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The fine structure of autonomic neurons in the wall of the human urinary bladder*

C. J. GILPIN, J. S. DIXON, S. A. GILPIN AND J. A. GOSLING

Department of Anatomy, Stopford Building, University of Manchester, Manchester M13 9PT

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INTRODUCTION

Although it is generally accepted that autonomic ganglia occur at the base of the urinary bladder (Gosling & Thompson, 1977; Klück, 1980) and in the vicinity of the ureterovesical junctions (Schulman, Escalante & Boyarsky, 1972), the presence of nerve cell bodies in other regions of the bladder wall has been denied by some workers (Ek, Alm, Andersson & Persson, 1977; Alm, 1978). However, a recent light microscopic study (Dixon, Gilpin, Gilpin & Gosling, 1983) has confirmed the existence of autonomic ganglia in samples removed from the lateral walls and dome of the human urinary bladder. In order to obtain more information on the morphological characteristics and intraganglionic relationships of intramural bladder ganglia, fresh biopsy specimens have now been examined using electron microscopy. The present paper describes the results of this study.

MATERIALS AND METHODS

Biopsy samples of urinary bladder were obtained at cystoscopy from 25 patients (10 males, 15 females) undergoing clinical investigation and treatment for a variety of conditions including bladder instability, bladder outlet obstruction and urinary frequency and incontinence. The age range of these patients was 20–68 years with a mean age of 55 years. From each patient samples were obtained from the dome and the right and/or left lateral walls. In addition, bladder samples from 13 adult patients (8 male, 5 female) ranging in age from 26 to 65 years and from 2 male neonates (aged 3 and 10 months), undergoing open bladder procedures, were included. Other specimens were obtained from 4 patients (3 males, 1 female) undergoing radical cystectomy (age range 28–66 years). In the latter patients, those areas of the 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 post-fixed for 30 minutes in 1% osmium tetroxide in the same buffer. The samples were subsequently dehydrated in ascending concentrations of ethyl alcohol, block-stained in a 2% alcoholic solution of uranyl acetate for 15 minutes and embedded in TAAB epoxy resin.

In order to identify those blocks which contained autonomic ganglia, sections 1 μm thick were stained with toluidine blue and examined using light microscopy. Thin sections were then cut from appropriate blocks using a diamond knife and a

* Reprint requests to Dr J. S. Dixon.

