Fertility tests

The libido and fertility of the treated and control males were tested at varying time intervals ranging from 3 to 125 days post-operatively. This was done by pairing each male overnight with 1 or 2 pro-oestrus females. Mating was confirmed by the presence of sperm in the vaginal smears and/or the presence of a copulatory plug the following morning. In the absence of these criteria, daily smearing of the females was performed to check for pseudopregnancy until they were killed or laparotomized.

In Tables 9, 10, 11a,b the results of these fertility tests are summarized. However, the animals were not actually tested on days exactly matching those indicated in these tables. Usually two matings were undertaken in the first week following the insertion of collars and thereafter at least at fortnightly intervals. For simplification, therefore, the implantations resulting have been allocated to the column most closely approximating the number of days post-operatively that these matings took place. In a few cases one result has been entered into the tables in more than one column, and in these cases the numbers are shown in brackets. However, for statistical analysis the duplications arising from this method have been removed. In instances where an individual male was allowed to mate with 2 females, the larger number of implantations has been entered into the tables.

Unilateral vas ligation

In 6 rats, a double ligation using 5/0 silk sutures was made at the prostatic end of the right vas deferens in the region where the collars were normally placed. The surgery in other respects was similar to that for the insertion of collars. The ligated site was not transected.

Sperm counts in the male tract

Spermatozoal counts were made as described in Section 3, p.44 ,3 days following unilateral vas ligation (6 rats) and the insertion of one 25% methoxamine hydrochloride collar (7 rats), or one 50% tyramine hydrochloride collar (6 rats) on the right vas and a drug-free collar on the left vas.

Sperm counts in the female tract

A minimum of two control counts were made on 4 rats as described elsewhere (p. 44). Further counts were undertaken on days 3 and 7 following the application of a 25% methoxamine hydrochloride collar (1 rat) or a 50% tyramine hydrochloride collar (3 rats) to the right vas and a control collar to the left vas.

Histology

The testes, segments of the epididymis and parts of the vas under the collar and between the collar and the cauda were removed under anaesthesia from the 4 rats used to estimate sperm counts in the female tract. The tissues were fixed in Bouin's for subsequent histological examination.

Motility of the cauda epididymis spermatozoa

Spermatozoa from the cauda epididymiswere examined immediately prior to fixation and their index of motility scored in the manner described earlier.

Blood pressure measurements from conscious rats

The method of inserting a permanent in-dwelling catheter was similar to that described by Popovič et al. (1960). Six rats were anaesthetized with ether and a 2 - 3 cm incision made in the ventral surface of the neck, using aseptic precautions. The right carotid was exposed and a polythene catheter (Internal diameter 0.76 mm and external diameter 1.2 mm, Portex) was then inserted into the carotid artery and advanced to the aorta. The outer end of the cannula was taken through the subcutaneous tissue using a trochar and exteriorized at the dorsal surface of the neck and clipped to the skin with two 12 mm Michel wound clips. An antibiotic mixture which contains bacitracin, neomycin and polymyxin (Polybactrin-Calmic Ltd.) was sprayed into the

operative site and the incision was closed with 12mm Michel wound clips. Transparent plastic dressing (Nobecutane - B.D.H. Ltd.) was then sprayed on to the wound. The animals were subsequently transferred to individual cages and allowed to regain consciousness. The catheter was flushed through periodically with heparinised 0.9% saline containing 0.05% chlorocresol.

The blood pressure was measured continuously for about 30 - 45 minutes from the carotid artery, after coupling the polythene tubing to a pressure transducer (Statham P23V). The pressure transducer was calibrated against a mercury manometer.

After measuring the control carotid blood pressure, 3 rats were re-anaesthetised with ether and collars containing 50% tyramine were implanted, one around each vas deferens as described earlier. The carotid blood pressure was then monitored in each animal individually after regaining consciousness, at various time intervals up to 2 days. Another rat was implanted with a single control collar around each vas deferens. Blood pressure was measured for about 30 minutes after which a second operation was done to replace the control collars with 50% tyramine collars. The blood pressure was then monitored at various time intervals from immediately after the second surgery up to day 2.

Statistics

The significance of differences between the treated and the control groups were assessed using Mann-Whitney or Wilcoxon rank-sum non-parametric tests (Seigel, 1956), taking $p < 0.05$ as significant.

RESULTS

Fertility tests

Control collars (1 around each vas)

All 5 control rats were fertile as shown by the normal number of implantations produced in the females with which they were mated (Table 9). In addition, no abnormalities were encountered amongst the litters born from two of the mated females.

Table 9

The effect of 50% norephedrine, 25% methoxamine and drug-free collars on the fertility of male rats. Each male was tested individually with 1 or 2 females at various times from day 3 after the insertion of collars around the vas deferens. In cases where 2 females were allowed to mate, the higher number of implantations resulting from the matings is inserted in the table. The parentheses indicate duplication of results.

Animal number	Number of implants						
	Days after operation						
	3	7	14	21	28	42	56
a) 50% norephedrine							
27	0	0	(0)	0	2	2	0
28	0	0	0	(0)	0	0	0
29	0	0	8	(8)	1	1	(1)
31	0	8	0	13	0	(0)	0
32	3	(3)	13	(13)	2	(2)	0
33	1	1	2	(2)	0	0	1
b) control							
10	14	(14)	14	14	14	(14)	14
11	13	13	(13)	(13)	14	12	12
17	13	13	14	14	(14)	12	12
40	14	(14)	12	(12)	7	13	(13)
41	11	(11)	14	14	8	(8)	8
c) 25% methoxamine							
30	0	0	(0)	2	(2)	3	0
46	0	0	0	(0)	0	0	1
80	0	0	0	0	0	(0)	(0)
81	0	(0)	0	0	(0)	0	0
82	0	0	(0)	0	0	2	2

25% methoxamine collars (1 around each vas)

Of the 15 rats in this group, only 5 survived (Table 9) and all were practically sterile (days 3 and 7, $p = 0.008$, day 14, $p = 0.028$, day 21, $p = 0.016$) (Fig. 11).

50% norephedrine collars (1 around each vas)

All 6 rats in this group were infertile at their first mating after insertion of the collars, but a subsequent partial return of fertility occurred in 3 rats on day 14 or 21 (Table 9) (days 3 and 7, $p = 0.002$, day 14, $p < 0.056$, day 21, $p = 0.094$). These 3 rats which spontaneously regained fertility, again became sterile 28 days after the operation. At autopsy on day 116-170, all the animals in the group had granulomas in both vasa deferentia proximal to the collars.

50% tyramine collars (1 around each vas)

Of the 6 rats studied, none were fertile at the first mating (Table 10) but spontaneous recovery of fertility was observed in 4. The average number of implants recorded for the group was significantly lower than that of control group on day 3 ($p = 0.004$), day 7 ($p < 0.02$) and day 21 ($p \leq 0.042$).

In 2 rats which recovered fertility, the collars were replaced by new 50% tyramine collars on day 58 and both animals subsequently become sterile once again. One regained fertility on day 76 but the other remained infertile for the rest of the study (100 days).

75% tyramine collars (1 around each vas)

Of the 7 animals in this group, one died as a result of a fight on day 8. All but one animal were permanently sterile and at autopsy were found to have bilateral granulomas (day 3, $p = 0.002$, day 7, $p = 0.006$, days 14 and 21, $p = 0.002$).

2 collars in the abdomen (50%, 75% tyramine and 50% norephedrine)

No significant reduction in fertility was seen in any of these groups (Figs. 12, 13). Furthermore, no gross abnormalities were noticed in the litters resulting from

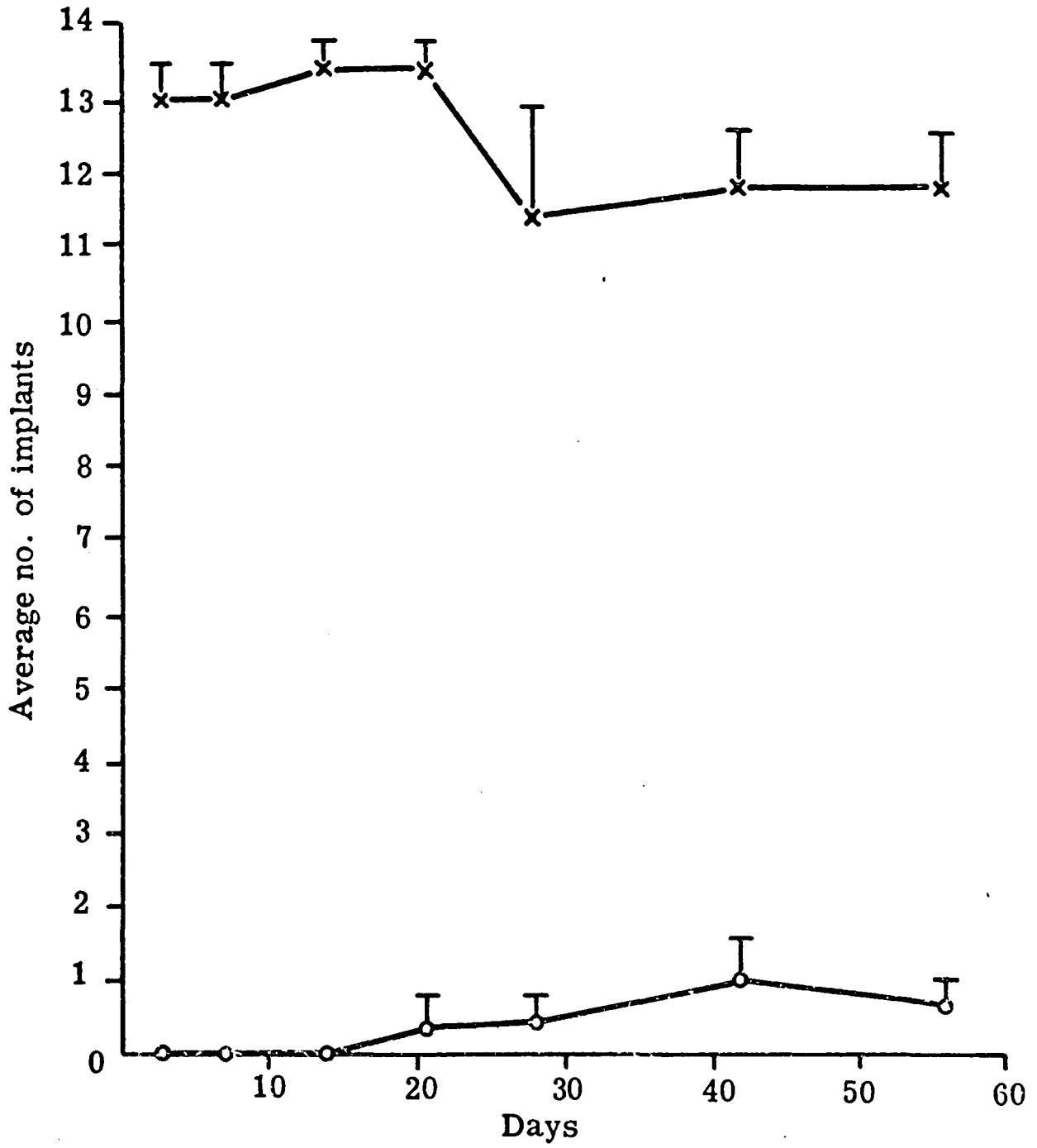


Fig. 11

The effect of collars containing 25% methoxamine or drug-free collars placed around the vas deferens on the fertility of male rats. Each male was paired individually with 1 or 2 females at various days from day 3 after operation. Each point is the mean of the following numbers of results: 25% methoxamine collars (o) n = 5, control collars (x) n = 5. The standard errors are represented by the vertical bars.

Table 10

The effect of 50% and 75% tyramine collars on the fertility of male rats. Each male was tested individually with 1 or 2 females at various times from day 3 after the insertion of these collars around the vas deferens. In cases where 2 females were allowed to mate, the higher number of implantations from the matings is inserted in the table. The parentheses indicate duplication of results.

