TOWARDS AN UNDERSTANDING OF THE ROLE OF INTRAVESICAL CAPSAICIN IN THE TREATMENT OF DETRUSOR HYPERREFLEXIA

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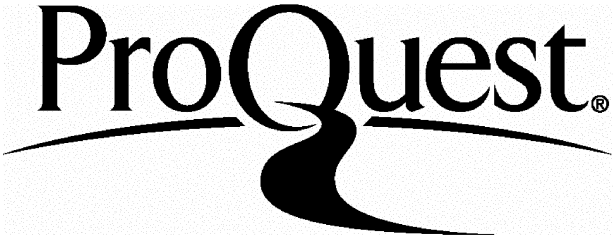

ABSTRACT

Towards an understanding of the role of intravesical capsaicin in the treatment of detrusor hyperreflexia

Capsaicin is the pungent extract of red-hot chilli peppers. Chillies were grown as early as 5000 BC in Mexico and have been used medicinally, for a variety of seemingly unrelated disorders, for thousands of years.

Animal experiments have demonstrated that capsaicin blocks a C-fibre mediated micturition reflex in spinal cats. The aim of this study was to investigate its role in the treatment of detrusor hyperreflexia due to spinal cord disease. Intravesical instillations of 1-2 mmol/l of capsaicin, dissolved as a powder in 30% alcohol in saline, were effective in 70% of patients with refractory detrusor hyperreflexia. After initial deterioration in voiding symptoms capsaicin caused an increase in the functional bladder capacity and decrease in the amplitude of hyperreflexic detrusor contractions. The beneficial effect of a single instillation lasted for 3-6 months. Even after repeated instillations over 5 years there was no evidence of pre-malignant or malignant changes in biopsies from bladders thus treated.

Suprapubic discomfort during instillations was reduced by the prior use of intravesical lignocaine (40ml of 2% for 20 mins) or by anaesthetising the bladder with iontophoresis of intravesical lignocaine (electromotive drug administration) before capsaicin.

Cryostat sections of flexible cystoscopic biopsies before and 6 weeks after capsaicin treatment were stained with the neuronal markers S 100 and PGP 9.5. By using computerised image analysis of lamina propria nerve densities ('MiniMOP' for S 100 and 'Seescan' imaging for PGP 9.5) it was found that

intravesical capsaicin caused a reduction in densities of the presumptive sensory suburothelial nerves. Early data using electron microscopy seemed to show a reduction in the densities of clear and dense cored vesicles after capsaicin treatment. These findings indicate that capsaicin causes a sensory denervation the bladder in these patients.

Intravesical capsaicin is a significant advance in Uro-Neurology and is likely to lead to the application of other vanilloids, such as resiniferatoxin, in the treatment of detrusor hyperreflexia.

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PREFACE

This thesis attempts to answer three questions.

1. What are the benefits of intravesical capsaicin in detrusor hyperreflexia?
2. What is its mechanism of action?
3. What are its long-term effects in this group of patients?

Detrusor hyperreflexia and detrusor sphincter dyssynergia affect the majority of patients with spinal cord lesions. Many find their urinary symptoms to be the most disabling aspect of their disease condition. Anticholinergics and clean intermittent self catheterisation (CISC) remain the cornerstone of therapy. Although about 70% respond to this combination the treatment is also ineffective in 30% of patients. Some patients find the antimuscarinic side effects intolerable and eventually stop their medication. Rather than subject them to major urinary reconstructive surgery or diversion, an effective but non-operative treatment would be of great value. We investigated the role of intravesical capsaicin in these patients. Capsaicin is the pungent ingredient of chilli peppers and is commercially available in its synthetic form as a dissolvable powder.

Till the early 1990s most experiments using capsaicin were performed on animals. This extensive body of literature improved our understanding enough for us to embark on human trials of this substance. All clinical and laboratory experiments described in this thesis are on human subjects and have direct clinical relevance. The author is indebted to the patients who agreed to participate and without whose co-operation this work would not have been possible.

The idea of using intravesical capsaicin to treat intractable detrusor hyperreflexia was that of Dr Clare Fowler. The first patient was treated in the Uro-Neurology department at Queen Square in 1990. Since then numerous others have received these instillations as part of an ongoing trial. Mr Vijay Chandiramani joined the department in 1993 and described the urodynamic changes during capsaicin instillations. He took the initial bladder biopsies and some of these tissues were used for the histological work described here. Thereafter the author continued this study as MRC Fellow between August 1995 and April 1997. He still holds an honorary contract at Queen Square and is actively involved with this ongoing MRC project. The author has treated most patients with capsaicin at some stage during their illness. In addition to bladder biopsies from patients thus treated he obtained biopsies from patients in the control group at the Institute of Urology, London. Mr Andrew Beckett at Queen Square processed these tissues and Professor F Scaravilli and Dr R Crowe supervised the immunohistochemistry. Professor D N Landon performed electron microscopy on the bladder biopsies and the initial data was analysed by him and the author, who provided all the biopsies. Since 1995, Dr M C Parkinson at UCL has been carefully examining the biopsies to exclude any cancer. The author researched the history of chillies with assistance from Dr D Wujastyk at the Wellcome Institute Library, London.

The word 'chilli' has been spelt as 'chili', 'chile' and 'chilly' by various authors. The spelling used here is derived from the *Oxford Dictionary for Writers and Editors*.

SECTION I

INTRODUCTION

CHAPTER 1

THE HISTORY OF CAPSAICIN

1.1 Chillies: a historical perspective

Chilli peppers have played an important role in the history of medicine. Given the contemporary controversy over the 'bio-piracy' of ancient Indian remedies like turmeric and neem by modern medicine (Agarwal and Narain, 1996), the history of chillies and its evolution from traditional to modern times is more than just of antiquarian interest. Their use as a counter-irritant in the treatment of various conditions was recognised centuries ago.

Red-hot chilli peppers (Fig. 1) are widely used in Indian cooking and it is popularly assumed that they originated in India. However this is not the truth; chillies or *Mirchi* as they are called in Hindi, were originally grown in Mexico and reached Spain and Portugal long before they were ever tasted in India (Achaya, 1994; Dasgupta and Fowler, 1997).

Capsicums were cultivated by the inhabitants of Tamaulipas mountains in Mexico as early as 5000 B.C (Tannahill, 1973) and 'chilli' is a Mexican word (Achaya, 1994). They were also grown in Peru around 2000 BC. The Incas put chillies in the waters of lakes in which they caught fish so that the fish were spiced even before they were cooked (Toussaint-Samat, 1992).

Chillies were mentioned in old prehispanic Aztec manuscripts or *tlacuilos* (information from the Internet: 'the Great Mexican Chile') and they have continued to be an integral part of the Mexican diet and culture.

It is unknown how chillies reached the Caribbean from Mexico; perhaps they were naturally indigenous, but it is thought that it was Columbus (Figs. 2, 3)

Fig. 1 Chilli or pepper plant. From an old water-colour, date and artist unknown. Reproduced with permission of the Wellcome Institute Library, London and the British Journal of Urology

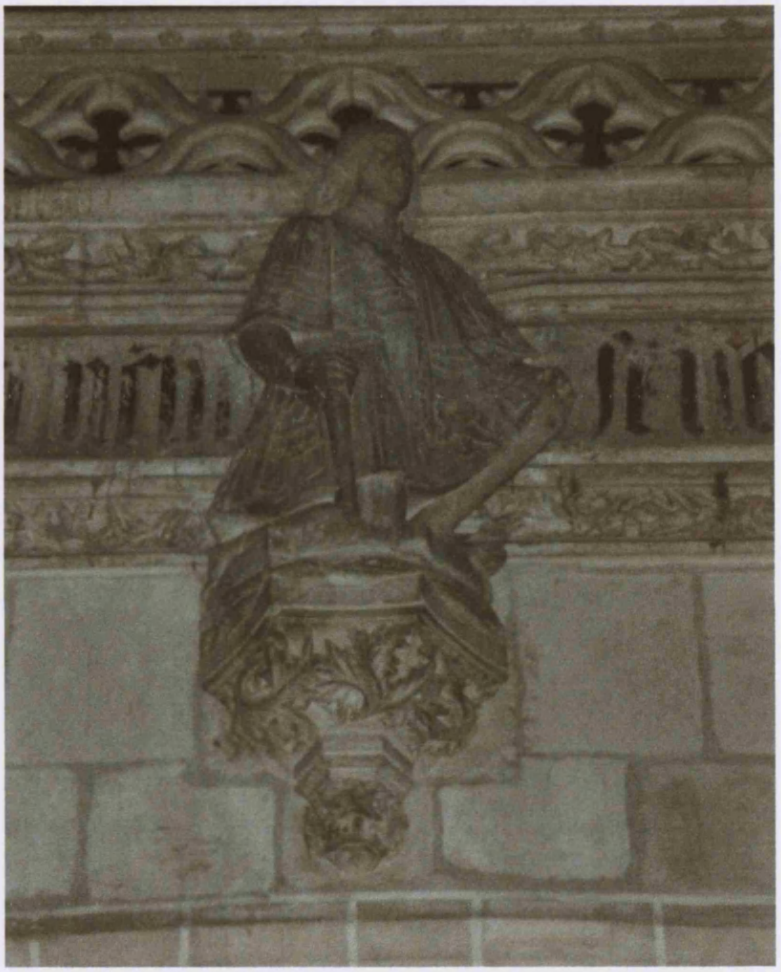


Fig. 2 Statue of Christopher Columbus in Toledo. This is where he met Queen Isabella of Spain before setting on his voyages. Photograph by the author

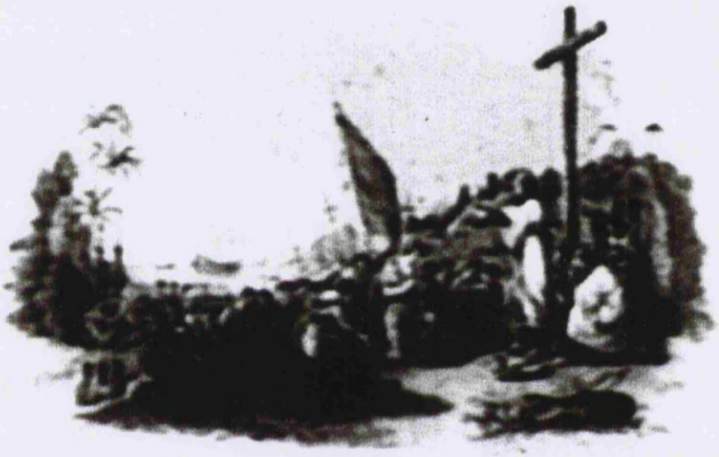


Fig. 3 Landing of Columbus in the New World. Engraving by J. Knight and W. Humphrys. Reproduced with the permission of the Wellcome Institute Library, London

who introduced them from the New to the Old World i.e., from the Caribbean to Europe. During his second voyage to the West Indies he is known to have remarked that chillies were 'better spice than our pepper' (*mejor que pimienta neustra)* and therefore they were subsequently named *pimiento* (Toussaint-Samat, 1992).

The first record of chillies as we know them now appears in a letter written by Dr. Diego Alvarez Chanca, physician to the fleet of Columbus on his second voyage, to Hernandez, the court surgeon to King Phillip of Spain in January 1494. This was sent under the care of Don Antoniode Torrens on 2 February 1494 and reached Spain on 8 April 1494 (De Ybarra, 1906). Chanca noticed chilli being used as condiment by the natives of Hispaniola (now Haiti and the Dominican Republic), who called it *Ahi* which in Castilian Spanish was converted to *Aji* or *Axi* (Dymock et al, 1891).

Lucien Guyot in the 17th century noticed chillies being widely used in the Sunda Islands and this indicates their spread to the east (Toussaint-Samat, 1992). It is a mystery as to why chillies never reached India before Columbus. It seems that they reached Portugal from the New World and were then taken by explorers to India whereupon they were rapidly accepted. Clusius states that chillies were brought to India from Pernambuco (in Brazil) by the Portuguese in the late 15th century (Dymock, 1891) (Fig. 4).

Chillies did not feature in traditional Indian medicine or cooking before the arrival of the Portuguese (Figs. 5, 6). Although *Maricha* is mentioned in the works of Charaka and Susruta (around the time of Christ) as a medicinal plant, this refers to black pepper (Garrison, 1929) which is Piper nigrum. The *Ain-i-Akbari* (a book from the Mughal period), refers only to black pepper to

impart pungency to the many recipes mentioned in it (Achaya, 1994). Piper nigrum (Linn) and Piper Longum (Linn) were both used medicinally in India and the latter looks a lot like chilli pepper. Perhaps it was this physical resemblance which later led to the ready acceptance of the chilli in India (Dasgupta and Fowler, 1997). The great south Indian composer Puranadasa (early 16th century) aptly describes the chilli as: "I saw you green, then turning redder as you ripened, nice to look at and tasty in a dish, but too hot if an excess is used..." (Achaya, 1994). Chillies are called *Mirchi* in India and black pepper is referred to as *Maricha* and it is the similar sounding names, which has led to the confusion regarding its origin (Dasgupta and Fowler, 1997).

Leonard Fuchs (1501-1566), the Bavarian naturalist, in his Historia Stirpium describes chilli pepper as originating in Calcutta as "Calcutta pepper" (Toussaint-Samat, 1992) which probably indicates that chillies had reached Eastern India by then. Rumphius states that the Indian term *'Achar'* for pickles is probably derived from *Axi* or *Achi.* Virey, in his "Histoire Naturelle des Medicamens" has expressed the singular notion that it is owing to an abuse of pickle that the inhabitants of hot climates suffer so much from liver complaints (Ainslie, 1826).

Fig. 4 Pernambuco in Brazil -this is where the Portuguese explorers found chillies, which were later taken to India. Taken from an old map and reproduced with the permission of the Wellcome Institute Library, London

Fig. 5 Vasco da Gama -the first Portuguese explorer to reach India on 20 May, 1498

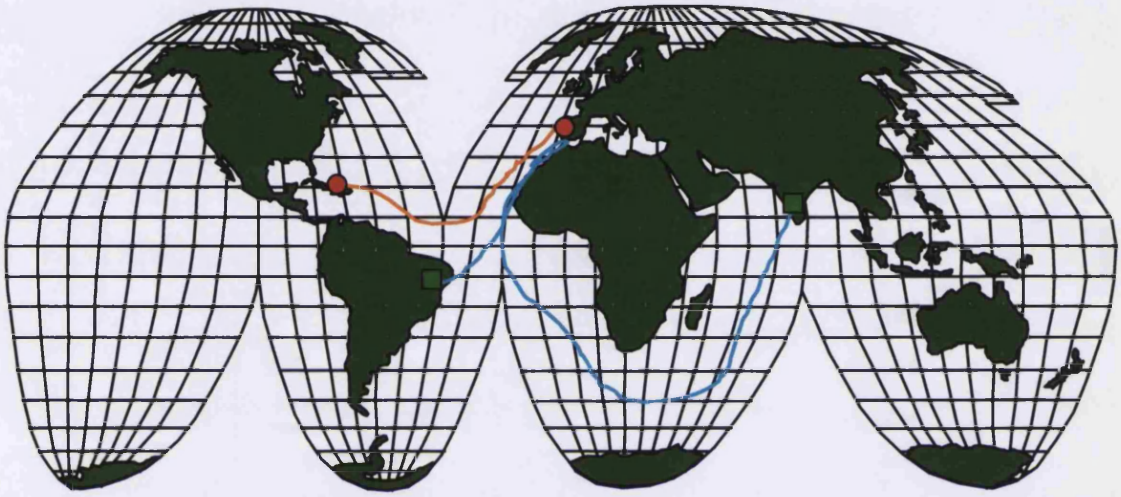


Fig. 6 Routes taken by Spanish (orange line) and Portuguese (green line) explorers

Meanwhile the nations of Europe were complaining about the Portuguese and Spanish monopoly of spices as trading in them was lucrative in those days. Spices had become extremely popular in Europe because prior to their introduction the diet of these regions was somewhat bland. In Spain chilli had become a popular ingredient in a cup of chocolate: "For every hundred cocoa beans mix two pods of chilli or Mexican pepper....". It has been said that the English were so incensed over the fact that the price of "pepper" had gone up from 15 pence to 40 pence a pound that London merchants were driven to establish the East India Company (Tannahill, 1973). Thus while the Spanish and Portuguese obtained most of their chilli peppers from the West Indies, the British imported them from India via the East India Company.
In the last 500 years chillies have become popular in many different parts of the world. They have been used for *harissa* by the Arabs, chilli sauce in Orissa, India, Moroccan and Brazilian salads, Tabasco sauce in Mexico (Fig. 7) and *tsiao yeou* by the Chinese (Fig. 8).

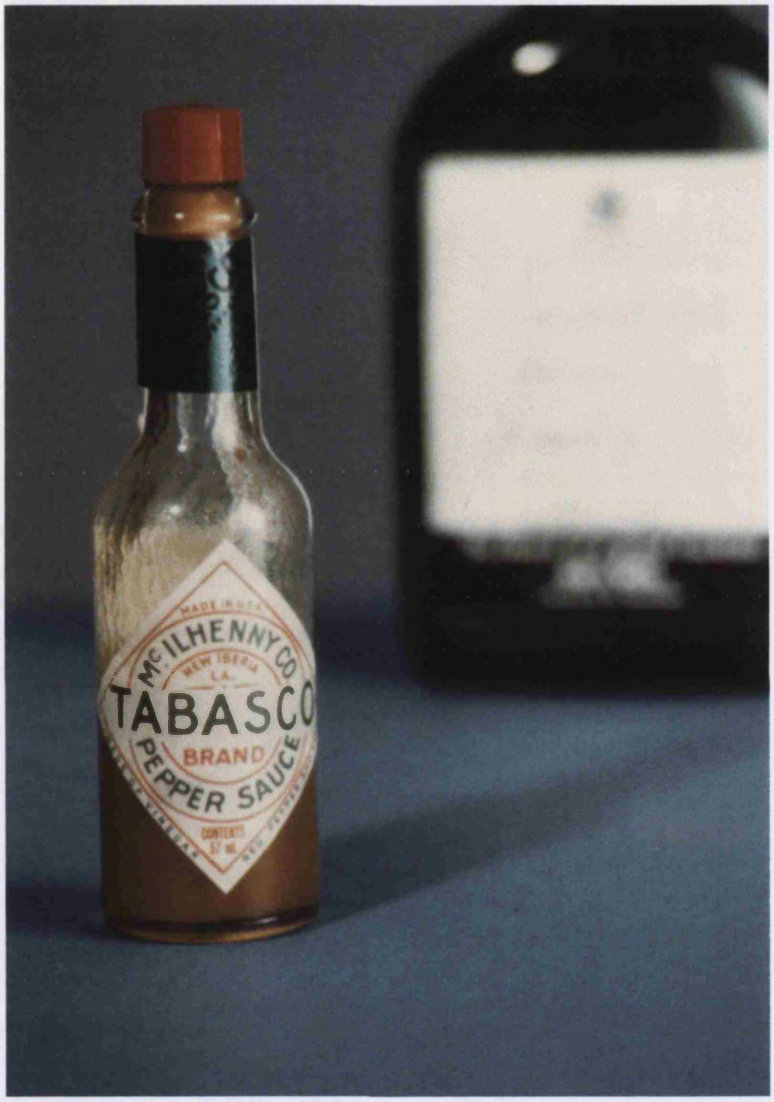


Fig. 7 Tabasco sauce has a capsaicin concentration of about 2 mmol. Only a few drops are added for culinary purposes

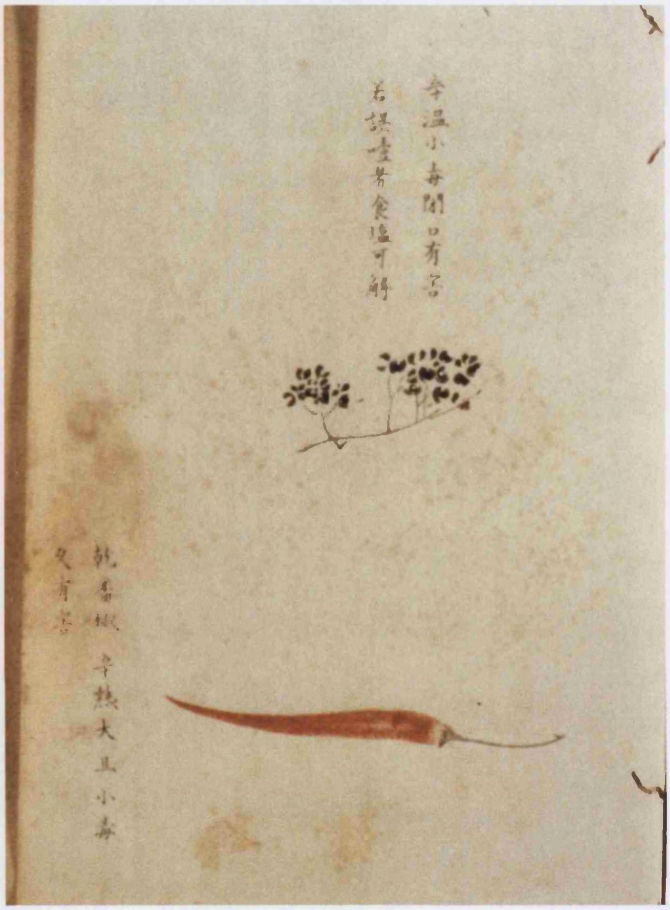


Fig. 8 Chilli pepper from an old book of Japanese water-colours. The writing on the sides is in Chinese. Reproduced with permission of the Wellcome Institute Library, London and the British Journal of Urology

1.2 Traditional uses of chilli peppers in medicine

Chillies have been used medicinally for hundreds of years although the pungent ingredient capsaicin was extracted only relatively recently. The concept of using a pungent substance to treat urinary symptoms (Dasgupta and Fowler, 1997) was known to the Indians as is evident from the Susruta Samhita (around the time of Christ) which includes an exhaustive Pharmacoepia (Fig. 9). Susruta catalogues 37 classes of medicines and peppers (*Pippali*) are classified under *Pippalyadigana* (Dutt, 1883). *Pippali* refers to Piper Longum which contains the pungent ingredient piperine not of course capsaicin. It was taken internally for a host of diseases including intractable urinary symptoms caused by diabetes (Dutt, 1883).

It is interesting that soon after the introduction of chilli peppers into India the same principle of using their pungency was employed to treat a host of unrelated diseases. Externally a chilli paste was used as a rubefacient and as a local stimulant for tonsillitis. Its application to the throat was said to hasten the separation of membranes in diphtheria. When made into a lozenge with tragacanth and sugar it was taken as a remedy for hoarseness. It has also been used as an adjunct to bitter tonics to treat loss of appetite and flatulence and in combination with rhubarb and ginger as a carminative. Along with opium and fried asafoetida it has been given for cholera. The whole plant steeped in milk has been applied to reduce swellings. In chronic lumbago a plaster of capsicum with garlic, pepper and liquid amber *(Silarasa)* was used as an effective counter-irritant. Similar repeated applications were used in rheumatism and gout (Nadkarni, 1982). There is also mention of it in the treatment of snakebite (Nadkarni, 1982).

In South America the Aztecs took chillies as a remedy for cough, in combination with honey; and in persistent cough they drank an infusion containing chillies and salt. The Tarahumara Indians regarded them as being useful in bronchitis and throat irritation. Chillies contain vitamins A and C and even after cooking they do not lose more than a third of the total vitamin C content although this is completely lost if they are dried.

In the West Indies chillies were made into an infusion with cinnamon and sugar to relieve the sinking feeling in the epigastrium felt by drunkards; this constitutes a most valuable drink for patients suffering from delirium tremens, as it satisfies the craving for stimulants (Dymock, 1891). In Africa they were applied as antiseptics, for enhancing wound healing and for parasitic infestations of the intestine. There is also mention of the rather extreme method of treating piles with red-hot chilli peppers (Toussaint-Samat, 1992). In Latin American countries rubbing chillies on fingers has been a common practice by mothers who wish to deter their children from the habit of sucking their thumbs.

In England, in the last century, chilli was used for dyspepsia, tympanitis and palsy. It was recommended for dropsies, for healing ulcers of the fauces, as a gargle for pharyngitis and in combination with hogs' lard as a liniment for paralytic limbs (Ainslie, 1826). It was applied in the form of plasters in rheumatic and neuralgic affections because of its rubefacient effect (Dymock, 1891). Pads dipped in a strong solution of the crushed pods were used, the treated area being subsequently covered with paraffin paper or oil silk. Sir James Sawyer (1891), Professor of Medicine at Queen's College, Birmingham found excellent results by using an "ethereal tincture" of

capsicum to treat chronic gout, rheumatism, bronchial catarrh and chronic bronchitis. He recommended pure ether instead of rectified spirit of wine in the preparation of this tincture: "I find an ethereal tincture of capsicum, by reason of the comparatively rapid evaporation of its ether, can be used more freely than an alcoholic tincture as an application to the skin. Furthermore, I think the solvent action of ether upon the sebaceous secretion of the skin makes ether a menstruum preferable to alcohol for drugs designed to affect cutaneous surfaces, or to produce therapeutic effect through the skin. If an ethereal tincture of capsicum be gently rubbed upon the back of a hand it will produce a feeling of warmth, with sensation of burning and pricking, in about a minute's time, together with an irregular and patchy hyperaemic redness, which may last for some hours...An excellent and powerful rubefacient liniment may be made of equal parts of ethereal tincture of capsicum, liquor ammoniae, oleum terebinthinae and oleum lini" (Sawyer, 1891). German physicians curiously mentioned it 'to be peculiarly injurious' in gonorrhoea (Ainslie, 1826).

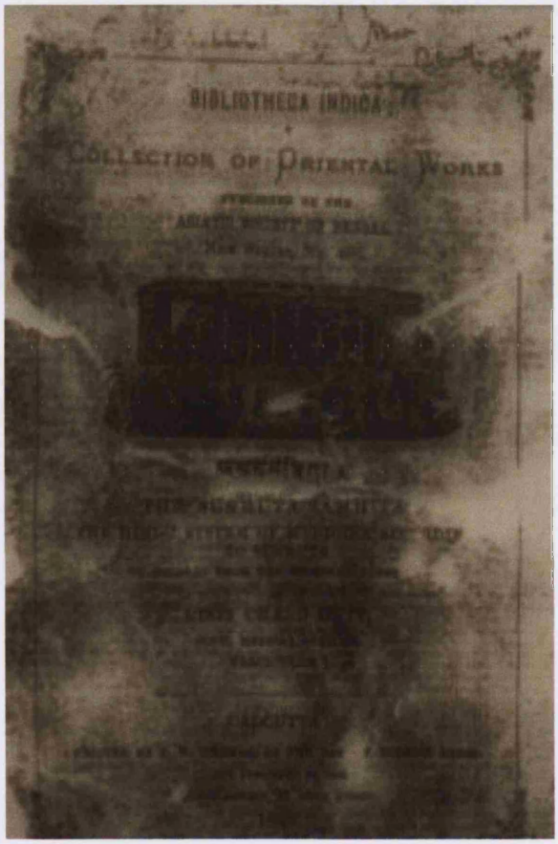


Fig. 9 An English translation of the Susruta Samhita, one of the traditional works of Indian medicine (written around the time of Christ)

CHAPTER 2

THE CHEMICAL PROPERTIES OF CAPSAICIN

2.1 The botany of chillies

Chillies belong to the Solanacea family and Capsicum species of which there are a wide variety, the two common ones being Capsicum annuum (Linn) and Capsicum frutescens (Linn) (Achaya, 1994). The difference between the annum and the frutescens species is the stem, which is shrubby in the latter (Ainslie, 1826). Historically the frutescens species it thought to correspond to the *capo-molago* of van Rheede (in the Hortus Indicus Malabaricus published as 12 volumes between 1678-1703) as opposed to the annum species which he calls the *Vallia-capo-molago* (Ainslie, 1826).

The hotness of chillies is measured in units called 'Scovilles'; 300-600 indicates a mild variety whereas 200,000-350,000 Scovilles represent some of the hottest chillies in the world. Wilbur Scoville introduced the 'Scoville' unit in 1912. He quantified the heat of peppers by placing their extracts on the human tongue. If a pepper has 50,000 Scoville units, this means that its alcoholic extract needs to be diluted 1:50,000 with water for it to cease to be hot tasting on the human tongue (Scoville, 1912). In general small chillies tend to be hotter than the larger ones and the varieties with broader shoulders are usually milder in flavour (Larkcom, 1995).

Based on their appearances chillies are further classified into different groups. The Hungarian Wax and Anaheim types, which also include the Antler and Inferno subtypes, are large, flattish mild chillies, often quite unlike their names. The Cayenne types are long, thin chillies, which are somewhat hotter and include the Scorpion, Thai Hot and Fire Candle varieties. Varieties

such as Serrano, Jalapeno and Manzano are popular in Mexico. A particularly hot type is the Bird's Eye chilli (Achaya, 1994). A few African chillies are round and a West Indian variety is colloquially known as *bouda a Man Jacques* or Madame Jacques's bum (Toussaint-Samat, 1992). The Habanero chilli was probably introduced into Mexico from Cuba and despite its innocuous appearance, being small and yellow, it is the hottest of all chillies (Larkcom, 1995).

2.2 The chemistry and physiology of capsaicin

Capsaicin is 8 -methyl -N -vannilyl -6 -nonenamide and was chemically synthesised as early as 1923 (Nelson and Dowson, 1923). However, Dymock (1891) mentions that it was extracted by Thresh from Capsicum minimum, using petroleum ether. It forms needles, which are insoluble in water but very soluble in alcohol and fat. The prismatic crystals of capsaicin begin to volatilise at 100°C and the pungency can be removed by heating with potassium bichromate and dilute sulphuric acid.

Capsaicin is an alkaloid and its molecular structure is similar to other derivatives of homovanillic acid (Fig. 10). Fluka Chemika-Biochemika (Switzerland) manufactures it in its synthetic form, Pelargonic acid vanillylamide. Capsaicin used in this study was purchased from this company.

The capsaicin content of chillies is about 0.14% [5 g of chillies contain 7 mg of capsaicin] (Sirasat and Khanolkar, 1960). Meyer has found that capsaicin is not, as is generally assumed, distributed in the entire capsicum fruit, but mainly occurs in the light yellowish-red placentae (Fig. 11) and their attachments (Dymock, 1891).

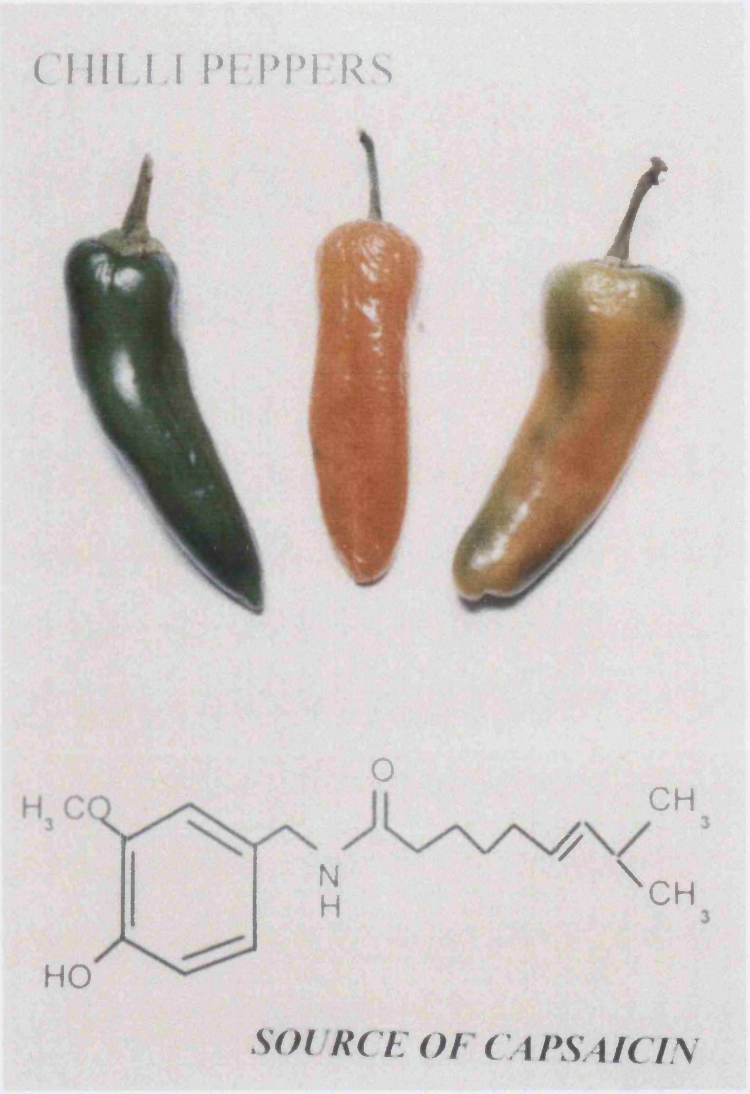


Fig. 10 The chemical structure of capsaicin. Figure reproduced with permission of Dr Clare Fowler

Fig. 11 Cross-section of red chilli showing the placentae in which capsaicin is largely concentrated. Photographed by Steve Durr

2.3 The effects of capsaicin

Direct application of capsaicin to the skin or tongue results in a burning sensation and sharp taste respectively. It causes an intensely painful sensation when applied to the eyes, nose, mouth and throat; a property that is exploited by riot control police. An extract from chillies, which contains *oleoresin capsicum,* is one of the elements used in the manufacture of teargas. Small pocket sized self defense sprays containing *oleoresin capsicum* are now available for use by police departments and federal law enforcement agencies in the USA and a spray containing a 10% concentration of this substance (MK-6C) has been legally sold to the residents of California since a new law was passed on 1 Jan 1996. Little canisters of the spray can be concealed in the palm of the hand or clipped to the belt while jogging or walking, for self-defence (information from the Internet).