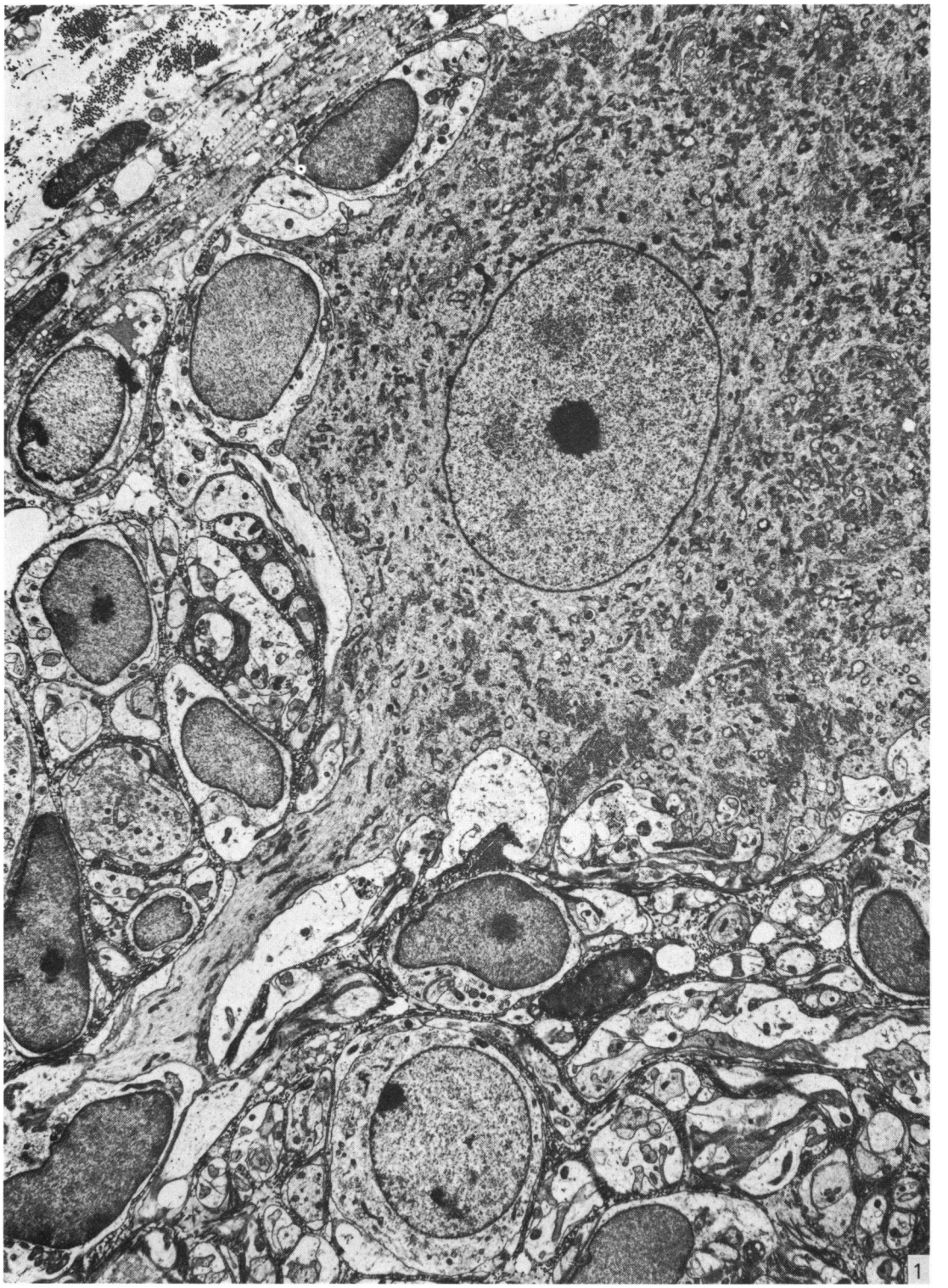

Fig. 1. An electron micrograph of a typical intramural ganglion cell containing a relatively large nucleus and prominent nucleolus. × 4350.

