

## **Presumptive sensory axons of the human urinary bladder: a fine structural study**

**J. S. DIXON AND C. J. GILPIN**

*Department of Anatomy, University of Manchester,  
Manchester M13 9PT, U.K.*

*(Accepted 16 May 1986)*

### **INTRODUCTION**

Previous neurohistochemical studies of the human urinary bladder have demonstrated a rich plexus of acetylcholinesterase-positive nerves extending throughout the muscle coat (Mobley, Elbadawi, McDonald & Schenk, 1966; Nyo, 1969; Ek, Alm, Andersson & Persson, 1977; Alm, 1978; Gosling, 1979; Klück, 1980; McConnell, Benson & Wood, 1982). These nerves are generally believed to represent the autonomic motor innervation to the smooth muscle cells of the detrusor. Similar enzyme-positive nerves have been demonstrated in the lamina propria adjacent to the urothelium of the human urinary bladder (Gosling, Dixon & Humpherson, 1983). Nerves in this situation are apparently unrelated to recognised neuro-effector target sites and it has been suggested that they represent the terminations of sensory neurons (Gosling *et al.* 1983). The present paper describes the results of a detailed fine structural study of suburothelial axons and their vesicle-containing varicose regions in the human urinary bladder. In addition comparison is made between suburothelial presumptive sensory terminals and those of autonomic motor axons located within the detrusor muscle.

### **MATERIALS AND METHODS**

Biopsy samples of urinary bladder were obtained at cystoscopy from 15 carefully selected patients (9 males, 6 females) undergoing clinical investigation for a variety of urological disorders, including urothelial tumours and urethritis. The cystoscopic examination and biopsy were considered essential for clinical diagnosis in each case. Each patient was neurologically and urodynamically normal, with a stable cystometrogram and no evidence of bladder trabeculation. Patients suffering from any form of 'sensory bladder disorder' were deliberately excluded from this study. The patients ranged in age from 20 to 68 years with a mean age of 52 years. From each patient samples were obtained from the dome and the right and/or left lateral walls. In addition, tissue samples from four patients undergoing total cystectomy were included in the present study (age range 45–66 years). In the latter patients, those areas of bladder wall showing macroscopic pathological features were carefully avoided.

After cutting into small pieces, each tissue sample was fixed for 2 hours in 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate, and then postfixed for 1 hour in 1% osmium tetroxide in the same buffer. The tissue pieces were subsequently block-stained in a 2% aqueous solution of uranyl acetate for 15 minutes before dehydration in ascending concentrations of acetone and embedding in EMIX epoxy resin. Thin sections were cut from selected blocks using a diamond knife and a

Reichert OmU4 ultramicrotome. The sections were mounted on uncoated copper grids, double stained with alcoholic uranyl acetate and lead citrate and examined in a Philips EM300 electron microscope.

For quantitative studies a total of 75 electron micrographs of subepithelial nerve varicosity profiles (15 from 5 separate patients) and a similar number of intramuscular nerve varicosity profiles were recorded at a known magnification of  $\times 16000$ , the electron microscope having been calibrated using a diffraction grating replica of known line spacing. Enlargements were prepared at a final magnification of  $\times 64000$  and the following variables were obtained from each micrograph. (1) Nerve varicosity profile area and axonal mitochondria profile area, measured using a planimeter. (2) The total number of small agranular and large granulated vesicle profiles were counted and recorded. (3) The diameter of each large granulated vesicle profile within each axonal varicosity profile was determined using a calibrated circle lattice. (4) The diameter of small agranular vesicle profiles was determined using a calibrated circle lattice.