This 'hot' sensation caused by capsaicin arises from the excitation of afferent nerve endings. There is a highly selective activation of a sub-class of somatovisceral afferents which contain unmyelinated C-fibre axons (Lynn, 1990). Topical or systemic administration of capsaicin results in the powerful activation of nociceptive fibres. It is also known from animal experiments that capsaicin, after an intense initial excitation of sensory neurons, makes them unresponsive to noxious chemical stimuli (Szolcsányi, 1984).

A lot of interest has centered on the afferent and efferent effects mediated by capsaicin sensitive sensory neurons (Jancsó et al, 1967; Szolcsányi 1982, 1983, 1984; Maggi and Meli, 1988). In general, a sensory neuron in addition to conveying sensory impulses to the central nervous system may also

release transmitters in the periphery by an axon reflex arrangement. This "sensory axon reflex /vasodilatation" is similar to Lewis' triple response in the skin. In addition to this axon reflex mechanism, capsaicin sensitive primary afferents (CSPA) are thought to release neurotransmitters at the same terminal, which is activated by the sensory stimulus. Although there is no direct evidence for this it has been proposed that chemical stimulation in the region of a capsaicin sensitive terminal may release the stored neuropeptides by depolarisation, resistant to local anaesthetics and tetrodotoxin. On the other hand, local anaesthetics or tetrodotoxin can abolish neurotransmitter secretion by ortho- or anti-dromic activation of sensory nerves (Maggi and Meli, 1988).

Cutaneous effects

The majority of C-fibres are known to be nociceptors. In the skin, nociceptive C-fibres of the 'mechano-heat' class are stimulated by capsaicin in all mammalian species tested including cat, rabbit and rat. Other types of C-fibre afferents i.e., mechanoceptors or cold sensitive thermoceptors are not excited. However excitation of warm-sensitive thermoceptors is known to occur. A-fibre afferents are generally unaffected with the exception of the relatively uncommon Aδ nociceptors in the rat hairy skin (Lynn, 1990). Following application of a capsaicin solution to rat skin, low frequency firing in C-polymodal nociceptors occurs with blood flow in the skin increasing upto 300%. Vasodilatation in response to saphenous nerve stimulation (antidromic vasodilatation) is significantly reduced by a single application of 33 mM of capsaicin (Lynn et al, 1992). In rats it appears to block the effector arm of the axon reflex since flare does not spread to the areas treated with it. Animal experiments have shown that the degree of neurotoxicity caused by capsaicin depends on the age of the animal. High doses given subcutaneously to newborn rats results in loss of more than half the unmyelinated fibres (Scadding, 1980) whereas when administered to adult animals the loss is significantly less (Jancso et al, 1985). The axonal loss in peripheral nerves caused by a topical application of capsaicin (Pini et al, 1990) seems to be independent of the animal's age. A concomitant depletion of neuropeptides in these nerves is also noticed (Jessel et al, 1978). Topical application of capsaicin to the human skin produces an initial burning, erythematous reaction which gradually diminishes over 24 hours leaving the skin unresponsive to histamine induced axon reflex vasodilatation

(Bernstein et al, 1981). Chronic administration of high doses of topical capsaicin causes a loss of the flare response to histamine, platelet activating factor (McCusker et al, 1989) and substance P [SP]. Topical capsaicin pretreatment also inhibits the axon reflex vasodilatation caused by somatostatin and vasoactive intestinal polypeptide [VIP] in human skin (Anand et al, 1983).

Gastrointestinal effects

It is well known that chillies cause a burning sensation in the tongue and reflex salivation. The burning sensation occurs on the apex of the tongue where there is a high density of SP containing nerves (Fuller, 1990). The salivation caused by capsaicin is blocked by the administration of anticholinergic agents (Duner-Engstrom et al, 1986). Capsaicin is also known to cause damage to the mucous membrane in rats (Sirasat and Khanolkar, 1960). Repeated exposure of the oral mucosa to capsaicin gradually leads to desensitization to its irritant action as is well known to those who eat 'hot' foods.

The effect of intra-gastric infusion of 5, 7.5 and 10 mg/hr doses of capsaicin on the DNA content of gastric aspirate has been studied. Lower doses have no effect but a dose of 10 mg/hr causes a significant increase of DNA content of the aspirate which is a good indicator of accelerated exfoliation of gastric surface epithelial cells in humans (Desai et al 1973). These observations indicate a dose-dependent effect of capsaicin on the exfoliation of gastric surface epithelial cells (Desai et al 1976). This effect of capsaicin occurs only during the period of intra-gastric infusion and does not persist during the hour after the infusion is stopped. A similar effect is noticed with an infusion of red chilli powder at a dose of 1.6 g/hr. Inhabitants of some tropical countries consume up to 3 g of chillies every day. The study by Desai et al in 1976 answers a few questions about the effect of this popular spice on the stomachs of these people.

Respiratory effects

The first effect of inhaled capsaicin to be detected in human studies was cough and this was suppressed by lignocaine applied locally to the larynx (Collier and Fuller, 1984). Capsaicin acts by stimulating the Bezold-Jarisch reflex and when given by a nebuliser causes a transient increase in respiratory drive, which is soon lost (Maxwell et al, 1987). In human subjects treated with atropine and propranolol the effect of the directly acting spasminogen leukotriene D4 (Ayala et al, 1988) can be reversed by inhaled capsaicin (Lammers et al, 1988) thus indicating a nonadrenergic-noncholinergic action of capsaicin. A transient increase in airway resistance measured by body plethysmography (Fuller et al, 1985) or forced oscillation occurs in response to inhaled capsaicin (Fuller, 1991). This response is not affected by antitussive doses of lignocaine (Fuller, 1991; Choudry et al, 1989) which indicates that the nerves or receptor sites involved may be different for capsaicin and lignocaine.

Effects when administered parenterally

It is difficult to perform studies of parenteral dosing of capsaicin in man. Low doses have been injected intravenously in human volunteers to study its effects on cardiovascular reflexes (Winning et al, 1986). Pain has been reported in the skin especially in the genitalia and chest. Surprisingly no detectable changes in pulse or blood pressure have been noticed. Studies in anaesthetised man are yet to be performed.

2.4 The ionic basis for the effects of capsaicin

It is well known that transmitter release from both central and peripheral endings of CSPA requires extracellular calcium ions (Maggi et al, 1989a). Capsaicin evoked depolarisation involves an increased conductance of cations, both Na^+ and Ca^{++} (Marsh et al, 1988). Removal of Ca^{++} from the medium does not prevent this action, however removal of Na^+ reduces the depolarising action of capsaicin on the rat vagus by about 60%. Wood et al have demonstrated that capsaicin activates a type of cation channel which is expressed on the membrane of primary afferents at both somal and axonal level (Wood et al 1988).

Peptide release by capsaicin requires substantially lower concentrations of Ca^{++} than is necessary for depolarisation (Maggi et al, 1988a). The peptide release evoked by capsaicin in low Ca^{++} medium is enhanced by removal of Mg^{++}. Mg^{++} ions also block the capsaicin-evoked uptake of radioactive Ca^{++} by cultured dorsal root ganglion (DRG) neurons (Wood et al, 1988). Since the action of capsaicin can be found on isolated membrane patches (Wood et al, 1988), intracellular messengers are unlikely to be primarily involved in its excitatory action on sensory afferents. A specific increase in cyclic-GMP follows capsaicin induced depolarisation of rat DRG neurons but elevated cyclic-GMP levels do not contribute to capsaicin-evoked ionic fluxes. No change is observed in cyclic-AMP levels in rat sensory neurons in culture (Wood et al, 1989).

The depolarising action of capsaicin and transmitter release are unaffected by blockers of voltage sensitive Ca^{++} channels of the N, L or T type (Maggi, 1991a); however the L type Ca^{++} channel agonist Bay K 8644 might enhance

the response to capsaicin (Dray et al, 1989). Nickel ions block the capsaicin-evoked uptake of radioactive Ca^{++} in neuronal culture (Wood et al, 1988). However nickel chloride does not affect the capsaicin induced Ca^{++} dependent release of neuro peptides (Maggi et al, 1988a).

It seems that capsaicin interacts with a specific receptor expressed on the plasma membrane of certain primary afferents leading to the opening of a cation channel which admits both Na^{+} and Ca^{++}. The resultant depolarisation generates an afferent impulse and the Ca^{++} entry leads to release of neuropeptides from the same terminals. The conduction of these impulses probably involves tetrodotoxin (TTX) sensitive fast Na^{+} channels. The release of neuropeptides is thought to be TTX resistant.

2.5 The therapeutic applications of capsaicin

Capsaicin has been used topically to treat a variety of disorders, which are seemingly unrelated. Most of the studies have been attempts to relieve pain due to different reasons. There are three important considerations in this regard:

1. The therapeutic uses of capsaicin in human beings have developed following animal experiments. It is however difficult to extrapolate directly from animals to humans. Most animal studies have used systemic treatment, direct application to nerve trunks or injection into tissues and the dosage used is usually about 10 times or more greater than in human trials. The biphasic action of capsaicin, which involves selective excitation followed by desensitization of C-afferents, is the underlying principle behind its use in man. It has been noted that pain relief with topical capsaicin often takes 1 -2 weeks to become apparent. This may either be because of the low concentrations used in humans or due to the fact that in some chronic painful conditions there is increased excitability of the central nervous system due to abnormal C-fibre inputs (Lynn, 1990).

2. The pungency of capsaicin is obvious to both administrator and recipient making blinding extremely difficult (Fowler et al, 1994). Lignocaine applied prior to capsaicin can act as a local anaesthetic without preventing desensitization of sensory nerve endings (Maggi, 1991a) however the pungency of capsaicin as opposed to vehicle alone is still a factor which cannot be eliminated.

3. Minimal systemic absorption occurs after topical application (single or repeated) of effective local concentrations of the drug and no permanent damage to tissues occurs in this way (Maggi, 1991a).

Table 1 Studies reporting the clinical efficacy of capsaicin

Disease	Site of application	Dose of capsaicin	Improvement	Reference
Cold and heat urticaria	Skin	1%	100%	Toth-Kasa et al, 1983
Psoriasis vulgaris	Skin	0.01-0.025%	68%	Bernstein et al, 1986
Cutaneous allergy	Skin	30 mM	100%	Lundblad et al, 1987
Post herpetic neuralgia	Skin Skin	0.025% 0.025%	75% 78%	Bernstein et al, 1987 Peter et al, 1988
Vasomotor rhinitis	Nasal mucosa Nasal mucosa	13 μM 10-100 μM	100% 100%	Marabini et al, 1988 Saria & Wolf, 1988
Cluster headache	Nasal mucosa	10 mM	77%	Sicuteri et al, 1989
Postmastectomy pain	Skin	0.025%	86%	Peter et al, 1989
Hypersensitive bladder	Intravesical	10 μM	100%	Maggi et al, 1989b Barbanti et al, 1995 Lazzeri et al, 1996
Detrusor hyperreflexia	Intravesical Randomised trial	1-2 mmol/l 1-2 mmol/l 1-2 mmol/l 1 mmol/l 1mmol/l 1mmol/l 1mmol/l	71%	Fowler et al,1992a; 1994 Chandiramani et al, 1996 Dasgupta et al, 1996a De Ridder et al, 1997 Cruz et al, 1997a Wiart et al, 1998 de Sèze et al, 1998
Loin pain/ haematuria synd	Intraureteral	1 mmol/l	100% (loin pain)	Bultitude, 1995
Spinal cord injury	Intravesical	2 mmol/l 1-2 mmol/ l 0.1-2mmol/l	90%	Geirsson et al,1995 Igawa et al, 1996 Das et al, 1996

CHAPTER 3

THE VANILLOID RECEPTOR

A peripheral vanilloid receptor in the urinary bladder of the rat was first characterised by Szallasi et al in 1993 (Szallasi et al, 1993a; 1993b). Specific binding of RTX is thought to represent the site of this receptor. Since homovanillic acid is the key structural motif shared by capsaicin and RTX their recognition site appears to be best termed the vanilloid receptor (Szallasi, 1994). This receptor has been compared to similar receptors in the dorsal root ganglia (DRG) as well as spinal cord of the rat. Capsaicin inhibits RTX binding to the receptors in the urinary bladder, DRG and spinal cord with similar potency. Interestingly RTX binds to the bladder in a non-cooperative manner as opposed to the apparent positive cooperativity of RTX binding in both DRG and spinal cord. This suggests heterogeneity in the properties of the vanilloid receptors at different sites. The binding of RTX to the central receptors seems to be of a higher affinity when compared to those of the periphery. The size of the central vanilloid receptors is 270 KiloDaltons as measured by radiation inactivation and the high affinity cooperative binding suggests a receptor structure with cooperating subunits. RTX binds to these receptors with a higher affinity than capsaicin.

In their study however, Acs et al (1994) failed to demonstrate receptor heterogeneity and suggested instead that the vanilloid receptors present at different parts of the primary afferent neuron are similar. They found that the specific binding of RTX to the urinary bladder, DRG, spinal cord, dorsal vagal complex and sciatic and vagal nerves in the rat all followed sigmoidal

saturation kinetics indicating positive cooperativity among the binding sites. They failed to show any major differences in the affinities of capsaicin and capsazepine in inhibition of RTX binding at different sites (Acs et al, 1994).

Activation of the vanilloid receptor causes a depolarisation, which is due to an increase in cation permeability. The response is biphasic with the immediate effect being stimulatory, sensed as a painful irritation and a peripheral release of neuropeptides including SP and CGRP. This is then followed by long lasting functional changes in the afferent C-fibres.

The characteristics of the vanilloid receptor appear to differ in different species of animals. In the guinea pig these receptors bind RTX with lower affinity than in the rat. The receptor density however is higher in guinea pigs. The binding sites in the hamster and the rabbit show low levels of specificity. This is in keeping with the marked sensitivity of the guinea pig to vanilloid actions and the relative resistance in the hamster and rabbit to homovanillic acid derivatives (Szallasi et al, 1994).

RTX induces a dose-dependent loss of vanilloid receptors in the urinary bladder and spinal cord of the rat (Goso et al, 1993). This effect is reversible in the capsaicin-sensitive neurons present in the bladder but not so in the spinal cord.

There also appear to be regional differences in receptor density in the same organ. In normal rats the receptor density is 1.7 fold higher in the neck than in dome of the urinary bladder. Pelvic and hypogastric nerve resections result in 90% and 25% loss of these RTX-binding sites respectively, whilst pudendal nerve section induces no such change (Szallasi et al, 1993b).

In post-mortem human spinal cord specific RTX-binding sites can be detected and their binding parameters are similar to those determined in the guinea pig spinal cord. It is not known as yet whether this receptor is operated by endogenous ligands or to which receptor superfamily, if any, it belongs (Szallasi et al, 1994).

Caterina et al have recently used an expression cloning strategy to isolate a cDNA encoding the capsaicin receptor, also called the vanilloid receptor subtype 1 or VR1 (Caterina et al, 1997). This is a non-selective cation channel whose amino-acid sequence is related to the transient receptor potential (TRP) family of proteins. When activated the channel opens, allowing an influx of calcium and sodium ions. About ten calcium ions enter for every sodium ion. This depolarises neuronal pain fibres initiating an impulse through the dorsal root ganglion to the brain. Activation of the same receptor channel explains why the mouth feels hot when eating chillies (Clapham, 1997).

Excessive ionic influx is also thought to be responsible for cell death caused by capsaicin. This cytotoxicity is necrotic and not apoptotic. It is the presence of the VR1 receptor, which determines whether a cell would die as a result of exposure to capsaicin. Even non-neuronal cells transfected with VR1 died when treated with capsaicin while those lacking the receptor did not (Caterina et al, 1997).

CHAPTER 4

THE EFFECTS OF CAPSAICIN ON THE URINARY BLADDER

4.1 Animal studies

In 1962, David Annis, in an Arris and Gayle lecture at the Royal College of Surgeons of England, described studies dealing with the physiological properties of the urothelium and one of these was the effect of intravesical instillation of a solution of capsicums on the dog urothelium. His experiments showed a decrease in the functional bladder capacity during and immediately after such instillation (Annis, 1962). This observation indicates that the immediate effect of intravesical capsaicin on the bladder is excitatory and is only later followed by an inhibitory effect. The response of the bladder to capsaicin is biphasic.

The majority of literature available regarding the effects of capsaicin on the urinary tract deals with animal experiments. This work has been done by Dr. Carlo Maggi's team in Italy and Dr. W C de Groat's team in Pittsburgh. Capsaicin sensitive primary sensory neurons supplying the rat urinary bladder have been found in two groups of spinal ganglia located in the T13-L2 and L6-S1 segments. These neurons terminate within Rexed's laminae I, V, X and in the dorsal gray commisure of the lumbosacral spinal cord.

Treatment of newborn rats with capsaicin causes a selective and permanent degeneration of unmyelinated sensory fibres containing immunoreactive SP. After giving capsaicin (50 mg/kg) to newborn rats, the SP content of the skin, oral and nasal mucosa decreases by 66-75% while that of the urinary

bladder reduces by 60-84%. The sensory innervation of the bladder in capsaicin treated rats has been studied using retrograde tracing and immunocytochemical techniques. Five days after injection of the fluorescent tracer True Blue into the bladder wall numerous labelled cells can be found in the dorsal root ganglia of the nerves supplying the bladder. In capsaicin treated rats the number of labelled cells reduces by over 50%. In addition SP is virtually absent in the same animals. In these rats a tendency towards developing urinary retention in later life has been noted and it has therefore been suggested that this is due to an impairment of the normal micturition reflexes by capsaicin (Sharkey et al, 1983). One day after intrathecal capsaicin 50% of rats develop a blockade of the micturition reflex (Durant and Yaksh, 1988). Systemic capsaicin is also known to inhibit the contractile response of the detrusor to topically applied capsaicin (Holzer et al, 1984). Administration of capsaicin to newborn rats produces a significant increase in bladder weight (Lee and Wong, 1989). Capsaicin pretreatment of adult animals increases the micturition threshold and bladder capacity indicating a possible functional impairment of the sensory arc of the micturition reflex (Santicioli et al, 1985).

In vivo studies in urethane anaesthetised rats have shown that topical capsaicin and SP produced similar excitatory effects in both quiescent and rhythmically contracting bladders, which have two components: tetrodotoxin (TTX) resistant tonic contractions followed by TTX sensitive phasic contractions (Maggi et al, 1984). The former is a myogenic contractile activity which is absent at birth but develops postnatally and the latter appears 10 days after birth and thereafter increases in parallel with the ability of the

bladder to respond to distension (Maggi et al, 1986a). The TTX sensitive component is abolished by spinal cord transection indicating the involvement of a supraspinal micturition reflex (Maggi et al, 1986b). The TTX resistant component on the other hand is thought to be due to neuropeptide release from the sensory nerves (Santicioli et al, 1987) and is thus thought to represent the "efferent" function of these capsaicin sensitive nerves (Maggi and Meli, 1988).

The SP antagonist pro4, trp7, 9, Leu11 SP-(4-11) selectively antagonises contractions produced by either capsaicin or SP on the isolated rat urinary bladder (Maggi et al, 1985). It has been suggested from observations in acute spinal rats that measurement of SP like immunoreactivity in the bladder may be a useful biochemical index for monitoring the effects of capsaicin (Maggi et al, 1987a).

Calcitonin gene related peptide (CGRP) is another presumptive sensory neurotransmitter, which can be depleted in the female rat urogenital tract by neonatal treatment with capsaicin (Ghatei et al, 1985). A similar reduction in CGRP immunostaining has been noted in adult guinea pigs (Su et al, 1986) and newborn rats (Wimalawansa, 1993). Lowering the pH of the surrounding fluid markedly increases the CGRP like immunoreactivity from isolated guinea pig detrusor and this increase is abolished by pretreatment with capsaicin (Geppetti et al, 1990). Hypertonic sodium chloride (Del-Bianco et al, 1992) and Veratridine (Tramontana et al, 1992) induce CGRP release from capsaicin sensitive nerves which is dependent on the influx of

extracellular calcium via channels which are insensitive to nifedipine, omega -conotoxin and ruthenium red.

Capsaicin also induces a loss of VIP from the dorsal horn of nerves supplying the urinary bladder (Gibson et al, 1986). Pituitary adenylate cyclase activating peptide (PACAP) is a VIP like substance which is found in the suburothelial nerves in close relation to CGRP and SP. Immunocytochemical studies have shown that capsaicin reduces PACAP densities in the rat urinary bladder (Moller et al, 1993; Fahrenkrug and Hannibal, 1998).

The ability of topical tachykinins like neurokinin B (NK-B) to activate the micturition reflex can be largely impaired in 2 month old rats pretreated with capsaicin (50 mg/kg) and it is possible that a NK-B receptor located on sensory nerves in the bladder wall, participates in this tachykinin induced activation of the micturition reflex (Maggi et al, 1987b). Thiorphan, a well-known inhibitor of enkephalinase enhances the capsaicin-induced contraction of detrusor strips, which is thought to be mediated by the release of endogenous tachykinins. Furthermore co-administration of Spantide (a SP inhibitor) and L-659, 877 (a neurokinin NK-2 selective receptor antagonist) can reduce the response to capsaicin in the rat isolated urinary bladder (Maggi et al, 1991b). A loss of NK-1 receptors in the sensory terminals of the urinary bladder after capsaicin has also been reported (Geraghty and Burcher, 1992). The NK-1 receptor antagonist GR 82, 334 blocks the chemonociceptive micturition reflex induced by topical application of capsaicin (Lecci et al, 1992). Intrathecal administration of the NK-2 receptor

antagonist SR 48,968 reduces bladder contractions induced by intravesical capsaicin while the NK-1 receptor antagonist RP 67, 580 demonstrates no such effect (Ishizuka et al, 1994). The same NK-2 receptor antagonist given intraarterially near the bladder counteracts bladder hyperactivity induced by intravesical capsaicin. These findings do seem to contradict each other to a certain extent.

Autoradiographic localisation studies of binding sites for SP, NK-A and CGRP have been performed in the rat urinary bladder. Dense labelling for SP and NK-A occurs in the smooth muscle and in addition SP is found around submucosal blood vessels. In these ligand-binding studies no differences occur between sections of bladder from capsaicin and vehicle pretreated rats. CGRP binding on the other hand is noted mainly in the epithelium and blood vessels but not over smooth muscle. The density of CGRP binding increases after chronic capsaicin pretreatment suggesting receptor upregulation (Banasiak and Burcher, 1994).

Topical bradykinin applied to the serosal surface of the rat urinary bladder causes low amplitude tonic contractions and high amplitude phasic contractions; these phasic contractions are reduced by systemic pretreatment with capsaicin (Lecci et al, 1995). In adult rats that have been neonatally treated with capsaicin a marked fall in c-fos activation in the intermediolateral gray column and the dorsal commisure of L5-S1 spinal segments by mechanical or chemical noxious stimuli has been noted (Cruz et al, 1994).

The functional role of capsaicin sensitive innervation of the rat bladder has been studied using transvesical cystometrogram. Desensitization by capsaicin (50-125 mg/kg, 4 to 60 days before experimentation) significantly increases both the volume and pressure thresholds for micturition, indicating functional impairment of the mechanisms which transmit volume information from the bladder to the central nervous system. However an increase of infusion rate above the physiological range produces micturition cycles with normal volume and pressure thresholds (Maggi et al, 1986c). These findings suggest that at high values of volume/pressure thresholds, micturition could be initiated by capsaicin resistant mechanisms and therefore there must be multiple sensory systems relaying volume/pressure signals from the bladder to the central nervous system. Maggi and Conte have also noted that these effects are more evident in urethane anaesthetised rats (Maggi and Conte, 1990). Other investigators have found that in conscious adult rats, although capsaicin does deplete both SP and CGRP, there are no functional changes in the micturition patterns and cystometric investigations. The same authors have noted increased responses to carbachol and electrical stimulation of detrusor after capsaicin treatment, which may indicate the development of supersensitivity to muscarinic stimulation (Malmgren et al, 1990). Maggi's findings in urethane anaesthetised rats have also been contradicted. In their study in rats, de Groat's group (Cheng et al, 1993) did not find significant changes in various parameters of urinary bladder function, including micturition volume, amplitude, duration and interval between reflex bladder contractions after pretreatment with capsaicin (125 mg/kg s.c 4 days before the experiment). A large dose of capsaicin (50 mg/kg) during the experiment

did cause an acute block of bladder activity that persisted for 8-15 hours. Capsaicin pretreatment was found to significantly reduce the arterial pressor responses accompanying reflex bladder contractions (Cheng et al, 1993). Cystometrograms carried out in rabbit urinary bladder before and after capsaicin have shown no change (Harrison et al, 1990), findings which are in direct contradiction to Maggi's results in rats. This raises the possibility of species related differences in response to capsaicin treatment.

There are significant species related differences with regard to the functions mediated by the capsaicin sensitive neurons in the urinary bladder (Maggi et al, 1987c). Within species there are regional differences in the motor response to the drug. Capsaicin induced contractions of isolated strips of the guinea pig bladder have been found to be more evident in the dome than in the bladder neck. This response in the dome can be antagonised by a SP antagonist whilst that in the neck can be blocked by anti CGRP serum (Maggi et al, 1988b).

Systemic capsaicin also causes an inflammatory response in the rat urinary tract and this leads to plasma extravasation, which can be measured by the leakage of Evans Blue around the vesical tissues. This "neurogenic inflammation" can be induced by antidromic electrical stimulation of the dorsal roots of nerves supplying the bladder and thus makes it a suitable method for mapping the organs where capsaicin sensitive nerve endings exert their function (Pinter and Szolcsanyi, 1995). Budenoside reduces this extravasation by capsaicin in the urinary bladder in a dose dependant fashion.

The effects of a number of other substances have been linked to capsaicin. Nicotine induces a contraction of the rabbit urinary bladder and this response is reduced by capsaicin (Kizawa et al, 1988). Cadmium chloride also induces contractions of the isolated rat urinary bladder by activation of capsaicin sensitive afferent nerves (Patacchini et al, 1988). Topical application of prostanoids on the serosal surface of the bladder in urethane anaesthetised rats activates reflex micturition and intravesical instillations of PGE2 lowers the threshold for reflex micturition; both these effects can be blocked by systemic capsaicin administration. N-formyl-methionyl-leucyl-phenylalanine (FMLP), a synthetic analogue of a chemotactic peptide derived from a variety of bacteria, activates capsaicin sensitive afferent nerves in the guinea pig urinary bladder via prostanoid generation. Subcutaneous treatment with acrylamide causes urinary retention and overflow incontinence along with abolition of the sensory nerve mediated response to capsaicin. Bladder nerves have been found to be depleted of SP and CGRP following acrylamide therapy (Abelli et al, 1991). Cystitis induced by cyclophosphamide in rats is reportedly mediated via capsaicin sensitive pathways (Ahluwalia et al, 1994).

The ability of the mast cell degranulating substance 48/80 to activate the 'afferent' and 'efferent' effects of capsaicin in the rat urinary bladder has been studied. In vitro, this substance produced a calcium dependant release of CGRP, which is prevented by capsaicin desensitization. In vivo, topical application of 48/80 on the serosal surface of the bladder produces contractions, which are hexamethonium sensitive. This effect too can be abolished by systemic capsaicin desensitization (Eglezos et al, 1992).

Of interest is the discovery of the neuropeptide secretoneurin, which is derived from secretogranin II (Sg II). This is one of the newest among the many neuropeptides stored in and released from capsaicin-sensitive C-afferents. Its distribution in the spinal cord of the rat and human post-mortem tissues seems to be very similar. Capsaicin treatment causes a marked depletion of secretoneurin in the substantia gelatinosa, but not in other immunopositive areas of the spinal cord and a loss of small (<25 microns) Sg II containing dorsal root ganglia neurons (Kirchmair et al, 1994).

When applied to the outer surface of the urinary bladder, capsaicin causes transient bradycardia and hypotension and this is thought to be due to activation of a spinal sympathetic cardiovascular reflex since neither atropine nor cervical vagotomy can abolish this response (Giuliani et al, 1988).

There is some controversy as to whether repeated treatment eventually leads to desensitization to capsaicin. Those who do believe it happens report that a second application of capsaicin to strips of guinea pig detrusor has no effect on the release of SP and CGRP like the first dose does (Maggi et al, 1988c). Recently it has been reported that repeated instillations of intravesical capsaicin in conscious female Sprague-Dawley rats do not result in self-desensitization (Ishizuka et al, 1995).

More recent studies have focused on the capsaicin like activity of some naturally occurring pungent substances like piperine, mustard oil, eugenol and curcumin on peripheral endings of visceral primary afferents. All test substances dose dependently contract the rat bladder and produce desensitization like capsaicin. Development of cross tachyphylaxis among

the natural pungent substances and capsaicin suggests a common site of action. The presence of an acrylamide linkage (as in capsaicin but not in the other compounds) does not seem to be essential for desensitization of sensory nerve terminals (Patacchini et al, 1990).

There are two so called capsaicin antagonists: ruthenium red and capsazepine. The former acts by a non-competitive mechanism whereas capsazepine acts as a competitive antagonist at the vanilloid receptor sites. Ruthenium red is thought to have a protective action towards capsaicin desensitization of sensory nerves (Maggi et al, 1988d) and reduces the capsaicin induced release of SP from sensory nerves (Maggi et al, 1988d). It does not however influence the initial excitatory effects of capsaicin on visceral afferents. This substance can be used as a pharmacological means of differentiating between the acute and chronic effects of capsaicin (Amann et al, 1990).

The contractility of the rat urinary bladder to electrical field stimulation (EFS) after sensory denervation with capsaicin has also been studied. EFS evoked contractions have two components: a cholinergic component which can be blocked by atropine and a purinergic one which can be blocked by desensitization of P2x purinoceptors with alpha beta methylene ATP. In capsaicin treated rats the field stimulation evoked contractions are significantly larger than those treated with vehicle and this difference remains after addition of alpha beta methylene ATP. However when treated with atropine the difference between the two groups at 8 and 16 Hz can be

abolished while the contractions induced by capsaicin are significantly larger at frequencies of 2 and 4 Hz (Ziganshin et al, 1995).

The behavioural response induced by intravesical instillation of capsaicin has been investigated in catheter implanted, freely moving male and female rats. Intravesical capsaicin (25 nmol/ rat) evokes an intense licking of the lower abdominal and perineal skin, which lasts for about 15 mins. This is reduced by pretreatment with capsaicin at a dose of 150 mg/kg but not by a lower dose of 50 mg/kg. Placing a ligature around the proximal urethra 24 hours prior to the experiment in order to avoid contact of capsaicin with the urethra can almost abolish this response. Intravesical lignocaine (100 mM) abolishes the licking as well as the micturition contractions induced by intravesical capsaicin (Lecci et al, 1994).

4.2 Capsaicin and resiniferatoxin (RTX): similarities and differences

RTX like capsaicin is a homovanillic acid derivative (Szallasi and Blumberg, 1989). RTX is 1000 times more potent than capsaicin. The behavioural responses to the excitatory and desensitising effects of both agents when given intravesically have been studied in the rat. Abdominal licking and head turning occur significantly more often in RTX treated rats when compared to vehicle; a second injection of RTX 60 mins later does not increase this response indicating desensitization. Cross-desensitization has also been noted between capsaicin and RTX. The cation channel blocker ruthenium red antagonises the excitatory and desensitising effects of both drugs indicating that both act through a common mechanism involving cation channel activation (Craft et al, 1993).

In rats, the magnitude and duration of action of intravesical RTX depend primarily on the dose and number of exposures; duration of exposure and interval between exposures are less important determinants (Craft and Porreca, 1994a). Histological examination of bladder tissue indicates that both capsaicin and RTX produce inflammation, which diminishes in a dose and time dependent manner.

RTX is much more potent than capsaicin and the cystometric changes induced by both drugs in Sprague-Dawley rats have been studied (Ishizuka et al, 1995). Instillations of RTX can facilitate micturition but repeated doses for over 6 days cause desensitization to RTX. This apparently does not occur with capsaicin.

In an interesting study Craft and Porreca have demonstrated that the local anaesthetic tetracaine attenuates the irritancy without having any effect on the desensitization of sensory afferents produced by RTX (Craft and Porreca, 1994b). This would indicate that one could safely instill a local anaesthetic like lignocaine into the bladder prior to capsaicin or RTX, which would then decrease the irritancy without actually affecting the 'deafferenting' effect.

RTX is now being considered as an alternative to capsaicin for therapeutic purposes. Recently reported human trials indicate that unlike capsaicin, the suprapubic discomfort during RTX instillation is minimal and the initial worsening of irritative urinary symptoms after capsaicin instillation is not noticed (Cruz et al, 1997b; Chancellor and de Groat, 1999).

CHAPTER 5

NEURONAL CHANGES DUE TO SPINAL LESIONS

5.1 The pathophysiology of detrusor hyperreflexia

In humans, urinary bladder reflexes are mediated through the sacral segments S2-4 and these are directly connected to the pontine centre by the spinobulbospinal pathways. The pontine centre (Barrington, 1925) receives further inputs from cortical centres. Detrusor hyperreflexia can result either from loss of the normal inhibitory input from higher centres onto the pontine micturition centre or following a spinal lesion due to interruption of the spinobulbospinal pathways which normally control physiological bladder behaviour.

The nerves to the lower limbs emerge from the spinal cord at a level higher than the nerves to the urinary bladder (Fig. 12). This means that suprasacral spinal lesions affect both the legs and the bladder. The effect of this for the patients can be most unfortunate since their bladder control deteriorates at the same time as their mobility worsens. In patients with detrusor hyperreflexia due to demyelinating disease like multiple sclerosis (MS), the severity of urinary symptoms are related to the extent of pyramidal dysfunction in the lower limbs so that both problems are thought to reflect the extent of spinal involvement (Betts et al, 1993).