Animal number	number of implants						
	Days after operation						
	3	7	14	21	28	42	56
a) 50% tyramine							
13	0	13	15	13	13	9	(9)
14	0	2	1	1	0	0	0
21	0	0	1	4	(4)	14	(14)
22	0	0	(0)	8	13	14	(14)
37	0	0	0	(0)	0	0	0
38	0	(0)	2	(2)	(2)	6	6
b) 75% tyramine							
15	0	0	-	--	-	-	--
16	0	0	0	0	0	0	0
20	0	0	0	4	(4)	0	0
45	0	0	(0)	(0)	0	7	(7)
55	0	0	(0)	(0)	0	0	0
34	0	(0)	(0)	3	0	0	0
35	0	0	(0)	0	0	0	0

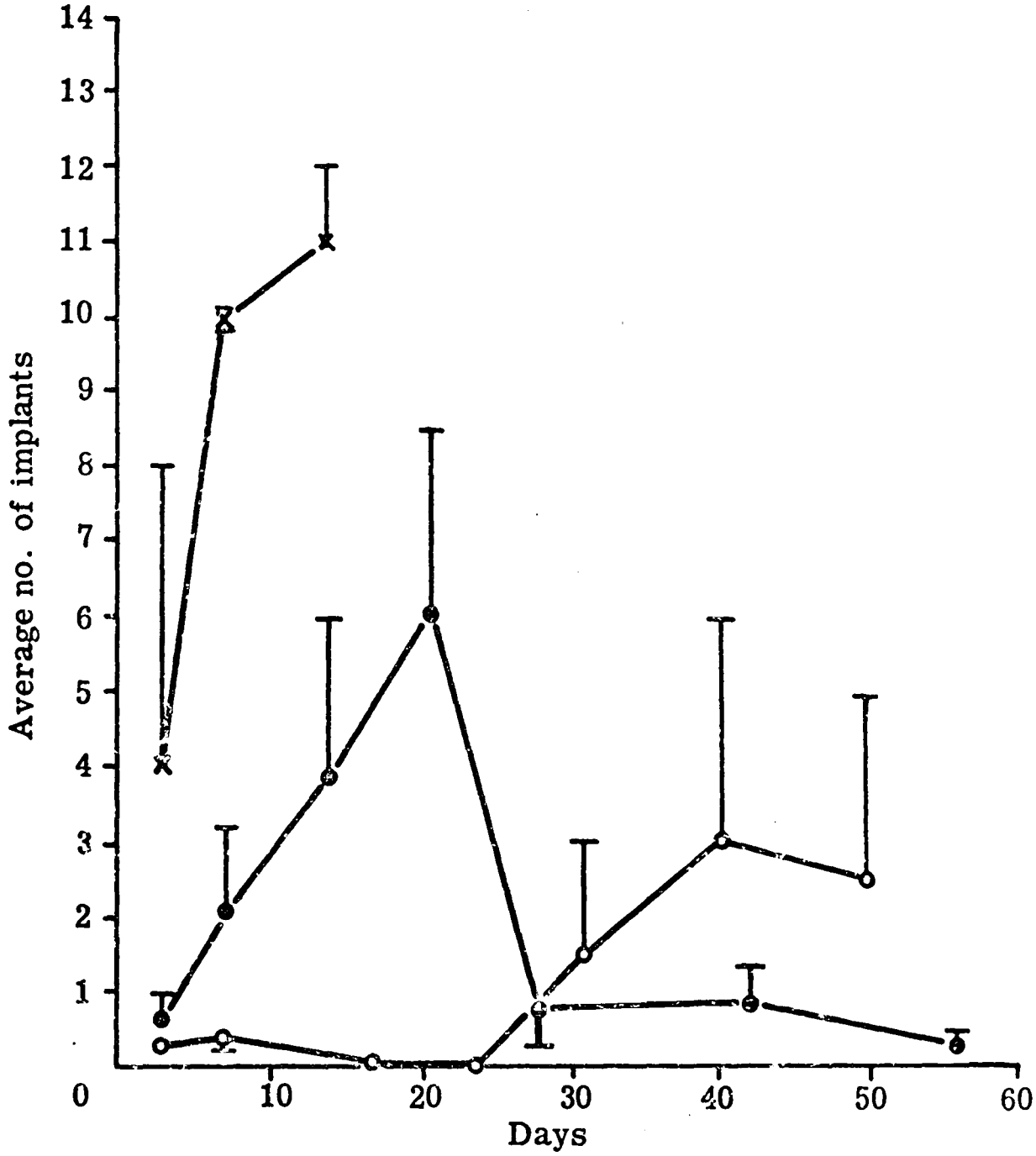


Fig. 12

The effect of 50% norephedrine collars on the fertility of male rats. 1) One collar was placed around each vasa (●) n = 6. 2) One collar was placed around each vasa and subsequently removed on day 10 (o) n = 4 - 10, 3) Two collars were placed in the abdominal cavity (x) n = 2. Each male was tested individually with 1 or 2 females at varying times from day 3 after the insertion of collars. The standard errors are represented by vertical bars.

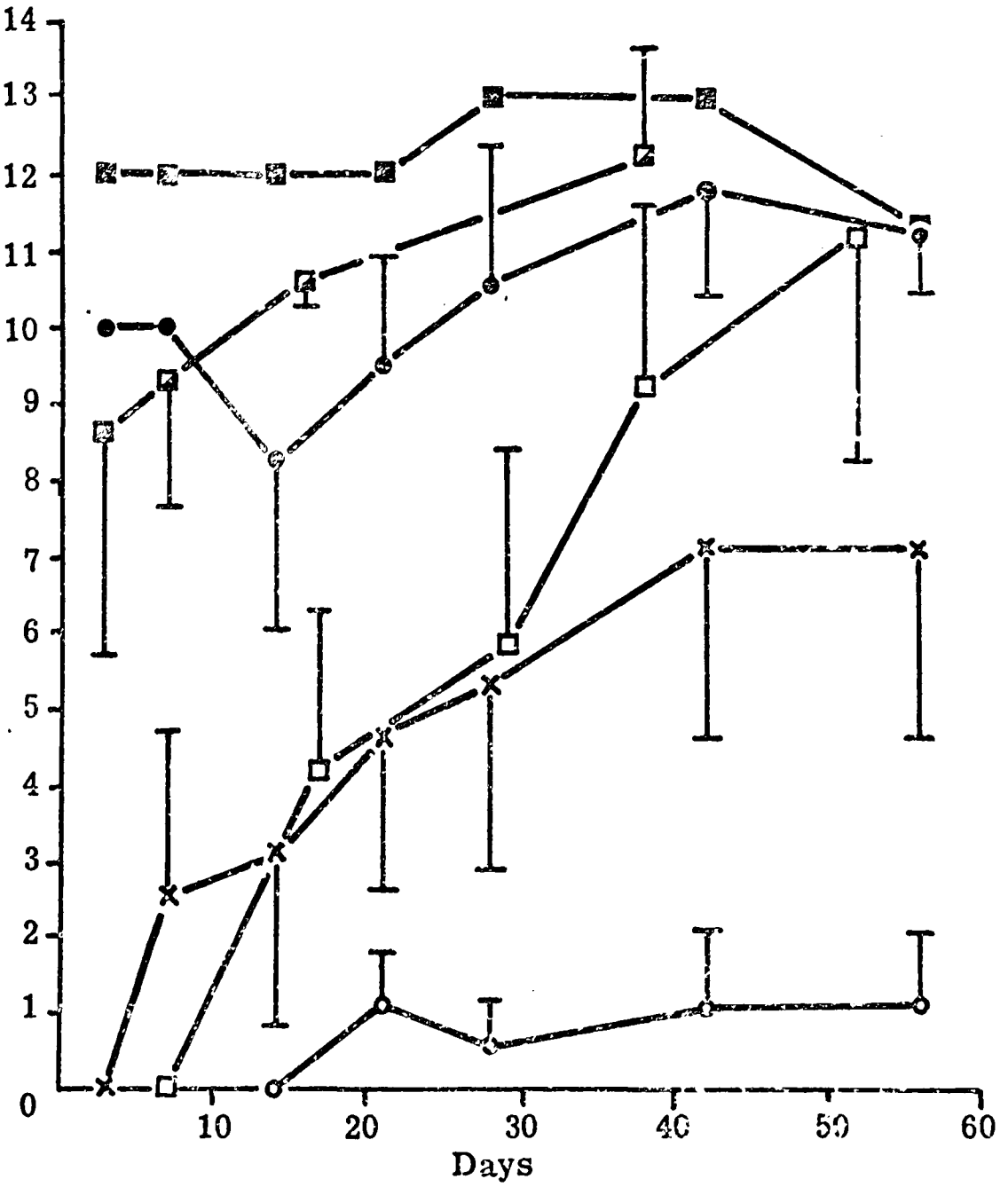


Fig. 13

The effect of 50% and 75% tyramine collars on the fertility of male rats. 50% tyramine collars were inserted as follows: 1) one around each vas (x) n = 6, 2) one around each vas and subsequently removed on day 10 (□) n=5-7, 3) one around a single vas, the contralateral side being untreated (◪) n = 3, 4) two in the abdominal cavity (●) n =2-4. 75% tyramine collars were inserted as follows: 1) one around each vas (○) n =6-7, and 2) two in the abdominal cavity (◙) n = 1. Each male was tested individually with 1 or 2 females at various times from day 3 after the insertion of collars The standard errors are represented by vertical bars.

the matings with those males in which 50% tyramine collars had been placed (Table 11a).

Removal of collars 10 days following insertion (50% tyramine and 50% norephedrine)

Of the 7 animals in the tyramine group, 2 had to be sacrificed due to the development of an unrelated skin rash. 4 of the remainder regained fertility as seen in Figure 13. The other had developed granulomas in both vasa deferentia.

In 6 of the 10 with norephedrine collars, removal was unsuccessful. Only one of the remaining four became fertile. The other 3 developed granulomas in the vasa deferentia (Fig. 12).

Norephedrine collars were also removed on day 21 from 2 animals who had not regained fertility in the main mating study (Table 9). No subsequent restoration of fertility was, however, observed..

50% tyramine collars (1 around single vas)

With the exception of one mating on day 3 which resulted in only 3 embryos, none of the 3 animals in the group were affected by the treatment (Fig. 13). On day 55, all had granulomas on the treated side under the collar.

Libido

A reduction in libido as judged by the criteria given on p.51 was not observed in any of the treated males.

Sperm counts in the male tract

50% tyramine collars

The most consistent effect seen in the 6 rats of this group, 3 days following the surgery, was a significant reduction ($p < 0.05$) in sperm numbers in the corpus and whole epididymis (Table 12). In the caput and cauda, the effects were inconsistent suggesting that the drug did not have the same effect in all the animals.

Table 11a

The effect of collars containing sympathomimetic drugs when placed in the abdominal cavity on the fertility of male rats. Each male was tested individually with 1 or 2 females at various times from day 3 after insertion of collars. In cases where 2 females were allowed to mate, the higher number of implantations resulting from the matings is inserted in the table. The parentheses indicate duplication of results.

Animal number	Number of implants						
	Days after operation						
	3	7	14	21	28	42	56
75% Tyramine							
36	12	12	(12)	12	13	(13)	11
50% Tyramine							
39	0	(0)	2	11	15	15	13
42	11	11	13	12	(12)	12	(12)
43	17	(17)	10	6	(6)	11	(11)
44	12	(12)	8	9	(9)	9	9
50% Norephedrine							
49	8	10	10	-	-	-	-
50	0	10	12	-	-	-	-

Table 11 b

Effect of drug-free or 50% tyramine collars on the blood pressure of conscious rats at various times after insertion. Because the record obtained from chronically implanted catheters was usually damped, the mean of the systolic and diastolic pressures is indicated in most cases. The values indicated were the closest available to the times mentioned in the table.

Animal number	Control Arterial Pressure mm Hg	Treatment	Post treatment arterial pressure (mm Hg)				
			15-30 min	1 hour	3 hours	1 day	2 days
150	120						
151	110						
152	142/132*	50% TA	182/176*	152/142	75	85	85
153	120/110*	50% TA	155	150	110	85	-
154	113	Control	110	-	-	-	-
154	110	50% TA	140	132	120	90	-
155	140	50% TA	165/150*	-	105	115	-

* Arterial pressure: systolic/diastolic

TA = tyramine hydrochloride collars.

Table 12

Effect of tyramine on the sperm distribution in the genital tract of male rats. A 50% tyramine collar was inserted around the right vas deferens. A drug-free collar was inserted around the left vas deferens. Sperm numbers in different regions of the right side compared with those of the left. Each result is the mean ± s.e.m. from 6 rats.

	Total sperm number (millions)	
	Control (left vas deferens)	50% tyramine (right vas deferens)
caput epididymis	123 ± 16	99 ± 13
corpus epididymis	33 ± 6	16 ± 2 *
cauda epididymis	202 ± 37	174 ± 3
whole epididymis	358 ± 49	289 ± 37 *
vas deferens	23 ± 3	14 ± 6

* significantly different from control at $p < 0.05$ (Wilcoxon test).

Table 13

Effect of methoxamine on the sperm distribution in the genital tract of male rats. A 25% methoxamine collar was inserted around the right vas deferens. A drug-free collar was inserted around the right vas deferens. Sperm numbers in different regions of the right side compared with those of the left. Each result is the mean ± s.e.m. from 7 rats.

	Total sperm number (millions)	
	Control (left vas deferens)	25% methoxamine (right vas deferens)
caput epididymis	80 ± 13	56 ± 12
corpus epididymis	3 ± 0.5	1 ± 0.6
cauda epididymis	89 ± 22	44 ± 8 *
whole epididymis	178 ± 41	102 ± 19
vas deferens	5 ± 2	18 ± 6

* signficantly different from control at $p < 0.05$ (Wilcoxon test).

25% methoxamine collars

In the 7 rats investigated 3 days after operation, there was a reduction in sperm numbers in all the segments of the epididymis and a substantial increase in the numbers present in the vas deferens of the treated side (Table 13). Yet only the change in numbers present in the cauda epididymis was statistically significant ($p < 0.05$).

Unilateral vas ligation

No significant change in sperm distribution was evident 3 days following unilateral vas ligation of 6 rats as shown in Table 14. However, a varying proportion of sperm was decapitated in the vas deferens of the ligated side of all the animals.

Histological observations

25% methoxamine collars

No obvious alteration in spermatogenesis was noticed in the testes of the 3 rats examined 7 days following the insertion of collars. However, some premature sloughing of rounded cells (presumably spermatids or spermatocytes) into the lumen was seen in a few tubules in 2 rats.

No disorganization of the epithelium of the epididymis was observed. The lumen contained spermatozoa in varying numbers depending on the region observed.

The portion of the vas deferens between collar and the epididymis showed no apparent change with regard to the structure of the epithelium, muscle wall or lumen. In contrast, the epithelial lining of the vas deferens immediately under the collar showed various degrees of damage. Partial to complete blockage of the lumen with fibrous material was apparent. No damage to the muscle layers was seen however (Fig. 14).