From these data the following values were obtained for each axonal varicosity profile. (1) Varicosity profile area (in  $\mu\text{m}^2$ ), calculated by subtracting total area of mitochondrial profile in each varicosity from the total varicosity profile area. (2) Total area of large granulated vesicle profiles (in  $\mu\text{m}^2$ ), determined by calculating the area of each granulated vesicle profile (using  $\pi r^2$ ) and summing. (3) Total area of agranular vesicle profiles (in  $\mu\text{m}^2$ ), determined by calculating the area of one agranular vesicle profile (using  $\pi r^2$ ) and multiplying by the number observed. All small agranular vesicle profiles were treated as being of the median diameter (47 nm) for this purpose. Both the small agranular and the large granulated vesicles appeared to be evenly distributed within the varicosities. Hence, using the above data the following were calculated.

(a) The proportion of varicosity profile area occupied by either type of vesicle profile (expressed as a percentage).

(b) The proportion of the total vesicle profile population represented by large granulated vesicle profiles (expressed as a percentage).

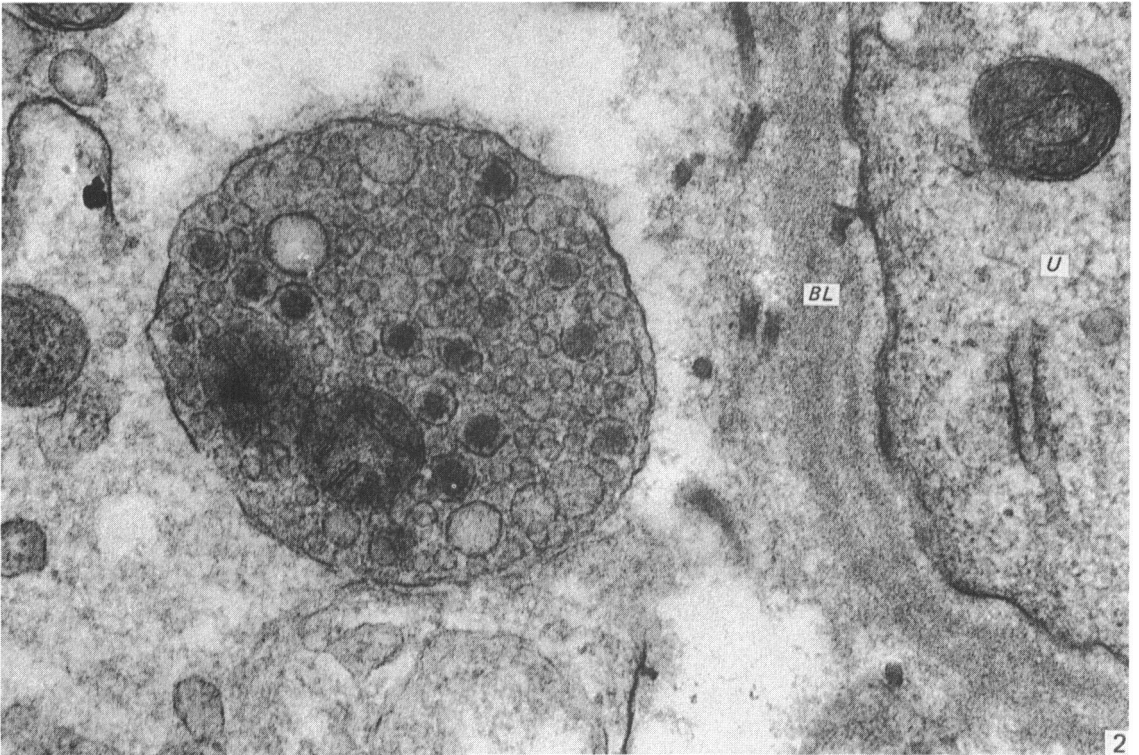
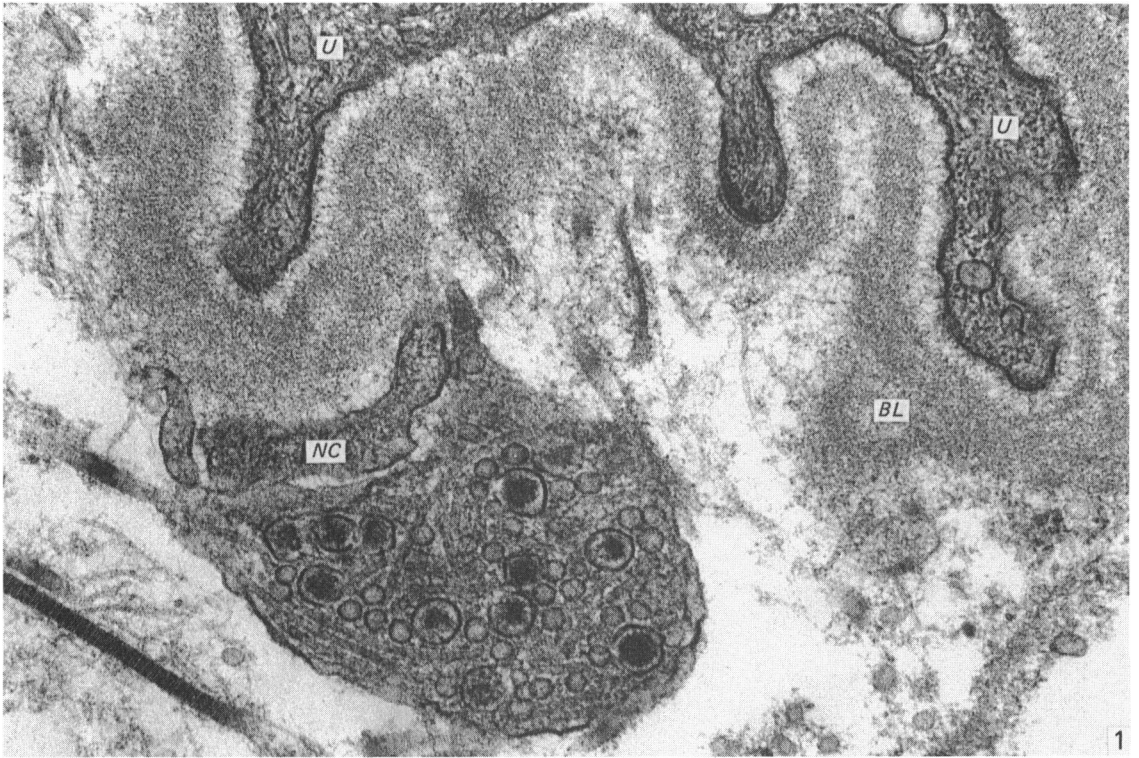
(c) The number of large granulated vesicle profiles per  $\mu\text{m}^2$  of varicosity profile area.