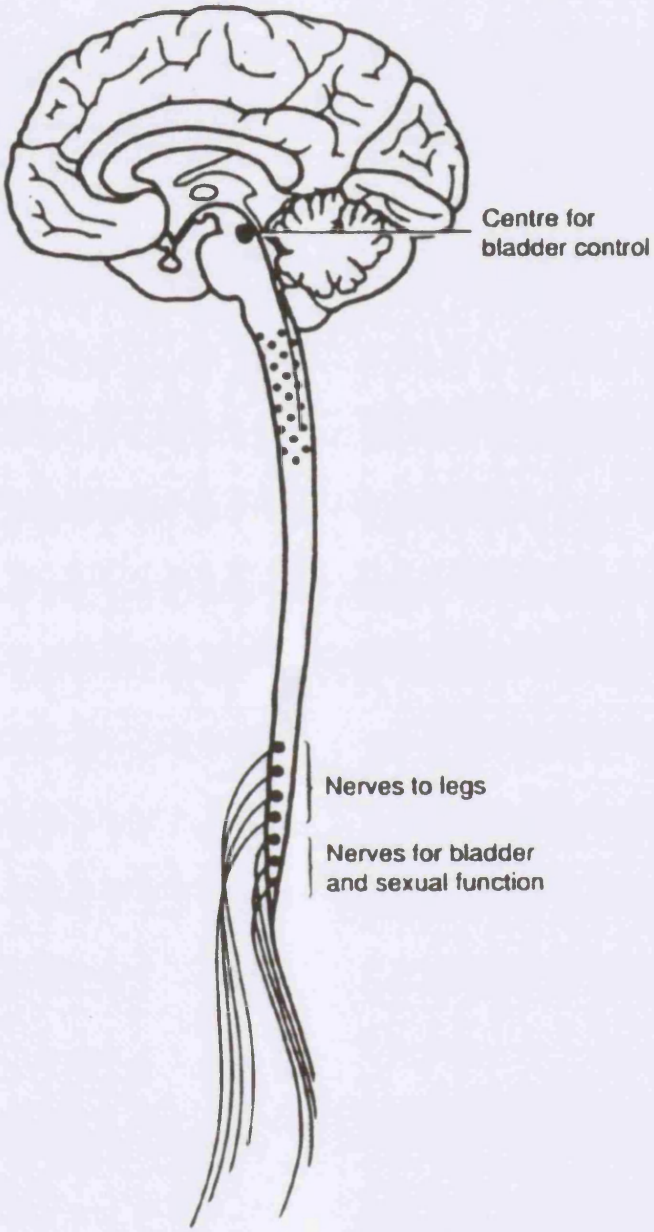


Fig. 12 Diagrammatic representation of the neural control of the bladder. Spinal cord disease as shown by the stippling affects the bladder as well as the legs. Reproduced with permission of Dr Clare Fowler

5.2 The recovery of urinary bladder function in spinal animals

Micturition in cats and rats with an intact neuraxis depends on a similar spinobulbospinal reflex. Electrophysiological studies in these animals (de Groat, 1975; de Groat et al, 1969; Mallory et al, 1989) have shown that micturition is mediated by a reflex pathway of long latency which passes through a relay centre in the rostral pons. A suprasacral transection blocks this reflex pathway and initially stops the release of urine. After a few weeks there is an emergence of spinal reflex mechanisms which initiate automatic micturition in chronic spinal animals (de Groat et al, 1990). In a series of experiments involving normal and spinal cats (cord transected at T8-T12) de Groat et al (1990) studied the afferent pathways from the bladder to the spinal cord using axonal tracing techniques. In normal animals both myelinated Aδ-afferents and unmyelinated C-afferents (60% of cats) were present. Neurophysiological techniques showed the latency of the C-fibres to be longer (180-200 ms) than that of Aδ-fibres (100-150 ms). Transection of the cord abolished both these reflexes immediately; however in chronic spinal cats 2-14 weeks later the C-fibre evoked reflex reappeared whereas the Aδ-evoked reflex never recovered (Fig. 13).

The role of these C-fibre afferents was further studied using capsaicin. In normal cats capsaicin administered systematically in large doses (30-45 mg/kg s.c) did not block reflex contractions of the bladder or the Aδ-evoked bladder reflex. However in chronic spinal cats (3-6 weeks after spinal transection), capsaicin in a dose of 20-30 mg/kg s.c completely blocked the rhythmic bladder contractions induced by bladder distension and C-fibre evoked long latency reflex firing recorded on vesical postganglionic nerves.

This effect occurred within 10-20 mins of injection and persisted for the entire duration of the experiment.

Similar experiments have also been performed in normal and chronic spinal rats (Cheng et al, 1987). In both categories of animals large doses of capsaicin (30-70 mg/kg s.c) blocked the micturition reflex within 10-15 mins and this persisted for 18-24 hours. Following recovery from the initial effect of capsaicin there was a residual effect, which became evident as a small increase in the bladder capacity and a reduction of arterial pressor responses associated with bladder contractions. This effect lasted for 4-7 days following the administration of capsaicin in both normal and chronic spinal rats.

In many species, neonatal micturition depends on somatic afferent input and voiding relies on an exteroceptive somato-bladder reflex triggered by stimulation of the perineal skin. This corresponds to a behavioural response whereby the mother licks the perineum of her newborn to initiate micturition. Separation of the mother from her offspring results in retention of urine. During postnatal development this excitatory somato-visceral response diminishes and is replaced by an inhibitory reflex whereby stimulation of the cutaneous regions corresponding to sacral dermatomes delays detrusor contraction. Spinal cord injury in adults causes in a re-emergence of the neonatal excitatory somato-bladder reflex (de Groat et al, 1998). This is associated with an expansion of the afferent terminals in the dorsal horn of the spinal cord, probably as a result of axonal sprouting (Steers, 1992).

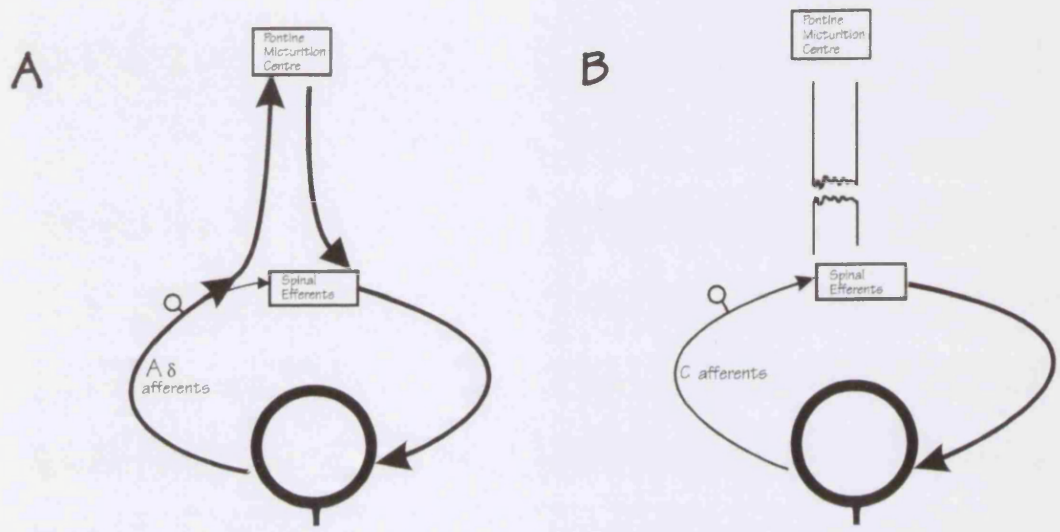


Fig. 13 Effect of spinal cord disease on bladder afferents. In the spinally intact animal the main afferent input from the bladder is via Aδ fibres. After a spinal lesion C fibres take over as the functionally significant afferents and have a direct effect at the sacral cord level. Based on animal studies by Dr de Groat and reproduced with permission of Dr Clare Fowler and the British Journal of Urology

5.3 The role of vasoactive intestinal polypeptide (VIP) in C-fibre afferent pathways in animals

In the cat VIP is present in up to 25% of the sacral dorsal root ganglion cells innervating the urinary bladder. Moreover the spinal cord at sacral levels contains VIP almost exclusively in the C-fibre afferent pathways (Honda et al, 1983). Immunohistochemical localisation and electron microscopy have confirmed these findings. These data together with the demonstration of the importance of the C-fibre afferents in initiating micturition in chronic spinal cats led to investigations on the effects of exogenous VIP on the micturition reflex (Kawatani et al, 1987).

The intrathecal administration of VIP in normal cats was found to depress reflex bladder contractions and firing of bladder postganglionic nerves. These effects were noted at doses of 1-10 μg, appeared 2-5 mins after injection and lasted for 5-30 mins depending upon the dose. When administered in smaller doses VIP had no effect. In chronic spinal cats VIP in doses of 0.1-1μg facilitated bladder activity whereas large doses (2-10 μg) still depressed vesical activity. It thus seems that the emergence of C fibre bladder reflexes is associated with the appearance of spinal excitatory actions of VIP, a putative C-fibre afferent transmitter.

Spinal cord transection also changed the distribution of VIP containing afferents in the sacral spinal cord. In normal cats VIP like immunoreactivity is most prominent in Lissauer's tract and in a band of fibres in the lateral lamina of the dorsal horn (de Groat, 1990). Transverse sections of the sacral cord in chronic spinal animals (below the level of transection) revealed that the band of VIP fibres in the lamina I was significantly wider than in normal or acute

spinal cats (85 μm as opposed to 40 μm). In addition VIP immunoreactivity in the ventrolateral lamina I of spinal cats formed a continuous band in the rostrocaudal axis unlike normal cats where VIP fibres were arranged in a discontinuous manner, the dorsoventral axons being spaced at 180-225 μm intervals along the length of the cord. Thus VIP seems to play an important role in chronic spinal cats in close association with the emergence of unmyelinated C-afferents. Its exact role in the bladders of these animals and the response to capsaicin remains unknown.

5.4 Detrusor hyperreflexia and the reorganisation of spinal reflex arcs in humans

Suprasacral spinal cord injury, MS, spinal arteriovenous malformations, tropical spastic paraparesis (TSP), transverse myelitis, spinal tumours, or any other cause of a cord lesion above S2-4 can affect the spinobulbospinal tracts and cause detrusor hyperreflexia. Little is known about the neurological mechanism of spinal reflexes in these patients (de Groat et al, 1990). Acute disconnection of the sacral cord from the pons results in detrusor arreflexia, which lasts for about six weeks although this period can be variable. Subsequently volume determined bladder reflex emptying becomes established. Patients with spinal cord disease are likely to have bladders of small functional capacity and develop uncontrolled detrusor contractions, which make them incontinent. Unlike animals, there is no direct proof in humans that the emergent reflex arc in these conditions is largely mediated via C-fibre afferents. It is a speculative hypothesis that spinal humans develop an unmyelinated C-fibre reflex similar to spinal cats or rats. Herein lies the justification of treating detrusor hyperreflexia with intravesical capsaicin. Since the C-fibres in animals were found to be capsaicin sensitive (de Groat et al, 1990) the use of this substance for intractable detrusor hyperreflexia in humans unresponsive to other conservative modes of treatment was tried at the National Hospital for Neurology and Neurosurgery (Fowler et al, 1992a). The results have so far been encouraging and provide strength to the above hypothesis.

In infants, voiding can be initiated by suprapubic or perineal stimulation. Just like in spinal animals, it seems that in humans, there is a re-emergence of an excitatory neonatal somato-bladder reflex, following spinal injury. This is exploited by paraplegic patients to facilitate bladder emptying ("trigger voiding") and supports the idea of reorganisation of sacral spinal centres following spinal cord injury (Steers, 1992).

Most immunohistochemical studies of the lower urinary tract in spinal cord disease have concentrated on the changes in the urethra. It seems that dense VIP immunoreactive nerves are found in the urethral smooth muscle in patients with thoracic lesions while dense adrenergic nerves are present at the same site in those with cervical injuries. However, quantitative differences in peptides like VIP in the external urethral sphincter of spinal injury patients as compared to control patients has not been noticed (Crowe et al, 1986). VIP immunoreactive nerves can be seen in normal amounts in the human bladder in 52% of MS patients while in 48% the amounts are reduced (Van Poppel et al, 1988a).

Since it is difficult to study the reorganisation at the spinal level that occurs in humans, most of our knowledge of the neuronal changes in spinal cord disease is restricted to information obtained from animal experiments.

CHAPTER 6

CAPSAICIN THERAPY FOR URINARY DYSFUNCTION

Following animal experiments a logical step was to move on to human therapy. Intravesical capsaicin (10 μmoles in saline) was used to relieve pain due to hypersensitive disorders (sensory urgency) of the lower urinary tract. On each instillation the patients felt a warm or burning sensation in the suprapubic region with an initial reduction in bladder capacity followed by a delayed improvement or disappearance of symptoms. Maggi et al reported a marked improvement in irritative urinary symptoms in four out of the five patients who received intravesical capsaicin instillation for hypersensitive bladders. The duration of clinical benefit was noted to last between 4 to 16 days and the findings were thought to indicate that capsaicin sensitive afferents determined the micturition threshold in humans (Maggi et al, 1989b). Although similar results were reported a few years later (Barbanti et al, 1995) a recent randomised study found capsaicin to be no better than placebo in relieving bladder pain (Lazzeri et al, 1996).

A pilot study of 10-250 μM of capsaicin in 1% alcohol in interstitial cystitis, showed subjective improvement in 4 of 5 patients and a trend towards decreased urinary SP level in 3 of 5 patients (Flood et al, 1997).

The first attempts to treat detrusor hyperreflexia (unstable bladder of neurological aetiology) with capsaicin were carried out at the National Hospital for Neurology and Neurosurgery in May 1990. At the time concentrations of 0.1-10 μmol/l were used but no clinical improvement was

noticed after intravesical instillations. On the advice of Dr. Bruce Lynn from the Physiology department at University College London, the dose was increased to 1 mmol/l of capsaicin. The success of the procedure in the first patient led to the use of the instillation in other patients with intractable hyperreflexia (Fowler et al, 1992a). Twelve patients with detrusor overactivity due to spinal cord disease and two other patients with detrusor overactivity of non-spinal origin were treated with intravesical instillations of 100 ml of 1-2 mmol/l of capsaicin. The response was biphasic with patients initially deteriorating for up to 2 weeks followed by clinical improvement or return to their previous state. Nine patients, all of whom had spinal cord disease, improved with this treatment and urodynamic studies in them showed an increase in mean±S.D bladder capacity from 106±57 to 302±212 ml and a fall in maximum detrusor pressure from 54±20 to 36±10 cm of water. There were no short-term side effects and the improvement in bladder function lasted for between three weeks to six months, after which patients came for repeat instillations. The effectiveness of capsaicin in improving continence appears to relate to the patients' overall neurological state because continence is achieved only in those patients who are less disabled and still able to walk (Fowler et al, 1994). A recent randomized controlled study of 1 mmol/L of capsaicin dissolved in 30% alcohol in saline against 30% alcohol alone, in patients with MS and spinal cord injury showed capsaicin to be effective independent of its vehicle (Wiart et al, 1998; de Seze et al, 1998).

Intravesical capsaicin causes a diuresis which is thought to be due to excitation of a "vesical-renal reflex". Intravesical instillation of a 10 μmolar solution of capsaicin increases the mean urinary output, glomerular filtration

rate, effective plasma renal flow, concentration of sodium and potassium in urine and prostaglandin (PG E2) excretion (Lazzeri et al, 1995).

At a dose of 2 mmols it has been found to be effective in increasing the cystometric capacity and/or decrease maximum detrusor pressure in 90% of patients with traumatic chronic spinal cord lesions. However only 30% of patients in this study obtained clinical benefit from the treatment. Immediately after capsaicin administration the ice water test became negative in 50% of these patients (Geirsson et al, 1995). A study from USA has reported symptomatic benefit from intravesical capsaicin in 60% of patients although the ice water test became negative in only a minority (Sedor et al, 1996; Das et al, 1996). Igawa et al found capsaicin to be beneficial in patients with spinal cord injury and autonomic dysreflexia. This is the only study reporting the use of general anaesthesia in these patients during capsaicin instillation (Igawa et al, 1995).

The above findings have been contradicted in a study from Denmark. Twelve patients with spinal cord disease and detrusor hyperreflexia underwent intravesical administration of 50 ml 2% lignocaine followed by either 100 ml 1 mmol/l capsaicin or 100 ml physiological saline for 30 min. Cross-over to the alternative treatment took place after 4 weeks. Burning sensation during capsaicin treatment prevented the study being conducted in a blind manner. No benefit was noticed after capsaicin treatment. Bladder biopsies taken 2 weeks after capsaicin showed more pronounced inflammation and immunohistochemical staining for SP and neuronal cell adhesive molecule showed small terminal axons and small nerve bundles in all of the biopsies (Petersen et al, 1999).

CHAPTER 7

NERVE DENSITY EVALUATION OF THE HUMAN URINARY BLADDER

Elbadawi has extensively described the autonomic intrinsic innervation of the bladder (Elbadawi, 1982). It seems that the main sensory innervation of the bladder exists as a plexus lying immediately beneath the urothelium (Dixon and Gilpin, 1987) and is therefore accessible to endoscopic biopsy.

Staining for acetylcholinesterase of endoscopic bladder biopsies has been extensively used for the diagnosis of bladder neuropathies (Parsons et al, 1980; Neal et al, 1982). The discovery of other neuronal markers (Trojanowski and Lee, 1983) and neuropeptides gave rise to other works on endoscopic biopsies (Gu et al, 1983).

So far quantitative nerve density evaluation of the urinary bladder has been performed using endoscopic cold punch biopsies. These biopsies have been compared to open bladder biopsies and good correlation has been found in the determination of mean nerve density scores by these two methods (Van Poppel et al, 1988b). Flexible fibreoptic cystoscopy has been used for over 15 years as a routine procedure for assessing urothelial changes and is almost painless under topical urethral anaesthesia in both sexes. It is now established as a minimally invasive diagnostic and therapeutic tool in urology (Fowler et al, 1984). Although biopsy forceps, which easily pass down the standard working channel, are available, bladder biopsies using this method have rarely been reported. To our knowledge we were the first to report the technique of nerve density evaluation using flexible cystoscopic biopsies (Dasgupta et al, 1997a).

A variety of stains can be used for assessing nerve densities in bladder biopsies. Two such stains are discussed here as they have been used in this study.

S 100

S 100 is a calcium binding protein closely associated with glial cells. In addition to Schwann cells (Stefansson et al, 1982), it visualises neuronal structures within the peripheral nerves with a distribution similar to that seen by neurofilament protein antibodies (Sato et al, 1984). S 100 was first described by Moore (Moore, 1965) and is so called because of its solubility in 100% ammonium sulphate at neutral pH. It may also stain some adrenergic nerves or fibres whose transmitter has not yet been discovered. Gu et al reported a convincing elevation of S 100 staining structures in neurogenic bladder (Gu et al, 1984), a finding confirmed in up to 30% of bladder biopsies from patients with multiple sclerosis (MS) (Van Poppel et al, 1989). Conventional histological appreciation of bladder biopsies is not affected by this method of staining. It is for these reasons that S 100 is often the preferred stain for nerve density evaluation in the suburothelium of the bladder (Van Poppel et al, 1988c). There has been concern that more nerves can be detected on frozen sections (acetylcholinesterase) than in fixed material (formaldehyde fixation for S100) but this is not true when the results of the two staining techniques are compared (Van Poppel et al, 1988b). Staining for neurofilaments (NF) seems to be an option but too much variability makes it unreliable for nerve density evaluation of the bladder (Van Poppel et al, 1988b).

PGP 9.5 [protein gene product]

Anti PGP 9.5 recognises a neuron associated antigen and is useful for quantitation of unmyelinated nerve fibre (UMNF) densities. Although electron microscopy (EM) has been the preferred method of determining UMNF densities, it is costly and time consuming. In their study Johnson et al have shown that UMNF density estimations using PGP 9.5 immunocytochemistry correlate excellently with those estimated by EM (Johnson et al, 1994). This technique also has the advantage of being quicker and cheaper. It enables the detection of minute axons (< 0.5 μm) and multiple axons per Schwann cell subunit but does not allow for the preparation of accurate fibre size histograms or the analysis of UMNF pathology. The analysis of nerve densities in the lamina propria of the bladder using a combination of S 100 and PGP 9.5 immunocytochemistry seems to be an attractive method for assessing the effects of a sensory deafferenting agent like capsaicin.

SECTION II

PATIENTS AND METHODS

CHAPTER 8

ETHICS, PROTOCOLS AND METHODS

8.1 Ethical approval

A medicine exemption certificate was obtained for the use of capsaicin for intravesical administration from the Medicines Advisory Committee. It was clearly stated that capsaicin was being obtained as a chemical, not a medicine and had no certification for use in humans. Its proposed use in detrusor hyperreflexia was therefore experimental. This was clearly explained to all patients treated with intravesical capsaicin.

Before embarking on this form of treatment in our centre, advice was sought from Professor A D Dayan, a toxicologist at St. Bartholomew's and Royal London School of Medicine and Dentistry, London. He scrutinised the literature on capsaicin and was of the opinion that the risk of developing bladder cancer in the long-term was minimal. Based on his advice a surveillance programme was established. Patients treated with intravesical capsaicin were informed of the possible unquantified risk of carcinogenesis, however minimal.

The following bodies approved the project:

1. **Study group**: The Joint Ethics Committee of the National Hospital for Neurology and Neurosurgery and Institute of Neurology, Queen Square, London.

2. **Control group**: The Joint Research Committee at the Institute of Urology, London and the Ethics Committee at University College London.

8.2 Selection criteria

The study involved **32 patients** over a period of five years: **20 in the study group** and **12 in the control group**. Patients in the **study group** fulfilled the following criteria:

1. Symptoms of detrusor hyperreflexia due to proven spinal cord lesion: frequency, urgency and urge incontinence.
2. Unsatisfactory response to oral anticholinergics or unable to tolerate the side effects of such medication.
3. Reasonably mobile with or without aids.
4. Able to perform CISC for incomplete emptying.
5. Clinically preserved mental function.

Patients in the **control group** fulfilled the following criteria:

1. Microscopic haematuria with no identifiable bladder pathology on flexible cystoscopy performed at the haematuria clinic as part of their investigations.
2. Normal flow rates.
3. No post-void residuals.
4. Neurologically normal.
5. Clinically preserved mental function –no quantitative tests were used.

8.3 Study protocol: capsaicin group

Informed consent was obtained from all patients. Pre-treatment evaluation consisted of history, frequency-volume charts, mid-stream urine cultures, intravenous urography in nine patients and upper tract ultrasonography in the others (to exclude upper tract dilatation). Patients with positive urine cultures were treated with antibiotics before entering the study.

Twenty patients with detrusor hyperreflexia due to spinal cord lesions (11 females, 9 males; mean 53 years (range 36-70 years); 12 MS, 4 tropical spastic paraparesis [TSP], 2 transverse myelitis [TM], 1 cervical myelopathy/cervical cord ischaemia [CM] and 1 complete spinal cord injury [SCI]) participated in the study. TSP is a myelopathy caused by the retrovirus Human T-lymphotropic virus type I (HTLV-I) in which the spinal white matter is primarily involved (Dasgupta and Hussain, 1999). All these patients had urge incontinence forcing them to use either pads (women) or uridom sheaths (men). The diagnoses are shown below.

Table 2 Diagnoses in patients treated with intravesical capsaicin

Diagnosis	Patients (n)	Males	Females
Multiple sclerosis (MS)	12	5	7
Tropical spastic paraparesis (TSP)	4	1	3
Transverse myelitis (TM)	2	1	1
Cervical myelopathy/ischaemia (CM)	1	1	0
Complete spinal cord injury (SCI)	1	1	0

All patients had two cystometries performed at 10-minute intervals using a Dantec Urodyn 5500 machine (Dantec, UK). A 10 French bladder catheter was used for filling the bladder with normal saline at room temperature; the intravesical pressure was monitored using a 5F catheter passed alongside the filling catheter and a similar rectal catheter was used for measuring the intra-abdominal pressure. Both the pressure lines were connected to external transducers positioned at the level of the upper border of the patient's symphysis pubis. The filling rate was 50 ml/min and the detrusor pressure was derived by subtraction of the intraabdominal pressure from the vesical pressure. The maximum cystometric capacity and detrusor pressure at capacity were recorded.

Bladder biopsies were taken from a rectangular area on the posterior wall of the bladder about 2 cm above the level of the ureteric orifices with the aid of a flexible cystoscope (Olympus, Keymed, Milton Keynes, UK). Twenty mls of lignocaine (2%) jelly (Instillagel, Farco-Pharma Gmbh, Cologne, Germany) was instilled into the urethra and left for 5 minutes prior to taking the biopsies. The procedure was always covered with prophylactic intravenous Gentamicin. A 21 SX flexible biopsy forceps which has jaws larger than the standard 19 SX forceps was used for taking the bladder biopsies (Figs. 14, 15). Patients were routinely questioned about the use of anticoagulants and aspirin, which were regarded as contraindications for obtaining biopsies. Strict asepsis was maintained during the entire procedure (Dasgupta et al, 1997a).

After a week the patients had an intravesical instillation of 100 ml of **1 mmol/l** of capsaicin in 30% alcohol in saline (0.3 g/l). Ms Sue Gerrard, Chief Pharmacist, purchased the capsaicin used at the National Hospital, as a powder from Fluka Chemika-Biochemika, Switzerland. 30% alcohol was used to dissolve the capsaicin powder. The solution was always freshly prepared in a fume cupboard in the Hospital Pharmacy and dispensed in 100 ml plastic packs. A Foley catheter (10-12 French; 10 ml balloon) was introduced into the urethra along with a bladder pressure measuring line. A rectal line was also put in so that the entire instillation could be carried out under cystometric monitoring.

Intravesical lignocaine (Phoenix Pharmaceuticals Ltd, UK) was used prior to capsaicin instillations in all the patients. This is in accordance with the standard protocol at the National Hospital since it has been argued that lignocaine substantially reduces the suprapubic burning sensation caused by capsaicin without affecting desensitization of the C-afferents (Chandiramani et al, 1996). The bladder was initially emptied and 40 mls of 2% lignocaine hydrochloride solution was instilled and left in the bladder for 20 minutes. The bladder was again emptied and the capsaicin solution was introduced slowly through the Foley catheter using two 50 ml syringes and left in for 30 minutes. All patients felt a suprapubic burning sensation and were asked to score this on an analogue scale of 0 ('no pain at all') to 10 ('worst pain imaginable') 5 minutes after starting the instillation and at 30 minutes.

Great care was taken to prevent leakage of capsaicin into the urethra and this was the reason for continuous monitoring of the detrusor pressure during

the instillation. Whenever the patients developed a detrusor contraction of over 20 cm of water, 5-10 ml of the capsaicin solution was withdrawn into the 50 ml syringe, which reduced the contractions and prevented any urethral leakage. A 50 ml syringe was always left attached to the Foley catheter to act as a safety valve against any sudden rise in detrusor pressure, in which case some of the capsaicin solution would flow back into the syringe. The bladder was emptied at the end of the procedure. This protocol has been described previously (Chandiramani et al, 1996).

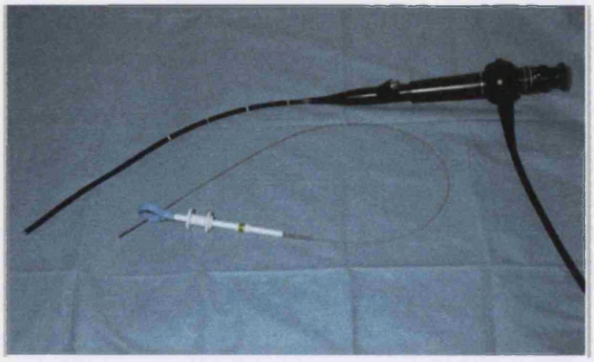
Patients returned to the department after 6 weeks when they were asked about their urinary symptoms and completed frequency-volume charts. The response to capsaicin was considered excellent if patients were continent most of the time, satisfactory if they had reduced episodes of urge incontinence and failure if there was no improvement. At the time this study began there were no validated questionnaires or tools to assess the lower urinary tract or quality of life in patients with neurogenic bladder dysfunction. Therefore a simple measure as described here was used in this study.

Two consecutive cystometries were performed, exactly as above and the flexible cystoscopic biopsies taken under local anaesthetic. On an average 3 biopsies were taken from each patient before and after capsaicin.

The response of the MS patients to treatment was also examined in relation to their degree of disability as assessed by their scores on a Kurtzke expanded disability status scale (EDSS) [higher the EDSS score more disabled the patient]. Since the Kurtzke score applies only to MS (Betts et al, 1993) other patients were not assessed on this scale.

Table 3 Kutzke EDSS of MS patients participating in the trial

Kurtzke EDSS	**Patients**
4.5	2
5	1
5.5	2
6	3
6.5	2
7	1
7.5	1
Total	12



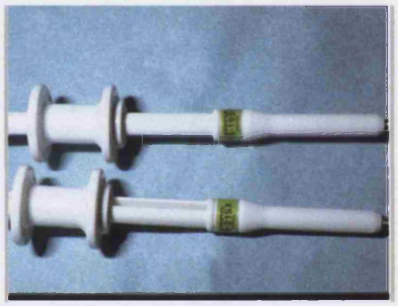
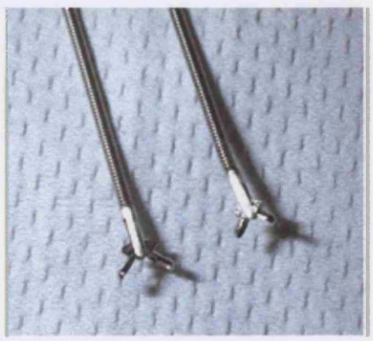


Fig. 14 The Olympus flexible cystoscope and the differences between the 21 SX and the 19 SX (standard) forceps. The 21 SX has larger jaws



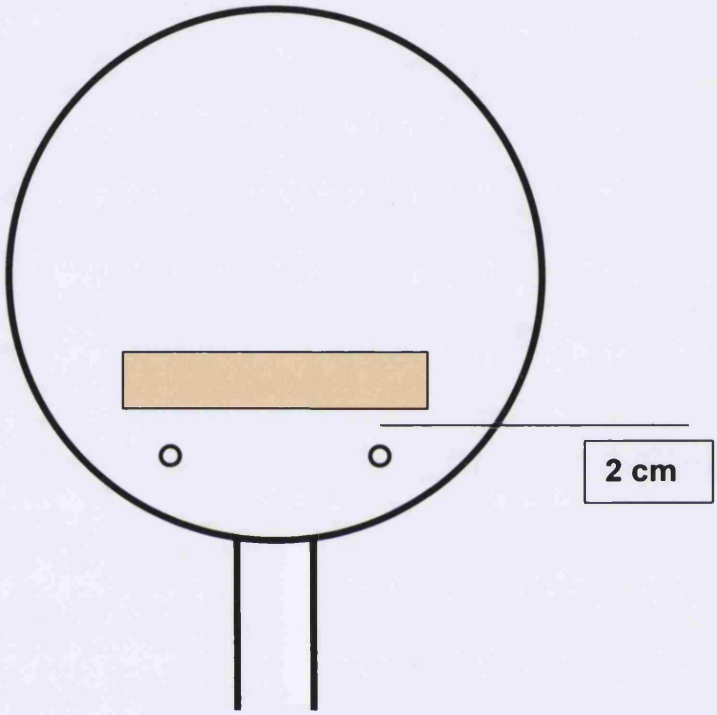


Fig. 15 Schematic diagram of rectangular area on the bladder from which biopsies were taken

8.4 Study protocol: repeated instillations of capsaicin

Some of the patients needed repeated instillations of capsaicin after the effect of the previous instillation wore off. They were given an intravesical instillation of 100 ml of 2 mmol/l of capsaicin in 30% alcohol in saline (0.6 g/l). Any increase in the amount of capsaicin lead to its precipitation in the solution and rendered it unsuitable for use. The solution was freshly prepared in the Hospital Pharmacy and dispensed in 100 ml plastic packs.

There was often a gap of a few weeks between the recurrence of incontinence and a further dose of capsaicin-which was usually because of social reasons as the majority of these disabled patients lived some distance away from the Hospital. Two patients had instillations of 2 doses at intervals of two weeks when they came to have their treatments repeated.

The instillation procedure was exactly the same as described in the previous section for initial instillation of 1 mmol/l of capsaicin. Bladder biopsies taken in the same manner as described in the last section, are being performed as a part of the ongoing study, but are not discussed in this thesis.

8.5 Study protocol: alcohol instillations

In two patients we waited for the beneficial effect of the last dose of capsaicin to wear off and then instilled only vehicle (30% alcohol in saline) into their bladders. It was not possible to perform a blinded study of capsaicin versus vehicle for two reasons:

1. Lack of informed consent from patients. The two patients treated with alcohol alone also did not wish to have bladder biopsies following alcohol instillations. This is discussed further in the results section.

2. The lack of pungency of the vehicle as opposed to the capsaicin solution made it obvious to both patient and administrator as to what was being used.

Both patients had instillation of 2 mmol/l capsaicin in 30% alcohol in saline 4 weeks after the instillation of vehicle only.

The author is aware that a French group has recently reported a blinded study of capsaicin versus its vehicle. de Sèze et al stress the importance of adhering to Fowler's protocol during the capsaicin instillations (de Sèze et al, 1998; 1999). In this protocol the capsaicin is withdrawn in to two 50 ml syringes from its 100 ml plastic container. The importance of using the syringes is to prevent any urethral leakage of capsaicin due to a detrusor contraction during instillation. This would not be possible if the capsaicin solution is instilled directly from its plastic container. We found that the pungency of capsaicin is quite evident to both patient and administrator while it is being withdrawn into the two syringes. Therefore we were unable to

perform a randomised, blinded study while adhering to Fowler's protocol as described from our department (Chandiramani et al, 1996).