50% tyramine collars

In 2 rats examined 8 days following the insertion of tyramine collars, some

Table 14.

Effect of unilateral vas ligation on the sperm distribution in the genital tract of male rats. The sperm numbers in different regions of the ligated side were compared with those of unligated side. Each result is the mean ± s.e.m. from 6 rats.

	Total sperm number (millions)	
	Control (left vas deferens)	Ligated (right vas deferens)
caput epididymis	102 ± 15	96 ± 22
corpus epididymis	19 ± 2	15 ± 3
cauda epididymis	249 ± 28	272 ± 39
whole epididymis	370 ± 38	382 ± 60
vas deferens	22 ± 66	24 ± 9

Table 15

Effect of 50% tyramine or 25% methoxamine collars on ejaculate sperm count of rats. Male rats were paired individually and the total sperm numbers in the mated females was estimated on the following day. Control sperm counts on different occasions are shown in the first column. Sperm numbers after insertion of these collars are shown in the fourth column.

Animal number	Total sperm count in uterus: 4-24 days before operation	Treatment	3-7 days after operation
7	1×10^6, 2×10^6	50% TA	0, 0
8	18×10^6, 12.5×10^6	50% TA	0, 0
11	50×10^6, 74×10^6	50% TA	0, 0
3	135×10^6, 60×10^6, 100×10^6	25% ME	0, 0

TA = tyramine hydrochloride

ME= methoxamine hydrochloride

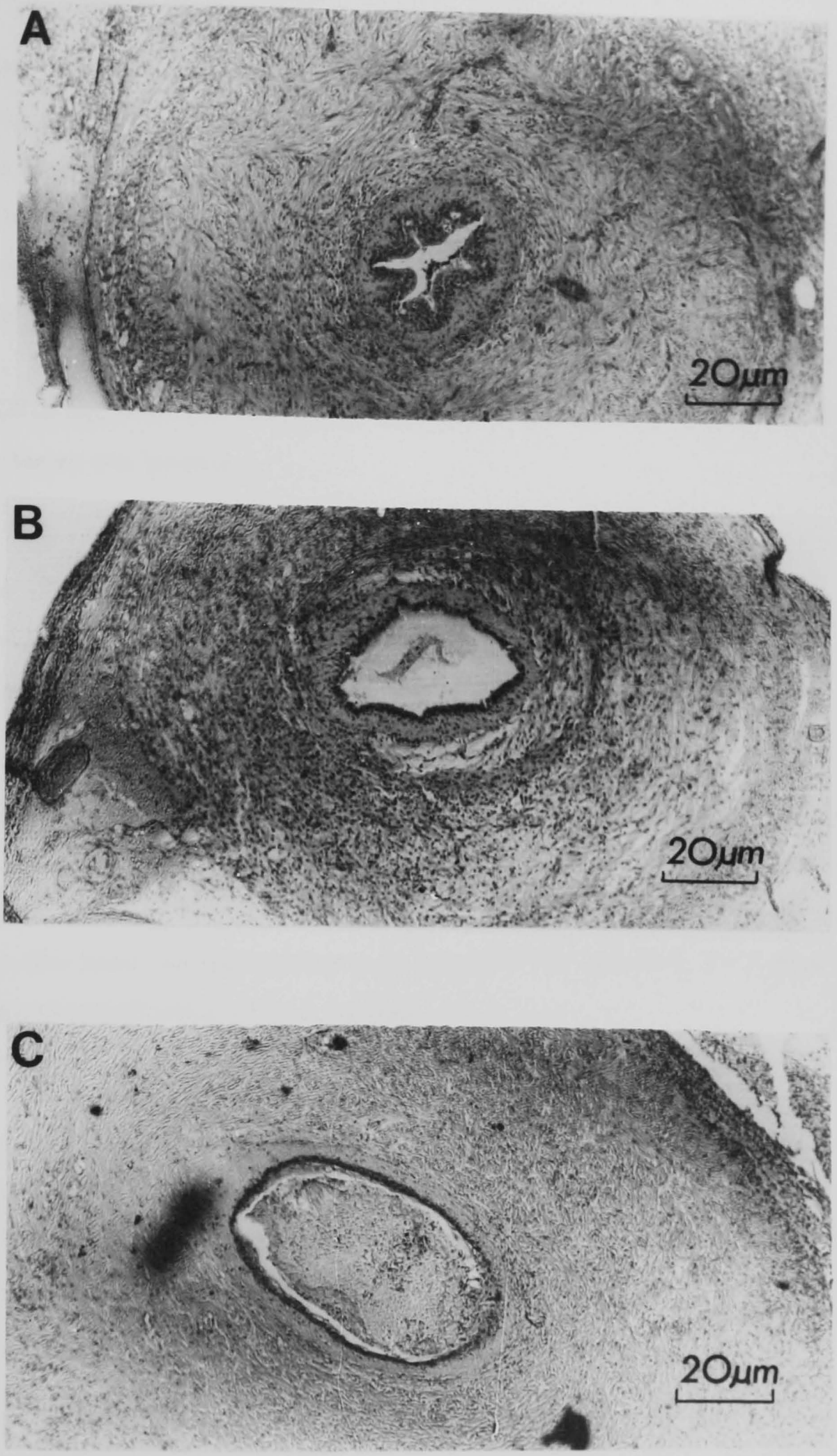


Fig. 14
Transverse section of rat vas deferens stained with haematoxylin and eosin. A. Untreated control. B. Section of vas under 50% tyramine collar 8 days after insertion. The epithelial lining shows only slight damage and a lumen is present. C. Section of vas under 25% methoxamine collar 8 days after insertion. The lumen is occluded completely with fibrous material. The epithelial lining surrounding the lumen is severely damaged.

exfoliation of spherical cells was seen in the majority of seminiferous tubules of the testes. Spermatogenesis appeared to be progressing normally.

No damage to the epithelium of the epididymis was observed. In one animal a few spermatids were also found amongst the spermatozoa in the lumen of the epididymis.

The appearance of the vas deferens between collar and epididymis was normal. Spermatozoa were seen in the lumen. Some damage to the epithelial lining (disintegration and sloughing) under the collar was noticed; but in all cases a lumen was present.

Motility of the epididymal spermatozoa

The cauda epididymal sperms were totally immotile 8 days following the insertion of either a 50% tyramine or a 25% methoxamine collar around each vas deferens.

Sperm counts in the female tract

Sperm numbers in the ejaculates before and after insertion of 50% tyramine or 25% methoxamine collars are shown in Table 15. Ejaculated sperm numbers even before the drug treatment ranged from 1 - 135 million between individuals. On the other hand, no spermatozoa were found in the ejaculate 3 - 7 days following the application of these collars.

Blood pressure measurements

The effect on blood pressure following insertion of collars is shown in Table 11b. A (transient) rise in blood pressure was evident following the insertion of tyramine collars but not with control collars. The rise in blood pressure was, however, short-lived (about 1 hr), and was followed by hypotension.

DISCUSSION

Application of collars containing 25% methoxamine, 50% and 75% tyramine or 50% norephedrine to the vasa of rats caused a loss of fertility which was permanent with methoxamine or at higher doses of tyramine. On the other hand, norephedrine or a lower dose of tyramine caused only a temporary inhibition

of fertility. None of these drugs reduced libido since successful matings nearly always occurred. The peak effect on fertility with the lower dose of tyramine was seen up to a week following the insertion of collars. There was then a spontaneous resumption of fertility which was probably due to exhaustion of the drug, since the replacement of the exhausted collars with new ones caused the animals to be infertile once again. A similar trend in fecundity was seen with norephedrine treatment. But a second phase of permanent infertility occurred, perhaps due to a mechanical obstruction in the vas deferens.

The rat testes is very sensitive and noticeable histological changes can be produced by quite mild stimuli, for instance, ischaemia (Steinberger et al., 1969), rise in temperature (Bowler, 1972; Fahim et al., 1975), ultrasound (Fahim et al., 1975), administration of vasoconstrictor drugs (Boccabella et al., 1972) or local anaesthetics (Banhawy et al., 1977). Thus it is possible that this exfoliation of spermatids observed in some seminiferous tubules could have resulted from the vasoconstrictor actions of sympathomimetics (Hoffbrand et al., 1973) which might have produced ischaemia or possibly even a disturbance of the function of the pampiniform plexus resulting in temperature changes Alternatively, severe contractions of the testicular capsule produced by sympathomimetics may have caused the shedding of some germ cells by increasing intratesticular pressure. Contractions of the testicular capsule have been described by Davis et al. (1971), Hargrove et al. (1976) and Firlit et al. (1975) following the administration of sympathomimetic drugs. Exfoliation of testicular germ cells is, however, unlikely to be the cause of sterility since the action of onset (3 days) and reversibility (7 - 10 days) was too rapid following respectively the application and removal of collars.

Since the collars caused a significant reduction in sperm numbers in the cauda (methoxamine) or in the whole epididymis (tyramine), it is likely that the sperm transport through the ductular system was accelerated. This could have resulted from rhythmic contractions in the vas (p.24) and in the epididymis (p. 31) induced by the drugs released from the collars. In spite of unaltered sperm production and the presence of sufficiently large numbers of sperm in

the cauda epididymides, none were present in the ejaculate. It seems, therefore, that such an absence of spermatozoa in the ejaculate could result from the spermatozoa outflow being mechanically blocked or by some deficiency in the mechanism which expel sperm at copulation.

In the case of methoxamine, a mechanical occlusion was produced in the vas beneath the collars by an ingrowth of fibrous material, as revealed by the histological studies. In contrast, such a blockage did not occur following the application of 50% tyramine collars, indicating that in this situation there must have been some failure in the ejaculatory process. This failure could have resulted from a depletion of endogenous noradrenaline in the adrenergic nerve plexus in the vas deferens (El-Badawi et al., 1962; Norberg et al., 1966) or receptor-specific desensitization (Wadsworth, 1974), either of which would lead to diminished contractions in it. Further experiments are needed, however, to confirm these hypothesized mechanisms. An alternative explanation for the lack of spermatozoa in the ejaculate would be a blockage resulting from sustained contraction in the vas created by this method of local administration of the drug. The tyramine collar did indeed cause piloerection, exophthalmia and hypertension. The development of hypertension would be expected from a vasoconstrictor drug. However, despite the sustained release method of application, the effect on hypertension was short-lived and it is unlikely that the general health of the animals was altered significantly.

Vas ligation did not cause a significant rise in sperm numbers in the ligated vas, but it did cause decapitation of spermatozoa, which is in agreement with the result of Kuwahara et al. (1975). Thus the methoxamine-induced rise in sperm numbers in the vas deferens cannot have been due to solely to obstruction and may indicate an additional effect, perhaps the expulsion of sperms from the epididymis. Furthermore, methoxamine treatment caused much less decapitation than vas ligation, again indicating that the effects of methoxamine cannot be entirely explained by occlusion of the vas deferens.

The indirectly-acting drugs, when administered into the peritoneal cavity or locally into one vas deferens, failed to reduce fertility. The lack of an

effect in the abdominal approach may perhaps be due to a reduced absorption of drugs from the collars as they are designed primarily to release the drugs via the inner surface. Indeed, a reduction of systemic absorption was evident by the development of side effects (piloerection, exophthalmia) to a lesser degree by this method of local application of collars.

The results of this study indicate that a reduction in fertility in male rats, without any loss of libido, has been produced by local application of collars containing 25% methoxamine, 50% or 75% tyramine or 50% norephedrine to both vasa deferentia. The sterility seen with norephedrine or the lower dose of tyramine was reversible, while the higher dose of tyramine and methoxamine caused permanent sterility. The main cause of infertility is the reduced number of spermatozoa appearing in the ejaculate. In addition, spermatozoa extracted from cauda exhibited no motility. It is therefore suggested that sterility is produced by 1) an occlusion of vas either by a mechanical block in the case of methoxamine, and 2) deficiency in the ejaculatory process.

SECTION 5.

LOCAL APPLICATION OF NOREPHEDRINE TO THE VAS DEFERENS OF RABBITS

METHODS

Animals

Nine sexually mature male Dutch rabbits, weighing 2 - 3 kilograms were used in the study. The animals were housed individually in wire-mesh cages and kept under constant environmental conditions, with free access to food (Diet HD1, Millers) and water. Prior to the experiment, the bucks were trained to ejaculate into an artifical vagina (Holborn surgical Instrument Company Ltd.) using a live doe as a mount. Collections were done roughly at weekly intervals before and after the operation with an additional collection on day 3 following the operation in drug treated rabbits.

Construction of collars

The collars were manufactured as described on p.60 but with a central hole of 3.2 mm diameter.

Insertion of collars

The rabbits were sedated by an intraperitoneal injection of 5 mg diazepam in 1 ml distilled water 30 minutes before the operation. Pentobarbitone sodium, 45 mg/kg was administered through the marginal ear vein to induce anaesthesia. The lower abdomen was then shaved and cleaned with Hibitane solution and a local anaesthetic, xylocaine (Astra Chemicals Ltd.), was irrigated around the cleaned area. A 3 -4 cm midline incision was made in the lower abdomen with the animals lying in a supine posture. The urinary bladder was then, if necessary, emptied manually and displaced towards the anterior end of the animal to reveal the ampullary region of both vasa deferentia. The vasal artery was carefully dissected free from the wall of the vas deferens and a collar was inserted around each vas deferens between the outer wall of the vas and the vasal artery. Six rabbits had collars containing 50% norephedrine hydrochloride placed

around each vas, the remaining three rabbits were used as controls and a drug-free collar was inserted in similar positions. The vasa deferentia were then returned to their normal positions in the abdominal cavity. The incision in the abdominal muscle layers was closed with 2/0 silk sutures and sprayed with an antibiotic mixture containing bacitracin, neomycin and polymyxin (Polybactrin, Calmic Ltd.). The skin incision was closed with 12 mm Michel wound clips and the animals allowed to recover consciousness. The day of the operation is taken as day 0.