## RESULTS

In specimens from both the dome and lateral walls of the bladder small nerve fibres containing from 1 to 8 axons ensheathed in neurilemmal cell cytoplasm were observed coursing through the subepithelial connective tissue. The majority of axon profiles were non-myelinated intervaricose segments containing organelles such as neurofilaments, neurotubules and occasional mitochondria. Very occasionally a myelinated axon was observed within these nerve bundles. Nerve bundles lying adjacent to the urothelium possessed fewer axons, many containing a single axon. These single axons tended to lie parallel to the basal lamina which separated the

Fig. 1. A suburothelial axon varicosity lies in close proximity to the basal lamina (*BL*) of the urothelium. Small agranular and large, granulated vesicles are tightly packed giving the axon an electron-dense appearance. *U*, basal urothelial cell; *NC*, neurilemmal cell.  $\times 56000$ .

Fig. 2. This suburothelial axon varicosity is totally devoid of a neurilemmal cell covering. *BL*, basal lamina; *U*, basal urothelial cell.  $\times 56000$ .



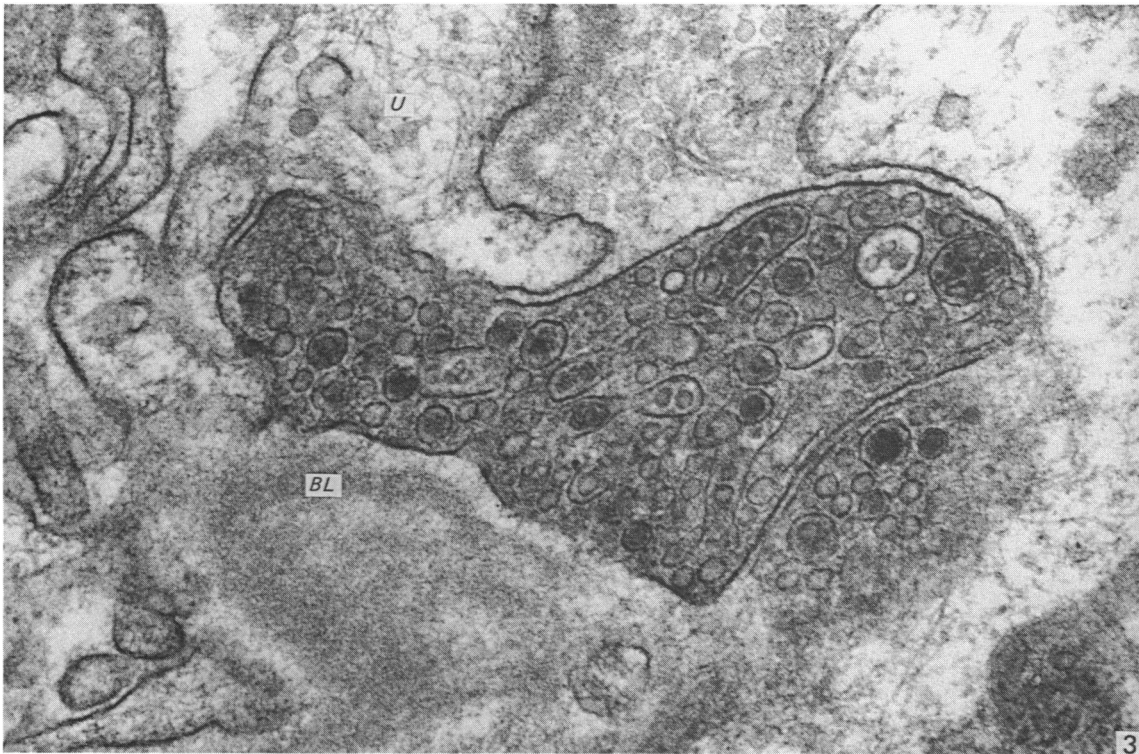


Fig. 3. A suburothelial axon has penetrated the urothelial basal lamina and lies in close proximity to a urothelial cell (*U*). *BL*, basal lamina.  $\times 56000$ .

Fig. 4. This vesicle-packed suburothelial axon lies in a groove at the surface of a connective tissue cell (*CT*). *BL*, basal lamina; *U*, urothelial cell.  $\times 56000$ .