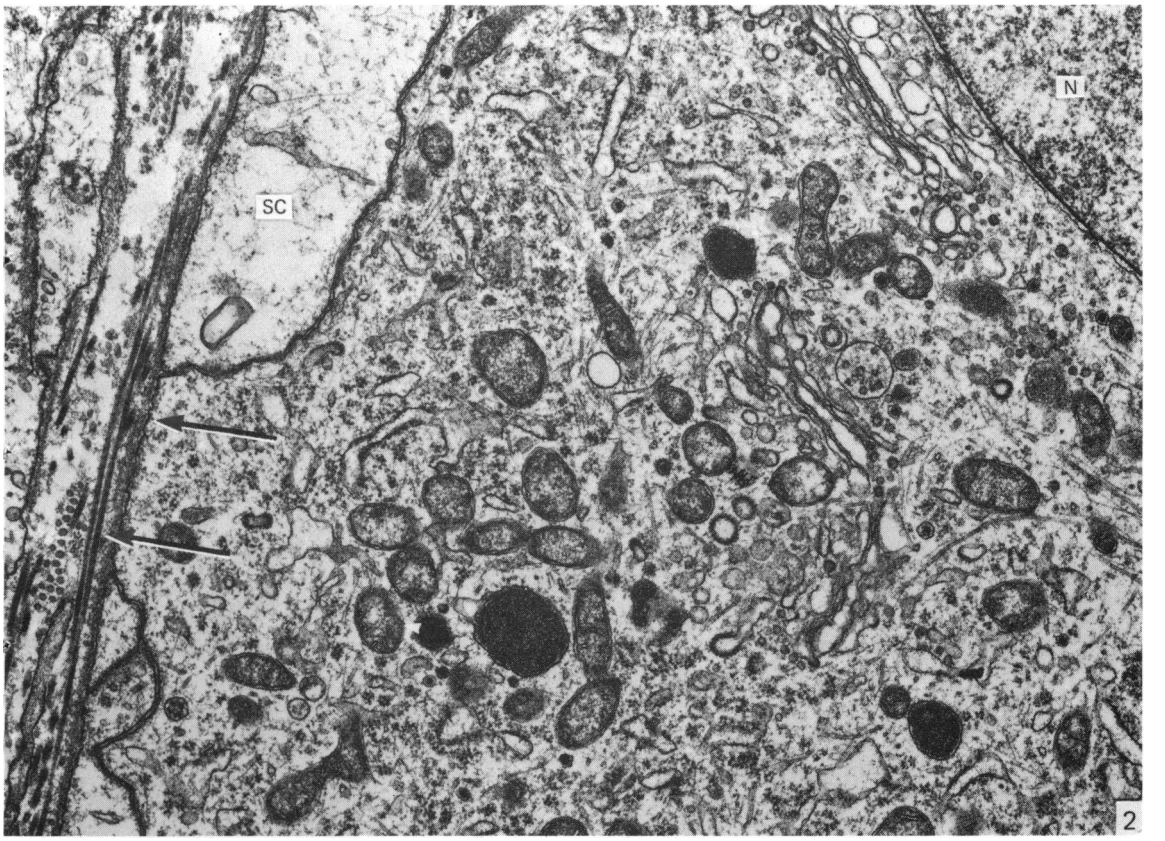

Fig. 2. An illustration of part of an intramural ganglion cell. The arrows indicate a gap in the satellite cell sheath (*SC*). N, nucleus. × 22 400.

Reichert Ultracut 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.

RESULTS

Approximately one in eight of the bladder biopsies examined in the present study contained one or more autonomic ganglia. The majority of the ganglia were small in size, and contained from one to six neurons; they occurred either in the lamina propria or embedded among the detrusor muscle bundles. Tissue obtained from radical cystectomy specimens revealed that the adventitia of the bladder contained larger ganglia composed of up to 20 nerve cells. Many of these adventitial ganglia were associated with large nerve trunks. All the ganglia examined possessed a thin capsule consisting of elongated connective tissue cells and interspersed collagen fibres.

The intramural ganglion cells were typical multipolar neurons, each with a large spherical nucleus and one or more dense nucleoli (Fig. 1). The nucleoplasm was finely granular with occasional condensations of chromatin around the periphery, adjacent to the porous nuclear membrane. The neuronal cytoplasm contained numerous stacks of Golgi membranes, clusters of granular cisternae and associated ribosomes, randomly scattered mitochondria, multivesicular bodies, dense (lysosome-like) bodies, microtubules and filaments (Fig. 2). Each neuron was invested by one or