Analysis of semen

The volume of the semen was measured immediately after the collection by means of a graduated syringe. A gelatinous material was present in most ejaculates, but by no means in all. If present, this 'gel' was removed before the semen was examined microscopically. The colour of the semen was noted and the pH was measured using indicator paper (pH range 6 - 8, Whatman - B.D.H.). A small drop of the ejaculate was removed and mixed with an equal amount of formol citrate (Dott et al., 1975) for subsequent estimation of the percentage live and dead spermatozoa.

The sperm motility was studied next by two methods. In the first a tiny drop of undiluted semen was placed on a warm (about 35°C), clean microscope slide and mounted with a cover glass. The motility of the spermatozoa was then assessed under the microscope, using a motility scale of 0 - 5 with 5 being vigorous motility with swirling bands of high opacity, and 0 being no motility of any type. In a second method, a drop of semen was diluted (usually 1:20) with warm (35°C) saline solution. A small drop of the diluted semen was examined as in method one, under the microscope and the percentage of motile cells (in intervals of 5%) was estimated on a scale of 0 - 4. The system of grading motility was as follows:- 0 = immotile, 1 = tails moving, no progressive motility, 2 = poor progressive motility, 3 = fair progressive motility and 4 = vigorous progressive motility. In order to examine the motility in a more uniform manner, a motility index was calculated for each semen specimen, from the sum of(grade of forward progression x % motile sperms).

An assessment of sperm numbers was made using an improved Neubauer haemocytometer. The semen sample was diluted with 10% formal-saline, and counts made in duplicate. The average of the two counts was expressed both in terms of numbers per millilitre and as total number per ejaculate.

The percentage of 'dead' (eosinophilic) spermatozoa was estimated by mixing an equal volume of aqueous nigrosin-eosin (Campbell et al., 1956) for 5 minutes, with the diluted semen sample. A drop of the mixture was smeared on two clean microscopic glass slides. The smears were allowed to dry in air and subsequently mounted with DPX. The slides were then scored under oil immersion (at a magnification of 1000x) for the percentage of eosinophilic spermatozoa, spermatozoa with atypical morphological features (coiled tails, two tails, elongated heads, decapitation) and the position of the cytoplasmic droplet in the spermatozoa (100 sperms were scored per slide).

Matings

42 - 66 days subsequent to surgery one rabbit with drug-free collars and three with drug-containing collars were allowed to mate. Immediately after copulation the females were removed and allowed to litter. The number of young in the litter was counted and they were examined for obvious abnormalities.

Histology

Immediately after the final semen collection (3 - 6 months) the rabbits were sacrificed by an overdose of Nembutal given through the marginal ear vein. Parts of the vas deferens (under collar and between collar and epididymis) and a piece from the cauda epididymis were removed and fixed in Bouin's for subsequent histological examination.

Statistics

The significance of differences between the treated and the control groups were assessed using Mann-Whitney rank-sum non-parametric tests (Seigel, 1956) taking $p < 0.05$ as significant.

RESULTS

Appearance and volume of semen

The colour of the semen varied from white to slightly brownish, but the insertion of collars had no noticeable effect on this. An occasional leucocyte and several kinds of particles (Metz et al., 1968) were also present before and after surgery in both groups.

The volume of the ejaculate varied from 0.1 to 1.2 ml. No change in the semen volume was apparent in the control group (Table 16). A significant reduction in volume (for up to 28 days) was, however, observed in those rabbits into which 50% norephedrine collars had been inserted ($p < 0.02$). After this period, the volume recovered to within the normal range (Table 17).

Sperm number

The presence of control collars produced no change in either total spermatozoa count in the ejaculate or in the sperm concentration (Table 16, Fig. 15). However, one of the rabbits in this group had an elevated sperm count for 2 weeks following surgery. On the other hand, a drastic reduction in sperm counts ($p < 0.002$) occurred for up to 21 days after insertion of norephedrine collars (Table 17). The maximum depression of spermatozoa numbers in the ejaculate was during the first two weeks (Fig. 16). The sperm count in one of the treated rabbits returned to normal from day 7 but this took 5 - 8 weeks in the others.

Sperm motility

Even in the preoperative period in both groups the degree of sperm motility varied between individual semen samples. This has previously been reported by Harrison et al. (1978). However, when the results were pooled it was clear that some animals produced semen with better motility than others (Table 18). All the semen samples up to 21 days following drug treatment were immotile. In two animals a tendency towards partial recovery was seen between 28 - 49 days.

Table 16. Mean volume, pH and sperm numbers of semen collected from 3 rabbits before and after insertion of drug-free collars. Each result is the mean ± s.e.m. of 3 observations.

	Days before operation					Days after operation			
	28	21	14	7	3	7	14	21	28
Semen volume (ml)	0.25 ± .15	0.22 ± .07	0.53 ± .33	0.3 ± .02	0.3 ± .10	0.28 ± .06	0.53 ± .24	0.35 ± .15	0.32 ± .02
Total sperm number in ejaculate x 10^6	22 ± 19	16 ± 11	35 ± 9	66 ± 42	36 ± 22	40 ± 17	22 ± 12	32 ± 22	49 ± 44
Sperm concentration per millilitre of ejaculate x 10^6	65 ± 39	67 ± 17	90 ± 29	208 ± 117	107 ± 37	150 ± 73	40 ± 8	147 ± 127	165 ± 150
pH	7.5 ± .74	8.3 ± 0	8.1 ± .10	7.8 ± .26	7.8 ± .15	7.7 ± .34	7.5 ± .26	7.5 ± .45	7.5 ± .45

Table 17. Mean volume, pH and sperm numbers of semen collected from 6 rabbits before and after insertion of 50% norephedrine collars. Each result is the mean ± s.e.m. of 3-6 observations.

	Days before operation		Days after operation									
	14	7	3	7	14	21	28	35	42	49	56	63
Semen volume (ml)	0.41 ± .15	0.35 ± .13	0.12 ± .02	0.17 ± .04	0.25 ± .14	0.52 ± .33	0.14 ± .04	0.36 ± .17	0.18 ± .04	0.23 ± .04	0.18 ± .07	0.23 ±
Total sperm nos. in ejaculate $\times 10^6$	53 ± 16	88 ± 35	0.37 ± .37	1.65 ± 1.4	0.02 ± .01	13 ± 12	1.7 ± 1.5	5.2 ± 4.4	10.4 ± 6	18.2 ± 8.2	1.38 ± 1	56 ± 20
Sperm conc. per ml of ejaculate $\times 10^6$	241 ± 107	234 ± 83	2.5 ± 2.5	8 ± 7.4	2.8 ± 2.7	7.3 ± 5.5	23.6 ± 16.4	21.7 ± 17.6	39.6 ± 18.2	71 ± 22	9.5 ± 6.8	130 ± 20
pH	7.6 ± .37	7.6 ± .43	7.2 ± .74	7.9 ± .19	7.7 ± .16	7.9 ± .14	7.8 ± .17	7.9 ± .21	7.5 ± .19	7.7 ± .22	7.9 ± .16	7.9 ± .18

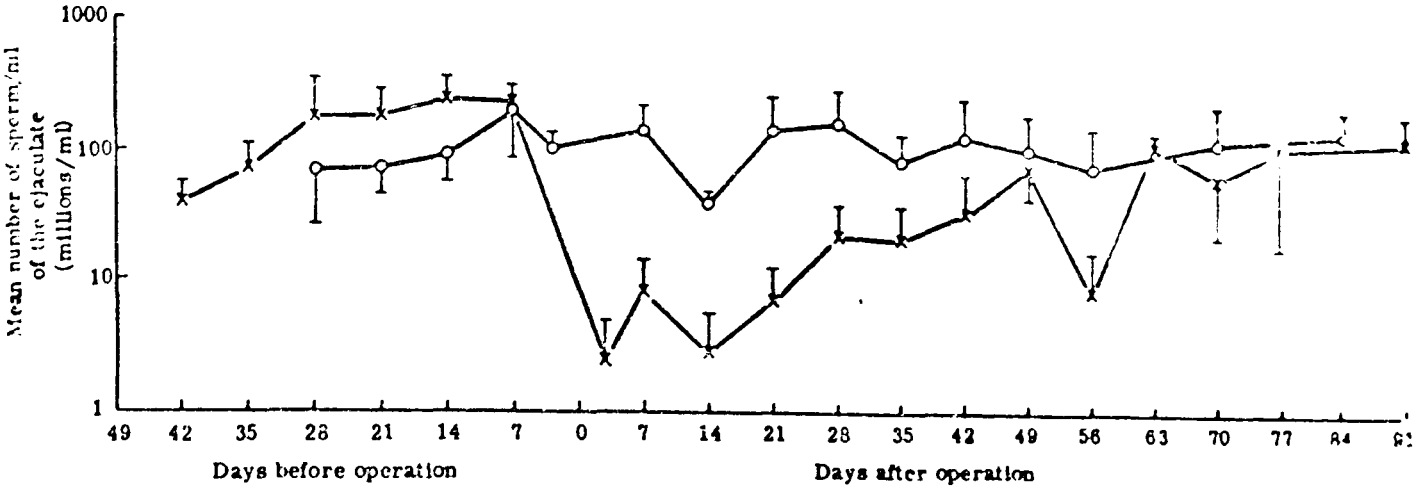


Fig. 15

Sperm output (mean ± s.e.mean) in numbers/millilitre of rabbits before and after insertion of a collar containing 50% norephedrine around each vas deferens (x, n = 3 - 6) or of a drug free collar around each vas deferens (o, n = 3). Semen collections were made approximately once a week.

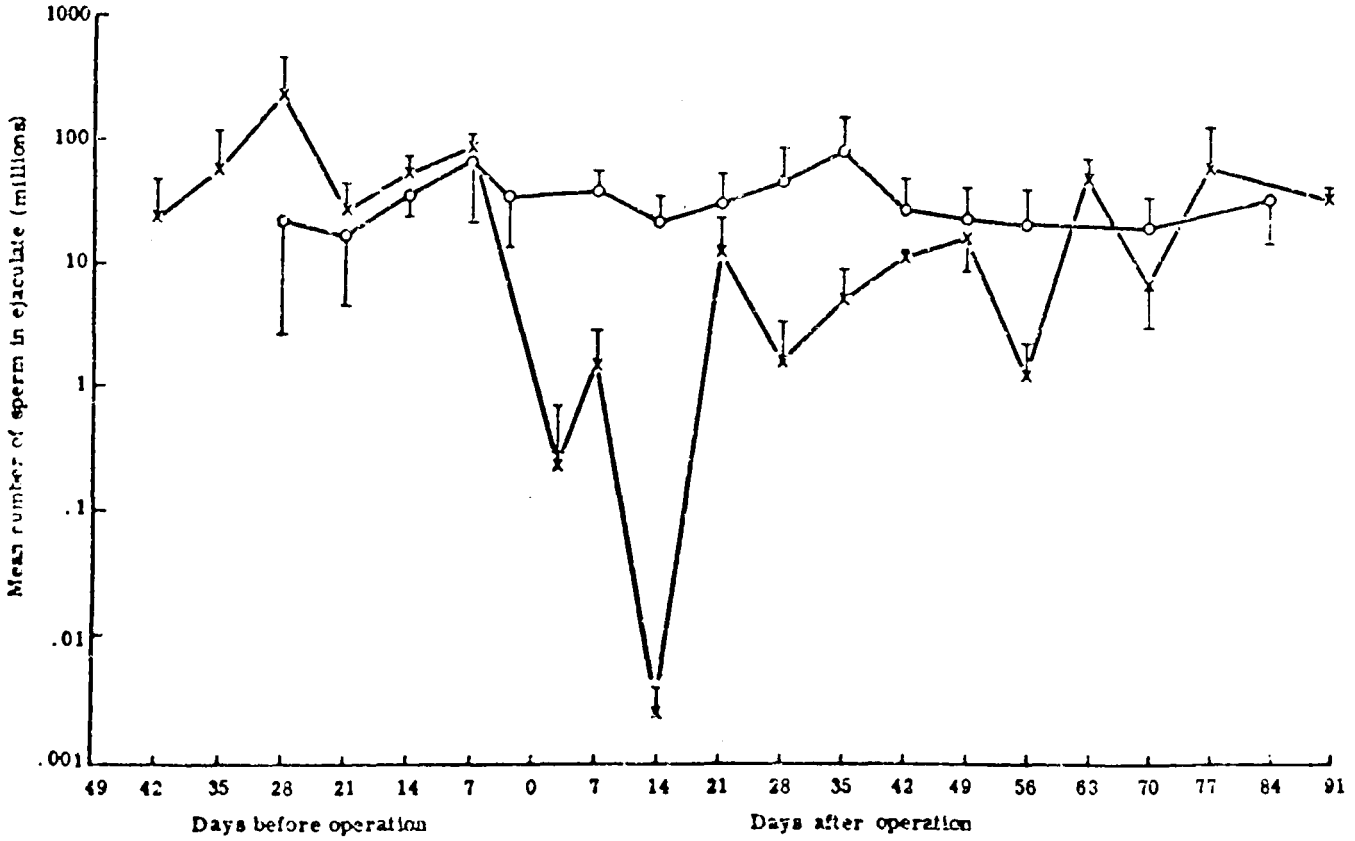


Fig. 16

Number of sperm (mean ± s.e.mean) in the ejaculate of rabbits collected approximately once a week before and after insertion of a 50% norephedrine collar around each vas (x, n = 3 - 6) or a drug free collar around each vasa deferentia (o, n = 3).