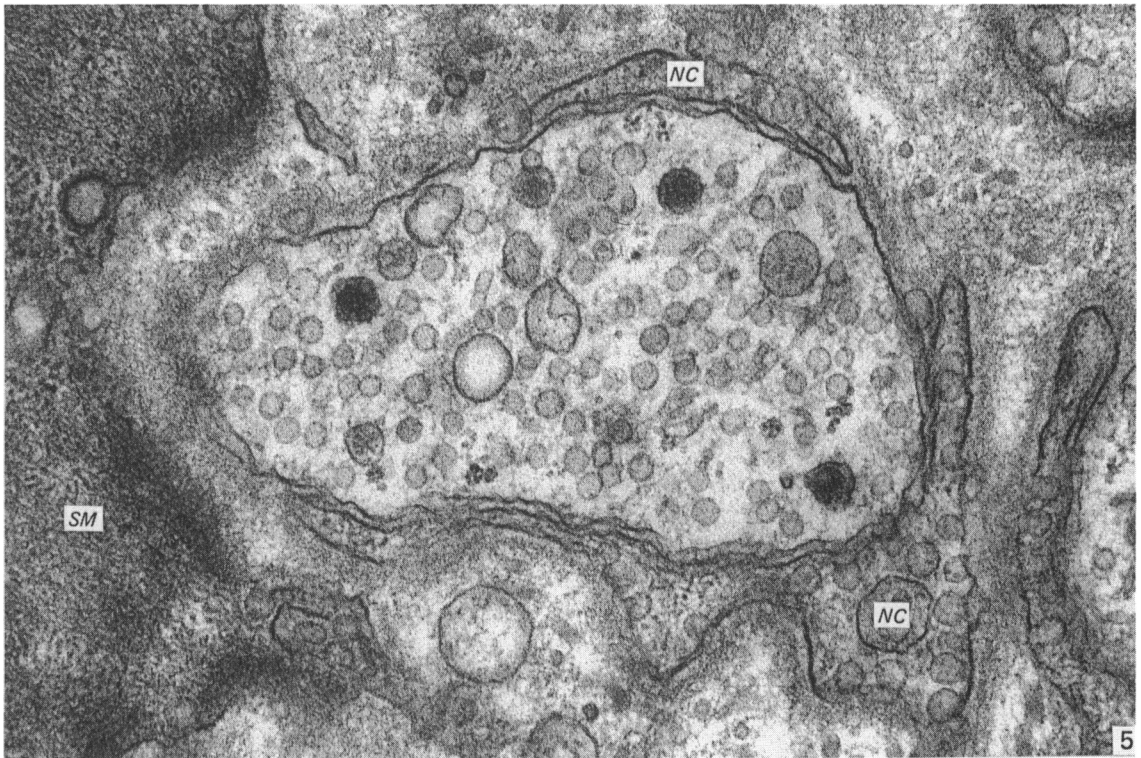


Fig. 5. This intramuscular axon varicosity is partially enclosed by a neurilemmal cell (NC), and lies adjacent to a detrusor smooth muscle cell (SM).  $\times 56000$ .

urothelium from the underlying connective tissue. Some of the suburothelial axons appeared varicose in section and were either partially or completely denuded of a neurilemmal cell covering (Figs. 1, 2). The axonal varicosities (Fig. 1) contained both small agranular vesicle profiles (median diameter 47 nm, range 39–54 nm) and large granulated vesicle profiles (median diameter 105 nm, range 62.5–187.5 nm). The axonal vesicle profiles were frequently closely packed together giving the varicosities an electron-dense appearance at low magnification. Vesicle-packed axonal varicosities were frequently observed lying within 100 nm of the urothelial basal lamina (Fig. 1). Very rarely a single vesicle-packed axon was observed to have penetrated the basal lamina and to lie in close association with a basal urothelial cell (Fig. 3). While the majority of subepithelial axonal varicosities appeared to be unassociated with other structures lying in the lamina propria, such as blood capillaries or fibroblasts, an occasional axon was observed occupying a groove at the surface of a connective tissue cell (Fig. 4). Such cells could be distinguished from neurilemmal cells by their lack of an investing basal lamina.

For comparative purposes axon varicosities lying among the smooth muscle cells of the detrusor were also examined. Intramuscular axons generally occurred in groups of two or three although single axons were not infrequent. Such axons were usually partially surrounded by neurilemmal cell cytoplasm, the naked region of axolemma lying in close proximity to the surface of a smooth muscle cell (Fig. 5). Intramuscular axonal varicosities contained two types of vesicle profile, namely small agranular vesicle profiles (median diameter 47 nm, range 39–54 nm) and larger vesicle profiles (median diameter 100 nm, range 47–187.5 nm), most of which con-

tained a central dense granule. The axonal vesicles were generally well separated from one another within the axoplasm of each varicosity. Close packing of vesicles similar to that observed in suburothelial axon varicosities was only very rarely observed. Furthermore, approximately one third of the intramuscular axon varicosity profiles examined contained only small agranular vesicle profiles. However, in such instances vesicles of the large granulated type may well have occurred outside the plane of section.