8.6 Study protocol: electromotive drug administration (EMDA) of lignocaine before capsaicin instillation

Towards the later part of the trial **eight patients** (4 males, 4 females; mean age 51 yrs; range 38-66) entered this arm of the study. At the time, they all suffered from detrusor hyperreflexia due to spinal cord disease (4 MS, 2 transverse myelitis, 1 cervical myelopathy, 1 TSP) and had failed to respond to treatment of their incontinence using a combination of oral anticholinergics and CISC. Five of the 8 patients had previously been treated with intravesical capsaicin (100 ml solution containing 2 mmol/L of capsaicin in 30% alcohol in saline) preceded by intravesical instillation of 40 ml of lignocaine 2% for 20 mins. Two were treatment failures, three responded for varying periods of time then reverted to their original hyperreflexic states. The 8 patients had no abnormality on upper tract screening by ultrasound. Those with positive urine cultures were treated with antibiotics before entering the study. All patients had medium fill cystometry (Urodyn 5500, Dantec) using normal saline at room temperature (infusion rate 50 ml/min) 2 weeks before intravesical capsaicin. The maximum cystometric capacity (ml) was recorded.

In the 5 repeat patients previous capsaicin instillations were carried out under continuous urodynamic monitoring as previously described (Chandiramani et al, 1996). At the time each patient scored suprapubic pain on a scale of 0 ('no pain at all') to 10 ('worst pain imaginable') (Huskisson, 1974) at 5 mins after starting the capsaicin instillation and at the end of the procedure (30 min unless discomfort forced premature discontinuation).

These 5 patients had subsequent instillations of capsaicin preceded by EMDA of lignocaine which was used for all capsaicin instillations in the 3 remaining patients and all 8 patients scored their pain in the same manner.

The drug solution for EMDA was prepared in a sterile kidney dish by mixing 75 ml of lignocaine hydrochloride 4% (NaCl free) with 75 ml of sterile water and 1.5 ml of 1: 1000 adrenaline giving a final solution of 150 ml of lignocaine 2% with adrenaline 1: 100000. Lignocaine by itself has a short duration of action intravesically (15-20 min) (Gürpinar et al, 1996) and adrenaline was therefore added to prolong the effect.

The urethra was lubricated with 20 ml of lignocaine 2% gel and the bladder catheterised using a 16 F balloon catheter with 3 side holes near the tip (Ag 9301), and containing the positive electrode. A 5 F catheter was passed beside this catheter to monitor intravesical pressure. The balloon of the 16 F catheter was inflated to 6 ml and snugged firmly into the bladder neck. A 5 F rectal catheter was inserted to measure the intra-abdominal pressure.

The bladder was flushed and drained with 500 ml of bi-distilled water and the solution of lignocaine and adrenaline instilled corresponding to a volume of 5-10 ml below the bladder capacity or to 150 ml, whichever was less. The lower abdomen of the patient was thoroughly cleaned with alcohol, covered generously with conductive gel and 2 dispersive electrodes (2 x 50 cm^2) soaked in 0.9% saline were placed on this area. Complete contact of the electrodes to the gel without intervening air bubbles was assured. A battery powered current generator, the Physionizer 30® (Physion SRL, Mirandola, Italy), was used for providing pulsed DC; it was connected to the positive electrode in the catheter and to the dispersive (negative) electrodes. Electric

current of 20 mA with a rise rate of 30 μA/sec was used for 15 mins, following which the leads were disconnected and the bladder was drained and flushed (Figs. 16, 17).

Immediately after EMDA, 100 ml of 2 mmol/L of capsaicin in 30% alcohol in saline was instilled into the bladder through the same catheter under urodynamic monitoring. The patients scored suprapubic pain at 5 mins and 30 mins after starting the instillations on a 10-point scale as described earlier. The bladder was then emptied and all catheters removed. Patients were discharged home immediately after the treatment.

Follow-up consisted of an appointment after 6 weeks when the response to treatment was assessed subjectively from the patients' history and objectively by the functional bladder capacity (ml) on medium fill cystometry with normal saline at room temperature (50 ml/min).

Capsaicin instillations preceded by EMDA of lignocaine were repeated once in 2 patients and twice in 1 patient when the effect of the previous dose declined.

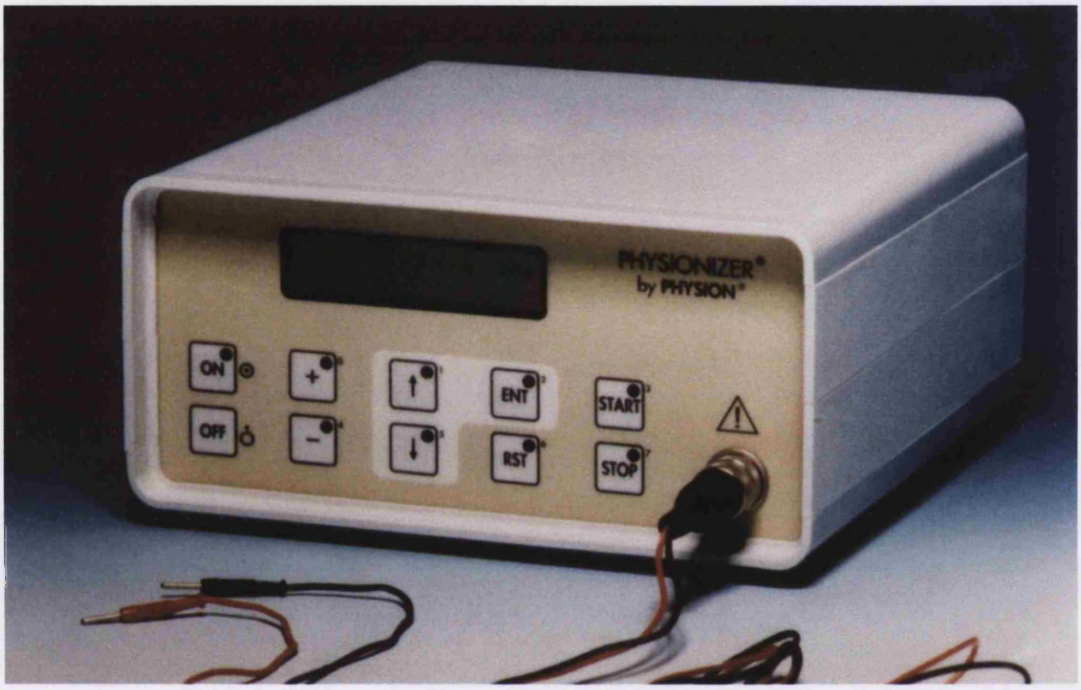


Fig. 16 The Physionizer. Reproduced with permission of Physion Srl, Mirandola, Italy

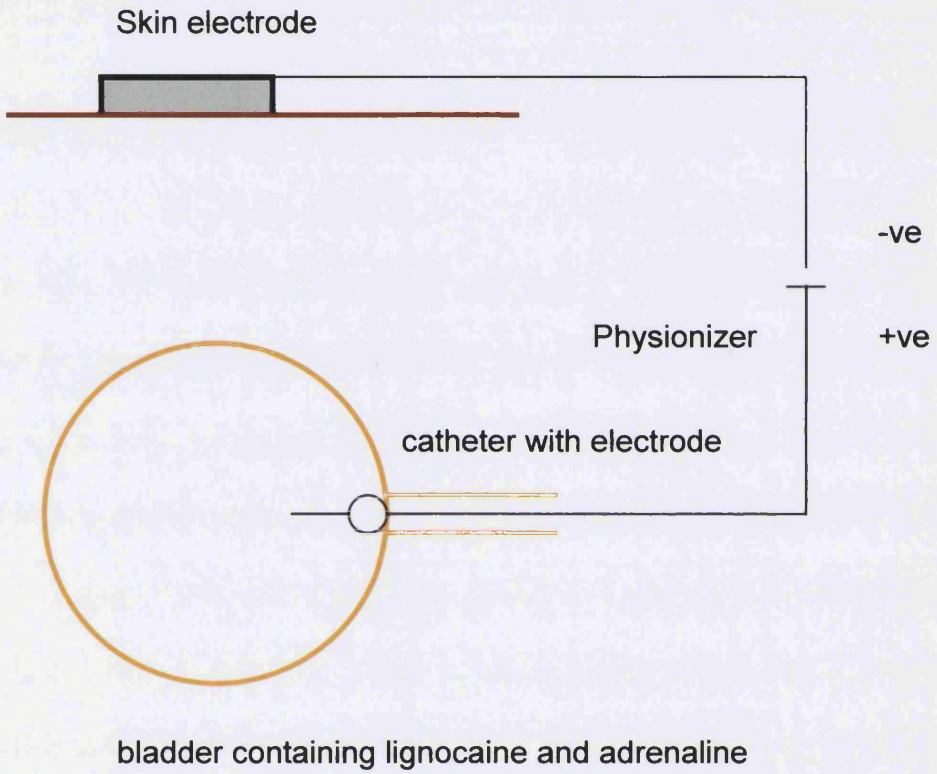


Fig. 17 Schematic diagram of EMDA of lignocaine and adrenaline before capsaicin

8.7 Study protocol: surveillance

The long-term effects of intravesical capsaicin are unknown. Based on the advice of Professor A D Dayan (please see page 75) a surveillance programme was established involving flexible cystoscopy and bladder biopsies in the 20 patients treated with intravesical capsaicin. The results of 5-year follow up in these patients are reported here.

Cystoscopy immediately after intravesical capsaicin can be a painful procedure mainly due to urethral irritation and was therefore not performed in every patient. However two of the male patients with transverse myelitis had reduced urethral sensation and agreed to have cystoscopies before, immediately after and 6 weeks after the capsaicin instillations.

8.8 Study protocol: control group

Informed consent was obtained from twelve control patients (9 males, 3 females; mean age 49 years, range 25-68 years) who attended the haematuria clinic at The Institute of Urology, London.

Only bladder biopsies were taken from this group of patients but none of them were given intravesical capsaicin.

All the biopsies were taken as day case procedures. The patients were given prophylactic Gentamicin and 10 ml of 2% lignocaine was instilled into the urethra 5 minutes prior to the procedure. They had flexible cystoscopy as a part of their routine workup for microscopic haematuria and biopsies were taken from the same part of the bladder as in the capsaicin group. The same instruments were used in the control and the capsaicin groups. On an average 3 biopsies were taken per patient.

CHAPTER 9

STAINING TECHNIQUES

9.1 Initial processing of bladder biopsies

The biopsies were transferred immediately into 4% paraformaldehyde in phosphate buffered saline [PBS]. The exact time of putting the biopsies into fixative was noted and they were left in this solution for 2 hours. The fixative was then poured off and 0.45 molar sucrose in PBS was added to the tissues which were then left overnight in a fridge at 4°C.

The biopsies were subsequently frozen and 10 μm sections were cut using a cryostat. Although minute (approximately 1mm x1mm x1mm) each of the biopsies yielded about 60 sections which were then transferred to glass slides (5 biopsies/slide).

The slides were each allocated a unique identification number in the Neuropathology Department at the Institute of Neurology, London. The observer assessing nerve densities from these sections was blind to the names of the patients corresponding to these numbers until the nerve densities of all samples had been assessed.

9.2 S 100 immunostaining

This was performed in Professor Francesco Scaravilli's Department at the Institute of Neurology, London.

The sections were rinsed in 70% and 90% ethanol and endogenous peroxidase blocked by 0.03% hydrogen peroxide in methanol for 10 mins. They were then washed in tap water for 10 mins and placed in Tris -buffered saline [TBS]. Normal swine serum at 1:20 dilution in TBS (Dako X0901, Dako Ltd, Cambridge, UK) was applied to the sections and left for 10 mins at room temperature. This solution was then tipped off from the slides and S 100 polyclonal antibody at 1:1600 dilution in TBS (Dako Z311) was added and left overnight at 4°C. The sections were then washed three times in TBS and incubated with biotinylated swine anti-rabbit antibody, diluted 1:500 in TBS with 40 μl /ml normal human serum (Dako E0353) for 30 mins, at room temperature. They were rewashed thrice with TBS and incubated with avidin -peroxidase conjugate diluted 1:500 in TBS (Dako P0364). The sections were again washed thrice in TBS, placed in PBS and incubated with diaminobenzidine [DAB] solution (50 mg DAB in 100 ml PBS buffer which was activated by adding 32 μl hydrogen peroxide immediately before use) for 10 mins. After washing with tap water counterstaining was performed using Mayer's haemalum for 30 secs. The sections were dehydrated through graded alcohols, cleared in xylene and mounted.

9.3 PGP 9.5 immunostaining

This was performed in the Anatomy Department of University College London. The slides were thawed at room temperature and 100 μl of polyclonal antiserum to PGP 9.5 was used per slide. The sections were initially incubated with the primary antibody, anti PGP antibody at 1: 1000 dilution (Ultraclone Ltd, Isle of Wight, UK) for 18 hours in a humid chamber. They were washed three times with PBS and incubated with the secondary antibody, anti IgG biotinylated species specific whole antibody raised in donkey at 1:250 dilution (Amersham Int, Little Chalfont,UK) for 1 hr. After three more washes with PBS they were incubated further with streptavidin fluorescin at 1:100 dilution (Amersham) for 1 hr. They were washed thrice with PBS, stained with pontamine sky blue (0.1% in 1% dimethyl sulfoxide/PBS) for a couple of minutes, washed twice with PBS and mounted using Cityfluor (Agar Scientific Ltd, Stansted, UK).

9.4 Electron microscopy

This arm of the study was performed in collaboration with Professor D N Landon and commenced in the later part of the trial period (about 2 years after the trial began). Flexible cystoscopic biopsies were obtained from **8 patients** with detrusor hyperreflexia (3 males, 5 females; mean age 55 yrs, range 46-70 yrs) before and 6 weeks after treatment with capsaicin. The samples were fixed in 3% glutaraldehyde in 0.1 molar sodium cacodylate buffer. They were postfixed in 1% osmium tetroxide, dehydrated in ascending alcohol (70-100%), treated with propylene oxide, infiltrated with agar 100 epoxy resin, polymerised for 48 hrs and sectioned at the midpoint. Trimmed ultrathin sections stained with urinyl acetate and lead citrate provided samples of full thickness of the lamina propria and epithelium. The sections were scanned by electron microscopy and all nerve profiles photographed at initial magnification of 6-10,000. Counts and measurements were made on these micrographs (n=510) and mean values of the following parameters were determined:

1. Number of axons/nerve
2. Number of Schwann cell units/nerve
3. Percentage of axons with greater than 10 clear vesicles
4. Percentage of axons with dense cored vesicles
5. Percentage of empty Schwann cell bands

9.5 Haematoxylin-eosin staining

All patients treated repeatedly with capsaicin were kept under surveillance by flexible cystoscopy and bladder biopsies at least once a year.

Ten micron sections (processed in the same way as in 9.1) were stained with haematoxylin-eosin (a standard technique) and examined at a magnification of x250 by the same histopathologist (Dr M C Parkinson).

CHAPTER 10

COMPUTERISED ANALYSIS OF NERVE DENSITIES

10.1 Computerised analysis of S 100 positive profiles

To assess nerve densities in the samples stained for S 100, we used the 'Mini MOP' version 2.1 (Kontron, Munich, Germany) (Fig. 18), consisting of a light emitting cursor attached to a microscope. One mm on a graticule was measured in 'MOP' units [B] under the microscope using the cursor. The area of lamina propria in a bladder section [A] was then measured in 'MOP' units by moving the lighted cursor. [A] / $[B]^2$ gave the area of lamina propria in sq.mm. The number of S 100 positive structures [Y] were counted in this area at a magnification of x250 using an overlying grid. The scores of S 100 positive profiles (nerves/sq.mm) were then calculated by the formula:

S 100 positive profiles (nerves/sq.mm) = $(Y \times B^2) / A$

At least five counts were performed per patient and the average of these counts determined. Statistical advice was sought at Queen Square and analysis performed by the author using the two tailed paired t-test (parametric) and Mann-Whitney U test (non-parametric), according to the distribution of the data.

Fig. 18 The 'MiniMOP' attached to its microscope.

The bottom picture shows the cursor used to measure the tissue area in 'MOP' units

10.2 Computerised image analysis of PGP 9.5 positive profiles

The 'Seescan' (Seescan Ltd, Cambridge, UK) computer was used for assessing nerve densities by PGP staining (Fig. 19). It was connected to a Zeiss immunofluorescent microscope, which transferred the image to a video monitor. The suburothelial nerves, which normally look green under the immunofluorescent microscope, appear as red structures in a blue background on the scanner. The computer "saw" the suburothelial nerves as red structures in its blue frame and by setting the "interactive threshold" (IT) the exact appearances of these nerves could be reproduced as a computerised image. The nerve density scores were expressed as "red %" and "red in frame".

Two independent observers (the author and Mr Vijay Chandiramani) took some of the readings on two separate occasions. The mean nerve density scores were compared to assess inter-observer variability.

Statistical advice was sought at Queen Square and analysis performed by the author using the two tailed paired t-test (parametric) and Mann-Whitney U test (non-parametric), according to the distribution of the data.

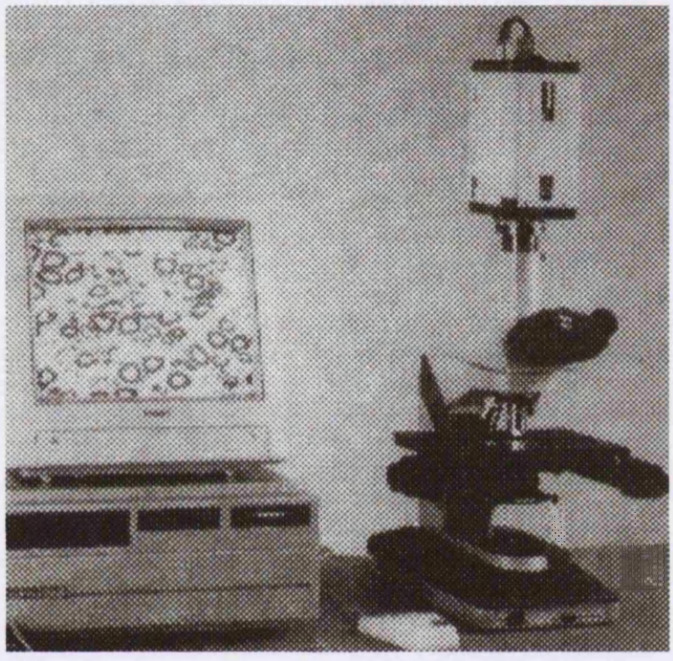


Fig. 19 The 'Seescan' computerised image analyser

SECTION III

RESULTS

CHAPTER 11

RESULTS: CLINICAL

11.1 The response of patients to capsaicin

All patients treated with capsaicin, complained of an initial suprapubic burning sensation lasting with intensity for over 5-10 minutes and then diminishing over the next 30 minutes. The burning sensation was reduced by the instillation of lignocaine before capsaicin. The majority of patients noted an initial deterioration in their urinary symptoms with worsening urinary incontinence for 2 days to 2 weeks after capsaicin instillation. Following this there was either a clinical improvement or a return to the patient's previous state. There were no systemic side effects.

The beneficial effect lasted for a period of 3-6 months on average after which the instillations were repeated.

Table 4 Results of capsaicin instillations in 20 patients

Diagnosis	Patients (n)	Successes	Failures
Multiple sclerosis	12	8	4
Tropical spastic paraparesis	4	4	0
Transverse myelitis	2	1	1
Cervical myelopathy/ischaemia	1	1	0
Complete spinal cord injury	1	0	1

11.2 Cystometric changes

The cystometric traces of a patient before, during and 6 weeks after capsaicin are shown in Fig. 20. During the instillations phasic detrusor contractions were noted which were reduced by intravesical lignocaine before capsaicin. In order to prevent urethral leakage of capsaicin, a small amount of the solution was withdrawn from the bladder using the 50 ml syringe attached to the catheter.

Of the 20 patients 14 (70%) responded to treatment. 11 (55%) patients had a good or excellent response and were mostly continent on intermittent catheterisation, while 3 (17%) had satisfactory improvement with decreased episodes of urge incontinence. 6 (30%) of the patients did not improve clinically although on urodynamics half of them had slight increase in bladder capacity (Fig. 21).

In the 14 patients who responded, the maximum cystometric capacity increased from [mean ± standard error] (standard deviation) 190 ± 18.65 (69.79) to 399 ± 39.61 (148.20) mls ($p<0.0002$; t-test). The amplitude of detrudor hyperreflexia decreased from 63 ± 7.49 (28.03) to 51 ± 7.04 (26.35) cms of water ($p<0.004$; t-test). These changes are depicted in Figs. 22, 23; Tables 5-7.

In the MS group, more disabled patients with higher EDSS scores had poorer response to capsaicin (Fig. 24).

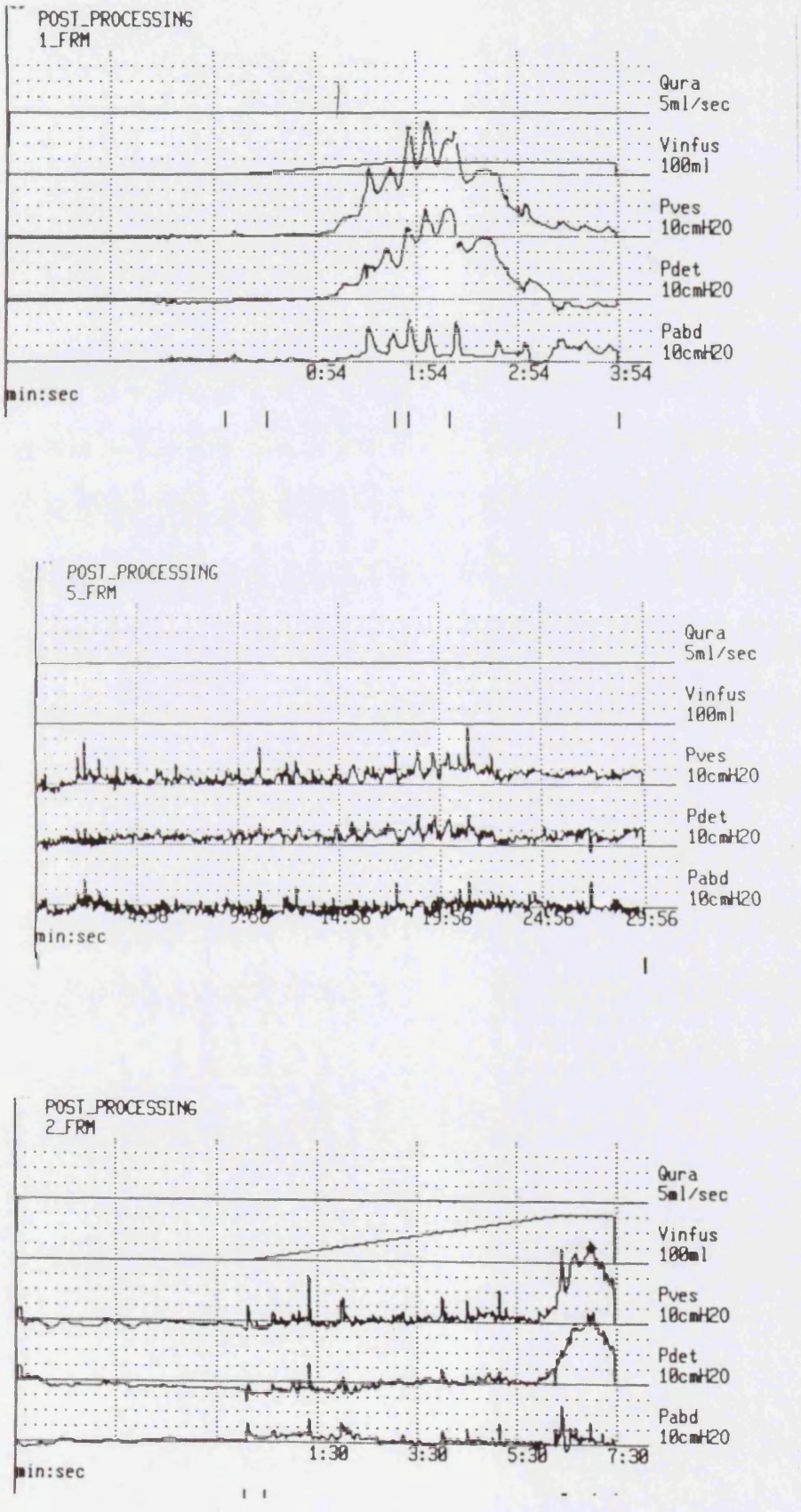


Fig. 20 Cystometries before, during and 6 weeks after capsaicin instillation

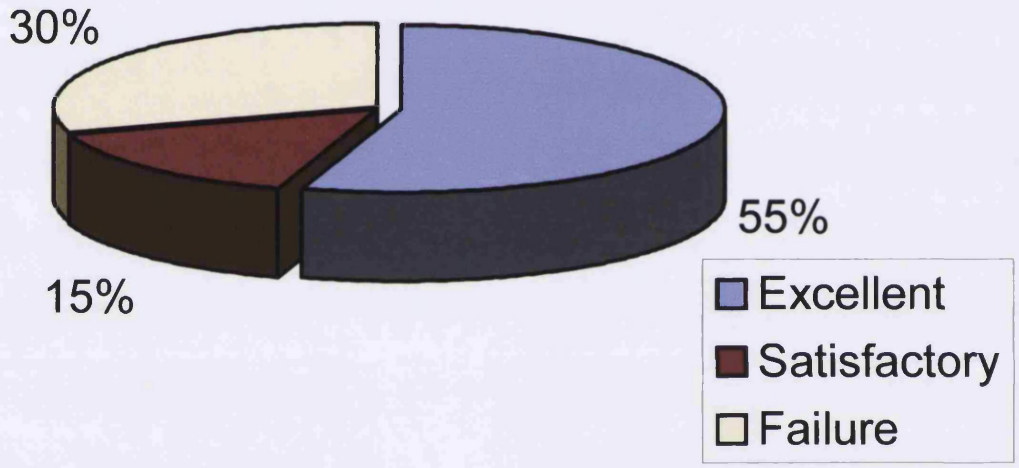


Fig. 21 Summary of clinical results

Table 5 Results of cystometry before and 6 weeks after capsaicin in responders

Patient	Diagnosis	Bladder capacity		Amplitude of hyperreflexia	
		Precaps	Postcaps	Precaps	Postcaps
1	MS	150	750	40	28
2	MS	260	550	45	20
3	MS	255	328	100	80
4	MS	338	428	24	20
5	MS	150	380	40	40
6	MS	240	370	40	28
7	MS	138	585	64	80
8	MS	137	460	110	80
9	TSP	250	398	60	32
10	TSP	180	282	110	100
11	TSP	178	277	37	30
12	TSP	165	316	80	51
13	TM	66	191	67	62
14	CM	155	275	65	60

Table 6 Results of cystometry before and 6 weeks after capsaicin in non-responders. The changes in bladder capacity ($p<0.91$) and amplitude of hyperreflexia ($p<0.38$) did not reach statistical significance

Patient	Diagnosis	Bladder capacity		Amplitude of hyperreflexia	
		Precaps	Postcaps	Precaps	Postcaps
1	MS	140	125	29	70
2	MS	235	150	45	40
3	MS	181	249	42	38
4	MS	70	62	89	67
5	TM	42	121	170	99
6	SCI	83	62	86	60

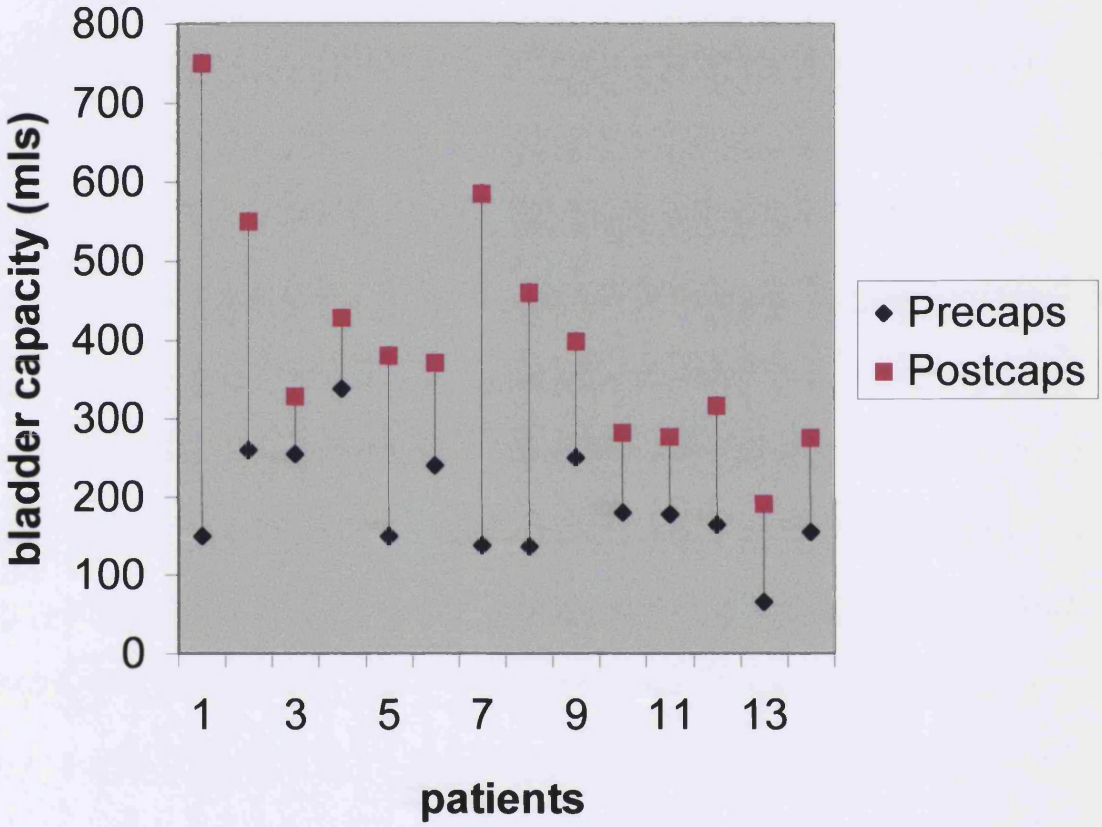


Fig. 22 Bladder capacity in responders

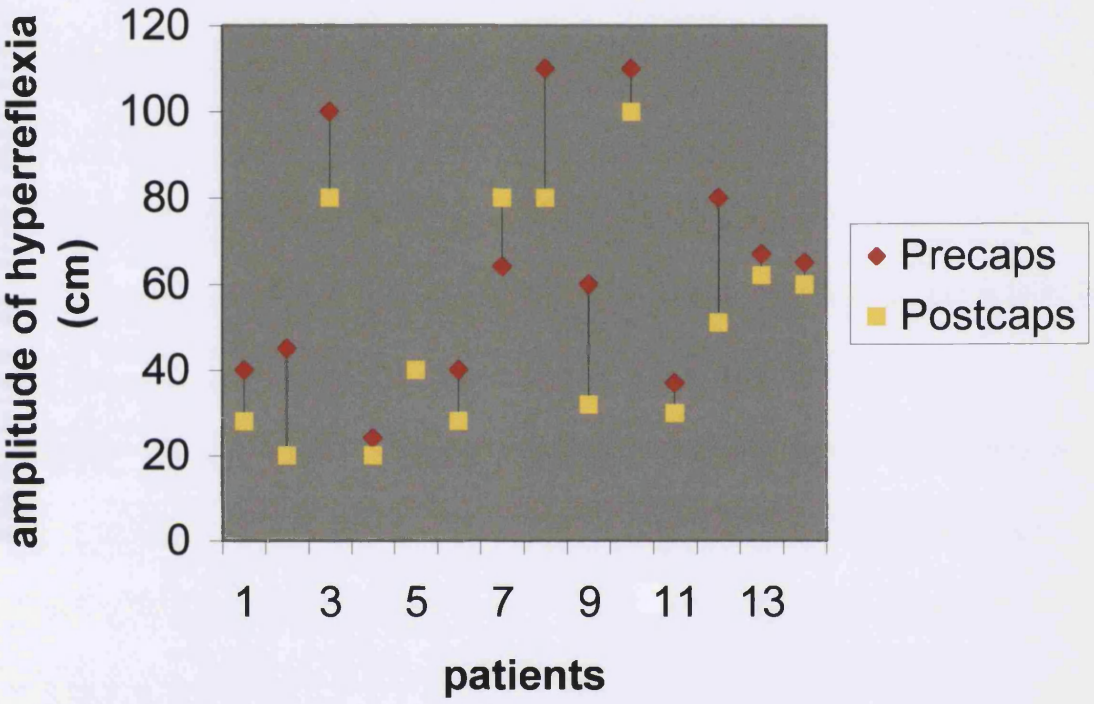


Fig. 23 Amplitude of detrusor hyperreflexia in responders

There was no change in the frequency volume charts of patients that did not improve with intravesical capsaicin instillations. Analysis of the charts in the 14 patients that did improve showed an increase in the mean residual urine volume on intermittent catheterisation and a decrease in urinary frequency.