Table 18

Motility of sperm in the ejaculate before and after insertion of 50% norephedrine collars. Each result in the mean from at least 2 ejaculates taken during the period indicated.

Animal number	Sperm Motility					
	Control		Norephedrine			
	0 - 28 days before operation		after operation			
			1-21 days		28-49 days	
	Motility score	Motility index	Motility score	Molitity index	Molitity score	Motility index
908	4	275	0	0	2	153
849	2.6	255	0	0	0.75	35
984	1.7	100	0	0	0	0
911	3	160	0	0	0	10
906	2.7	120	0	0	0	0
981	0	0	0	0	*	

* Animal died due to respiratory infection.

Eosinophilic sperm

The treatment produced an elevation of the number of eosinophilic (presumably dead) spermatozoa (Table 19) for up to 21 days. An apparent recovery was later seen in two of the treated animals.

Sperm abnormalities

An increased proportion of spermatozoa with abnormalities (especially decapitation) was observed in the semen collected from the treated animals during the initial period following operation (Table 20). Some sperm had a sharp bend between the head and the midpiece and appeared to be in the process of becoming decapitated. A similar situation has been described by Glover (1958) following experimental cryptorchidism. This indicates that the separation of the head has occurred in the mature spermatozoa and was not a spermiogenic defect created by the drug.

Immature sperm

An apparent increase in the number of spermatozoa containing cytoplasmic droplets was seen in the ejaculate collected from the drug-treated group (Table 21).

Fertility tests

Females were mated with males containing drug-free (1 rabbit) and drug-containing collars (3 rabbits), between 42 - 66 days after operation. At this time the sperm count in the treated animals had returned to within the normal range. In each of these four matings there was a normal number in the litter (4 - 8) and no abnormalities were seen (Table 22).

Libido

Throughout the experimental period there was no apparent reduction in libido in the rabbits except for one of the drug-treated animals, whose libido declined from 42 days after surgery.

Table 19.

Percentage of eosinophilic (presumably dead) sperm in the ejaculate before and after insertion of 50% norephedrine collars. Each result is the mean from at least 3 ejaculates taken during the period indicated.

Animal number	Control	Norephedrine after operation	
	0-28 days before operation	1-21 days	28-49 days
908	5	39	29.5
849	24	30	27.25
984	33	- *	34
911	9	40	32
906	15	- *	38.66
981	36	- *	- *

* insufficient sperm ejaculated for assessment of percentage of dead sperm.

Table 20

Percentage of sperm with abnormal morphology in the ejaculate before and after insertion of 50% norephedrine collars. Each result is the mean from at least 3 ejaculates taken during the period indicated.

Animal number	Percentage of sperm abnormalities		
	Control	Norephedrine after operation	
	0-28 days before operation	1-21 days	28-49 days
908	0	6	14.25
849	5.3	15	14
984	11.3	- *	25.66
911	2	13	27
906	5.6	- *	20.66
981	24	- *	- *

* insufficient sperm ejaculated for assessment of abnormalities.

Table 21

Percentage of sperm with cytoplasmic droplets in the ejaculate before and after insertion of 50% norephedrine collars. Each result is the mean of at least 3 ejaculates taken during the period indicated.

Animal number	Percentage of immature sperm		
	Control	Norephedrine after operation	
	0-28 days before operation	1-21 days	28-49 days
908	16	22	27
849	6	31	30.75
984	14	- *	12.3
911	5	20	15
906	38.3	- *	30
981	7	- *	- *

* insufficient sperm in the ejaculate for assessment of percentage of immature sperm.

Table 22

Fertility of bucks 42 - 66 days following the insertion of control or 50% norephedrine collars.

Animal number	Treatment	Number in litter
881	Control	5
908	50% norephedrine	8
849	50% norephedrine	4
984	50% norephedrine	6

Histology

In the testis no impairment of spermatogenesis was observed in either group 3 - 6 months after surgery. No sloughed germinal cells were evident in the lumen of the seminiferous tubules.

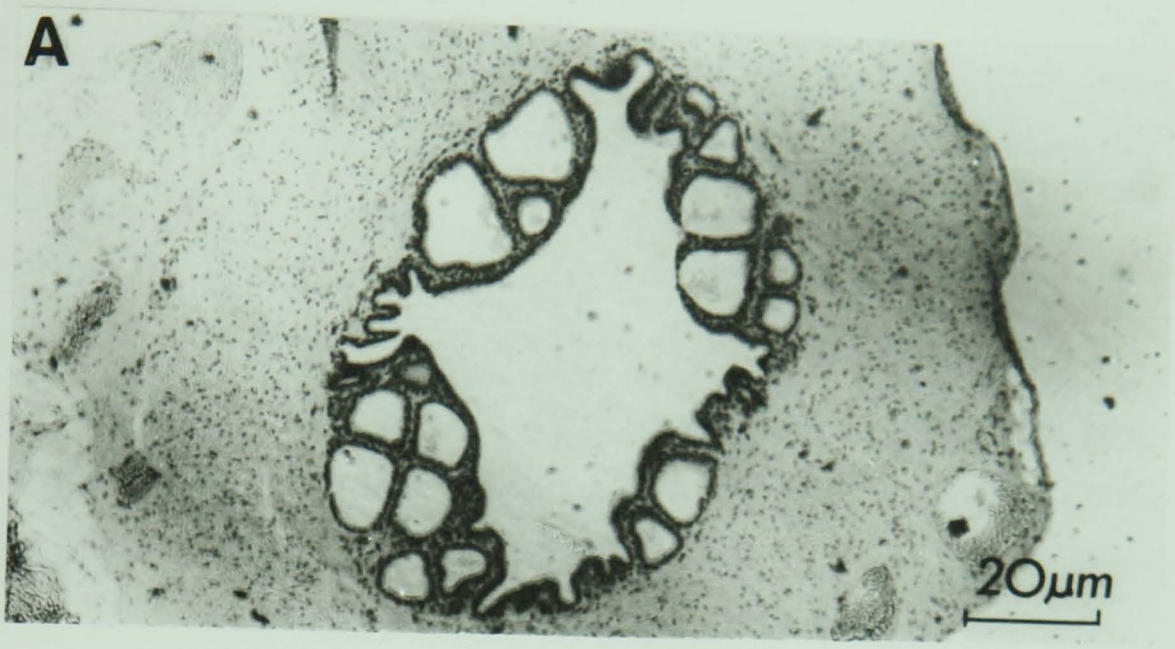
The epithelium of the cauda epididymides appeared normal with numerous spermatozoa in the lumen of the tubules. The wall of the epithelial lining of the vas deferens both under collar and in the region between collar and epididymis showed no obvious damage. The lumen contained varying numbers of spermatozoa in different animals (fig. 17)

DISCUSSION

The results of the present experiments show that local administration of a sympathomimetic drug, norephedrine, to the vas deferens of rabbits results in a significant reduction in semen volume and ejaculated spermatozoa numbers. As shown in Figure 16, the effect is short-lived (about 21 days) with its maximal effect during the first 2 weeks.

The fact that the effect on sperm output occurred so rapidly (3 days) and that no reduction in libido was evident demonstrates that the action of the drug is at an extragonadal site. Sympathomimetic drugs (Birmingham, 1968; McLeod et al., 1973; Ventura et al., 1973), including norephedrine(see p. 26) cause rhythmic contractions of isolated vasa deferentia. If such contractions were produced by this method of local administration, it is possible that large numbers of spermatozoa from the lumen of the vas and perhaps also from the cauda epididymis would be voided in the urine resulting in ejaculates virtually devoid of spermatozoa. A regular spontaneous expulsion of sperms, presumably by micturation or emission (Holtz et al., 1972) is reported to occur in rabbits.

Alternatively, production of azoospermic or oligospermic ejaculates immediately after the application of collars may have been caused by some obstruction to the passage of spermatozoa. No permanent block is, however, created by the drug as indicated by the following observations: (1) subsequent resumption of normospermic ejaculates and (2) no occlusion was evident in



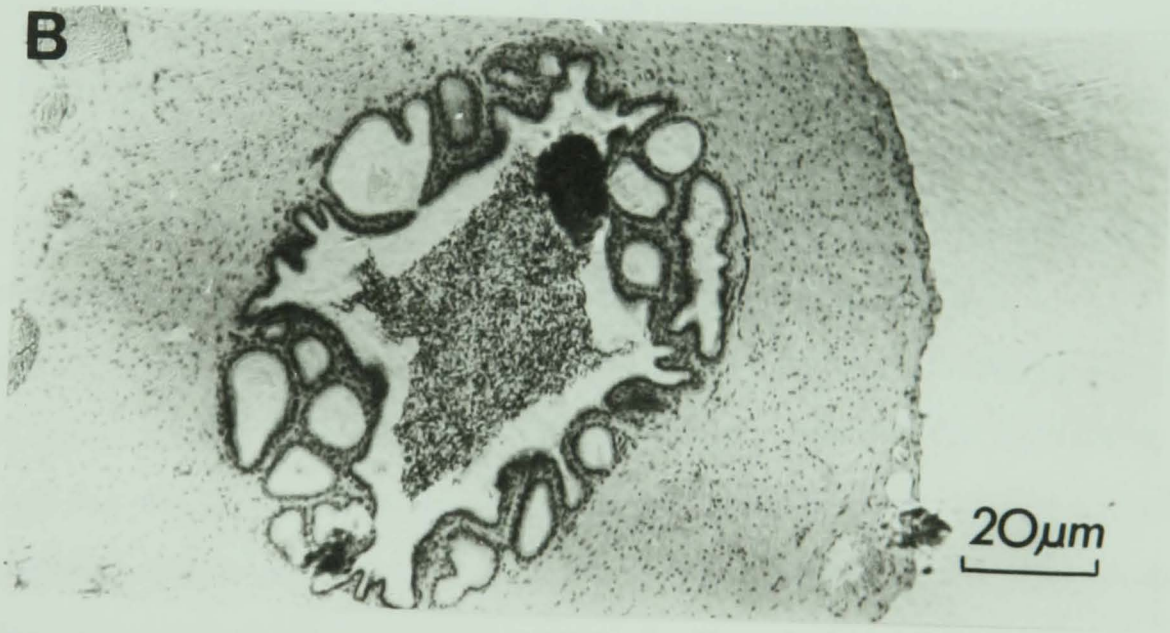


Fig. 17

Transverse section of rabbit vas deferens stained with haematoxylin and eosin.

A. Section of vas under control collar 4 months after its insertion.

B. Section of vas under 50% norephedrine collar 5 months after insertion. The lumen and the epithelial lining surrounding it are normal.

the lumen of the vas from the histological studies made at a later stage. It seems possible, however, that a temporary blockage may have resulted from a sustained contraction of the section of the vas under the collars in the period during which norephedrine was being released. This would have blocked the passage of sperm at ejaculation.

Yet another explanation would be that drug treatment reduces the naturally occurring contractions in the vas and accessory glands at ejaculation. This is suggested by the parallel reduction in sperm numbers and semen volume seen in the treated animals. A similar effect has been reported in bulls following daily administration of adrenaline (van Demark et al., 1958). A deficiency in the contractile mechanism could have resulted from either depletion of the transmitter or receptor-specific desensitization (Wadsworth, 1974).

The treatment also produced an increased number of immature sperm in the ejaculate and caused a variety of morphological abnormalities. Although motility improved and the proportion of dead sperm declined as sperm numbers returned to normal levels, the incidence of morphological abnormalities was not reduced. Nevertheless, the production of normal litter sizes (Table 22) by the does mated with these treated males suggests that this degree of deterioration in the semen was not sufficient to impair fertilizing competence.

It may be concluded from the present study that collars containing norephedrine, when applied locally to the vas deferens, reduce the fertilizing capacity of the male; primarily by reducing the sperm numbers in the ejaculate. Any substantial reduction in sperm numbers in the ejaculate leads to a decrease in fertility, because of the tremendous loss of sperm during their transport in the female tract. This effect of norephedrine was temporary, in other words it produced a reversible effect.

GENERAL DISCUSSION

Local application of sympathomimetic drugs to the vas deferens of anaesthetised rats using a slow-releasing formulation caused rhythmic contractions lasting several days. The amplitude of these contractions was smaller than that observed in organ bath experiments, presumably because only that part of the vas deferens underneath the collar was exposed to the drug. However, it was expected that the mechanism underlying the induction of the repeated contractions would be similar *in vivo* and *in vitro*.

The sperm numbers in the epididymis and in the ejaculate was reduced following local application of sympathomimetic drugs either to the epididymis or to the vas deferens. Such a reduction in the epididymis could have been caused by continuous expulsion of sperm by rhythmic contractions of the type observed *in vitro*. These sperm might perhaps have been voided in the urine or spontaneously ejaculated, although no evidence was obtained to support these theories. It was not clear whether the reduction in the epididymal sperm numbers would have been sufficient to produce azoospermia or whether an additional antifertility effect was being produced by the drugs. This could perhaps have resulted from a blockage of the normal transport by mechanical obstruction or by sustained spasm or through a deficiency in the mechanism of emission. Such a deficiency could have been induced through a depletion of endogenous transmitter, or by either receptor-specific desensitization or inhibition of the release of the transmitter by pre-synaptic receptor mediated inhibition.