### *Quantitative studies*

Quantitative measurements were carried out on 75 suburothelial axonal varicosities and 75 intramuscular axonal varicosities. Analysis of data within each group revealed a non-normal distribution and therefore the following group results are presented in the form: median (range).

#### *Suburothelial nerve varicosities*

The area of nerve varicosity profiles was  $0.78 \mu\text{m}^2$  (0.15–3.03) and of this area 33.84% (8.55–52.02%) was occupied by vesicle profiles of either type (small agranular and large granulated). Large granulated vesicle profiles formed 10.09% (1.75–57.14%) of the total vesicle profile population and there were 11.43 (1.96–34.29) large granulated vesicle profiles per  $\mu\text{m}^2$  of varicosity profile.

#### *Intramuscular nerve varicosities*

The area of nerve varicosity profiles was  $0.73 \mu\text{m}^2$  (0.22–4.17  $\mu\text{m}^2$ ) and of this area 18.81% (2.35–55.98%) was occupied by vesicle profiles of either type (small agranular and large granulated). Of the 75 varicosity profiles examined 24 (32%) contained no large granulated vesicle profiles. Of the total vesicle profile population the large granulated vesicle profiles represented 2.41% (0–29.7%) with 1.75 (0–17.86) large granulated vesicle profiles per  $\mu\text{m}^2$  of varicosity profile.

#### *Statistical comparison between suburothelial and intramuscular axonal varicosities*

A statistical comparison of the variables measured for the suburothelial and intramuscular axon varicosities was carried out. In only one group was the data normally distributed and therefore the non-parametric Mann–Whitney ‘U’ test was employed throughout for significance testing between groups.

Suburothelial varicosity profile areas were not significantly different from those of the intramuscular varicosities ( $P = 0.33$ ). However there was a marked difference in their content of large granulated vesicle profiles. Both the proportion of large granulated vesicle profiles (as a percentage of the total vesicle profile population) and the number of large granulated vesicle profiles per  $\mu\text{m}^2$  of varicosity profile were significantly greater in suburothelial axon varicosities ( $P < 0.01$  in both cases).

Subjective assessment suggested a difference in the ‘packing density’ of axonal vesicle profiles contained in the suburothelial and intramuscular axonal varicosity profiles. Quantitative evaluation of this variable showed that the area of varicosity profile occupied by vesicle profiles of both types was significantly greater in the suburothelial axons than in the intramuscular axons ( $P < 0.01$ ).

## DISCUSSION

The present fine structural study has shown that both the suburothelial and intramuscular nerve plexuses of the human urinary bladder contain many single axons that possess vesicle-packed varicosities. Axons in both situations contain two types of axonal vesicle, namely small (approximately 50 nm in diameter) agranular vesicles together with larger (approximately 100 nm in diameter) granulated vesicles. However, when a careful comparison is made between suburothelial and intramuscular axon terminals, several distinct morphological differences are apparent. Firstly there is a marked difference in the relative proportions of the two types of vesicle profiles, suburothelial varicosities containing over 10% of large granulated vesicle profiles compared with less than 2.5% in the intramuscular axons. Secondly the 'packing density' of the vesicle profiles is considerably greater in suburothelial varicosities, over 30% of terminal area being occupied by axonal vesicle profiles of either type compared with less than 20% for intramuscular varicosities. Furthermore many of the suburothelial axons are completely devoid of a neurilemmal cell covering, a feature which is relatively rare for intramuscular axons.