Table 7 Analysis of frequency volume charts before and 6 weeks after capsaicin in the 14 patients who improved

Frequency volume charts	**Before capsaicin instillation**	**After capsaicin instillation**
Volume on CISC (mls)	145	234
Daytime frequency	10.3	5.1
Nocturia	2.4	1.3

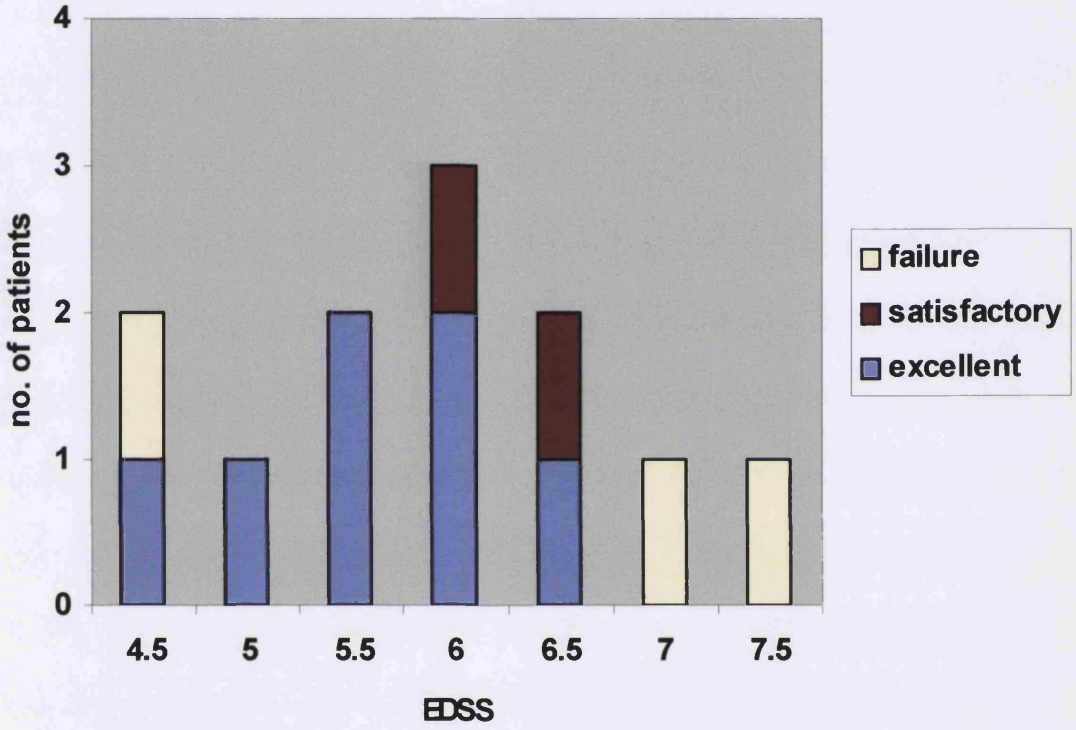


Fig. 24 Kurtzke EDSS and response to capsaicin treatment in MS patients. More disabled patients with higher EDSS scores had poorer response to capsaicin

11.3 Results of repeated capsaicin instillations

All 14 responders have had repeated instillations of capsaicin with similar improvement in their lower urinary tract symptoms on each occasion. The median followup was of 19 months although the instillations took place over a 5-year period and thus some of the initial patients were reviewed for 5 years. The two patients who had two instillations over two weeks reported better subjective improvement than the benefit obtained on the occasions when they received the usual single doses. The maximum number of instillations over the study period has been 17 in a male patient with MS (patient 2 - Fig. 25). No systemic side effects have been reported by any of the patients. The suprapubic discomfort during capsaicin became progressively less in patients who had repeated treatments.

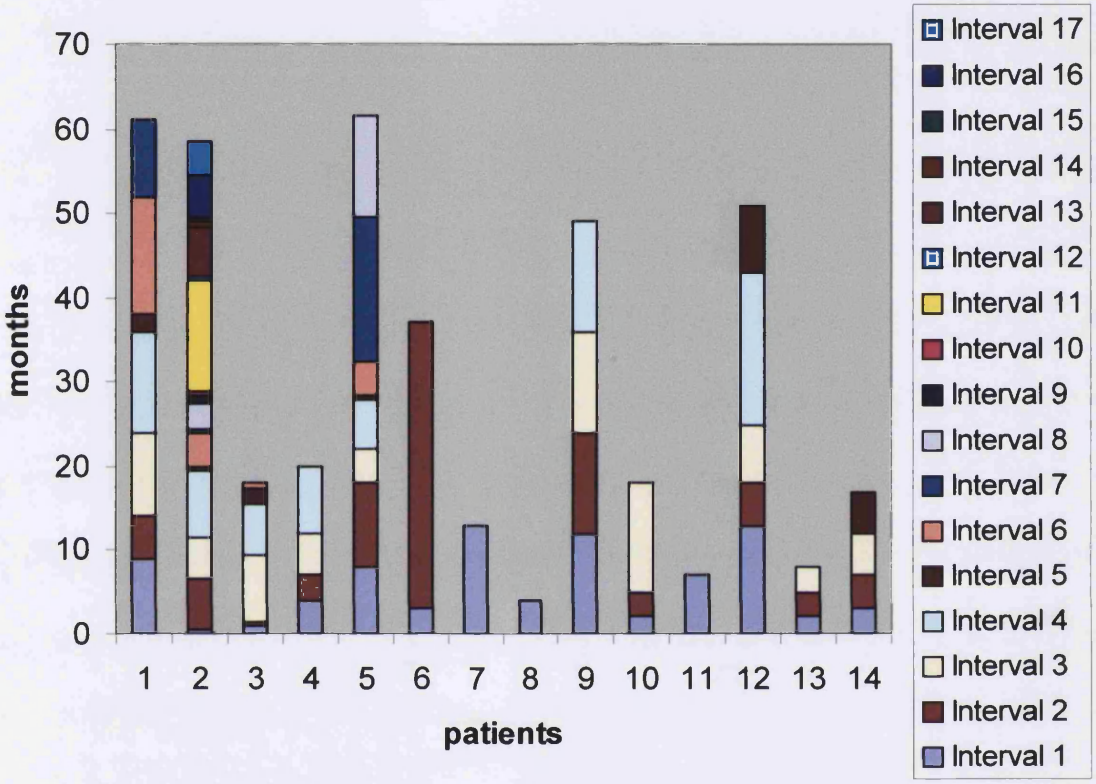


Fig. 25 Repeated instillations of capsaicin in 14 patients over 5 years. The intervals do not necessarily indicate the duration of response as there was often a gap of a few weeks between the recurrence of incontinence and a further instillation of capsaicin- usually due to social reasons as the majority of these disabled patients lived some distance away from the Hospital. Patients 2 and 3 had two instillations at two weekly intervals on some occasions

CHAPTER 12

RESULTS: EFFECTS OF VEHICLE INSTILLATIONS

12.1 The effect of alcohol only on the bladder

Two patients who received alcohol and saline instillations had marked worsening of their symptoms for 4-5 weeks followed by no clinical or urodynamic improvement. They had increased frequency, urgency and incontinence, which was particularly worse at night. They also complained of persistent suprapubic pain. Their bladder control became so much worse that neither of them agreed to have further bladder biopsies. At this stage they were given further treatment with 2 mmol/l of capsaicin in 30% alcohol in saline and showed the same improvement as they had earlier. The significant deterioration of these patients after instillation of vehicle alone deterred us from trying this on other patients in this series.

CHAPTER 13

RESULTS: LIGNOCAINE AND EMDA

13.1 Capsaicin preceded by lignocaine alone

Hyperreflexic bladder contractions soon after capsaicin instillations were observed in the 5 patients who had capsaicin preceded by lignocaine alone (Figure 26A). About 5-10 ml of capsaicin was withdrawn into the 50 ml syringe when such a contraction occurred in order to prevent leakage of the solution into the urethra. Involuntary rectal contractions were also noted during the instillations but these did not appear to affect the patient. In 3 of these 5 patients the capsaicin instillations had to be stopped at 15, 15 and 20 mins because of severe discomfort in two and in one female patient with cervical myelopathy, due to autonomic dysreflexia (sweating, headache and blood pressure rising from 160/70 to 190/106 mm of Hg) during the procedure; her blood pressure returned to its previous level soon after this. The pain scores during capsaicin instillations in the 5 patients were 6.8±2.8 (mean±standard deviation) at 5 mins and 3.2±1.9 at the end of the procedure. These patients had initial worsening of their urinary symptoms for 3-14 days followed by improvement in 3 patients, two of whom had tolerated the instillations for the entire 30 mins. In the female patient with cervical myelopathy there was no urinary incontinence for a period of 3 weeks while the 2 other responders benefited for periods of 3 and 4 months respectively. Two of the 5 patients failed to achieve a satisfactory response; in both the treatments had to be terminated prematurely.

13.2 Capsaicin preceded by EMDA of lignocaine

Following EMDA, minimal or no hyperreflexic contractions were recorded in any of the patients during capsaicin instillations (Fig. 26B). There was no need to withdraw the capsaicin solution intermittently and as a result of which every patient was given the full 100 ml for 30 mins treatment time. The same rectal contractions were noticed during the instillations as were seen without EMDA. The patient with cervical myelopathy, who had formerly been so highly symptomatic during the instillation, had no sweating or symptoms of autonomic dysreflexia. She has had 2 further instillations of capsaicin preceded by EMDA without any discomfort or change in blood pressure.

in the 5 patients who had previous capsaicin instillations after lignocaine alone, the pain scores during capsaicin instillations after EMDA of lignocaine were 0.6±0.4 at 5 mins and 0.4±0.5 at the end of the procedure (Fig. 27). The pain scores in the 3 other patients who had capsaicin after EMDA of lignocaine as their initial treatment were 0, 1, 2 at 5 mins and 0, 3, 0 at 30 mins respectively (Dasgupta et al, 1998a).

After the capsaicin all patients had worsening of their urinary symptoms for 3-21 days. Of the 8 patients 6 (75%) responded to treatment, 5 became fully continent and 1 had decreased episodes of urge incontinence during the day. The responders included the 2 patients who had previously failed to respond to capsaicin preceded by lignocaine alone. Patients were continent while using CISC and did not need condom catheters or pads during the period of improvement. The maximum cystometric capacity increased from 177±64.9 ml before capsaicin to 395±188.3 ml after capsaicin (Fig. 28).

The duration of benefit was 3 months in 3 patients, 6 months in 2 patients

and 8 months in 1 patient. There was no improvement in 2 patients (both females with MS) who went on to have indwelling catheters.

The repeated instillations of capsaicin after EMDA of lignocaine were also performed without any patient discomfort and were followed by similar improvement.

No systemic side effects were observed. One patient had urethral pain for 3 days due to leakage of capsaicin while removing the balloon catheter.

Urinary tract infection occurred in 1 patient and was treated with a course of antibiotics while in another patient there was initial difficulty in deflating the balloon catheter, probably due to a failure of the valve mechanism.

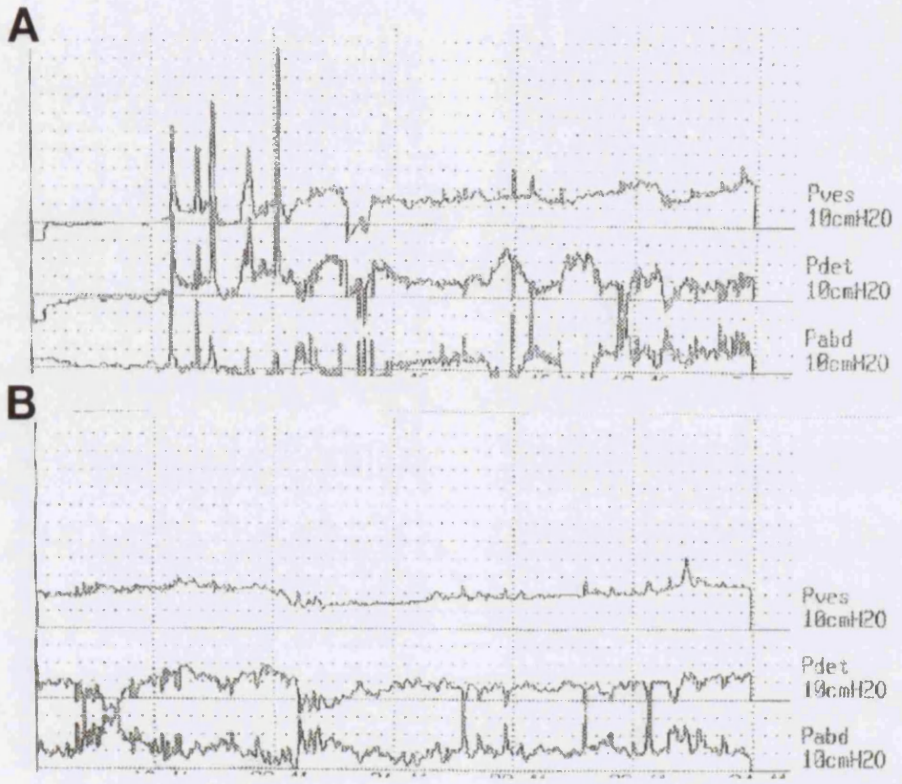


Fig. 26 EMDA of lignocaine before intravesical capsaicin

A Intermittent bladder and rectal contractions in a patient during capsaicin instillation which was preceded by intravesical lignocaine for 20 mins. Each division on the X-axis represents 5 mins

B EMDA of lignocaine before intravesical capsaicin abolished the phasic vesical contractions in the same patient. The rectal contractions continued. The detrusor pressure is the subtracted abdominal pressure from the vesical pressure and therefore shows 'pseudo' detrusor contractions.

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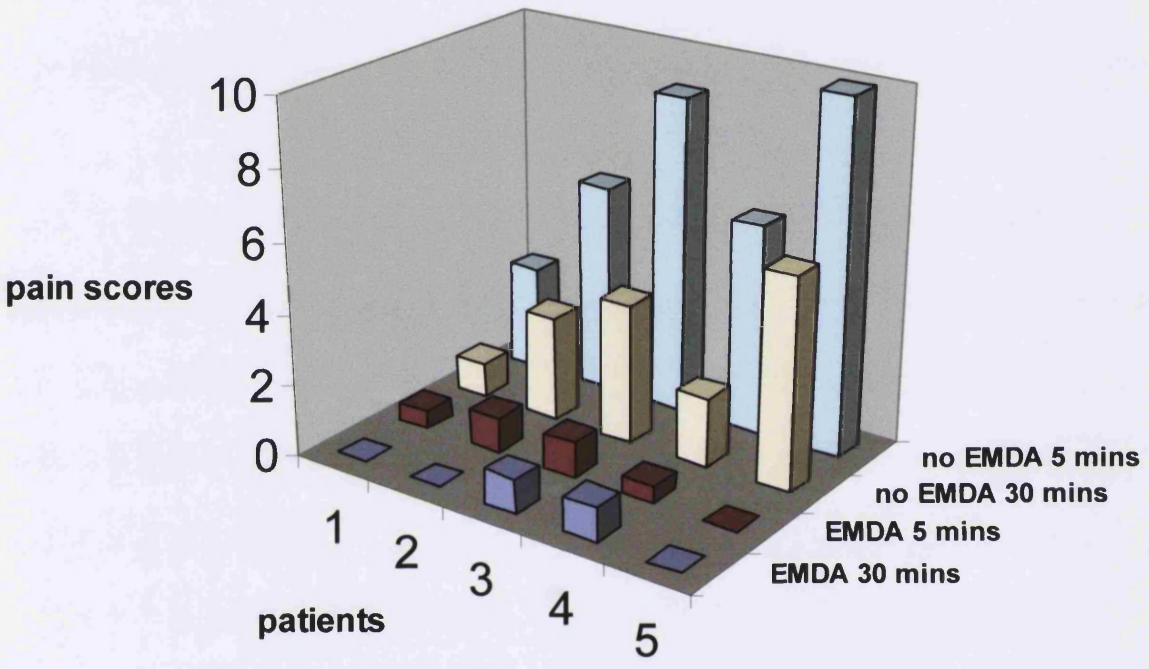

Fig. 27 Pain scores with and without EMDA. Reproduced with permission of the Journal of Urology

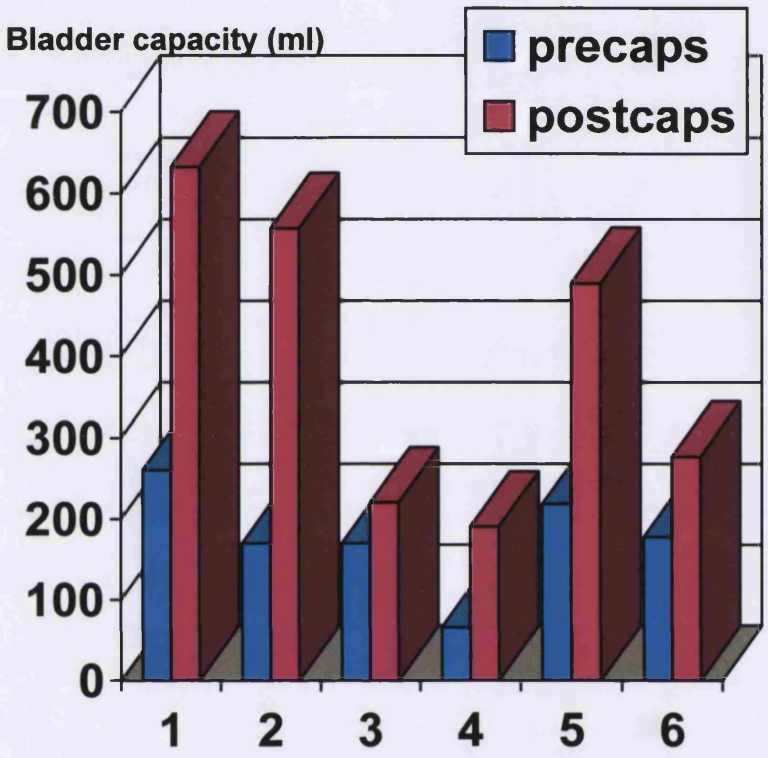

Fig. 28 Bladder capacities in the patients responding to EMDA followed by capsaicin. Reproduced with permission of the Journal of Urology

CHAPTER 14

RESULTS: NERVE DENSITIES

14.1 Flexible cystoscopic biopsies

These are the first reported results of flexible cystoscopic biopsies for nerve density evaluation of the urinary bladder (Dasgupta et al, 1997a). The biopsies although very small (on average 1mm x1 mm x1mm) produced excellent samples for neuronal evaluation and up to 60 sections per specimen (Fig. 29). Surprisingly 48% of the samples also contained detrusor muscle.

Although the biopsies were taken under local anaesthetic, none of the patients complained of undue discomfort or pain and all of them agreed to have the procedure done repeatedly. The control patients rated the biopsies were no more uncomfortable than the flexible cystoscopy itself. This is probably because the samples taken were so minute. All patients had haematuria lasting for between 1-20 hours, which then ceased spontaneously. None had urinary tract infections due to this operative intervention.

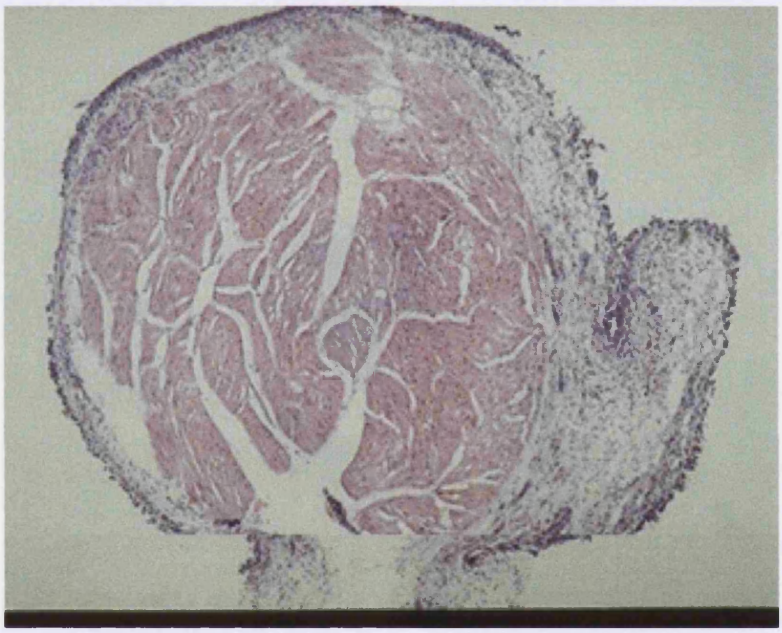

Fig. 29 A flexible cystoscopic biopsy -taken with an Olympus cystoscope and 21SX forceps under local anaesthetic. Lamina propria and detrusor are clearly seen (x40). Reproduced with permission of the British Journal of Urology

14.2 Effect of capsaicin on S 100 positive nerves

Early results of this arm of the study were reported at the ICS in Athens (Dasgupta et al, 1996b). The S 100 positive profiles in the lamina propria appeared as brown, thread like structures of various thickness and were sometimes seen to be arranged in bundles. Biopsies from **17 of the 20 patients** treated with capsaicin were stained by this method. Results from **13 patients** (7 males, 6 females; 10 MS, 2 transverse myelitis and 1 cervical myelopathy) are presented here. Results of the biopsies from the **4 TSP patients** are discussed separately. Of the 13 patients 9 responded to capsaicin while the treatment failed in 4 patients-3 with MS and 1 with transverse myelitis.

1. The mean score (nerves/sq.mm) of the S 100 positive profiles in the control group (12 patients) with no identifiable bladder pathology was 83 ± 3.18 (mean ± standard error of mean).
2. In patients with hyperreflexic bladders who responded to capsaicin (9 patients) the mean scores (nerves/sq.mm) of the S 100 positive profiles were found to be reduced from 100 ± 12.19 before to 66 ± 9.39 ($p<0.01$, t-test), 6 weeks after treatment.
3. In those who did not respond to capsaicin (4 patients) the mean scores (nerves/sq.mm) of the S 100 positive profiles were 82 ± 9.31 before and 103 ± 14.53, 6 weeks after treatment with no statistically significant difference between them ($p<0.36$, t-test). These changes are shown in Table 8 and Figs. 30-33.

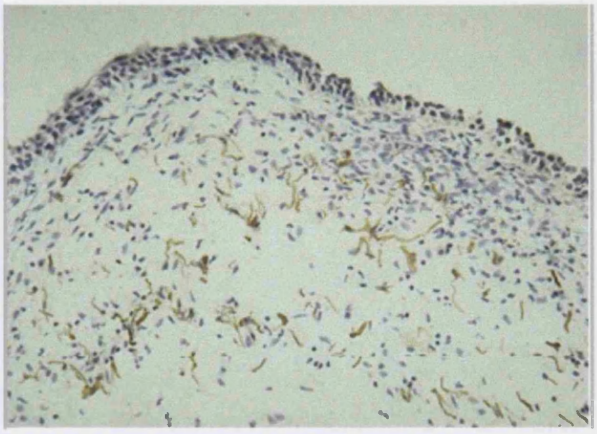

Fig 30A Nerve densities with S 100 immunostaining -before capsaicin in detrusor hyperreflexia. The brown structures are the nerves in the suburothelium. Reproduced with permission of British Journal of Urology (x250)

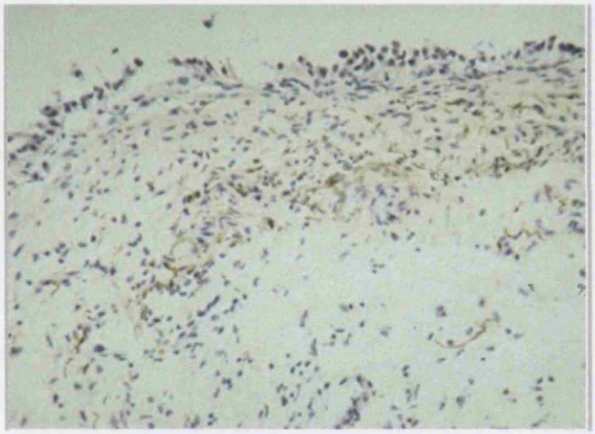

Fig 30B Nerve densities in a control patient

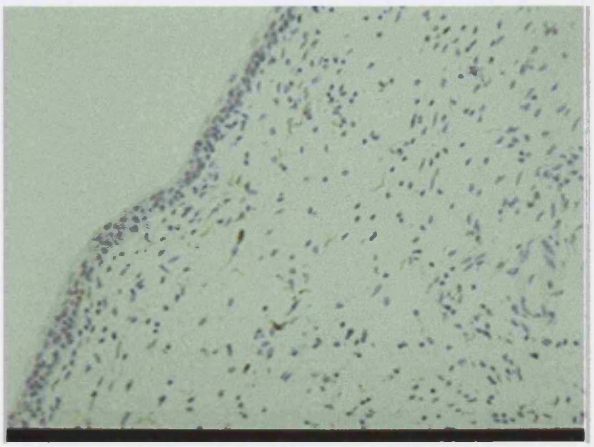

Fig 30C Nerve densities 6 wks after capsaicin-same patient as in 30A

Table 8 Mean densities of S 100 positive profiles (nerves/sq.mm)

S 100 positive profiles	Controls(12)	Responders (9)		Non responders (4)	
nerves/sq.mm	normal bladders	precapsaicin	postcapsaicin	precapsaicin	postcapsaicin
mean	83	100	66	82	103
[range]	[63-101]	[60-163]	[22-120]	[58-102]	[64-134]

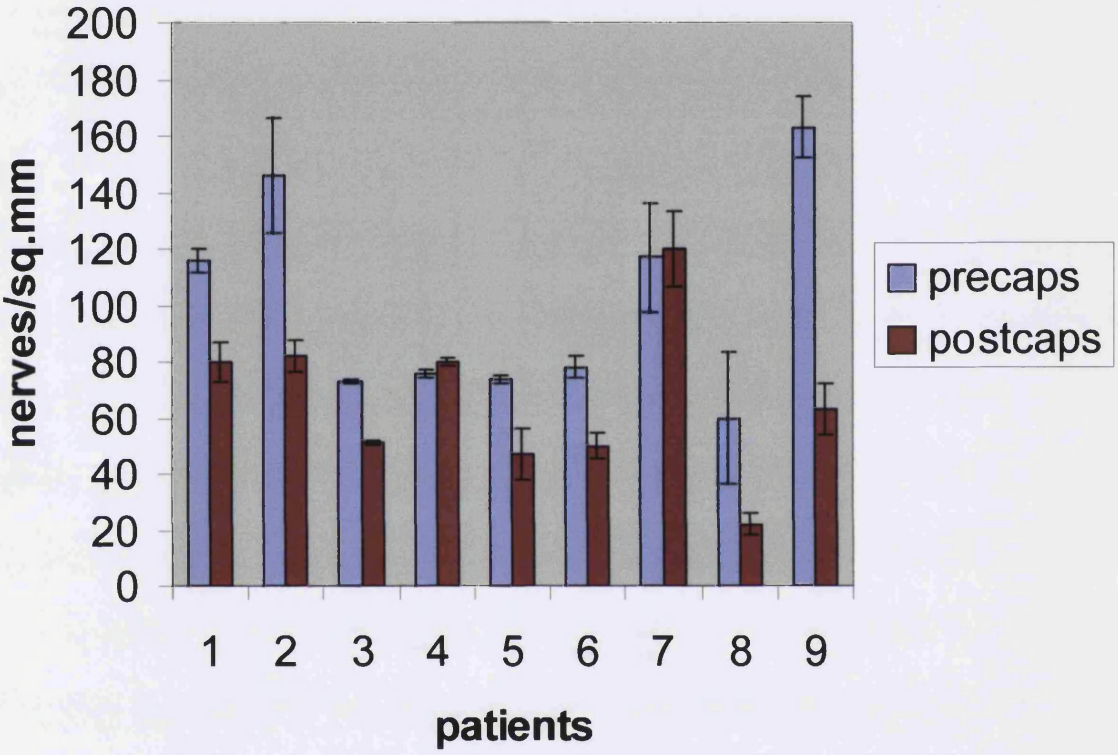

Fig. 31 S 100 positive profiles before and 6 weeks after capsaicin in responders. Bar charts denote means and the respective standard deviations. Adapted with permission of the British Journal of Urology International

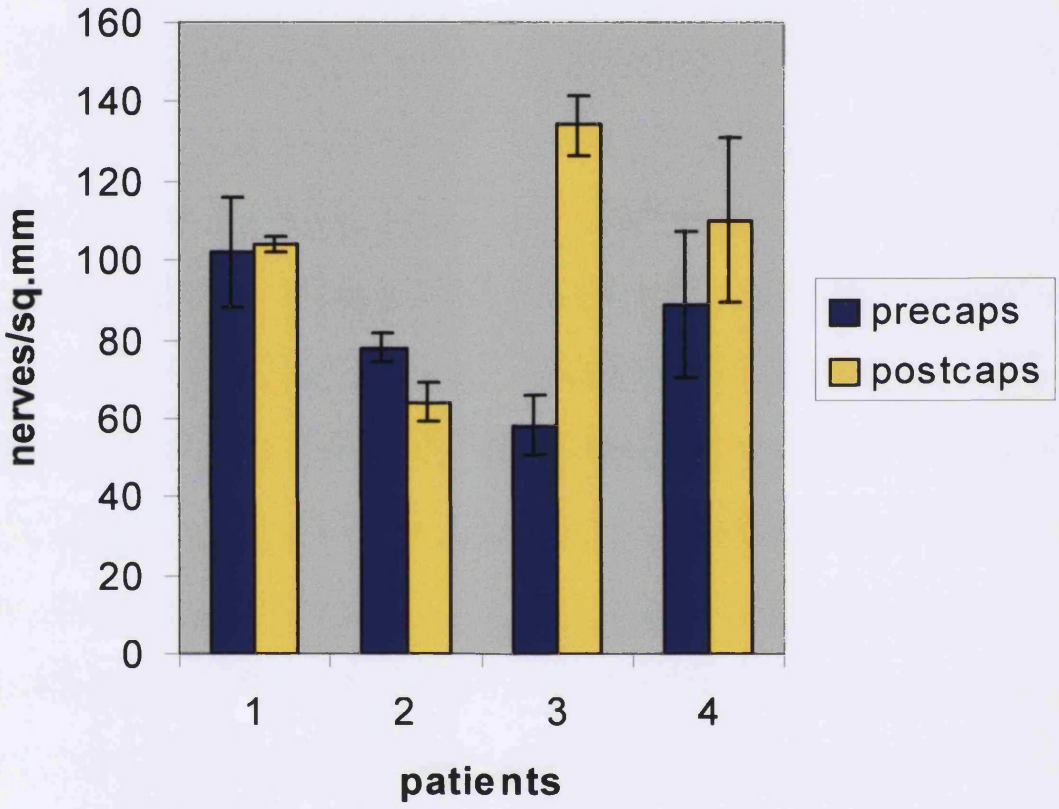

Fig. 32 S 100 positive profiles before and 6 weeks after capsaicin in non-responders.

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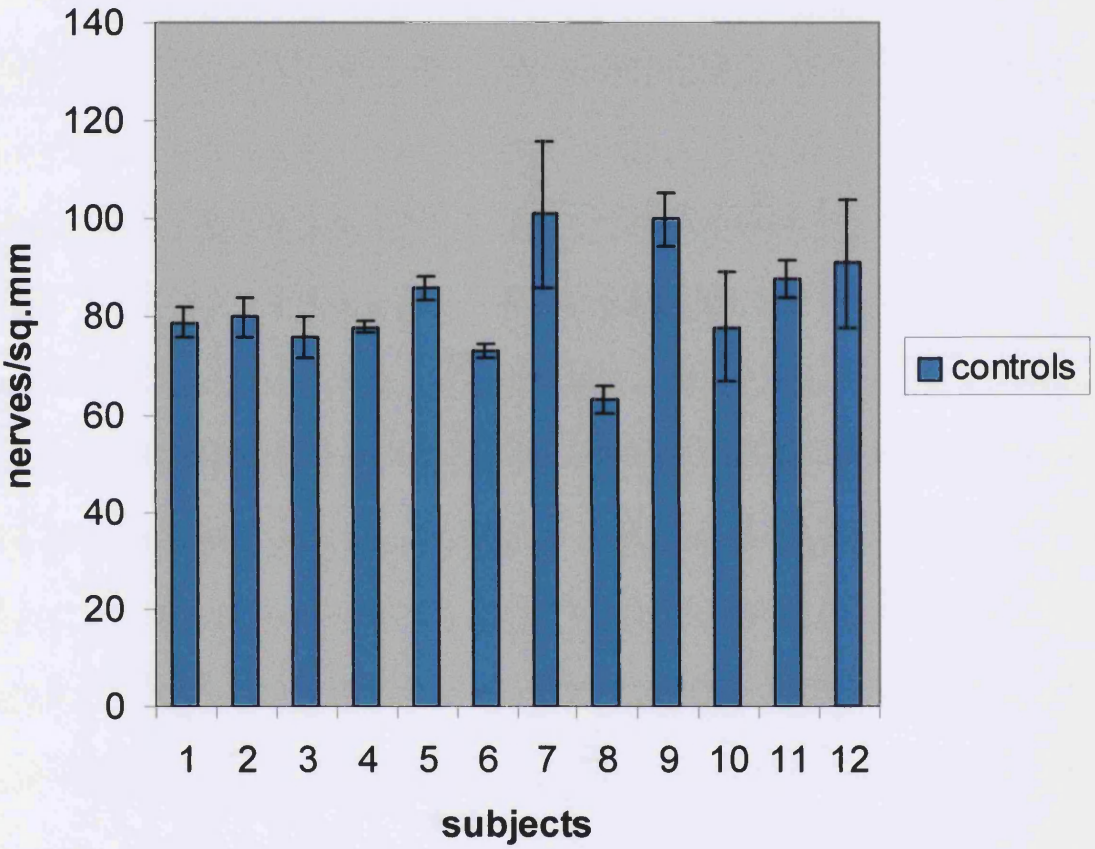

Fig. 33 S 100 positive profiles in control subjects. They were not treated with capsaicin.