Production of oligospermia would by itself be sufficient to bring about infertility. But, in addition, there was a decrease in the quality of sperm obtained either from the rat epididymis or from rabbit and rat ejaculates. Even if sperm numbers had not been reduced, this effect would presumably have resulted in sperm incapable of fertilizing ova. The effects of the drugs on the numbers and quality of the sperm are sufficient to account for the observed infertility in the male rats following the application of the drugs.

Silastic rods or collars containing sympathomimetic drugs provide a method of inducing rapid azoospermia without depressing libido or spermatogenesis. Of course, this particular technique of local application would not be a practicable contraceptive device in the human, but it demonstrates that it is in principle possible to produce an antifertility effect using sympathomimetic drugs. Should an improved delivery system, involving entirely biodegradable materials, be developed or drugs be found which have specific affinities to the vas or epididymis, then the approach used would provide an elegant method of male contraception.

REFERENCES

Ågmo, A. (1976) The number of spermatozoa in spontaneous ejaculates of rats. J. Reprod. Fert. 48, 405-407.

Alexander, N.J. (1975) Immunologic effects of vasectomy in Rhesus monkeys. In Control of Male Fertility (Eds. Sciarra, J.J., Markland, C. & Speidel, J.J.) p. 189-195. Harper & Row: New York.

Alexander, N.J. & Schmidt, S.S. (1977) Incidence of antisperm antibody levels and granulomas in men. Fert. Steril. 28, 665-667.

Al-Zuhair, A.G.H., Dixon, J.S. & Gosling, J.A. (1976) Studies on the autonomic innervation of the guinea-pig seminal vesicle and ductus deferens. J. Physiol., Lond. 257, 9-11P.

Amann, R.P. & Griel, L.C. (1974) Fertility of bovine spermatozoa from rete testis, cauda epididymides and ejaculated semen. J. Dairy Sci. 57, 212-219.

Amann, R.P., Johnson, L., Thompson, D.L. Jnr. & Pickett, B.W. (1976) Daily spermatozoal production, epididymal spermatozoal reserves and transit time of spermatozoa through the epididymis of the Rhesus monkey. Biol. Reprod. 15, 586-592.

Amann, R.P., Kavanaugh, J.F., Griel, L.C. Jnr. & Voglmayer, J.H. (1974) Sperm production of Holstein bulls determined from testicular spermatid reserves, after cannulation of rete testis or vas deferens and by daily ejaculation. J. Dairy Sci. 57, 93-99.

Ambache, N., Dunk, L.P., Verney, J. & Zar, M.A. (1972) Inhibition of postganglionic motor transmission in vas deferens by indirectly acting sympathomimetic drugs. J. Physiol., Lond. 227, 433-456.

Ambache, N. & Zar, M.A. (1971) Evidence against adrenergic motor transmission in the guinea-pig vas deferens. J. Physiol., Lond. 216, 359-389.

Ansbacher, R., Williams, A. & Mumford, D.M. (1975) Vas ligation: Sperm antibodies. In Control of Male Fertility (Eds. Sciarra, J.J., Markland, C. & Speidel, J.J.) p. 189-195. Harper & Row: New York.

Anton, P.G., Duncan, M.E. & McGrath, J.C. (1977) An analysis of the anatomical basis for the mechanical response to motor nerve stimulation of the rat vas deferens. J. Physiol., Lond. 273, 23-43.

Anton, P.G. & McGrath, J.C. (1977) Further evidence for adrenergic transmission in the human vas deferens. J. Physiol., Lond. 273, 45-55.

Arora, K., Dinakar, N. & Prasad, M.R.N. (1975) Biochemical changes in the spermatozoa and luminal contents of different regions of the epididymis of Rhesus monkey, Macaca mulatta. Contraception 11, 689-699.

Ball, R.Y. & Mitchinson, M.J. (1977) Degradation of phagocytosed spermatozoa by mouse peritoneal macrophages in vitro. J. Reprod. Fert. 49, 409-411.

Banhawy, M.A., Rashed, R.M.A., Bowler, K. & Stacey, M.J. (1977) The effect of a single injection of mepivacaine hydrochloride on spermatogenesis in the rat. J. Reprod. Fert. 51, 477-479.

Batra, S.K. (1974) Sperm transport through vas deferens: review of hypotheses and suggestions for a quantitative model. Fert. Steril. 25, 186-202.

Baumgarten, H.G., Holstein, A.F. & Rosengren, E. (1971) Arrangement, ultrastructure and adrenergic innervation of the smooth musculature of the ductuli efferents, ductus epididymides and ductus deferens of man. Z. Zellforsch. 120, 37-79.

Bedford, J.M. (1963) Morphological changes in rabbit spermatozoa during passage through the epididymis. J. Reprod. Fert. 5, 169-177.

Bedford, J.M. (1966) Development of the fertilizing ability of spermatozoa in the epididymis of the rabbit. J. exp. Zool. 163, 319-329.

Bedford, J.M. (1975) Maturation, transport and fate of spermatozoa in the epididymis. In Handbook of Physiology (Eds. Hamilton, D.W. & Greep, R.O.) Section 7, 5, p. 303-317. Williams & Wilkins: Baltimore.

Bedford, J.M. (1976) Adaptation of the male reproductive tract and the fate of spermatozoa following vasectomy in the rabbit, Rhesus monkey, hamster and rat. Biol. Reprod. 14, 118-142.

Bedford, J.M., Calvin, H. & Cooper, G.W. (1973) The maturation of spermatozoa in the human epididymis. J. Reprod. Fert. Suppl. 18, 199-213.

Bennett, M.R. (1972) Autonomic neuromuscular transmission. University Press: Cambridge.

Birmingham, A.T. (1968) The human isolated vas deferens: its response to electrical stimulation and to drugs. Br. J. Pharmac. 24, 692-693P.

Birmingham, A.T. (1970) Sympathetic denervation of the smooth muscle of the vas deferens. J. Physiol., Lond. 206, 645-661.

Birmingham, A.T. & Freeman, M.A. (1971) Separation of the components of the response of isolated vas deferens to electrical stimulation. J. Physiol., Lond. 222, 19-20P.

Boccabella, A.V., Salgado, E.D. & Alger, E.A. (1962) Testicular function and histology following serotonin administration. Endocrinology 71, 827-837.

Bowler, K. (1972) The effect of repeated applications of heat on spermatogenesis in the rat: a histological study. J. Reprod. Fert. 28, 325-333.

Briggs, M. & Briggs, M. (1974) Oral contraceptives for men. Nature, Lond. 252, 585-586.

Brodie, N. (1975) Male sterilization employing an intra-vas device. Int. J. Fert. 20, 197-203.

Brotherton, J. (1976) Difference in size between spermatozoa from the cauda epididymides and the caput epididymides of rat. J. Reprod. Fert. 48, 365-366.

Brown-Woodman, P.D.S., Salamon, S. & White, I.G. (1974) Effect of α-chlorohydrin on the fertility of rams. Acta Eur. Fertil. 5, 193-197.

Bruschini, H., Schmidt, R.A. & Tanagho, E.A. (1977) Studies on the neurophysiology of the vas deferens. Invest. Urol. 15, 112-116.

Buckner, C.K., Bohuski, K. & Ryan, C.F. (1975) Influence of temperature and cocaine responses of the isolated mouse vas deferens to adrenergic amines. Arch. int. Pharmacodyn. Ther. 216, 19-27.

Bunge, R.G. (1972) Plasma testosterone levels in man before and after vasectomy. Invest. Urol. 10, 9-13.

Burnstock, G. & Holman, M.E. (1962) Effect of denervation and reserpine treatment on transmission at sympathetic nerve endings. J. Physiol., Lond. 160, 461-463.

Bustos-Obregon, E. (1976) Ultrastructure and function of the lamina propria of mammalian seminiferous tubules. Andrologia 8, 179-185.

Calvin, H.I. & Bedford, J.M. (1971) Formation of disulphide bonds in the nucleus and accessory structures of mammalian spermatozoa during maturation in the epididymis. J. Reprod. Fert. Suppl. 13, 65-75.

Campbell, R.C., Dott, H.M. & Glover, T.D. (1956) Nigrosin eosin as a stain for differentiating live or dead spermatozoa. J. agrc. Sci., Camb. 48, 1-8.

Clermont, Y. (1958) Contractile elements in the limiting membrane of the seminiferous tubules of rat. Expl. Cell Res. 15, 438-440.

Coffey, D.S. & Freeman, C. (1975) Vas injection: a new non-surgical procedure to induce sterility in human males. In Control of Male Fertility (Eds. Sciarra, J.J., Markland, C. & Speidel, J.J.) p. 147-160. Harper & Row: New York.

Cohen, J. (1977) Reproduction, Butterworths: London.

Cooper, G.W. & Bedford, J.M. (1971) Acquisition of surface charge by the plasma membrane of mammalian spermatozoa during epididymal maturation. Anat. Rec. 169, 300-301

Cooper, T.G. & Hamilton, D.W. (1977) Observations on the destruction of spermatozoa in the cauda epididymides and proximal vas deferens on non-seasonal male mammals. Am. J. Anat., 149, 93-110.

Cooper, T.G. & Orgebin-Crist, M.C. (1975) The effect of epididymal and testicular fluids on the fertilizing capacity of testicular and epididymal spermatozoa. Andrologia 7, 85-93.

Cooper, T.G. & Orgebin-Crist, M.C. (1977) Effect of ageing on the fertilizing capacity of testicular spermatozoa from the rabbit. Biol. Reprod. 16, 258-266.

Coppola, J.A. (1969) An extragonadal antifertility agent. Life Sci. 8, 43-48.

Cross, B.A. & Glover, T.D. (1958) The hypothalamus and seminal emission. J. Endocr. 16, 385-395.

Cummins, J.M. (1976) Effects of epididymal occlusion on sperm maturation in the hamster. J. exp. Zool. 197, 187-190.

Da Silva, M.C., Gimeno, M.F. & Gimeno, A.L. (1975) Physiologic and pharmacologic studies on the motility of isolated guinea-pig cauda epididymides. Fert. Steril. 26, 1250-1256.

Davis, J.R. & Langford, G.A. (1971) Comparative responses in the isolated testicular capsule and parenchyma to autonomic drugs. J. Reprod. Fert. 26, 241-245.

Dott, H.M. & Foster, G.C. (1975) Preservation of differential staining of spermatozoa by formal citrate. J. Reprod. Fert. 45, 57-60.

Duncan, M.E. & McGrath, J.C. (1976) Observations on the origin of the complex mechanical response to motor nerve stimulation by rat vas deferens. J. Physiol., Lond. 259, 54-55P.

Dym, M. (1977) Male reproductive tract. In Histology. (Eds. Leonwess & Greep, R.O.) p.979-1038. Blakiston: New York

Dym, M. & Romrell, L.J. (1975) Intraepithelial lymphocytes in the male reproductive tract of rats and Rhesus monkeys. J. Reprod. Fert. 42, 1-7.

El-Badawi, A. & Schenk, E.A. (1962) The distribution of cholinergic and adrenergic nerves in the mammalian epididymis. A comparative histochemical study. Am. J. Anat. 121, 1-14.

Ericsson, R.J. (1975) Chaemosterilants. In Control of Male Fertility (Eds Sciarra, J.J., Markland, C. & Speidel, J.J.) p. 262-269. Harper & Row: New York.

Euler, U.S.v & Hedqvist, P. (1975) A tentative transmission mechanism for the twitch elicited in guinea-pig vas deferens by field stimulation Med. Hypotheses 1, 214-216.

Evans, B., Gannon, B.J., Heath, J.W. & Burnstock, G. (1972) Long lasting damage to internal male genital organs and their adrenergic innervation of rats following chronic treatment with antihypertensive drug, guanethidine. Fert. Steril. 23, 657-667.

Ewing, L.L., Stratton, L.G. & Dejardins, C. (1973) The effect of testosterone polydimethyl siloxane implants upon sperm production, libido and accessory sex organ function in rabbits. J. Reprod. Fert. 35, 245-253.

Fahim, M.S., Fahim, Z., Der, R., Hall, D.G. & Harmen, J. (1975) Heat in male contraception (hot water 60°C, infra-red microwave and ultra-sound). Contraception 1, 549-562.

Fahim, M.S., Fahim, Z., Harmen, J., Thompson, I. Montie, J. & Hall, D.G. (1977) Ultrasound as a new method of male contraception. Fert. Steril. 28, 823-831.

Farnebo, L. & Malmfors, T. (1971) ^{3}H-noradrenaline release and mechanical response in the field stimulated mouse vas deferens. Acta physiol. scand. Suppl. 371, 1-18.

Farrell, N.E. (1968) Fine structure of nerve fibres in smooth muscle of the vas deferens in normal and reserpinized rats. Nature, Lond. 217, 279-281.

Fernández-Collazo, E., Videla, E. & Pereyra, J.C. (1971) Elimination of spermatozoa in the urine of isolated male rats. J. Reprod. Fert. 27, 145-147.

Firlit, C.F., King, L.R. & Davis, J.R. (1975) Comparative responses of the isolated human testicular capsule to autonomic drugs. J. Urol. 113, 500-504.

Flechon, J. & Hafez, E.S.E. (1975) Spermiation and epididymal maturation of spermatozoa in the bonnet macaque (Macaca Radiata) as viewed by scanning electron microscopy. Fert. Steril. 26, 1219-1227.

Flickinger, C.J. (1972) Ultrastructure of rat testis after vasectomy. Anat. Rec. 174, 477-494.