The fine structure of the intramuscular axon varicosities is similar to that of cholinergic nerve terminals described in other tissues (Burnstock, 1975). Thus it may be assumed that the intramuscular varicosities represent the cholinergic motor innervation to the detrusor muscle (Daniel, Cowan & Daniel, 1983). However the functional significance of the structurally different suburothelial nerves remains unresolved. Urothelial cells of the human urinary bladder are not known to possess any secretory activity and therefore the suburothelial axons are unlikely to be secretomotor in function. An alternative hypothesis is that the suburothelial nerves may have a trophic influence upon the urothelial cells (Alm *et al.* 1978) although there appears to be no supportive experimental evidence. Thus the most probable function for the suburothelial nerves of the human urinary bladder is that they represent the terminations of afferent sensory neurons. A similar hypothesis has been proposed for free-ending nerves that are known to occur beneath the bladder urothelium of many animal species (Elbadawi, 1982). A functional difference between the suburothelial nerves and those innervating smooth muscle cells is supported by their different fine structure as outlined above.

It is interesting to note that substance P has been shown to occur in submucosal nerves of the human urinary bladder (Islam *et al.* 1983). Furthermore it has been proposed that substance P-containing nerves occurring close to, or within, surface epithelia may represent peripheral endings of primary sensory neurons (Hockfelt, Kellerth, Nilson & Pernow, 1975). In this context it has recently been shown that treatment of rat urinary bladder with capsaicin not only reduces the level of substance P in the bladder wall (Holzer, Bucsics & Lembeck, 1982), and abolishes substance P-containing nerves in the bladder as demonstrated immunocytochemically (Sharkey, Williams, Schulzberg & Dockray, 1983), but also markedly alters the sensory threshold of micturition, causing urine retention (Sharkey *et al.* 1983; Santicioli, Maggi & Meli, 1985).

Electron microscopic immunocytochemical methods have demonstrated that large granulated vesicles are the storage site of neuropeptides in cholinergic nerve terminals (Johansson & Lundberg, 1981). A similar function may be performed by the large granulated vesicles which the present study has demonstrated to occur in relatively high numbers in the suburothelial, presumptive sensory axons of the human urinary

bladder. Clearly immunocytochemical studies at the fine structural level are required to determine the precise location of substance P in these nerves and such studies are currently under way in our laboratory. Furthermore vaso-active intestinal polypeptide has also been shown to be present in the subepithelial nerves of the human urinary bladder (Gu *et al.* 1982) although it is not yet known whether this neuropeptide coexists with substance P in the same nerve axon or whether distinct populations of suburothelial axons occur. Immunocytochemical electron microscopy using labelling with gold particles of two different sizes should resolve this question.

Finally, in order to obtain more information on the functional role of subepithelial nerves in the human urinary bladder, additional studies are currently in progress on biopsy material from patients with specific neurological damage which is known to involve the afferent innervation.

#### SUMMARY

The mucosa of the human urinary bladder possesses an extensive plexus of suburothelial nerve fibres which are believed to be sensory in nature. Many of these presumptive sensory nerves occur as single axons whose vesicle-packed varicose regions are totally devoid of neurilemmal cell covering and occasionally penetrate the urothelial basal lamina. The axonal vesicles are of two types, small agranular vesicles (median diameter 47 nm, range 39–54 nm) and large granulated vesicles (median diameter 105 nm, range 62.5–187.5 nm).

When compared statistically with intramuscular axon varicosities the suburothelial varicosities are shown to possess a significantly greater packing density of axonal vesicles and to contain a significantly greater proportion of large granulated vesicles. The latter finding may reflect the presence of substance P, a neuropeptide known to occur in primary sensory nerves.