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14.3 Effect of capsaicin on PGP 9.5 positive nerves

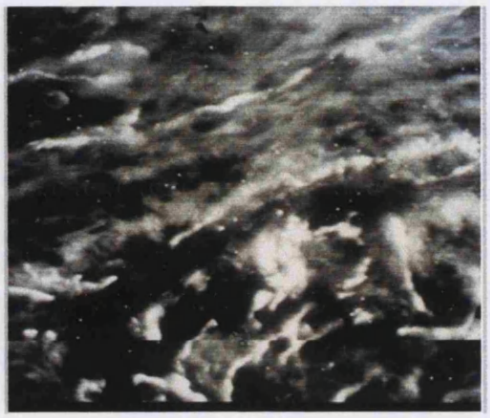
PGP staining gave the nerves a fluorescent green appearance. Neuronal vesicles and ganglia in the detrusor could be seen but these were not counted in this study. Biopsies from **15 of the 20 patients** treated with capsaicin were stained by this method (these were exactly the same patients as in the S 100 group except that biopsies from two of the responders-one with MS and one with transverse myelitis were found to be inadequate). Results from **11 patients** (6 males, 5 females; 9 MS, 1 transverse myelitis and 1 cervical myelopathy) are presented here. Results of the biopsies from the **4 TSP patients** are discussed separately. Of the 11 patients 7 responded to capsaicin while the treatment failed in 4 patients-3 with MS and 1 with transverse myelitis.

1. The mean "red%" in the control group (12 patients) with no identifiable bladder pathology was 2.82 ± 0.29 (mean ± standard error).
2. In patients with hyperreflexic bladders who responded to capsaicin (biopsies available from 7 patients; inadequate samples in 2 others), the mean "red%" was found to reduce from 3.41 ± 1.06 to 1.15 ± 0.32 ($p<0.04$, t-test), 6 weeks after treatment.
3. In those who did not respond the mean "red%" was 5.29 ± 0.35 and 5.36 ± 0.42 before and 6 weeks after treatment with no statistically significant difference between them ($p<0.36$). The difference in red% between responders and non-responders precapsaicin just failed to reach signifiance ($p=0.058$, Mann-Whitney U test), being skewed by a red% of

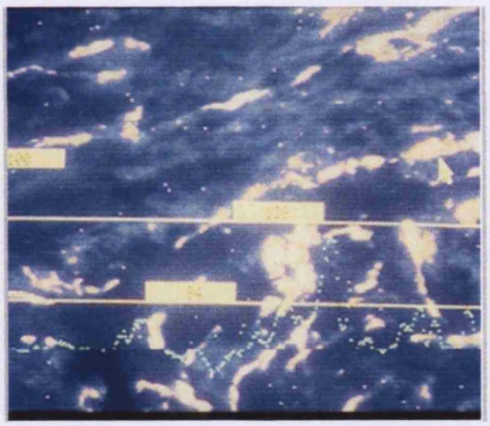
9.67 in one patient. However postcapsaicin this difference was highly significant (p=0.006, Mann-Whitney U test).

4. The mean "red in frame" in the control group (12 patients) with no identifiable bladder pathology was 714.17 ± 72.57 (mean ± standard error).
5. In patients with hyperreflexic bladders who responded to capsaicin the mean "red in frame" was found to reduce from 824.71 ± 246.34 to 297.86 ± 83.53 ($p<0.05$, t-test), 6 weeks after treatment.
6. In those who did not respond the mean "red in frame" was 1353.5 ± 83.43 and 1372 ± 101.65 before and 6 weeks after treatment with no statistically significant difference between them ($p<0.40$, t-test) (Dasgupta et al, 2000). These changes are shown in Table 9 and Figs. 34-41.

There was good correlation ($r=0.9$; Pearson co-efficient) between readings taken by the two independent observers as shown in Figs. 42, 43.

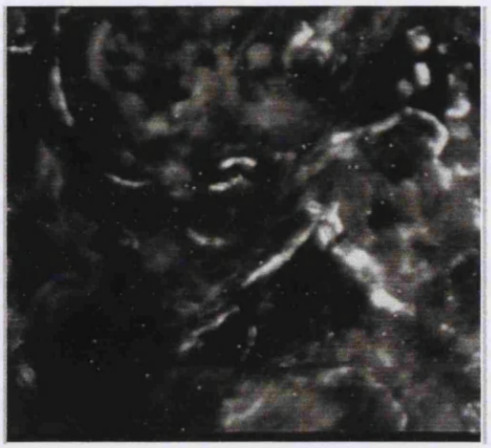


A



B

Fig. 34 Precapsaicin: 'Seescan' images of lamina propria nerves before capsaicin in an MS patient
A As seen under the immunofluorescent microscope
B Computerised analysis of the same image. The numbers and the graph indicate the interactive threshold (IT)



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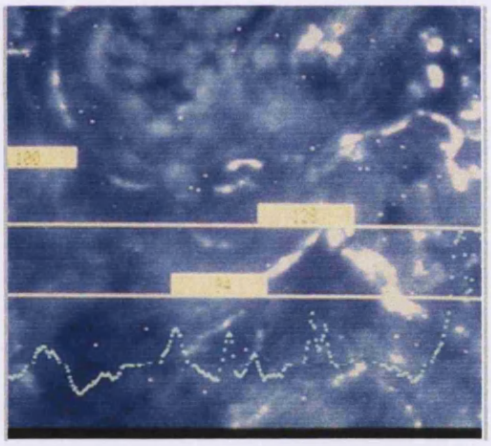

B

Fig. 35 Postcapsaicin: 'Seescan' images of lamina propria nerves 6 wks after capsaicin
A As seen under the immunofluorescent microscope
B Computerised analysis of the same image showing reduction in the nerve density
Same patient as in Fig. 34

Table 9 Mean densities of PGP 9.5 positive profiles ("red %" and "red in frame")

Nerve density	Controls (12)	Responders (7)		Non responders (4)	
mean[range]	normal bladders	precapsaicin	postcapsaicin	precapsaicin	postcapsaicin
Red %	2.82 [1.13-4.89]	3.41 [1.40-9.67]	1.15 [0.25-1.98]	5.29 [4.27-5.87]	5.36 [4.14-6.02]
Red in frame	714.17 [283-1223]	824.71 [450-2286]	297.86 [64-570]	1353.5 [1112-1496]	1372 [1075-1535]

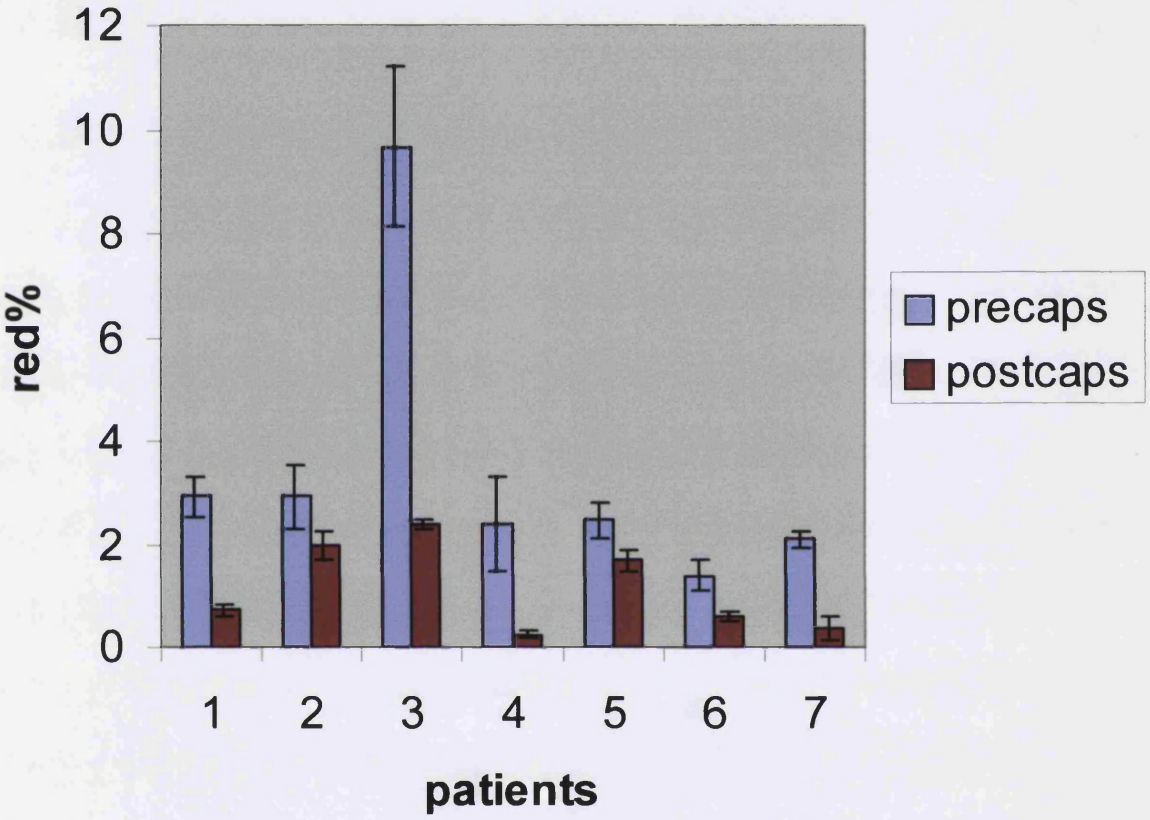

Fig. 36 Change in "red%" in responders. Adapted with permission of the British Journal of Urology International

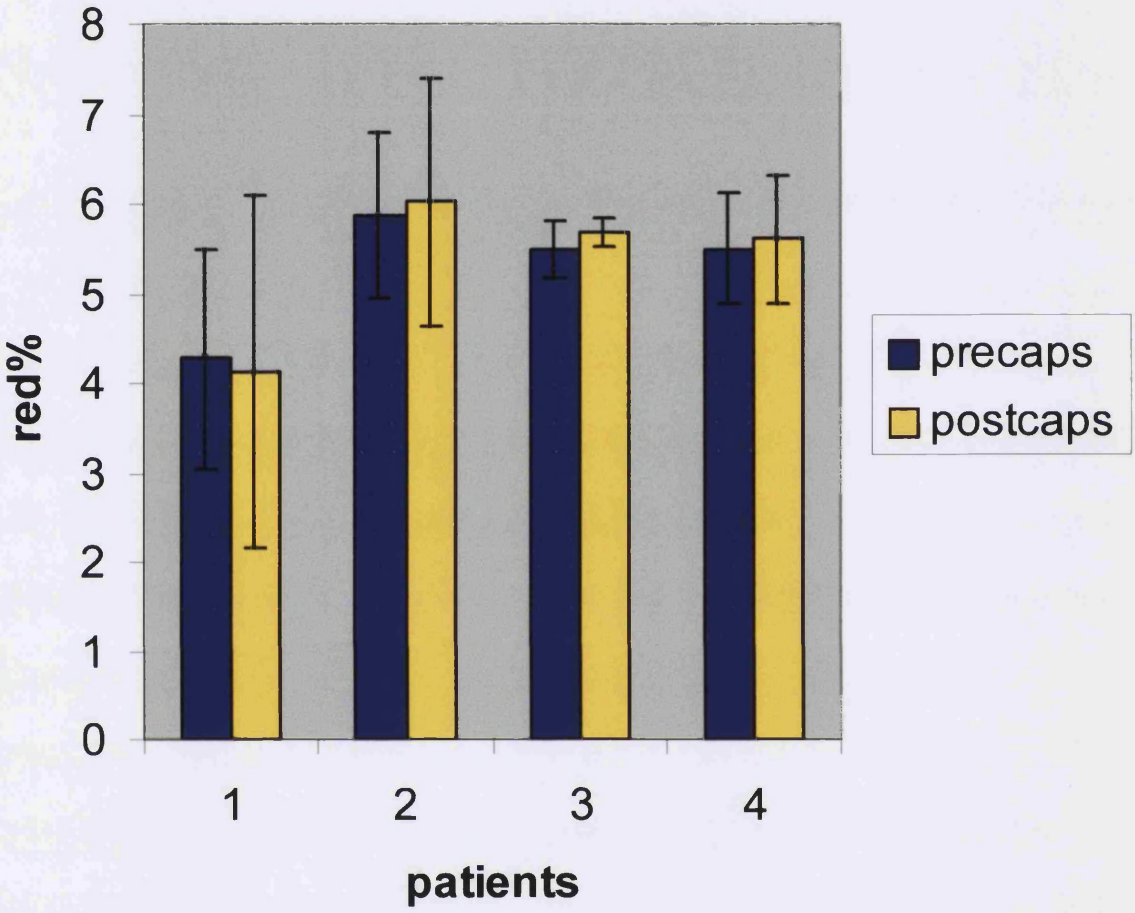

Fig. 37 Change in "red%" in non-responders. Adapted with permission of the British Journal of Urology International

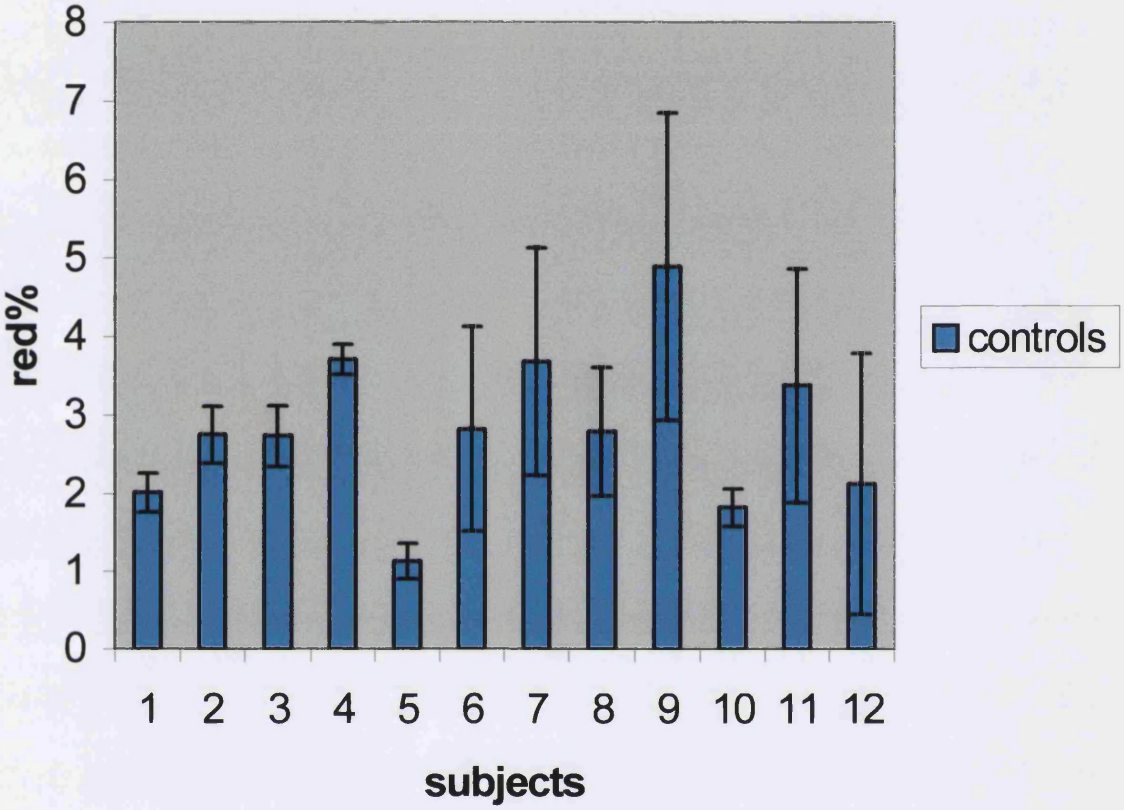

Fig. 38 "Red%" in control subjects. Adapted with permission of the British Journal of Urology International

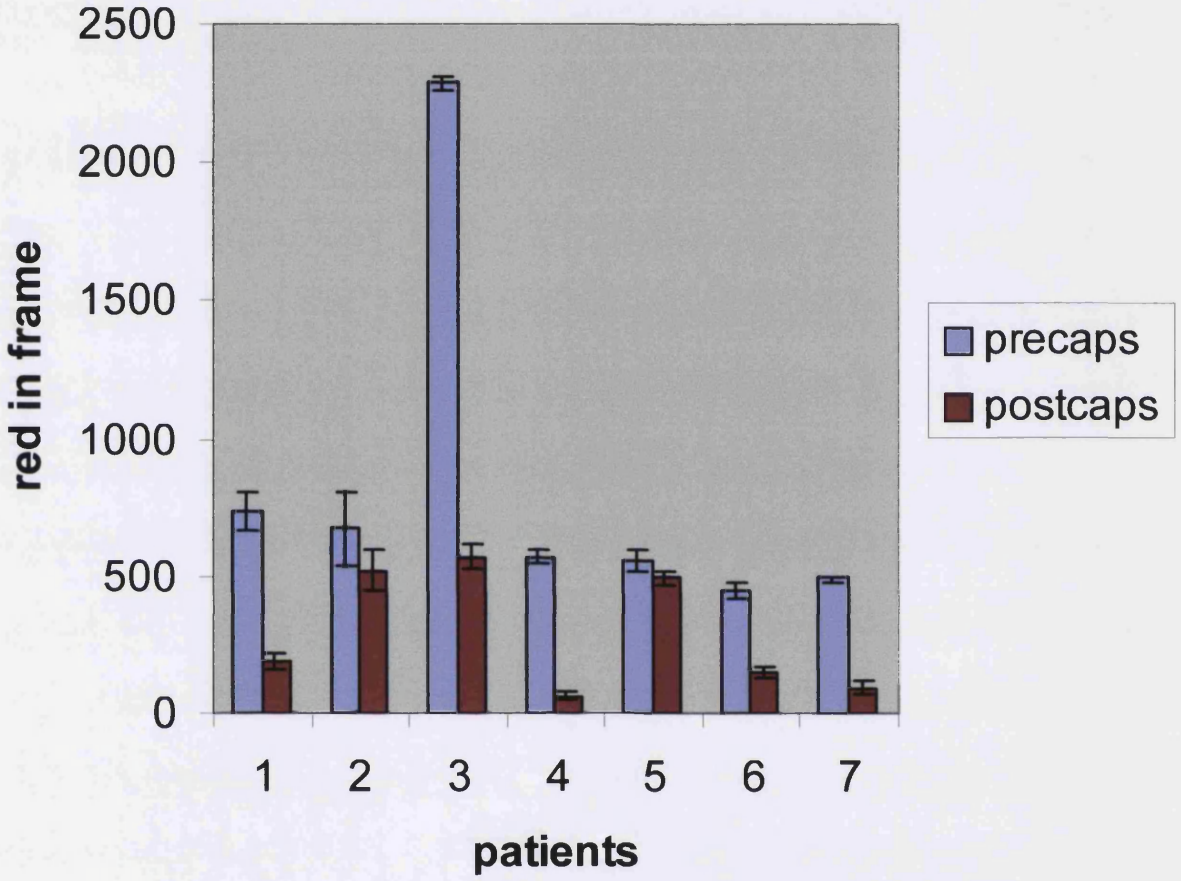

Fig. 39 Change in "red in frame" in responders. Adapted with permission of the British Journal of Urology International

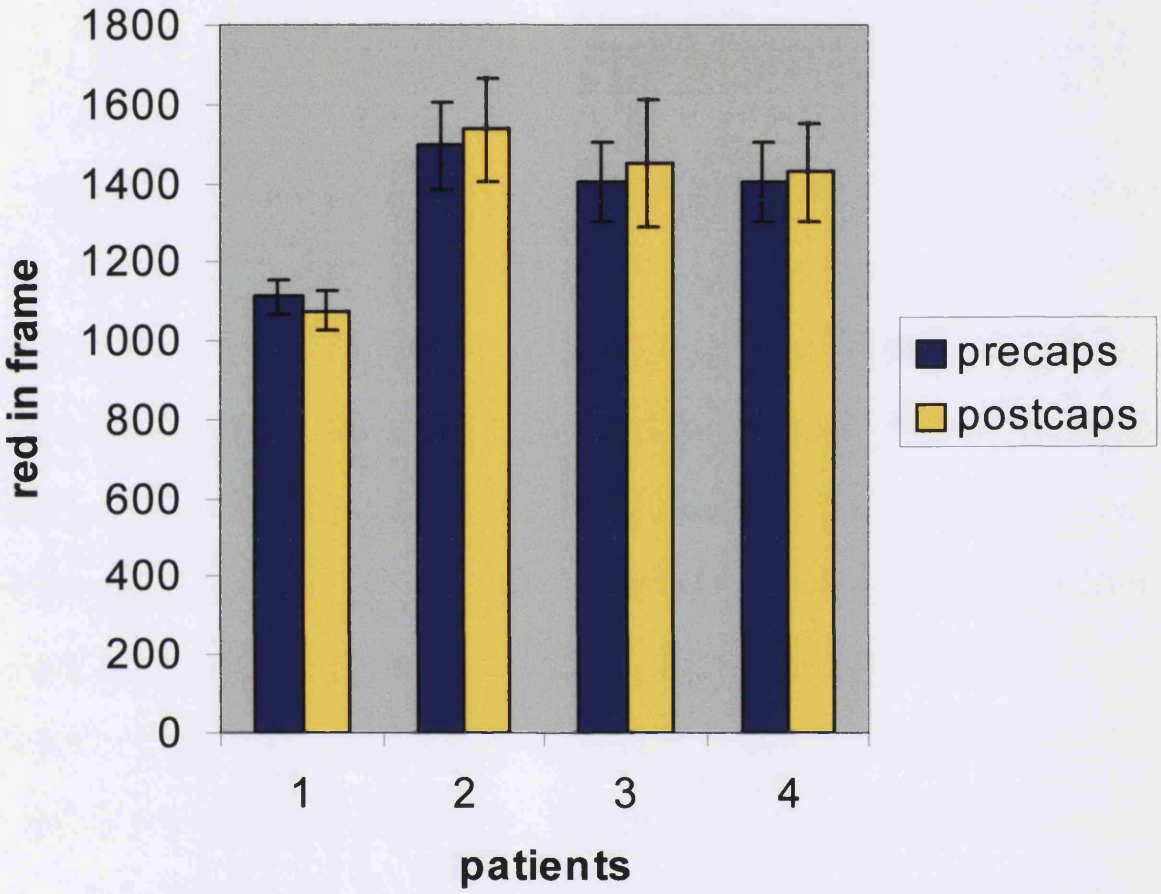

Fig. 40 Change in "red in frame" in responders. Adapted with permission of the British Journal of Urology International

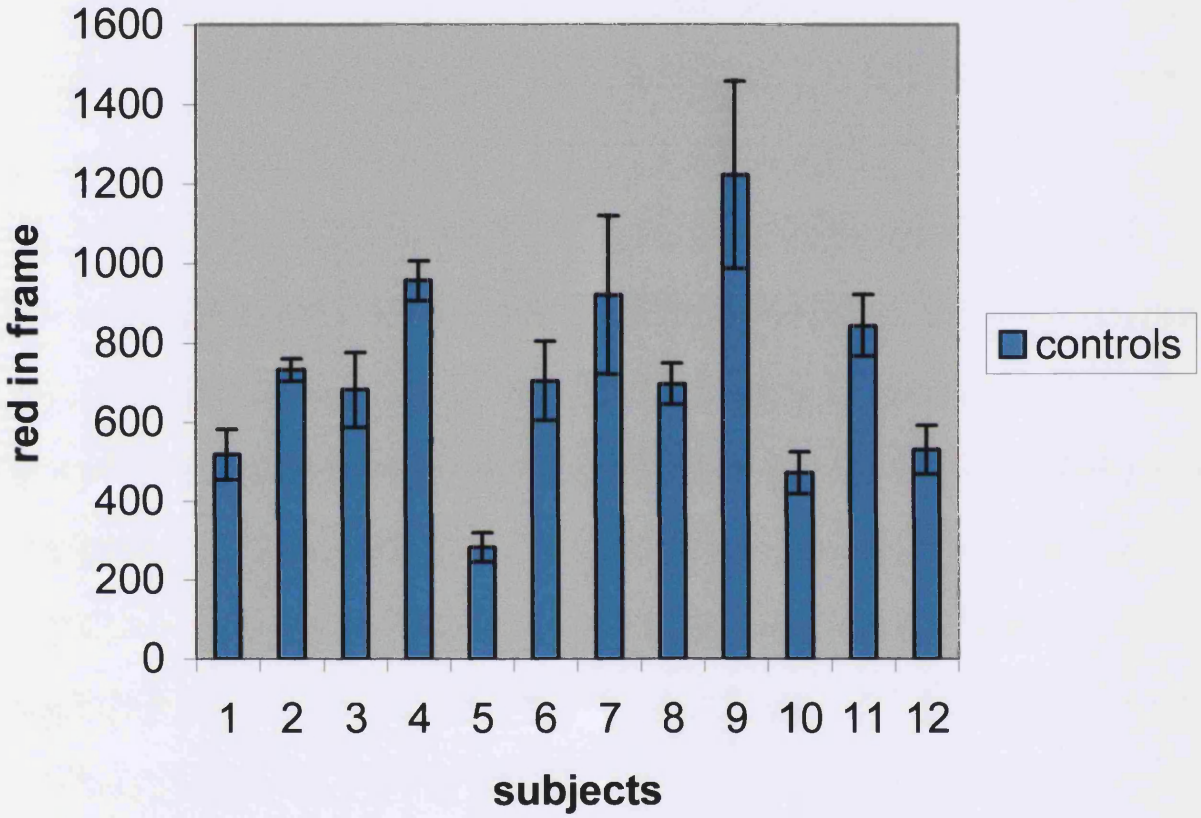

Fig. 41 "Red in frame" in control subjects. Adapted with permission of the British Journal of Urology International

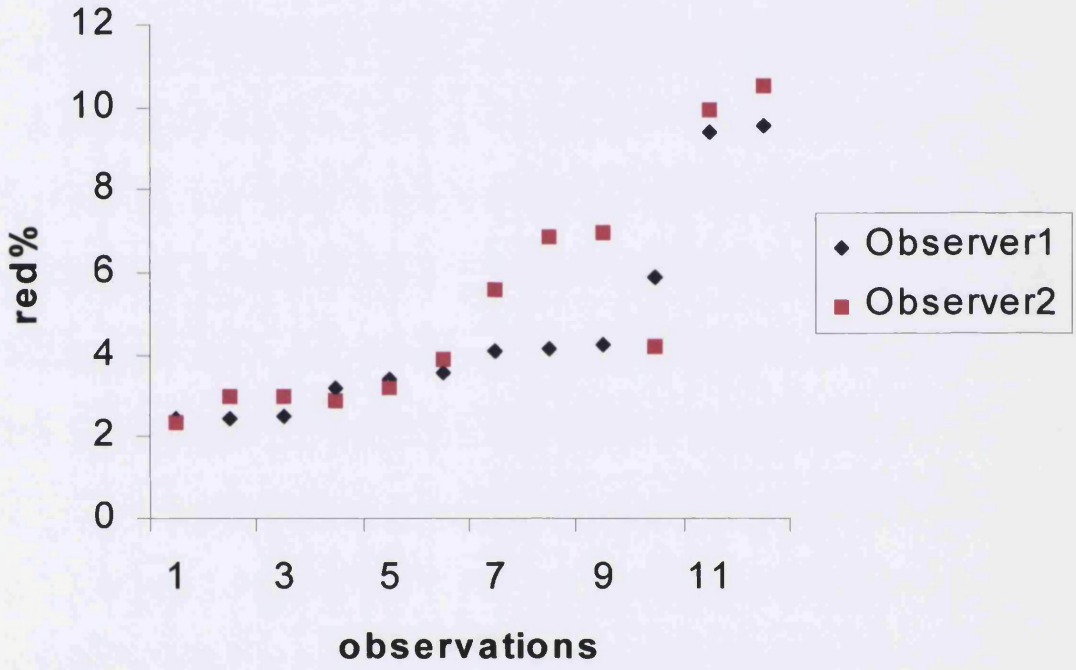

Fig. 42 Comparison of "red%" analysis by 2 independent observers.

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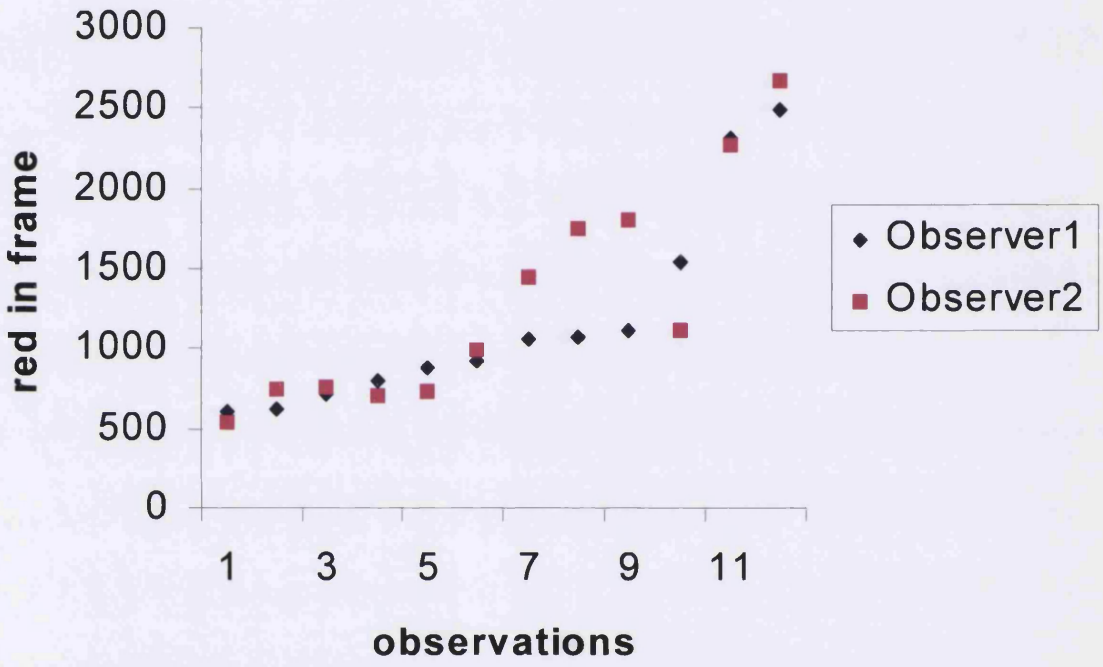

Fig. 43 Comparison of "red in frame" analysis by 2 independent observers.

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14.4 Neuronal appearances in TSP and the effect of capsaicin

Of particular interest were the curious neuronal appearances of the bladder in patients with TSP. Compared to those with MS and normal bladders the sub-urothelial S 100 positive profiles in TSP were considerably thickened. All 4 patients responded to capsaicin (please see Table 5) and there was a reduction in the mean nerve density scores 6 weeks after treatment as shown in Table 10 and Figs. 44-46.

Compared to those with MS and normal bladders the sub-urothelial PGP 9.5 positive nerves in TSP were considerably thickened and had "sausage roll" like appearances in some of the sections. These are the first reported appearances of nerves in the human bladder in this condition (Dasgupta et al, 1996c; Dasgupta and Hussain, 1999).