Flickinger, C.J. (1975a) Fine structure of the rabbit epididymis and vas deferens after vasectomy. Biol. Reprod. 13, 50-60.

Flickinger, C.J. (1975b) Fine structure of the rabbit testis after vasectomy. Biol. Reprod. 13, 61-67.

Fredholm, B. & Hedqvist, P. (1973) Increased release of noradrenaline from stimulated guinea-pig vas deferens after indomethacin treatment. Acta physiol. scand. 87, 570-572.

Free, J.M. (1975) Development of a reversible intravasal occlusive device. In Control of Male Fertility (Eds. Sciarra, J.J., Markland, C. & Speidel, J.J.) p. 124-139. Harper & Row: New York.

Frick, J., Bartsch, G. & Marberger, H. (1976) Steroidal compounds (injectable and implants) affecting spermatogenesis in man. J. Reprod. Fert. Suppl. 24. 35-47.

Furness, J.B. (1974) Transmission to the longitudinal muscle of the guinea-pig vas deferens: the effect of pretreatment with guanethidine. Br. J. Pharmac. 50, 63-68.

Furness, J.B. & Iwayama, T. (1972) The arrangement and identification of axons which innervate the vas deferens of guinea-pig. J. Anat., Lond. 113, 179-196.

Furness, J.M., McLean, J.R. & Burnstock, G. (1970) Distribution of adrenergic nerves and changes in neuromuscular transmission in the mouse vas deferens during postnatal development. Dev. Biol. 21, 491-505.

Gaddum, P. (1968) Sperm maturation in the male reproductive tract: development ofmotility. Anat. Rec. 161, 471-482.

Gillespie, J.S. & McGrath, J.C. (1975) The effect of lysergic acid diethylamide on the response to field stimulation of the rat vas deferens and the rat and cat anococcygeus muscle. Br. J. Pharmac. 54, 481-483.

Glover, T.D. (1958) Experimental induction of seminal degeneration in rabbit. Stud. Fert. 10, 80-93.

Glover, T.D. (1961) Disintegrated spermatozoa from the epididymis. Nature, Lond. 190, 185-186.

Glover, T.D. (1973) Aspects of sperm production in some East African mammals. J. Reprod. Fert. 35, 45-53.

Glover, T.D. (1976) Investigations into the physiology of the epididymis in relation to male contraception. J. Reprod. Fert. Suppl. 24, 95-114.

Glover, T.D. & Nicander, L. (1971) Some aspects of structure and function in the mammalian epididymis. J. Reprod. Fert. Suppl. 13, 39-50.

Gosling, J.A. & Dixon, J.S. (1972) Differences in the manner of autonomic innervation in the muscle layers of the guinea-pig ductus deferens. J. Anat. 112, 81-91.

Gupta, A.S., Kothari, L.T. & Devpura, T.D. (1977) Vas occlusion by tantalum clips and its comparison with conventional vasectomy in man: reliability, reversibility and complications. Fert. Steril. 22, 263-269.

Hamilton, D.W. (1975) Structure and function of the epithelium lining the ductuli efferents, ductus epididymides and ductus deferens in the rat. In Handbook of Physiology (Eds. Hamilton, D.W. & Greep, R.O.) Section 7, 5, p. 259-301. Williams & Wilkins: Baltimore.

Hargrove, J.L. & Ellis, L.L. (1976) Autonomic nerves versus prostaglandins in the control of rat and rabbit testicular capsular contractions in vivo and in vitro. Biol. Reprod. 14, 651-657.

Hargrove, J.L., Seeley, R.R., Johnson, J.M. & Ellis, L.L. (1973) Prostaglandin-like substances: inhibition and maintenance of rabbit testicular contractions in vitro. Proc. Soc. exp. Biol. Med. 142, 205-209.

Harrison, R.A.P., Dott, H.M. & Foster, G.C. (1978) Effect of ionic strength, serum albumin and other macromolecules on the maintenance of motility and the surface of mammalian spermatozoa in simple medium. J. Reprod. Fert. 52, 65-73.

Hedqvist, P. (1973) Prostaglandin action on noradrenaline release and mechanical response in stimulated guinea-pig vas deferens. Acta physiol. scand. 90, 86-93.

Heller, C.G., Nelson, W.O., Hall, I.C., Henderson, E., Maddock, W.O., Jungik, E.C., Paulsen, C.A. & Mortimer, G.E. (1950) Improvement in spermatogenesis following depression of human testes with testosterone. Fert. Steril. 1, 415-422.

Henderson, R.L. & Zar, M.A. (1976) Motor transmission in the vas deferens of guanethidine-treated guinea-pig. Br. J. Pharmac. 57, 454P.

Hepperlen, T.W., Dalske, H.F. & Lacy, S.S. (1976) Effects of prostaglandins, nitrofurnatoin and Escherichia coli on response of human vas deferens to norepinephrine.. Fert. Steril. 27, 275-281.

Hermo, L., Lalli, M. & Clermont, Y. (1977) Arrangement of connective tissue components in the walls of seminiferous tubules of man and monkey. Am. J. Anat. 148, 433-446.

Hib, J. (1976) Effects of autonomic drugs on epididymal contractions. Fert. Steril. 27, 951-956.

Hib, J. (1977) The 'in vivo' effects of oxytocin and vasopressin on spontaneous contractility of the rat epididymis. Int. J. Fert. 22, 63-64.

Hoffbrand, B.I. & Forsyth, R.P. (1973) Regional blood flow changes during norepinephrine, tyramine and methoxamine infusions in the anaesthetized Rhesus monkey. J. Pharmac. exp. Ther. 184, 656-661.

Hoffer, A.P. (1976) The ultrastructure of the ductus deferens in man. Biol. Reprod. 14, 425-443.

Holtz, H. & Foote, R.H. (1972) Sperm production, output and urinary losses in the rabbit. Proc. Soc. exp. Biol. Med. 141, 958-961.

Holtz, H. & Foote, R.H. (1974) Cannulation and recovery of spermatozoa from the rabbit ductus deferens. J. Reprod. Fert. 39, 89-92.

Hooker, R.H. & Gilmore, D.P. (1972) Obstruction of vasa deferentia of the male rat and rabbit with silastic medical adhesive. Int. J. Fert. 17, 14-16.

Hrdlicka, J.G., Schwartzman, W.A., Hasel, K. & Zinsser, H.H. (1967) The new approach to reversible seminal diversion. Fert. Steril. 18, 289-296.

Hulka, J.F. & Davis, J.E. (1972) Vasectomy and reversible vas occlusion. Fert. Steril. 23, 683-696.

Igboeli, G. & Foote, R.H. (1968) Maturation changes in bull epididymal spermatozoa. J. Dairy Sci. 51, 1703-1705.

Inomata, H. & Suzuki, T. (1971) Cholinergic spontaneous junctional potentials in guinea-pig vas deferens. Tohoku J. exp. Med. 105, 197-198.

Iversen, L.L. (1967) The uptake and storage of noradrenaline in sympathetic nerves. University Press: Cambridge.

Jackson, H. (1969) Chemical interference with spermatogenesis and fertility. In Advances in Reproductive Physiology. (Ed. McLaren, A.) 4, p. 69-98. Lagos Press: London.

Jacobowitz, D. & Koelle, G.B. (1965) Histochemical correlations of acetylcholinesterase and catecholamines in postganglionic autonomic nerves of the cat, rabbit and guinea-pig. J. Pharmac. exp. Ther. 148, 225-237.

Jenkins, D.A., Marshall, I. & Nasmyth, P.A. (1977) An inhibitory role of noradrenaline in the mouse vas deferens. Br. J. Pharmac. 61, 649-655.

Jhaver, P.S., Davis, J.E., Lee, H., Hulka, J.F. & Leight, G. (1971) Reversibility of sterilization produced by vas occlusion clip. Fert. Steril. 22, 263-269.

Johnson, A.L. & Howards, S.S. (1975) Intratubular hydrostatic pressure in testis and epididymis before and after vasectomy. Am. J. Physiol. 228, 556-564.

Johnson, A.L. & Howards, S.S. (1976) Intratubular hydrostatic pressure in testis and epididymis before and after long-term vasectomy in guinea-pig. Biol. Reprod. 14, 371-376.

Johnsonbough, R.E., Connell, K.O., Engel, S.B., Edson, M. & Sade, J. (1975) Plasma testosterone, luteinizing hormone and folicle stimulating hormone after vasectomy. Fert. Steril. 20, 329-330.

Jones, M.E.L. & Spriggs, T.L.A. (1975) Noradrenaline and motor transmission in the vas deferens of the mouse. Br. J. Pharmac. 53, 323-331.

Jones, R.C., Rowlands, I.W. & Skinner, J.D. (1974) Spermatozoa in the genital ducts of the African elephant Loxodonta Africana. J. Reprod. Fert. 41, 189-192.

Kihlström, J.E. (1966) Diurnal variations in the spontaneous ejaculations of the male albino rat. Nature, Lond. 209, 513-514.

Kimball, F.A., Frielink, R.D. & Proteus, S.E. (1978) Fertility of male rats treated with 15(S)-15-methyl-prostaglandin F2α methyl ester-containing silastic implants. Fert. Steril. 29, 109-111.

Kimura, Y., Adachi, K., Kisaki, N. & Ise, K. (1975) On the transportation of spermatozoa in the vas deferens. Andrologia 7, 55-61.

Kinson, G.A. & Layberry, R.A. (1975) Long-term endocrine responses to vasectomy in the adult rat. Contraception 11, 143-150.

Kirton, K.T., Ericsson, R.J., Roy, J.A. & Forbes, A.B. (1970) Male antifertility compounds: efficacy of U-5897 in primates (Macaca mulatta) J. Reprod. Fert. 21, 275-278.

Knight, T.W. (1974) A qualitative study of factors affecting the contractions of the epididymis and ductus deferens in the ram. J. Reprod. Fert. 40, 19-30.

Knoll, J., Somogyi, G.T., Iiles, P. & Vizi, E.S. (1972) Acetylcholine release from isolated vas deferens of the rat. Naunyn Schmiedeberg s Arch. Pharmacol. 274, 198-202.

Koch, V.J., Lorenz, F., Danehl, K., Ericsson, R., Hasan, S.H., Keyserlingk, D.V., Lubke, K., Mehring, M., Römmler, A., Schwertz, V. & Hammerstein, J. (1976) Continuous oral low dosage cyproterone acetate for fertility regulation in the male?-A trend analysis in 15 volunteers. Contraception 14, 117-135.

Kothari, M.L. & Pardanani, D.S. (1967) Temporary sterilization of the male by intravasal contraceptive device (IVCD) - a preliminary communication Indian J. Surg. 29, 357-363.

Kuwahara, M. & Frick, J. (1975) The ligation of the male reproductive organs and the role of the spermatic cyst. Andrologia 7, 1-14.

Lakomaa, E. & Kihlström, J.E. (1972) An adenohypophyseal extract influencing the output of semen in rabbits, guinea-pigs and mice. J. Reprod. Fert. 31, 469-472.

Lambiase, J.T.Jnr. & Amann, R.P. (1969) The male rabbit v. changes in sperm reserves and resorption rate induced by ejaculation and sexual rest. J. Anim. Sci. 28, 542-549.

Lane-Petter, W.L. (1972) The Laboratory Rat. In the U.F.A.W. Handbook on the Care and Management of Laboratory Animals. p. 204-211. Churchill Livingstone: Edinburgh & London.

Langer, Z.(1970) The metabolism of [^{3}H] noradrenaline released by electrical stimulation from the isolated nictitating membrane of the cat and from the vas deferens in the rat. J. Physiol., Lond. 208, 516-546.

Leeson, T.S. (1962) Electron microscopy of the rete testis of the rat. Anat. Rec. 144, 57-67.

Levin, R.J. (1975) Masturbation and nocturnal emissions - possible mechanisms of minimising tetratozoospermia and hyperspermia in man. Med. Hypotheses 1, 130-131.

Lino, R.F., Braden, A.W.H. & Turnbull K.E. (1967) Fate of unejaculated spermatozoa. Nature, Lond. 213, 594-595.

Lubicz-Nawrocki, C.M. (1976) The effect of metabolites of testosterone on the development of fertilizing ability by spermatozoa in the epididymis of castrated hamsters. J. exp. Zool. 197, 89-95.

Lubicz-Nawrocki, C.M., Lau, N.I.F. & Chang, M.C. (1973) The fertilizing life of spermatozoa in the cauda epididymides of mice and hamster. J. Reprod. Fert. 38, 165-168.

Lumbell, I. & Frischer, R. (1976) The current status of male and female sterilization procedures. Proc. R. Soc. Lond. 195, 516-546.

Mann, T. (1970) The biochemical characteristics of spermatozoa and seminal plasma. In The Human Testis. (Eds. Rosemberg, E. & Paulsen, C.A.) p. 469-494. Plenum Press: New York & London.

Martan, J. (1968) Factors affecting spontaneous ejaculation of male guinea-pigs. J. Reprod. Fert. 17, 161-163.

Martan, J. (1969) Epididymal histochemistry and physiology. Biol. Reprod. 1, 134-154.

Martan, J. & Risley, P.L. (1963) The epididymis of mated and unmated rats. J. Morph. 113, 1-15.

Martins, T., Valle, J.R. & Porto, A. (1940) Pharmacology in vitro of the human vas deferens and epididymis: the question of the endocrine control of the motility of the male accessory genitals. J. Urol. 44, 682-698.