#### REFERENCES

- ALM, P. (1978). Cholinergic innervation of the human urethra and urinary bladder. *Acta pharmacologica et toxicologica* **43**, Suppl. 2, 56–62.
- ALM, P., ALUMETS, J., BRODIN, E., HAKANSON, R., NILSSON, G., SJOBERG, N.-O. & SUNDLER, F. (1978). Peptidergic (substance P) nerves in the genito-urinary tract. *Neuroscience* **3**, 419–425.
- BURNSTOCK, G. (1975). Ultrastructure of autonomic nerves and neuroeffector junctions; analysis of drug action. In *Methods in Pharmacology*, vol. 3, *Smooth Muscle* (ed. E. E. Daniel & D. M. Paton), pp. 113–117. New York: Plenum Press.
- DANIEL, E. E., COWAN, W. & DANIEL, V. P. (1983). Structural bases for neural and myogenic control of human detrusor muscle. *Canadian Journal of Physiology and Pharmacology* **61**, 1247–1273.
- EK, A., ALM, P., ANDERSSON, K.-E. & PERSSON, C. G. A. (1977). Adrenergic and cholinergic nerves of the human urethra and urinary bladder. A histochemical study. *Acta physiologica scandinavica*, **99**, 345–352.
- ELBADAWI, A. (1982). Neuromorphological basis of vesicourethral function. I. Histochemistry, ultrastructure and function of intrinsic nerves of the bladder and urethra. *Neurourology and Urodynamics* **1**, 3–50.
- GOSLING, J. A. (1979). The structure of the bladder and urethra in relation to function. *Urological Clinics of North America* **6**, 31–38.
- GOSLING, J. A., DIXON, J. S. & HUMPHERSON, J. A. (1983). *Functional Anatomy of the Urinary Tract: An Integrated Text and Colour Atlas*. Edinburgh: Churchill Livingstone.
- GU, J., ISLAM, K. N., RESTORICK, J., MCGREGOR, G. P., ADRIAN, T. F., BLOOM, S. R. & POLAK, J. M. (1982). Peptidergic innervation of the human urinary tract. *Journal of Pathology* **138**, 89–90.
- HOCKFELT, T., KELLERTH, J.-O., NILSON, G. & PERNOW, B. (1975). Experimental immunohistochemical studies on the localisation and distribution of substance P in cat primary sensory neurons. *Brain Research* **100**, 235–252.
- HOLZER, P., BUCSICS, A. & LEMBECK, F. (1982). Distribution of capsaicin sensitive nerve fibres containing substance P in cutaneous and visceral tissue of the rat. *Neuroscience Letters* **31**, 253–257.



- ISLAM, K. N., GU, J., MCGREGOR, G. P., SHUTTLEWORTH, K. E. D., BLOOM, S. R. & POLAK, J. M. (1983). Morphological evidence of physiological functioning peptide-containing nerves in the human urinary bladder. *Proceedings of the 13th Annual Meeting of the International Continence Society, Aachen*, pp. 247-249.
- JOHANSSON, O. & LUNDBERG, J. M. (1981). Ultrastructural localization of VIP-like immunoreactivity in large dense-core vesicles of 'cholinergic-type' nerve terminals in cat exocrine glands. *Neuroscience* **6**, 847-862.
- KLÜCK, P. (1980). The autonomic innervation of the human urinary bladder, bladder neck and urethra. A histochemical study. *Anatomical Record* **198**, 439-447.
- MCCONNELL, J., BENSON, G. S. & WOOD, J. G. (1982). Autonomic innervation of the urogenital system. Adrenergic and cholinergic elements. *Brain Research Bulletin* **9**, 679-694.
- MOBLEY, T. L., ELBADAWI, A., McDONALD, D. F. & SCHENK, E. A. (1966). Innervation of the human urinary bladder. *Surgical Forum* **27**, 505-506.
- NYO, M. M. (1969). Innervation of the bladder and urethra. *Journal of Anatomy* **105**, 210.
- SANTICIOLI, P., MAGGI, C. A. & MELI, A. (1985). The effect of capsaicin pretreatment on the cystometrograms of urethane anesthetized rats. *Journal of Urology* **133**, 700-703.
- SHARKEY, K. A., WILLIAMS, R. G., SCHULTZBERG, M. & DOCKRAY, G. J. (1983). Sensory substance P innervation of the urinary bladder. Possible site of action of capsaicin in causing urine retention in rats. *Neuroscience* **10**, 861-868.