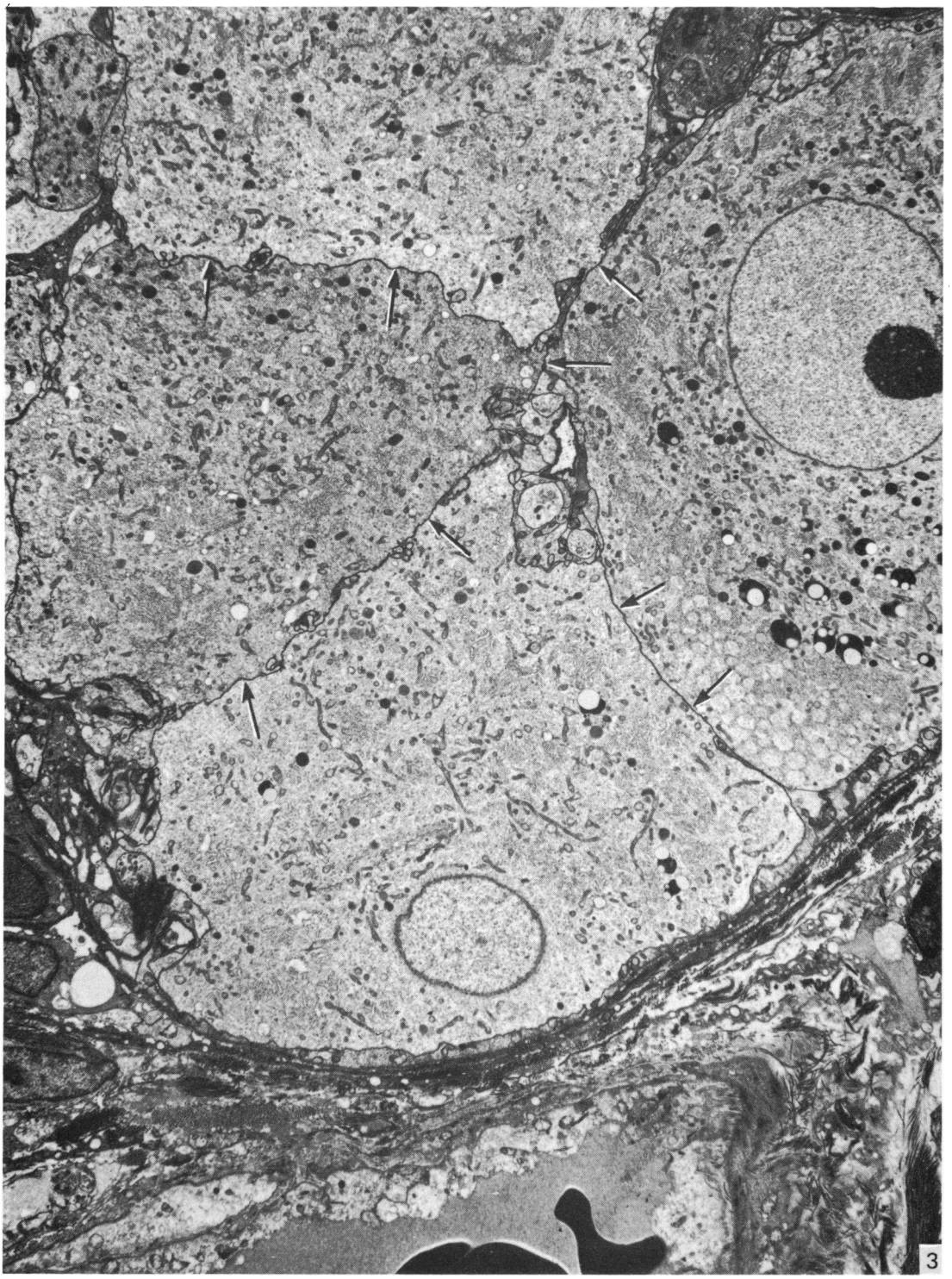

Fig. 3. In this illustration of a group of four intramural ganglion cells, regions occur in which adjacent cells are in close apposition (arrows) without intervening satellite cell cytoplasm. × 4500.

more layers of satellite cell cytoplasm, although quite frequently areas of nerve cell plasma membrane were devoid of satellite cell covering (Fig. 2). Over these regions, only the basal lamina separated the plasmalemma from the stroma of the ganglion.

In some of the larger ganglia, groups of two to five neurons were observed lying in close proximity to one another (Fig. 3). In such groups extensive areas of the cell membranes of adjacent nerve cells were in direct apposition, without intervening satellite cell cytoplasm. Over these regions a gap varying from 15 to 20 nm in width separated the apposing plasmalemmas (Fig. 4). Usually the apposing membranes were without any morphological specialisation although occasionally regions were observed at which there was an increased density of subjacent neuronal cytoplasm. Very rarely, the intercellular cleft contained periodic electron-dense bands, disposed perpendicular to the nerve cell plasma membranes (Fig. 5). These bands were regularly spaced, approximately 20 nm apart, and in most instances appeared to be in contact with the apposing plasmalemmas.