Table 10 Neuronal changes in TSP patients before and after capsaicin

Patient	S100 precaps	S100 postcaps	Red% precaps	Red% postcaps
1	98	49	10.06	5.04
2	78	79	3.28	3.05
3	250	133	10.31	9.34
4	156	145	9.96	9.48
Mean ± SE	**145 ± 38.6**	**101 ± 22.6**	**8.4 ± 1.7**	**6.7 ± 1.6**

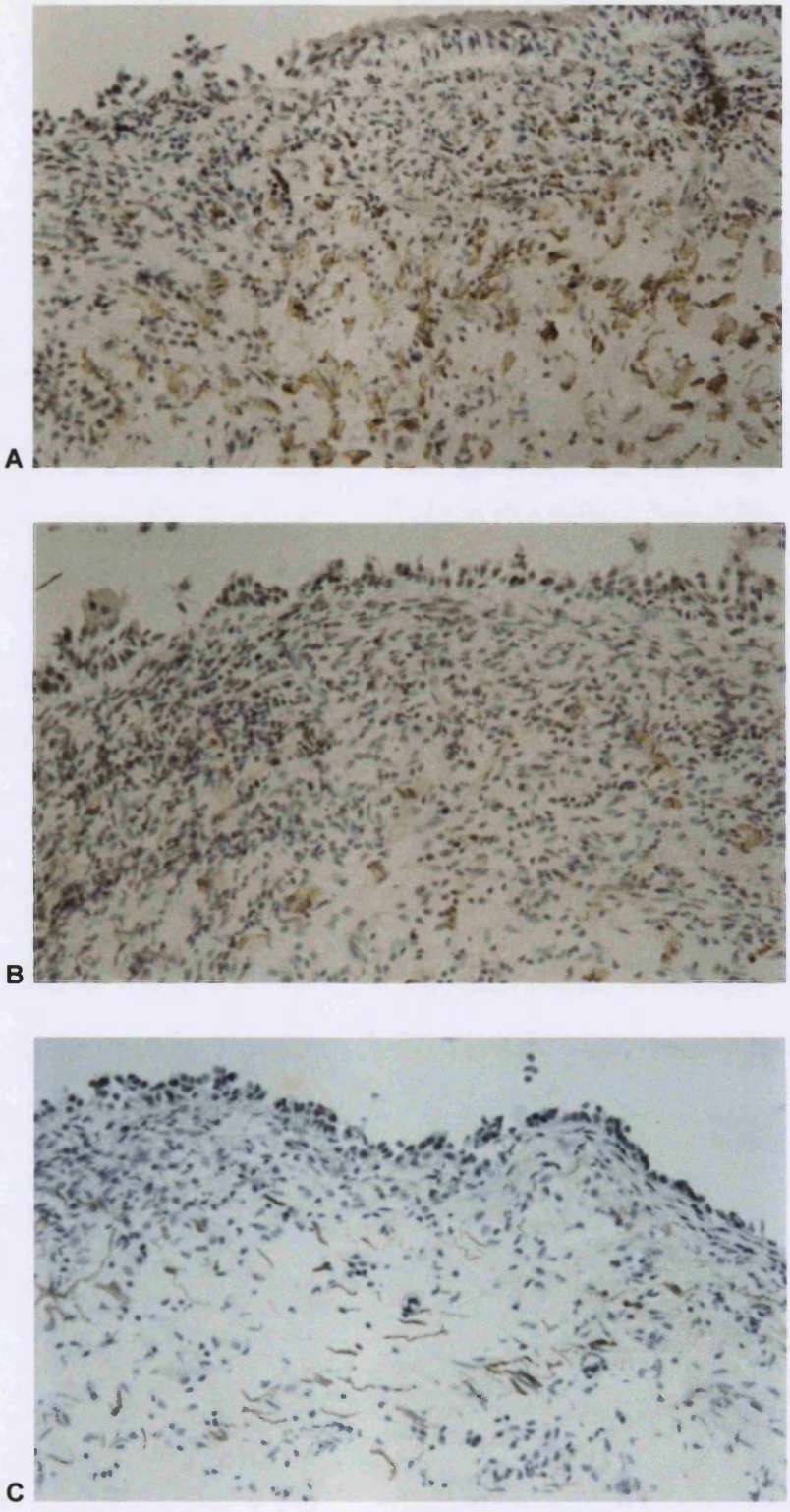


Fig. 44 Neuronal changes in TSP -S 100 immunostaining

A Thickened suburothelial nerves in a TSP patient before capsaicin

B Reduced suburothelial nerves in a TSP patient after capsaicin

C Suburothelial nerves in an MS patient for comparison (S100 staining; x250)

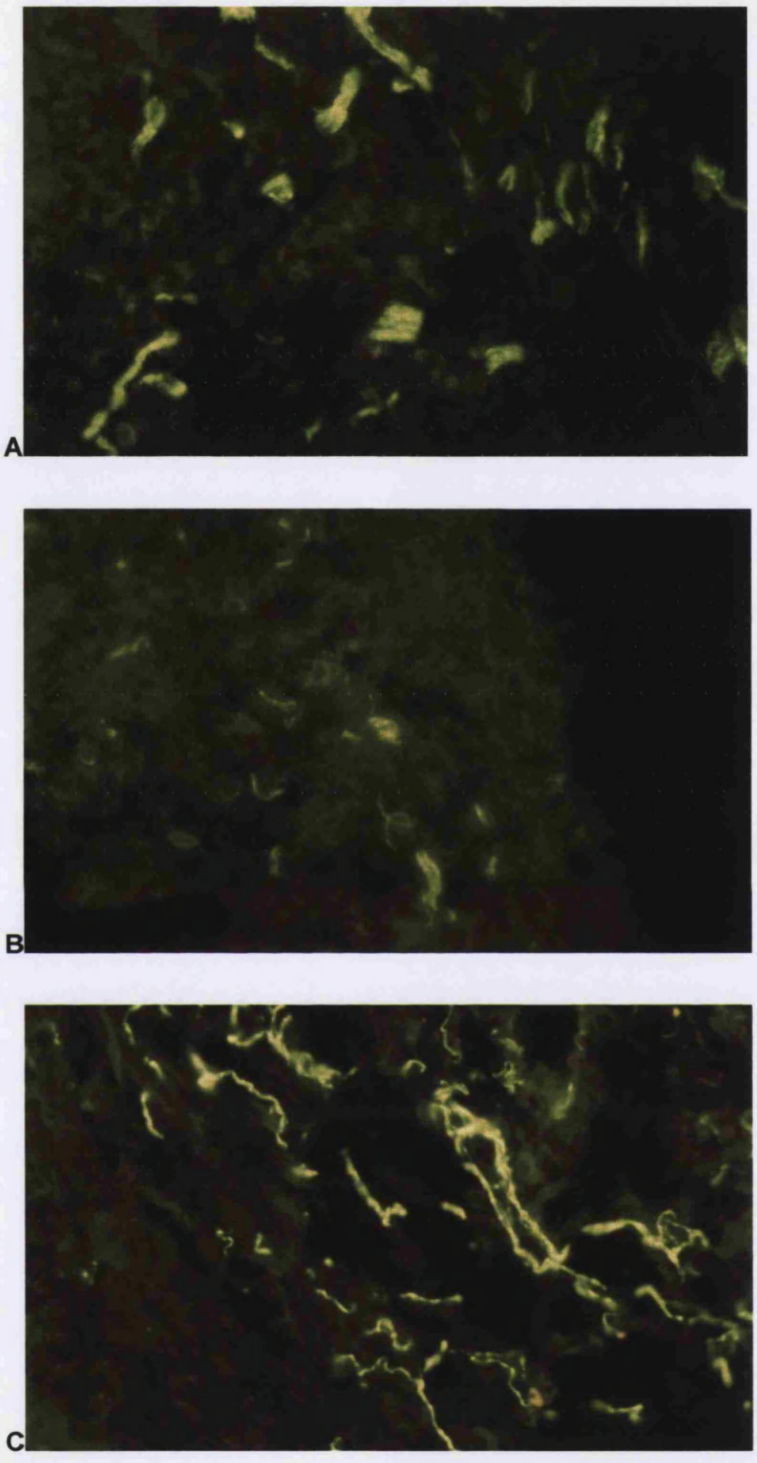


Fig. 45 Neuronal changes in TSP -PGP 9.5 immunostaining

A Thickened suburothelial nerves in a TSP patient before capsaicin

B Reduced suburothelial nerves in a TSP patient after capsaicin

C Suburothelial nerves in an MS patient for comparison (PGP staining; x250)

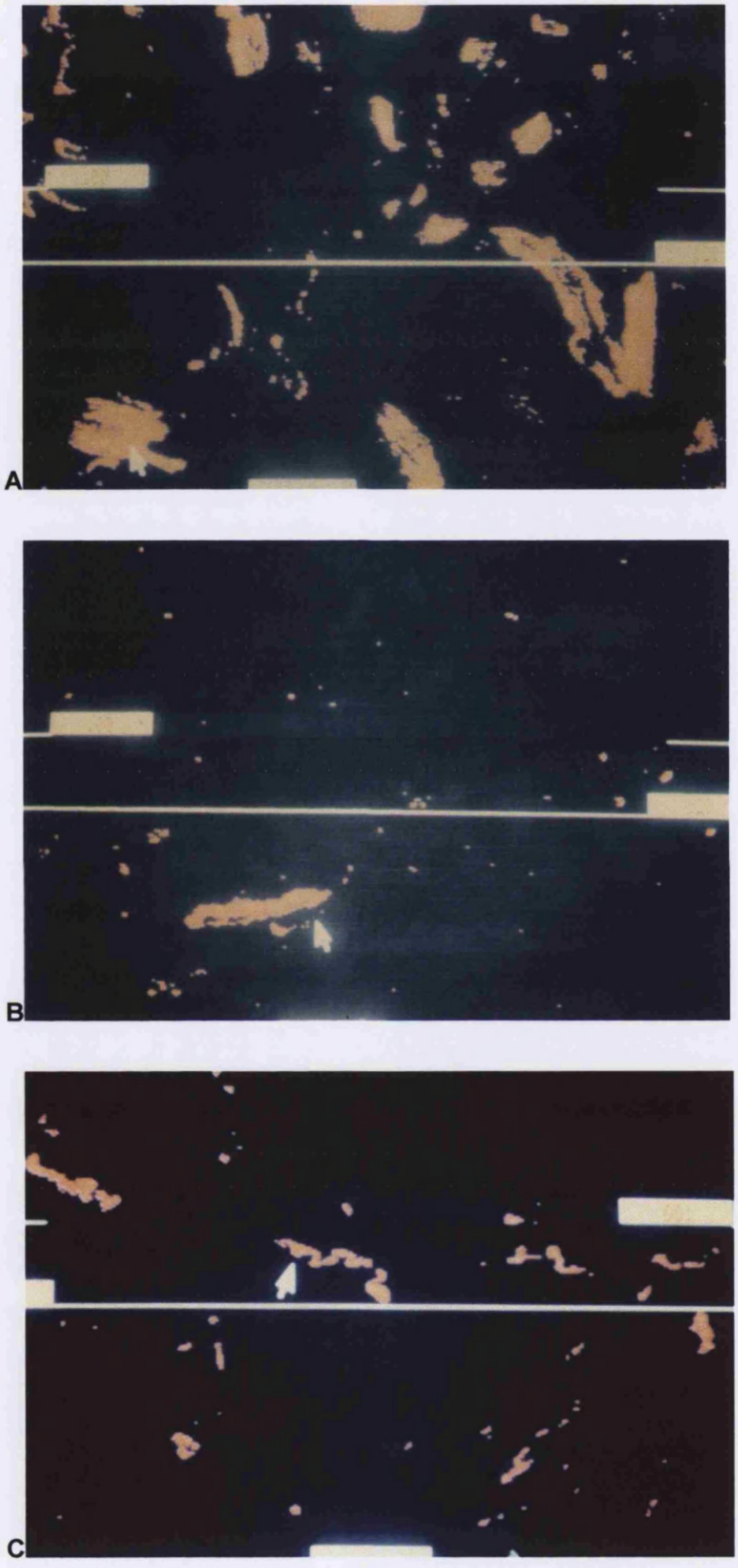


Fig. 46 Neuronal changes in TSP -'Seescan' analysis
A Thickened suburothelial nerves in a TSP patient before capsaicin
B Reduced suburothelial nerves in a TSP patient after capsaicin
C Suburothelial nerves in an control patient for comparison.
46C reproduced with permission of the British Journal of Urology

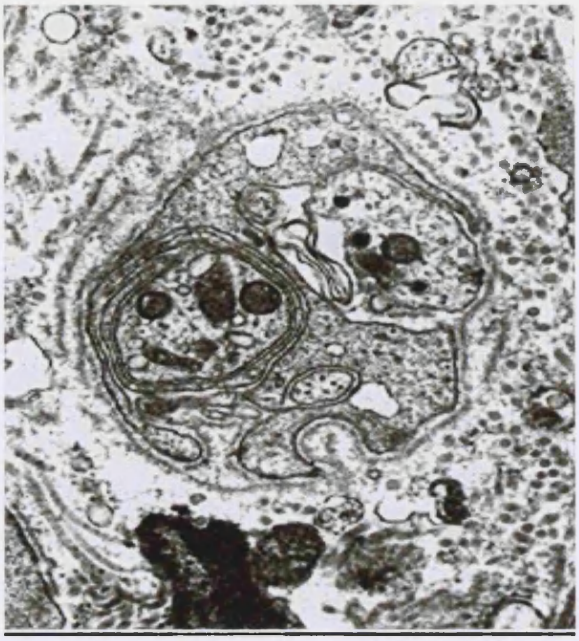
14. 5 Electron microscopy

The suburothelial neurons appeared as axons containing both pale and dense cored vesicles surrounded by Schwann cells. In patients who achieved continence after treatment, preliminary results indicated that the number of axons with >10 clear vesicles, the number of axons with dense cored vesicles and the Schwann cell profiles/nerve had reduced (Dasgupta et al, 1997b). This pilot study is continuing, as the sample size here was not large enough to reach statistical significance on a Wilcoxon ranked test. The results are summarised as:

Table 11 Ultrastructural changes as determined from electron micrographs

Pt	Diagnosis	Response	axons/nerve (mean ± SE)		Schwann cells/nerve (mean ± SE)	
			precaps	postcaps	precaps	postcaps
1	CM	Continent	3.45 ± 0.39	2.88 ± 0.24	1.58 ± 1.02	1.27 ± 0.71
2	MS	Continent	3.55 ± 0.28	5.52 ± 0.48	1.26 ± 0.58	1.45 ± 0.91
3	TSP	Continent	7.43 ± 0.58	6.48 ± 4.19	1.22 ± 0.56	1.21 ± 0.54
4	TSP	Continent	4.28 ± 0.44	2.83 ± 0.37	2.00 ± 1.51	1.09 ± 0.34
5	TM	Continent	4.26 ± 0.59	3.45 ± 0.26	1.05 ± 0.21	1.20 ± 0.70
6	TM	Failure	3.24 ± 0.34	5.00 ± 0.51	1.10 ± 0.29	1.10 ± 0.30
7	MS	Failure	3.29 ± 0.22	3.83 ± 0.24	1.28 ± 0.68	1.99 ± 0.93
8	SCI	Failure	7.61 ± 0.43	7.20 ± 0.63	1.24 ± 0.56	1.65 ± 1.45

There was some evidence of Schwann cell loss 6 weeks after capsaicin as viable axons surrounded only by basal lamina and no Schwann cells were seen in some sections (Fig. 47). Some of the samples also contained small diameter axons (Fig. 48) and empty Schwann cell bands. No changes were seen in the urothelial cells or in any intracellular organelles such as mitochondria. The results are depicted in Figs. 49-51.



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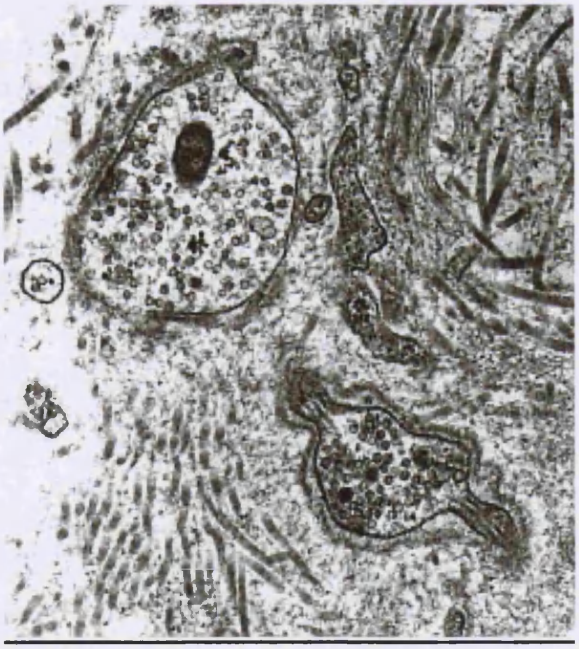
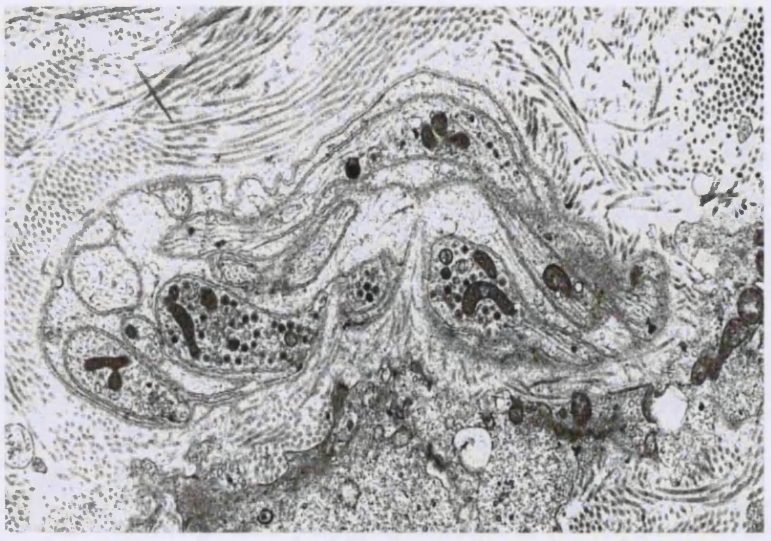


B

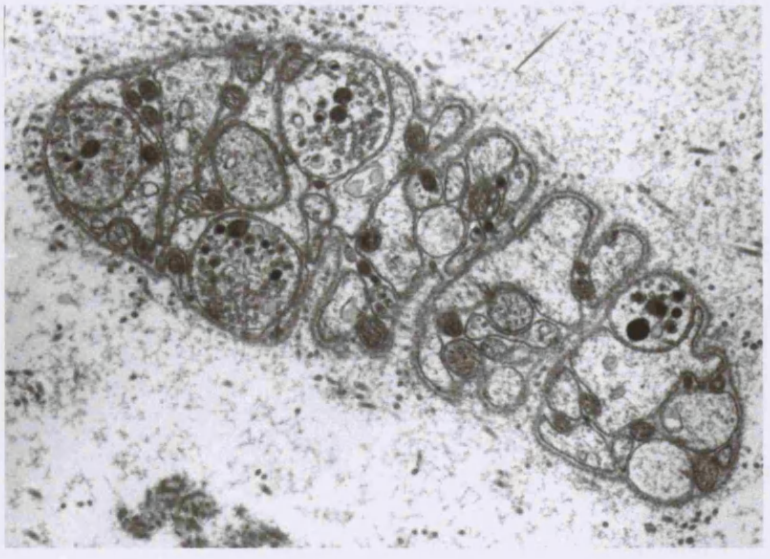
Fig 47 Suburothelial nerves on electron microscopy

A Precapsaicin: showing a suburothelial nerve with axon containing vesicles and Schwann cell and basal lamina surrounding the axons

B Postcapsaicin: showing viable axons surrounded by basal lamina only and absence of Schwann cells



A

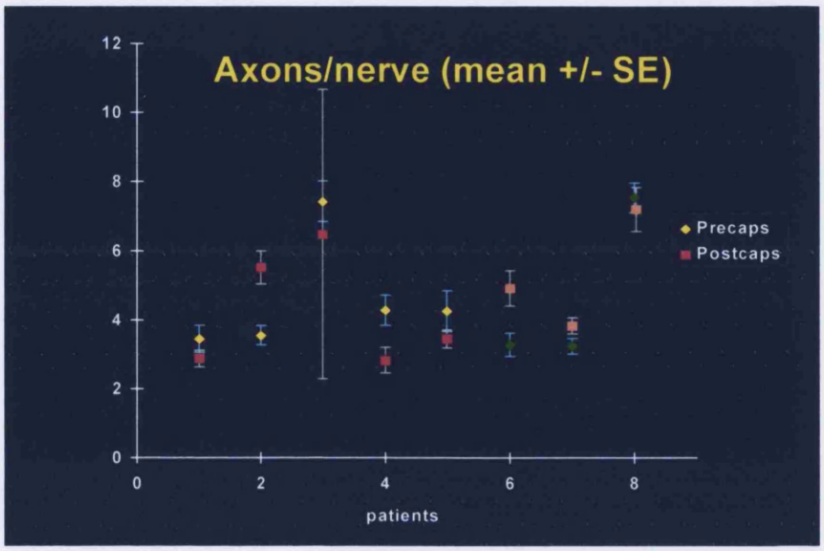


B

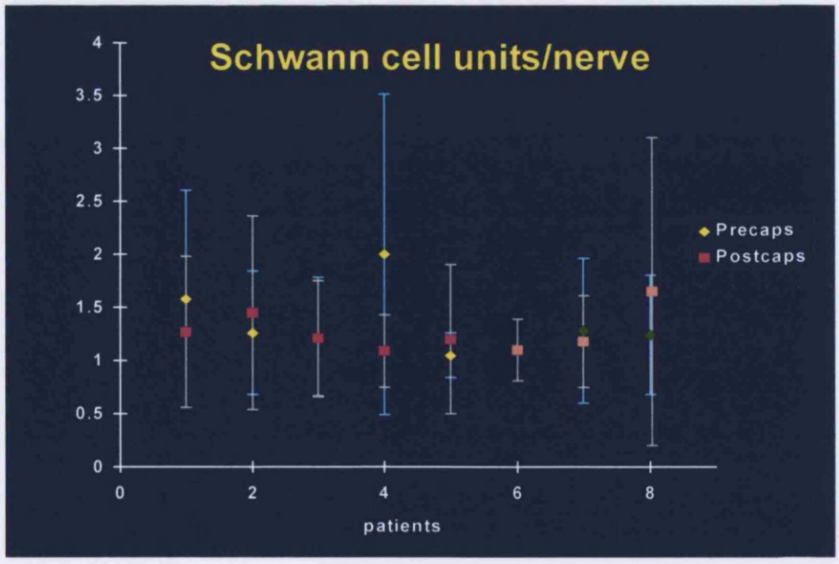
Fig. 48 Suburothelial nerves on electron microscopy

A Precapsaicin: suburothelial neuron with axons surrounded by Schwann cells

B Postcapsaicin: suburothelial neuron with some small diameter axons

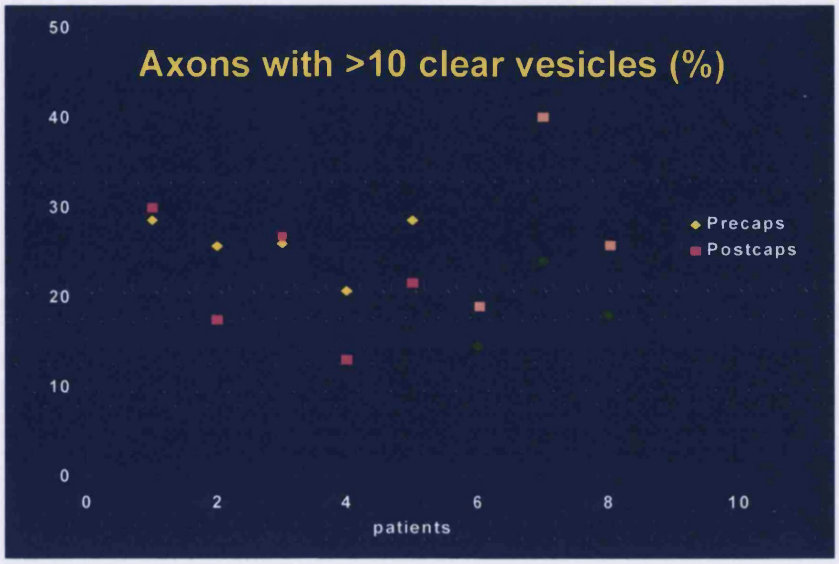


A

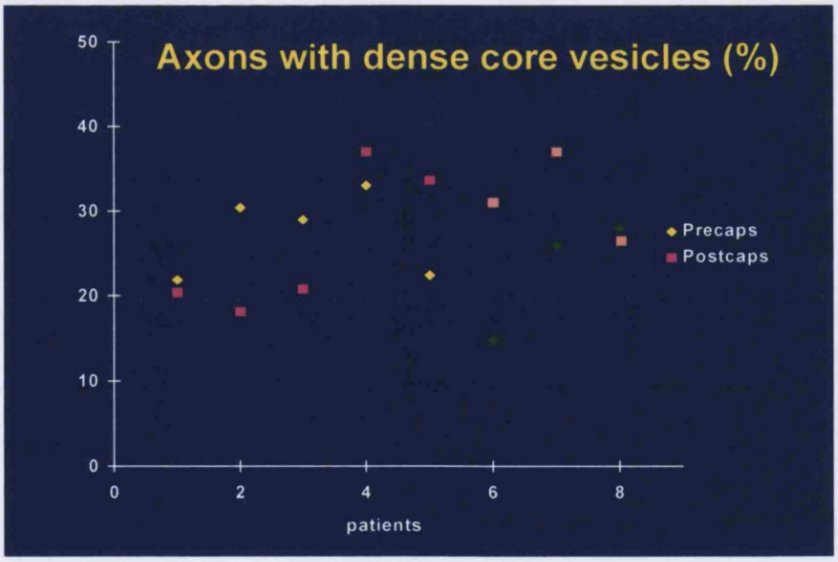


B

Fig. 49 A, B Quantification of electron microscopic changes before and after capsaicin treatment (mean ± SEM). The first 5 patients are responders and the last 3 are non-responders

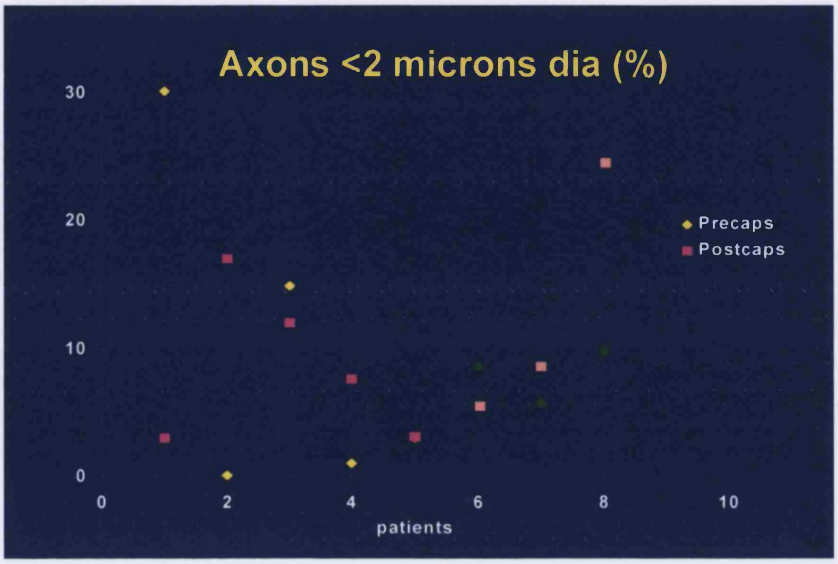


A

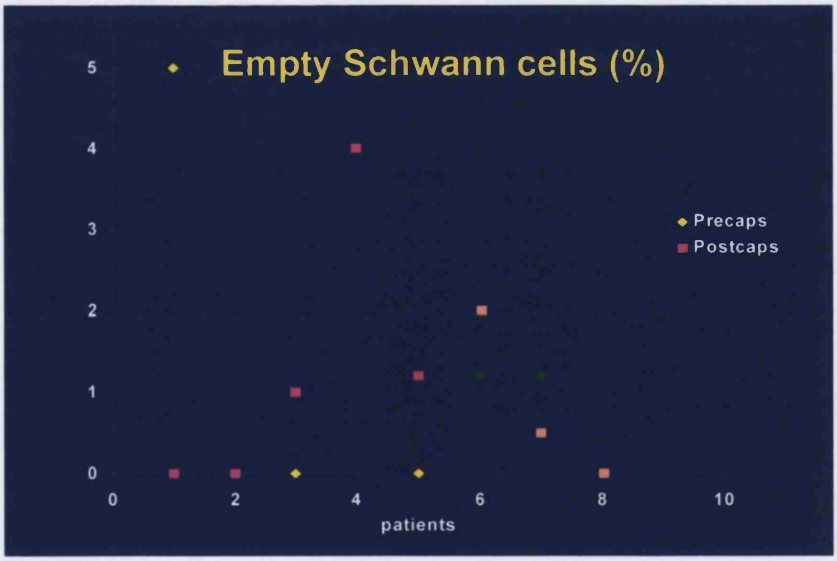


B

Fig. 50 A, B Quantification of electron microscopic changes before and after capsaicin treatment. The first 5 patients are responders and the last 3 are non-responders. Grouped analysis indicates small reductions in axons with more than 10 clear vesicles and axons with dense cored vesicles in those responding to capsaicin



A



B

Fig. 51 A, B Quantification of electron microscopic changes before and after capsaicin treatment. The first 5 patients are responders and the last 3 are non-responders. These are preliminary results

CHAPTER 15

RESULTS: SURVEILLANCE

None of the 20 patients treated with intravesical capsaicin were found to have any urothelial lesions on cystoscopy. All the biopsies examined were benign (Fig. 52) and none showed any form of metaplasia, dysplasia, flat carcinoma-in-situ, papillary or solid invasive cancer. Some had evidence of chronic inflammation but this was seen both in the pre and post capsaicin samples. A few of the sections had von Brunn's nests but again these were present in samples irrespective of the phase of treatment. There were no histopathological differences between tissues from responders and non-responders. Since the tissues were frozen, the illustrations are not as sharp as normally seen in paraffin sections (Dasgupta et al, 1997c; 1998b).

Cystoscopy in the two men immediately after capsaicin showed severe inflammation of the bladder wall as compared to the appearances before the instillations. The inflammatory reaction had settled down completely when cystoscopy was performed 6 weeks later (Fig. 53). Of these two men one was continent and performing CISC while the other failed to respond even after three instillations of capsaicin.

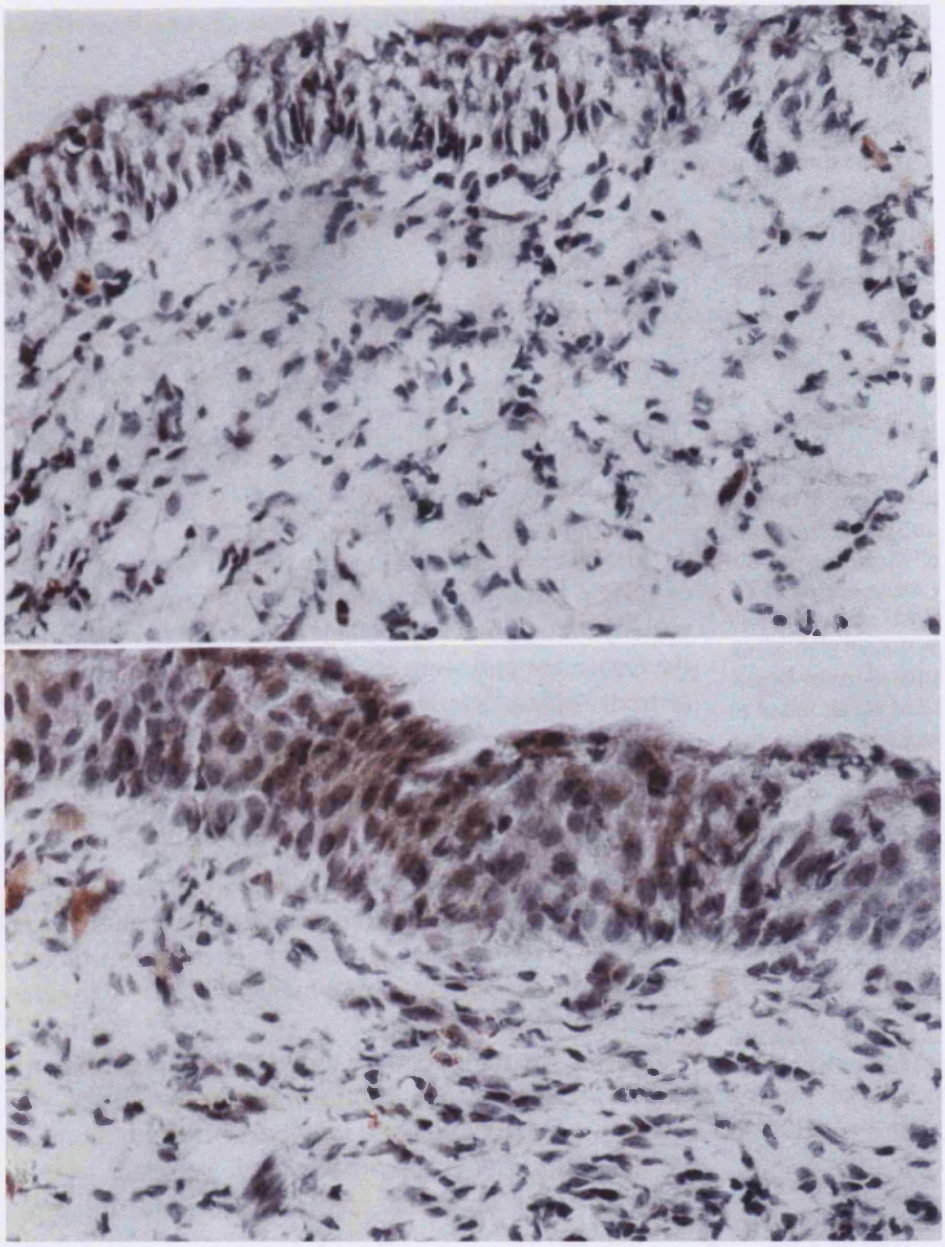


Fig. 52 Results of surveillance. Bladder biopsy from a patient with MS before (above) and 6 weeks after capsaicin (below). H.E. X250. This patient had 17 capsaicin instillations over 5 years. Reproduced with permission of European Urology

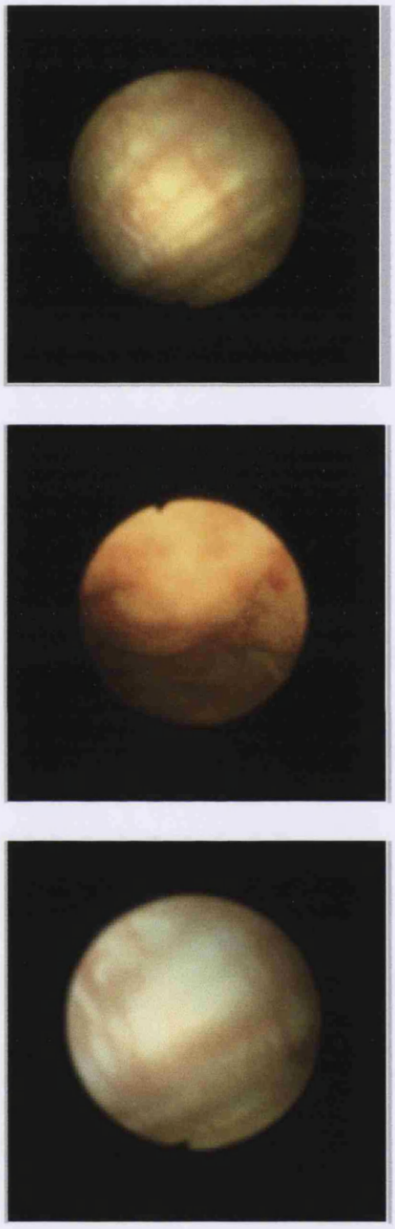


Fig. 53 Cystoscopic appearances of the bladder before **(A),** immediately after **(B)** and 6 weeks after intravesical capsaicin **(C).** Reproduced with permission of European Urology

SECTION IV

DISCUSSION

CHAPTER 16

DISCUSSION

16.1 Clinical response to capsaicin

Detrusor hyperreflexia is a common urological complication of suprasacral spinal cord disease. Detrusor sphincter dyssynergia resulting in incomplete bladder emptying is often found in conjunction with hyperreflexia. The majority of patients can be managed with a combination of anticholinergic medication and CISC (Fowler et al, 1992b) but some do not respond to this treatment and remain severely troubled by their incontinence (Dasgupta and Haslam, 1999). Moreover some patients are unable to tolerate the side effects of oral anticholinergics. These patients can be offered long term catheterisation, a urosheath (in men), major reconstructive surgery or urinary diversion. All these treatments have potential short and long-term side effects. A satisfactory and simple method like intravesical capsaicin for rendering the bladder less hyperreflexic is therefore of great value. As a result of clinical trials performed in our department it has been established that intravesical capsaicin is indeed an effective treatment in some of these patients (Fowler et al, 1992a).