Mather, E.C. (1975) Selection of an animal model for vas deferens surgical research. In Control of Male Fertility. (Eds. Sciarra, J.J., Markland, C. & Speidel, J.J.) p. 95-108. Harper & Row: New York.

Matthews, M. & Adler, N.T. (1977) Facilitative and inhibitory influences of reproductive behaviour on sperm transport in rats. J. Comp. physiol. Psychol. 91, 727-741.

McLeod, D.G., Reynolds, D.G. & Demaree, G.E. (1973) Some pharmacological characteristics of the human vas deferens. Invest. Urol. 10, 338-341.

Melin, P. (1970) In vivo recording of contractile activity of male accessory genital organs in rabbits. Acta physiol. scand. 79, 109-113.

Metz, C.B., Hinsch, G.W. & Anika, J.L. (1968) Ultrastructure and antigens of particles from rabbit semen. J. Reprod. Fert. 17, 195-198.

Mitsuya, H., Asci, J., Suyama, K., Ushida, T. & Hosoe, K. (1960) Application of X-ray cinematography urology: I. Mechanism of ejaculation. J. Urol. 83, 86-92.

Nakanishi, H., Doetuchi, M. & Takeda, H. (1970) Resistance of mechanical and electrical responses to hypogastric nerve stimulation to α-blocking agents in guinea-pig vas deferens. Jap. J. Pharmac. 20, 61-70.

Neumann, F. (1977) Pharmacology and potential use of cyproteron acetate - a review. Horm. Metab. Res. 9, 1-13.

Nicander, C. (1965) Resorption phenomena in the epididymis. Int. J. Fert. 8, 866 (Abs.)

Norberg, K.A., Risley, P.L. & Ungerstedt, U. (1967) Adrenergic innervation of the male reproductive ducts in some mammals. II Effects of vasectomy and castration. Experientia 23, 392-394.

Orbach, J. (1961) Spontaneous ejaculations in rat. Science, N.Y. 134, 1072-1073.

Orgebin-Crist, M.C. (1967) Sperm maturation in rabbit epididymis. Nature, Lond. 216, 816-818.

Orgebin-Crist, M.C., Danzo, B.J. & Davis, J. (1975) Endocrine control of the development and maintenance of sperm fertilizing ability in the epididymis. In Handbook of Physiology (Eds. Hamilton, D.W. & Greep, R.O.) Section 7, 5, p. 319-338. Williams & Wilkins: Baltimore.

Orgebin-Crist, M.C., Danzo, B.J. & Cooper, T.G. (1976) Re-examination of the difference of epididymal sperm viability on the epididymal environment. J. Reprod. Fert. Suppl. 24, 115-128.

Orgebin-Crist, M.C. & Jahad, N. (1977) Delayed cleavage of rabbit ova after fertilization by young epididymal spermatozoa. Biol. Reprod. 16, 358-362.

Owman, C.H. & Sjöberg, N.O. (1972) The importance of short adrenergic neurones in the seminal emission mechanism of rat, guinea-pig and man. J. Reprod. Fert. 28, 379-387.

Patanelli, D.J. (1975) Suppression of fertility in the male. In Handbook of Physiology. (Eds. Hamilton, D.W. & Greep, R.O.) Section 7, 5, p. 245-258. Williams & Wilkins; Baltimore.

Pennefather, J.N. (1976) The effects of cocaine and diphenhydramine upon the reactivity of rat vas deferens to supramaximal doses of noradrenaline and other agonists: the mode of action of cocaine. Eur. J. Pharmac. 35, 333-339.

Pennefather, J.N., Vardolov, L. & Heath, P. (1974) Regional variation in the response of the rat vas deferens to field stimulation, to noradrenaline and to tyramine. J. clin. exp. Pharmac. Physiol. 1, 451-462.

Phadke, A.M. & Padakone, K. (1964) Presence and significance of auto-antibodies against spermatozoa in the blood of men with obstructed vas deferens. J. Reprod. Fert. 7, 163-170.

Popovic, N.A., McLeod, D.G. & Borski, A.A. (1973) Ultrastructure of human vas deferens. Invest. Urol. 10, 266-277.

Popović, V. & Popović, P. (1960) Permanent cannulation of aorta and vena cava in rats and ground squirrels. J. appl. Physiol. 15, 727-728.

Potts, I.F. (1957) The mechanism of ejaculation. Med. J. Aust. 1, 495-497.

Prasad, M.R.N., Singh, S.P. & Rajalakshmi, M. (1970) Fertility control in male rats by continuous release of microquantities of cyproterone acetate from subcutaneous silastic capsules. Contraception 2, 165-178.

Ramos, A.S. Jnr. & Dym, M. (1977) Ultrastructure of ductuli efferents in monkeys. Biol. Reprod. 17, 339-349.

Rajalakshmi, M., Arora, R., Boss, T.K., Dinakar, N., Gupta, G., Thompan, T.N.R.V., Prasad, M.R.N., Kumar, T.C.A. & Moudgal, N.R. (1976) Physiology of the epididymis and induction of functional sterility in the male. J. Reprod. Fert. Suppl. 24, 71-94.

Reddy, P.R.K. & Rao, J.M. (1972) Reversible antifertility action of testosterone propionate in human males. Contraception 5, 295-301.

Reid, B.L. & Cleland, K.W. (1957) The structure and function of the epididymis. I. The histology of rat epididymis. Aust. J. Zool. 5, 223-243.

Rhodes, P. (1971) In Birth Control (Eds. Head, J.J. & Lowenstein, O.E.) p. 9-11. University Press: Oxford.

Richardson, D.W. & Eastell, R. (1976) Distribution of sperm in the reproductive tract of eleven men. Presented at the Annual conference of the Society for the Study of Fertility. Sheffield.

Richardson, D.W. & Short, R.V. (1977) Time of onset of spermatogenesis in boys. Presented at the Annual conference of the Society for the Study of Fertility. Dublin.

Risley, P.L. (1958) The contractile behaviour in vivo of the ductus epididymides and vasa efferentia of the rat. Anat. Rec. 130, 471.

Robert, E.D. & Oester, Y.T. (1970) Absence of supersensitivity to acetylcholine in innervated muscle subjected to a prolonged pharmacologic block. J. Pharmac. exp. Ther. 174, 133-140.

Robinson, P.M. (1969) A cholinergic component in the innervation of the longitudinal smooth muscle in the guinea-pig vas deferens. The fine structural localization of acetylcholine esterase. J. Cell Biol. 41, 463-476.

Rodger, J.C. & White, I.G. (1976) Comparison of sperm numbers released in the urine and ejaculates of the brush-tailed possum. J. Reprod. Fert. 46, 502-503.

Roosen-Runge, E.C. (1958) Motions of the seminiferous tubules of rat and dog. Anat. Rec. 109, 413.

Rotman, A & Creveling, C.R. (1976) A rationale for the design of cell-specific toxic agents: the mechanism of action of 6-hydroxydopamine. FEBS, 72, 227-230.

Roussel, J.D., Stallcup, O.T. & Austin, C.R. (1967) Selective phagocytosis of spermatozoa in the epididymis of bulls, rabbits and monkeys. Fert. Steril. 18, 509-516.

Russel, L. & Clermont, Y. (1976) Anchoring device between Sertoli cells and later spermatids in rat seminiferous tubules. Anat. Rec. 185, 259-277.

Sannowiya, P. & Demoraes, S. (1977) Responsiveness of guinea-pig vas deferens to phenyl ephrine and norepinephrine in vivo. Eur. J. Pharmac. 46, 59-62.

Schmidt, S.S. (1975) Complications of vas surgery. In Control of Male Fertility. (Eds. Sciarra, J.J., Markland, C. & Speidel, J.J.) p. 78-83. Harper & Row: New York.

Setty, B.S., Dasgupta, P.R. & Kar, A.B . (1972) Chemical occlusion of the vas in rat. Contraception 6, 329-333.

Shirai, S., Sasaki, K. & Rikimura, A. (1973) A histochemical investigation of the distribution of adrenergic and cholinergic nerves in the human male genital organs. Tohoku J. exp. Med. 111, 281-291.

Siegal, S. (1956) Non-parametric statistics for the behavioural science. McGraw-Hill: New York.

Simeone, F.A. & Young, W.E. (1931) A study of the function of the epididymis. IV The fate of non-ejaculated spermatozoa in the genital tract of the male guinea-pig. J. exp. Biol. 8, 163-175.

Simon, A. & Van Meanen, E.E. (1976) Dopamine receptors and dopaminergic nerves in the vas deferens of the rat. Arch. int. Pharmacodyn. Ther. 222, 4-15.

Sjöstrand, N.O. (1965) The adrenergic innervation of the vas deferens and the accessory male genital glands. Acta physiol. scand. Suppl. 257, 1-82.

Skinner, J.D. (1971) The effect of season on spermatogenesis in some ungulates. J. Reprod. Fert. Suppl. 13, 29-37

Smith, G. (1962) The effects of ligation on the vasa efferentia and vasectomy on testicular function in the adult rat. J. Endocr. 23, 385-399.

Steinberger, E. & Tjioe, D.Y. (1969) Spermatogenesis in rat testes after experimental ischaemia. Fert. Steril. 20, 639-649.

Stjärne, L. (1976) The twitch contraction in guinea-pig isolated vas deferens. Result of co-operative interaction of potassium and noradrenaline released from adrenergic nerves in close junctions? Acta physiol. scand. 98, 512-414.

Stone, T.W. (1977) Haloperidol inhibits contractions of the vas deferens. J. Pharm. Pharmac. 29, 312-313.

Swanson, L.V. & Hafs, H.D. (1968) Testicular and epididymal sperm numbers in unilaterally vasoligated rabbits. Proc. Soc. exp. Biol. Med. 131, 763-768.

Swedin, G. (1971) Studies on neurotransmission mechanisms in the rat and guinea-pig vas deferens. Acta physiol. scand. suppl. 369, 1-34.

Taylor, G.S. & Einhorn, U.F. (1972) The effect of prostaglandins on the junctional potentials in the mouse vas deferens. Eur. J. Pharmac. 20, 40-48.

Terner, C.M. & Smith, B.R. (1975) Changes in lipase and phosphatase activities of rat spermatozoa in transit from the caput to the cauda epididymis. J. Reprod. Fert. 45, 1-8.

Tesh, J.M. & Glover, T.D. (1969) Ageing of rabbit spermatozoa in the male tract and its effect on fertility. J Reprod. Fert. 20, 287-297.

Tingari, M.D. & Lake, D.E. (1972) Ultrastructural evidence for resorption of spermatozoa and testicular fluids in the excurrent ducts of the testis of the domestic fowl, Gallus Domesticus. J. Reprod. Fert. 31, 373-381.

Toyama, Y. (1976) Actin-like filaments in the sertoli cell junctional specilization in the swine and mouse testis. Anat. Rec. 186, 477-491.

Tsunoda, J. & Chang, M.C. (1976) Fertilitizing ability *in vivo* and *in vitro* of spermatozoa of rats and mice treated with α-chlorodydrin. J. Reprod. Fert. 46, 401-404.

Van Demark, N.L. & Baker, F.N. (1958) The effect of daily injections of epinephrine on semen production of bulls. J. Anim. Sci. 12, 956.

Vanwelkenhuyzen (1966) Cited by Batra, S.K. (1974) Sperm transport through vas deferens: review of hypotheses and suggestions for a quantitative model. Fert. Steril. 25, 186-202.

Vare, A.M. & Bansel, P.C. (1973) Changes in canine testes after bilateral vasectomy - an experimental study. Fert. Steril. 24, 793-797.

Ventura, W.P., Freund, M., Davis, J. & Pannati, C. (1973) Influence of norepinephrine on the motility of the human vas deferens: a new hypothesis of sperm transport by the vas deferens. Fert. Steril. 24, 68-77.

Vreeburg, J.T.M., Bielska, M. & Ooms, M. (1976) Maturation and survival of spermatozoa in the epididymis of pregnenolone treated hypophysectomized rats. Endocrinology 99, 824-830.

Vreeburg, J.T.M., Van Andel, M.V., Kort, W.J. & Westbroek, D.L. (1974) The effect of hemicastration on daily sperm output in the rat as measured by a new method. J. Reprod. Fert. 41, 355-359.

Wadsworth, R.M. (1973) Abolition of neurally evoked motor responses of the vas deferens by 6-hydroxydopamine. Eur. J. Pharmac. 21, 386-387.

Wadsworth, R.M. (1974) Excitatory and inhibitory effects of noradrenaline on the isolated guinea-pig vas deferens. Clin. exp. Pharmac. Physiol. 1, 135-145.

Whalen, R.E. & Luttage, W.G. (1969) Contraceptive properties of the anti-androgen, cyproterone acetate. Nature, Lond. 223, 633-634

Wohl, A.J., Hausler, L.M. & Roth, F.E. (1967) Studies on the mechanism of antihypertensive action of diazoxide: in vitro vascular pharmaco-dynamics. J. Pharmac. exp. Ther. 158, 531-539.

Wortman, R.N. (1975) In Population Reports, Series D, 2, p. 26-39. University Medical Centre, Washington.

Wyker, R. & Howards, S.S. (1977) Micropuncture studies of the motility of rete testis and epididymal spermatozoa. Fert. Steril. 28, 108-112.

Zucker, I. (1966) Effects of antiandrogen on the mating behaviour of male guinea-pig and rats. J. Endocr. 35, 209-210.

Zysk, J.R., Bushway, A.A., Whistler, R.L. & Carlton, W.W. (1975) Temporary sterility produced in male mice by 5-thio D-glucose. J. Reprod. Fert. 45, 69-72.