That 72% of patients with phasic detrusor hyperreflexia showed some response with clinical and urodynamic confirmation of lessening of detrusor hyperreflexia, is evidence of functionally important capsaicin sensitive afferents in the bladder of spinal man. A combined analysis of data from six series in 131 patients has shown a mean increase in bladder capacity from 144 ml (range 72-195 ml) before to 267 (range 185-321 ml) after capsaicin

treatment (Chancellor and de Groat, 1999). Experience to date with intravesical capsaicin has shown that it is most likely to be effective in those who have some residual power in their lower limbs. In a dual centre study involving London and Leuven we found that fewer patients in Leuven achieved complete continence as compared to those in London possibly because the patients in Leuven were more disabled (De Ridder et al, 1997). We would recommend that capsaicin be avoided in the severely disabled bed bound patients as it is unlikely to produce any sustained benefit in these individuals. In addition patients must not be cognitively impaired and must be properly motivated to try and overcome their incontinence. They must also be able to perform CISC.

It seems possible to be able to predict patterns of failure, which may help patient selection in future studies. We have previously reported that patients with poor bladder compliance on cystometry have no clinical or urodynamic improvement following intravesical capsaicin instillation (De Ridder et al, 1997). This is not surprising as the pathogenesis of poor bladder compliance is different from detrusor hyperreflexia (McGuire, 1994) Patients with sensory urgency and idiopathic detrusor instability also do not obtain any clinical benefit. These patients find the procedure to be exceptionally uncomfortable despite the use of local anaesthetic. Our results in treating patients with sensory urgency (De Ridder et al, 1997) do not match those obtained by Maggi (Maggi et al, 1989b). We used a higher concentration of capsaicin than Maggi et al, which may explain our different findings in this group.

This study has shown that capsaicin remains effective when given repeatedly to patients. Two patients had better subjective improvement in their urinary incontinence after two successive capsaicin instillations 2 weeks apart.

Considering that the dose and the number of exposures to the neurotoxin are more important parameters in determining the treatment outcome (Craft and Porreca, 1994a), this form of treatment (i.e. 2 successive instillations) in at least some patients who fail to have a sustained response to a single dose, is recommended.

In his editorial comment in the Journal of Urology, Maggi criticised our dual centre study between London and Leuven for lacking a placebo-controlled arm (De Ridder et al, 1997). This problem seems to have been overcome by the randomized trials conducted in France (Wiart et al, 1998). However we feel that at capsaicin concentrations of 1-2 mmol/l it is not possible to conduct randomized trials using Fowler's protocol of instillations, whereby the capsaicin solution is withdrawn from its plastic container into two syringes and instilled slowly by attaching these syringes to the urethral catheter. The pungency of capsaicin is quite obvious while withdrawing the solution into syringes. This method of instillation prevents leakage of the solution into the urethra, which can be extremely painful for the patient.

The author has corresponded with Dr Wiart regarding the exact methodology used in his randomized trail. Dr Wiart's team instilled capsaicin and alcohol (vehicle) directly from their plastic bags in a blinded fashion, resulting in pain and urethral leakage of both solutions (Dr L Wiart, personal communication). We feel that once such leakage occurs, the pungency of the capsaicin solution would be obvious thus making blinding impossible. Therefore although a randomized trial would have been ideal we have been unable to conduct it.

16.2 EMDA

Most investigators currently instill solutions containing 1-2 mmol/L of capsaicin for 30 mins to treat detrusor hyperreflexia. Although the outcome is often favourable, pain during and sometimes after the instillations and autonomic dysreflexia may limit the use of capsaicin (Cruz et al, 1997a). The concentrations used in hypersensitive bladders are about 100 times lower than in hyperreflexia. However a recent randomized study found capsaicin to be no better than placebo for relieving bladder pain and the authors suggested a higher concentration of capsaicin for painful bladders might be more effective (Lazzeri et al, 1996). Such an increase in concentration would necessitate effective anaesthesia during the procedure in these patients. From the literature two aspects relating to the use of intravesical capsaicin become apparent:

(1) Varying degrees of discomfort occur in most patients and in some the procedure is not tolerated for the full 30 mins

(2) Although the prior use of intravesical 2% lignocaine reduces the discomfort and the bladder overactivity caused by capsaicin (Chandiramani et al, 1996), variable volumes of the solution may still have to be withdrawn to reduce hyperreflexia and prevent urethral pain due to leakage.

Electromotive drug administration (EMDA) is a technique incorporating several electrokinetic phenomena (Stephen et al, 1994). For chloride salts of local anaesthetics, which are strongly ionised, the main force responsible for accelerated drug delivery is iontophoresis (Banga and Chien, 1988). Drug administration rates can be determined using equations derived from Faraday's Law (Fontanella et al, 1997).

Canine studies using methylene blue have shown a greater penetration of the dye into the bladder wall using EMDA as compared to controls without EMDA (Gürpinar et al, 1996a) suggesting that more profound local anaesthesia of the bladder with EMDA of lignocaine (which has a similar molecular weight to methylene blue) is due to deeper penetration of the drug into bladder tissues.

The scientific basis for EMDA and the rationale for using salt free lignocaine 2% to anaesthetise the bladder has been previously been described in detail (Fontanella et al, 1997). The presence of NaCl in the solution virtually ensures ineffectual local anaesthesia since these ions are highly mobile and there is charge competition between them and the lignocaine ions.

EMDA of lignocaine has been shown to be effective for cystodistension in the treatment of interstitial cystitis (Gürpinar et al, 1996b; Fontanella et al, 1992), for invasive procedures such as bladder biopsies (Lugnani et al, 1993) and even Transurethral resection of bladder tumours (Fontanella et al, 1997).

Although the instillation of lignocaine 2% before capsaicin made the procedure easier it did not provide adequate local anaesthesia in all patients. We considered the possible options to overcome this situation:

(1) To lower capsaicin concentration in the instillate: There is evidence from early work by Fowler et al that doses below 1 mmol/L are ineffective in detrusor hyperreflexia (Fowler et al, 1992a).

(2) To perform the procedure under general anaesthesia: This has been reported by Igawa and colleagues in patients with spinal cord injury (Igawa et al, 1996) but the agents used would not relax the detrusor and bladder spasms during the instillations would persist.

(3) To perform the procedure under epidural anaesthesia: A logical choice but which requires skilled specialist assistance.

(4) To perform the procedure following EMDA of local anaesthesia: The final option was to anaesthetise the bladder with EMDA of lignocaine and epinephrine. This is a simple office-based technique (Gürpinar et al, 1996b) which does not require specialised training. Recent studies have shown this method to be safe and free from systemic side effects. The mean maximum serum concentration of lignocaine after EMDA (25 mA for 25 mins), with higher total charge than used in this study (20 mA for 15 mins) was reported as 0.33 μg/ml (Fontanella et al, 1997) which is well below the range achieved for cardiac arrhythmias (1.5-5 μg/ml) (Gürpinar et al, 1996b). Allergy to local anaesthetics and frank haematuria are absolute contraindications to this procedure and relevant history should therefore be obtained from all patients scheduled for EMDA.

We know that the maximum tolerable intravenous dose of capsaicin in humans is 4 μg/kg (Winning et al, 1986); about 0.3 mg in the average adult. It can thus be postulated that the maximum amount of capsaicin entering the vasodilated bladder tissues (capsaicin causes marked inflammation) when a concentration of 2 mmol/L (60 mg/100 ml) is instilled, is probably only 1-2 mg; much in excess of this amount would cause notable systemic symptoms which have never been reported. This fraction, small and unknown as it is, should provide a valid reference point to assess standardised treatments for different degrees and aetiologies of detrusor hyperreflexia, but it is not consistent. Localised bladder pathologies and technical problems described

in the literature virtually guarantee that a specific protocol applied to a series of patients will result in substantial variations of capsaicin uptake.

It is known that patients with detrusor hyperreflexia have a higher risk of developing urinary tract infections and mechanical trauma due to catheterisation can cause further injury to the urothelium. Damaged urothelium is more permeable than normal urothelium and it is possible that this pathology will cause some variation in capsaicin uptake by the bladder.

The intravesical volume of a standard solution has a major impact on the uptake of its solute. If one patient tolerates the full 100 ml of a capsaicin solution (~ 105 sq. cm surface area) while another can hold only 50 ml (~ 65 sq. cm surface area), the first patient will be exposed to nearly double the amount of capsaicin taken up by the second patient.

Phasic detrusor hyperreflexia and suprapubic discomfort requiring withdrawal of a quantity of capsaicin during instillation (Chandiramani et al, 1996) reduces the total volume and contact time of capsaicin with the urothelium which will lead to further variation in the dose of capsaicin taken up by the bladder. The various factors, which may influence the uptake of intravesical capsaicin, are described in the appendix.

In contrast to previous clinical experience (Maggi et al, 1989b; Fowler et al, 1994; Geirsson et al, 1995; Cruz et al, 1997a) EMDA almost eliminates hyperreflexia and discomfort during capsaicin treatment and becomes an easier procedure with better patient compliance; the prescribed volume of capsaicin remains within the bladder for the optimum length of time without any need for withdrawal of solution or premature termination of treatment. This would explain the longer duration of benefit noted after the use of EMDA

in our patients and the fact that the two patients who failed to respond to previous capsaicin treatment subsequently responded to capsaicin after EMDA of lignocaine. Although there is no direct evidence it is possible that EMDA before capsaicin makes the bladder more permeable to capsaicin itself through the phenomenon of electroporation (Prausnitz et al, 1993).

The rectal contractions noted during capsaicin instillation are probably due to reflex neural activity (through a sacral arc) and not systemic absorption. They occur irrespective of the use of EMDA. This supports the concept that EMDA acts locally on the bladder (as the vesical trace on cystometry remains almost 'flat') while the rectal contractions are no different from those seen during capsaicin instillation preceded by lignocaine alone. It seems that since the rectum is not anaesthetised, the reflex effect of intravesical capsaicin on the rectum (similar to the phasic detrusor contractions) continues in spite of effective vesical anaesthesia; the nerve fibres mediating this response are unknown.

Autonomic dysreflexia is expected to occur during capsaicin instillations in patients with spinal cord injury particularly involving the cervical cord (Igawa et al, 1996) and can be a life threatening complication. Our early results with EMDA indicate that it might be a useful means of controlling this side effect thus allowing capsaicin to be effectively used in these patients.

It was decided to apply less total charge (current x time) to our patients (300 mA.min) than that used by Fontanella et al (625 mA.min) (Fontanella et al, 1997). Whereas urologists performing TUR bladder tumours may resect deeply into the bladder wall, in patients receiving intravesical capsaicin the

sensory nerves are thought to lie superficially in the lamina propria. Therefore less penetration of local anaesthetic in these patients is required. One advantage of EMDA remains unexplained. Although the local anaesthesia was expected to last for about 40-60 mins it is unknown why none of the patients felt suprapubic pain after this effect wore off.

The use of higher doses of capsaicin in hypersensitive bladders has been suggested (Lazzeri et al, 1996) and EMDA may provide adequate local anaesthesia in these patients during such treatment.

16.3 Flexible cystoscopic biopsies

Although the average size of a flexible cystoscopic biopsy is one-third to one-fourth that of a cold punch biopsy, the size is not related to the quality of tissue obtained and up to 60 sections can be obtained from each sample for light microscopy. The nerve density scores in the control patients were comparable to scores of 20 to 30 nerves on a high power field of 0.35 sq. mm reported previously using cold punch biopsies (Van Poppel et al, 1988b). Previous control data on nerve densities have included patients with bladder cancer who underwent rigid cystoscopy for tumour resection or follow-up and had biopsies from normal areas of the bladder during these procedures (Van Poppel et al, 1988b; 1988c). As transitional cell cancer of the bladder is a multifocal disease selecting these patients as controls is probably not the best option. Since patients with microscopic haematuria have flexible cystoscopies as a part of their investigations this group of patients was chosen for the control samples. Obtaining biopsies were no more uncomfortable in them than the flexible cystoscopies themselves, the likely explanation being the very small size of the samples.

Although the procedures were carried out using local anaesthesia none of the patients complained of undue discomfort or pain. Flexible cystoscopic biopsies are performed as outpatient procedures and are suitable for repeated use in patients. We recommend this simple technique for studying the sensory innervation of the human urinary bladder in health and in disease.

16.4 Effect of capsaicin on sensory nerves

Various studies, mainly in animals, have reported the effects of capsaicin as being neurally mediated. Capsaicin sensitive afferents are known to be present in the bladder of various animal species-a term which refers to those neurones that are initially stimulated and then subsequently desensitized by capsaicin (Maggi and Meli, 1988). These are mainly small, unmyelinated C fibres although occasionally Aδ thinly myelinated nerve fibres are also affected (Nagy et al, 1983).

There is as yet no direct evidence that a C fibre mediated reflex becomes functional on the bladders of humans with spinal cord disease. However the fact that patients with spinal cord disease did do well after capsaicin, supports the hypothesis. The selectivity of capsaicin's action on certain sensory nerve endings suggests that capsaicin and similar associated vanilloids interact at specific receptor sites to produce their effect. Studies carried out using resiniferatoxin have identified such a vanilloid receptor in the pig dorsal root ganglia (Szallasi and Blumberg, 1991). It has also been shown that nerve growth factor (NGF) regulates the response of adult dorsal root ganglion cells at least in vitro to capsaicin (Winter et al, 1988). The cells in a NGF deprived culture were unresponsive to capsaicin but capsaicin sensitivity was restored after replacement of NGF. Immunostaining shows increased NGF expression in the urothelium, most marked in patients with sensory urgency (Lowe et al, 1997) and increased NGF levels may explain the sensitization of nociceptor fibres in the bladder. At present the interaction between NGF and capsaicin in detrusor hyperreflexia remains unknown.

There have been various studies performed which show that direct topical application of capsaicin to nerves results in excitation of capsaicin sensitive afferents which is soon followed by a block in nerve conduction (mainly C fibres) (Petsche et al, 1983). Capsaicin also depletes the nerve terminals of neuropeptides, particularly substance P and is known to block the axonal transport of neuropeptides (Gamse et al, 1982). Systemic capsaicin treatment has also been shown to cause degeneration of axons but this effect is very much dependent on the dose of capsaicin used (Hoyes and Barber, 1981). Morphological changes induced by topical application of capsaicin in humans have not been studied systematically and its effects are ascribed to 'desensitization' (Nitti, 1994). It has been postulated that high doses of intravesical capsaicin, as used in detrusor hyperreflexia, may be acting directly on the bladder afferents (Fowler et al, 1994) since these nerve terminals are known to lie just below the urothelium (Dixon and Gilpin, 1987). These vesical nerves contain various neuropeptides and on co-localization studies and form an 'intrinsic' system similar to that of the small intestine (Smet et al, 1995).

This is the first study to show the depletion of presumptive sensory nerves in the suburothelial layer of the human urinary bladder, in response to high doses of intravesical capsaicin (Dasgupta et al, 2000). The clinical effect is not permanent and lasts for 3-6 months when the treatment has to be repeated.

The nerves in the lamina propria are unrelated to recognised effector sites and are therefore thought to have a sensory function. A recent study however demonstrated that some of these suburothelial fibres might not be sensory.

Co-localization techniques with double-label immunohistochemistry have shown that only about 26 ± 10% of suburothelial CGRP-immunoreactive nerves also contain SP and neurokinin A (NKA), indicating their sensory nature. The other nerves may be partly sympathetic, parasympathetic or sensory (Smet et al, 1997). The same study also demonstrated an increase in the density of CGRP and SP immunoreactive nerves within the suburothelium in women with idiopathic detrusor instability relative to control women with no symptoms of urgency and frequency (Smet et al, 1997). It is however important to note that the patients in that study were different from those reported here and the exact nature of suburothelial nerves in detrusor hyperreflexia remains undetermined. It is possible that the changes described in our study may in fact be involving a specific population of sensory nerves while others expressing different classes of neuropeptides are unaffected.

A study of the pulpal neurons of the lower incisors of mice treated with capsaicin subcutaneously on the second day of life showed damage to the unmyelinated axons as well as their Schwann cells when compared to similar nerves from a control group of untreated mice (Hiura and Ishizuka, 1992). Pini et al demonstrated a long-term reduction in the number of C-fibre nociceptors following capsaicin treatment of the saphenous nerve of adult rats. They noted a reduction in the density of C-fibres and a number of unusually small C-fibres on cross sections of the nerve as seen by EM (Pini et al, 1990).

The exact mechanism of capsaicin induced unmyelinated fibre damage is unclear but various reports indicate that it may be related to disturbances in

cellular ionic gradients. In an interesting animal study capsaicin when applied by superfusion was found to block potassium currents in cultured rabbit glial cells. The rate of blockade increased with increasing concentrations of capsaicin (1-100mM). Unlike tetraethylammonium ions, which reduced the outward current, capsaicin blocked both the inward and outward potassium currents (Baker and Ritchie, 1994). In addition, it is known that capsaicin-evoked depolarisation involves the opening of a cation channel which admits both sodium and calcium (Maggi et al, 1989a). Although it is difficult to extrapolate from these animal tissue culture studies no similar data exists in humans. Since Schwann cell integrity seems to be important for effective transmission of impulses along unmyelinated axons, it is possible that damage to these cells induced by capsaicin indirectly causes axonal degeneration due to a loss of trophic substances produced by these cells. The vanilloid receptor has not yet been identified in the human bladder but it can be speculated that it is present on afferent C-fibres in the suburothelium, which degenerate as a result of exposure to high doses of intravesical capsaicin. Perhaps in the non-responders, capsaicin failed to get through the urothelium and the fact that the nerve densities had not changed significantly would support this view. An alternative explanation could be the presence of different receptor sub-types in non-responders. The ionic flux as described, does not occur and the nerves do not degenerate. Antibodies to the VR1 receptor are commercially available and attempts to localise this receptor on human bladder biopsies are ongoing.

There are no validated and universally accepted methods for measurement of lamina propria nerve density. Some authors have described a quantitative

assessment, which involves counting the numbers of stained fibres per high power field (0.35 mm^2) at a constant magnification of x250 and expressing nerve densities as nerves/sq.mm (Van Poppel et al, 1989). A recent study from our department has looked at the variability in nerve densities in normal human bladder biopsies, when counts were performed by two independent observers and by the same observer on two different occasions, one month apart. Similar counts by different observers showed up to 12% variation, while counts by the same observer on two occasions showed a variation of around 2%. We also found that an increase in bladder filling from 100 mls to 350 mls at the time of biopsy resulted in a 22% reduction in nerve densities. The 'MiniMOP' method yields good count-recount validity but we believe that strict adherence to a standardised protocol while comparing lamina propria nerve densities is important (Hussain et al, 1998).

The present study has shown that in a group of patients with spinal cord disease, a greater reduction in nerve density in response to intravesical capsaicin does not necessarily imply a better or longer lasting response to capsaicin. In two of the responders, however, who did not show reduced mean S 100 profile densities after treatment, the duration of benefit was 3 and 2 months respectively; in the latter this period of response was the shortest recorded in the series.

Although the biopsies were obtained from the same area of the bladder at fixed bladder volumes, wide variation in mean profile densities between patients was found in both the capsaicin treated and age-matched control groups. This possibly reflects the minute size of the biopsy samples obtained with a flexible cystoscope. These biopsies are difficult to preserve and cut

and tissue damage during processing might to an extent explain the observed variation. Overall, capsaicin caused the mean S 100 positive profile densities in neurogenic bladders to fall to or below the control range although in one MS patient the profile density was lower than the control values even before capsaicin and was further reduced by intravesical capsaicin treatment. In those who did not respond there was no significant difference in the mean S 100 positive profile densities before and after treatment except in one female patient with MS who was found to have an increase in the densities post capsaicin. The difference between the grouped mean nerve densities in the non-responders pre and post-capsaicin was not statistically significant.

This would also appear to be the first attempt to quantify ultrastructural changes of human vesical nerves before and after capsaicin by electron microscopy. Neuronal degeneration following application of topical capsaicin to rat peripheral nerve has previously been reported; the same study showed a number of small diameter axons in the post capsaicin tissues (Pini et al, 1990). These small diameter axons were also seen in the suburothelial neurons of patients treated with capsaicin but the exact significance of this finding is not known. Although it has been speculated that some of the dense cored vesicles seen in the axons of suburothelial neurons on electron microscopy contain sensory peptides like CGRP and substance P, this has never been conclusively proven (Dixon and Gilpin, 1987). Further studies using immune-electron microscopy may help to assess the contents of these vesicles and the effects of intravesical capsaicin on them.

It is interesting to note that there is consistency between the three different methods of studying the suburothelial nerves. Both S 100 and PGP 9.5 staining demonstrated reduced nerve densities in those who responded to treatment with capsaicin. Likewise the EM data, although preliminary, appears to show a reduction in clear and dense cored vesicles post capsaicin. These findings together provide further evidence for the sensory nature of these neurons and of capsaicin being a de-afferenting agent.

The unusual appearances of the suburothelial nerves in TSP deserve mention. Thickening of these neurons in TSP is quite marked and in some of the bladder sections the nerves appear as "sausage rolls" (Dasgupta et al, 1996c). This was an incidental observation during the study and is the first such description. Such florid hyperplasia is not commonly seen in other neurogenic bladders due to MS, transverse myelitis or spinal cord injury and certainly not in normal bladder biopsies. Perhaps they represent "neurofilamentous masses" in the bladder as have been described in the spinal cord. The cause for this finding is unexplained but whether viral particles are responsible is yet to be ascertained (Dasgupta and Hussain, 1999).

16.5 Intravesical lignocaine and capsaicin

Intravesical lignocaine is now instilled prior to capsaicin instillation in all patients. The improvement this has made to the treatment protocol has been described previously (Chandiramani et al, 1996) but briefly, patient discomfort can be substantially reduced and the instillation procedure made far simpler as the frequent and repetitive detrusor contractions seen during capsaicin instillations are markedly reduced by lignocaine. We considered whether topical local anaesthetic would interfere with the effects of intravesical capsaicin but local anaesthetic blocks axon conduction and does not affect generator potentials in the nerve terminals (Fitzgerald, 1983) and hence has a different site of action. In addition some of the initial patients in our department underwent intravesical capsaicin instillation both without and with intravesical lignocaine pre-treatment. The urodynamic improvement in these patients remained unchanged irrespective of whether lignocaine had been used or not (Fowler et al, 1994; Chandiramani et al, 1996). In animal studies, Craft and Porreca have found that intravesical local anaesthesia does not reduce or alter the desensitization effects of intravesical resiniferatoxin (Craft and Porreca, 1994b).

Capsaicin has a biphasic mode of action on C-fibres; it initially stimulates and then impairs their function. Local anaesthetics reversibly inhibit all nerve fibres but small fibres such as C afferents are affected to a greater degree (Fontanella et al, 1997). Our study confirms that lignocaine can block nociception without affecting the beneficial effect of capsaicin as the percentage of patients responding to capsaicin is similar to that in previous reports (Fowler et al, 1994).

16.6 Capsaicin and its vehicle

It could be argued that alcohol itself causes the nerve density changes which were observed. Little is known about the effect of alcohol on the human bladder. There have been reports of two patients in whom undiluted Bonney's blue was mistakenly put intravesically. This solution consists of a 1:1 mixture of brilliant green and crystal violet diluted in 90% alcohol or industrial methylated spirit and was originally developed by Victor Bonney for preparation of vagina or skin during gynaecological operative procedures (Bonney and Browning, 1918). Its constituents are potentially toxic to epithelia and for this reason the solution needs to be diluted in water to a 0.5% concentration before being put into the bladder. The two patients who had the undiluted solution put into their bladders complained of persistent symptoms of suprapubic pain, urgency, marked urinary frequency and incontinence, which were refractory to a wide array of conservative and operative procedures. Both the patients were referred to the Medical Protection Society for medico-legal reasons (Christmas et al, 1989).

In their study on hypersensitive bladders Maggi and associates used capsaicin solutions at 3 strengths: 0.1μM, 1 μM, 10 μM and at the highest concentration the solution contained 0.1% ethanol in saline; 300 times less than that used in our solution. In our study high doses of capsaicin were dissolved in 30% alcohol as lower concentrations of alcohol make capsaicin precipitate in solution. Maggi's team reported that putting vehicle alone (0.1% ethanol in saline) into the bladder produced a slight but not statistically significant increase in the first desire to void (Maggi et al, 1989b).

Capsaicin in concentrations of 1 or 2 mmol/L is dissolved in a 30% alcohol in saline solution and instillations of 30% alcohol in saline (i.e. capsaicin's vehicle) were carried out in some patients to investigate the possibility that the beneficial effect noticed was due to the alcohol component of the solution. In two patients in whom bladder symptoms had improved with previous intravesical capsaicin instillation, 30% ethanol in saline instillation was carried out after the benefit of the last capsaicin instillation had worn off. There was a marked worsening of bladder symptoms for 4-5 weeks after the instillation of vehicle alone. This emphasises the point that the beneficial effect of capsaicin instillation is independent of the alcohol content of its vehicle.

These findings have been confirmed in a recent randomized controlled study of 1 mmol/L of capsaicin dissolved in 30% alcohol in saline against 30% alcohol alone, in patients with MS and spinal cord injury (Wiart et al, 1998; de Sèze et al, 1998). These authors mention the need for using a standardised instillation protocol for capsaicin (de Sèze et al, 1999) which was developed in our department. It seems difficult to perform a blinded-randomised study by adhering to this protocol, as the capsaicin solution has to be withdrawn by the administrator in to two 50 ml syringes, making its pungency quite obvious.

Ideally we should have studied the effect of 30% alcohol on suburothelial nerve densities. Unfortunately bladder biopsies after treatment with alcohol alone could not be obtained due to lack of consent from the patients.

The effects of alcohol on suburothelial nerve fibres may be resolved by studies comparing spinal and normal control animals. In female rats intravesical saline has negligible effect on the bladder mucosa whereas 1 mM of capsaicin in 30% alcohol causes thinning of the urothelium,

submucosal oedema and diminished GP 51 (a glycoprotein found in the urothelium) staining. These effects are more marked than that seen after instillation of 30% alcohol alone (Byrne et al, 1998). A similar animal model could be used to study the effects of alcohol on nerve densities.

16.7 The long-term safety of intravesical capsaicin

This is the first histopathological study reporting the effects of intravesical capsaicin on the human urinary bladder in patients followed up clinically over a 5-year period. Vanilloids are rapidly evolving as effective therapy for intractable detrusor hyperreflexia unresponsive to conventional medical treatments. The bladders of paraplegic patients are not normal and they have a greater risk of urinary tract infection and bladder cancer than in normal persons. It is therefore important to carefully evaluate the effects of any new intravesical substance on these bladders.

Despite the acceptance of capsaicin as an exciting tool in neuro-urology there is some uncertainty regarding its role as a possible carcinogen in man. This is based on initial genotoxicity, carcinogenecity and epidemiological studies in animals and humans (Diaz Barriga Arceo et al, 1995). There is a report that capsaicin may have been associated with tumours in an experiment in the mouse and it has been claimed to be genotoxic in V79 cells; the latter activity might have been due to the involvement of an intermediate phenoxy radical (Lawson and Gannett, 1989; Toth and Gannett, 1992). To complicate matters, an inhibitory effect of capsaicin on mouse lung tumour development has also been reported (Jang et al, 1989). A case control study from Mexico has suggested a 17 fold increase in the risk of gastric cancer in people consuming large amounts of chilli (Lopez-Carrillo et al, 1994) although the series was small and confounding factors were not controlled; the results have subsequently been criticised over the Internet as being based on insufficient evidence. Others have claimed a protective effect

of chillies against gastric and colonic cancer in Indians and Malays (Kang et al, 1992). The available evidence is therefore conflicting and confusing. Capsaicin is an irritant, and although the evidence is weak, it has also been suggested that it may have a tumour promoting action in a laboratory experiment (Agrawal et al, 1986).

There is as yet no data about the risk of tumorigenesis in the human bladder if capsaicin were instilled for therapeutic reasons. It is not possible to extrapolate from the conflicting in vitro and animal studies to man. We have shown that intravesical capsaicin seems to be a safe treatment over the duration of this study.

The inflammation caused by capsaicin obviously settles down within a few weeks of treatment and the urothelium reverts back to its original appearance. The intravesical instillations of capsaicin are intermittent and this may reduce any risk compared to experiments involving chronic continuous exposure to chillies. These findings are reassuring, as it is likely that chillies will continue to be used as an effective method of treating detrusor hyperreflexia.

It is however important to remember that the effects of chemical carcinogens may not become apparent for 10 years or more and so continuing follow up with annual urine cytology and flexible cystoscopy will be maintained in this study. The importance of lower urinary tract surveillance cannot be overemphasised in this situation and annual follow up of these patients is being continued.

SECTION V

CONCLUSIONS

CHAPTER 17

CONCLUSIONS AND FUTURE TRENDS

The introduction of capsaicin is one of the most fascinating advances in neuro-urology. Most of the patients treated in the reported studies had failed to respond to other conservative therapies and would possibly have needed other alternatives such as suprapubic catheterisation or urinary diversion. Capsaicin should therefore be regarded as a useful treatment in patients with detrusor hyperreflexia before surgical options are considered.

This study has shown that intravesical capsaicin acts by causing a reduction in the nerve densities in the lamina propria of the human urinary bladder. Its effects as a de-afferenting agent are noticed both on the axons and Schwann cells.

The effectiveness of repeated instillations of capsaicin persists for up to 5 years. During this period of follow-up it has also been found to be safe as none of the patients in this series have developed any premalignant or malignant lesions.

EMDA of lignocaine to anaesthetise the bladder before capsaicin instillation, significantly reduces suprapubic pain and makes the use of capsaicin in detrusor hyperreflexia, more acceptable.

The next few years are likely to see the introduction of other vanilloids to treat detrusor hyperreflexia. One such substance is resiniferatoxin (derived from Euphorbia, a cactus-like plant) which is about 1000 times more potent

than capsaicin but less pungent. Human studies with resiniferatoxin are in progress and early results have already been reported (Cruz et al, 1997b; Chancellor and de Groat, 1999).

We are indeed fortunate to have chillies both for our *curries* and urology.

SECTION VI

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CONTRIBUTION TO THE SCIENCE OF URO-NEUROLOGY

Systematic animal experiments using capsaicin started almost 40 years ago. It is only in the late 1980s that the first human trial of intravesical capsaicin was reported. Our department pioneered its use in detrusor hyperreflexia. Since then there has been considerable international interest in the use of this substance for various bladder disorders in humans, but mainly in detrusor hyperreflexia.

This is claimed to be the first detailed description of the long-term clinical effects of intravesical capsaicin instillation. It is the first report of a simple method of obtaining bladder biopsies under local anaesthetic for nerve density evaluation and the first description of the neuronal changes in the human bladder following capsaicin treatment. It is also the first attempt to describe the safety of intravesical capsaicin instillations over a 5-year period. This work has received 4 awards and has led to the publication of 6 papers, 3 book chapters and 20 abstracts in international meetings.

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APPENDIX

DIFFUSION OF CAPSAICIN INTO BLADDER WALL

The principles of nonequilibrium thermodynamics are used to develop equations in terms of solute flux (J_s), volume flux (J_v), transmembrane hydrostatic pressure (ΔP_m) and osmotic pressure differential across a membrane ($\Delta\pi_s$). The "practical transport equations" describe a single solute migrating through a membrane.

$$J_V = L_p\left(\Delta P_m - \sigma\Delta\pi_s\right) \qquad (1)$$

$$J_s = C_s(1-\sigma)J_v + \omega\Delta\pi_s \qquad (2)$$

where C_s is the average concentration of the solute, L_p is the hydraulic permeability, σ is the reflexion coefficient, and ω is the solute permeability (sieving coefficicent).

When $J_v \sim 0$ (capsaicin-alcohol-normal saline),

$$J_s = D_s / \Delta x \cdot \Delta C_s = \omega\Delta\pi_s \qquad (3)$$

where D_s is the diffusion coefficient and Δx is the membrane thickness.

Relating to membrane permeability, P

$$J_s = P \cdot \Delta C_s \quad \text{(diffusive flux)} \qquad (4)$$

If n bladders are infused with different volumes (V) retained for different times (*t*) they may be visualised as a series of concentric spheres, of surface areas, A, and radii, *r*. Then, the mass transport (*m*) of capsaicin into bladder tissues is:

$$m_1 = A_1 \cdot t_1(P \cdot \Delta C_s)$$

$$m_2 = A_2 \cdot t_2(P \cdot \Delta C_s)$$

$$m_n = A_n \cdot t_n(P \cdot \Delta C_s)$$

$$= \frac{3V_n}{r_n} \cdot t_n(P \cdot \Delta C_s)$$

Unless V, (and hence A and *r*) *t* and C_s are standardised, capsaicin delivery into the bladder wall will vary in direct proportion to changes in these independent variables.