Nerve terminal regions

Frequent axosomatic and less common axodendritic synapses (Fig. 6) were observed on intramural ganglion cells. The majority of these nerve terminal regions contained clusters of small (40–60 nm diameter) agranular vesicles, a few larger (80–100 nm diameter) dense cored vesicles and occasional mitochondria (Fig. 6). Very infrequently, a second type of axon profile was observed lying in close proximity to the surface of a ganglion cell. This type was packed with a heterogeneous population of small (40–60 nm diameter) agranular vesicles and large (110–170 nm diameter) membrane-bound vesicles. Some of these larger vesicles appeared to be empty while others contained a finely granular electron-dense material (Fig. 7). Clusters of mitochondria, occasional irregularly shaped multivesicular bodies and electron-dense myelin figures were also observed in this type of axon (Fig. 7).

DISCUSSION

The results of the present study have shown that intramural ganglion cells of the bladder wall possess fine structural characteristics which are similar in many respects to parasympathetic nerve cells observed in other locations (Dixon, 1966; Fehér, Csányi & Vajda, 1979). These intramural neurons presumably correspond to the acetylcholinesterase-positive ganglion cells observed using light microscopy by Dixon *et al.* (1983). While the presence of such an enzyme is not exclusive for cholinergic neurons, it seems reasonable to classify these cells as presumptive cholinergic in type in view of their rich enzyme content and fine structure. Similar enzyme-positive cells have also been observed in the pelvic plexus (Gosling & Thompson, 1977; Klück, 1980) and it is probable that the intramural neurons of the bladder represent an extension of this peripheral innervation.

In some of the intramural ganglia examined in the present study a most unusual feature has been noted. It consists of the intimate association of neurons whose plasma membranes lie in close apposition, without intervening satellite cell cytoplasm. For the most part the apposing nerve cell membranes appear to lack any structural specialisation. However, very occasional regularly spaced dense bands extend between the outer leaflets of adjacent membranes to give an appearance reminiscent of the septate junctions observed in invertebrate epithelia (Gilula, Branton & Satir, 1970) and the mammalian cerebellum (Gobel, 1971; Sotello &

Llinás, 1972). At present it is not known whether such junctions represent regions which function as high or low resistance pathways between adjacent cells. Nevertheless the close apposition of some intramural neurons in the wall of the bladder raises the possibility of cell-to-cell interaction without the involvement of neurotransmitter substance. To the authors' knowledge this type of close interrelationship between neurons has not been recorded previously in any other peripheral autonomic ganglia.

Two morphological types of nerve terminal regions have been observed in association with intramural ganglion cells. The first and commonest type contains mainly small (40–60 nm) agranular vesicles together with a few large (80–100 nm) granulated vesicles, and appears to be similar to those classified as cholinergic by many workers (Richardson, 1964). However, on a cautionary note, vesicle morphology can no longer be used to infer the type of neurotransmitter with such precision in view of the wide variety of substances, including peptides, which are now known to be present in the autonomic nervous system.

The second type of terminal is characterised by numerous large vesicles containing material of variable electron density, together with a few agranular vesicles, multivesicular bodies, mitochondria and dense myelin bodies. Nerve profiles of this type may represent degenerating axons (Cook & Burnstock, 1976), or alternatively they may have an inhibitory role (see below).

It is interesting to note that terminals of the noradrenergic type (i.e. those containing the characteristic small dense-cored vesicles) have not been observed in any of the intramural bladder ganglia examined in the present investigation. While it is possible that the small dense cores are extracted by the type of fixation procedure employed in the present work, this negative finding is in accord with the results of a previous fluorescence study (Dixon *et al.* 1983) in which intramural ganglion cells of the human bladder have been reported to be unrelated to catecholamine-containing nerves. This organisation contrasts with that in other parasympathetic ganglia such as those of the myenteric plexus (Gabella, 1972), the cardiac ganglia (Ellison & Hibbs, 1976) and the pelvic ganglia (Klück, 1980), in which noradrenergic terminals have been observed in synaptic relationships with cholinergic neurons. Such terminals are generally assumed to provide an inhibitory influence on ganglion cells, and to depress neuronal transmission (Trendelenburg, 1961; Volle, 1966). The absence of this type of autonomic terminal on the intramural bladder ganglion cells raises the possibility that some other type of inhibitory nerve is involved if inhibitory influences are to occur in this location. In this context, the second type of terminal presently observed may perform such an inhibitory role. However, axons of this type are extremely rare and none has been observed forming a synaptic relationship with a

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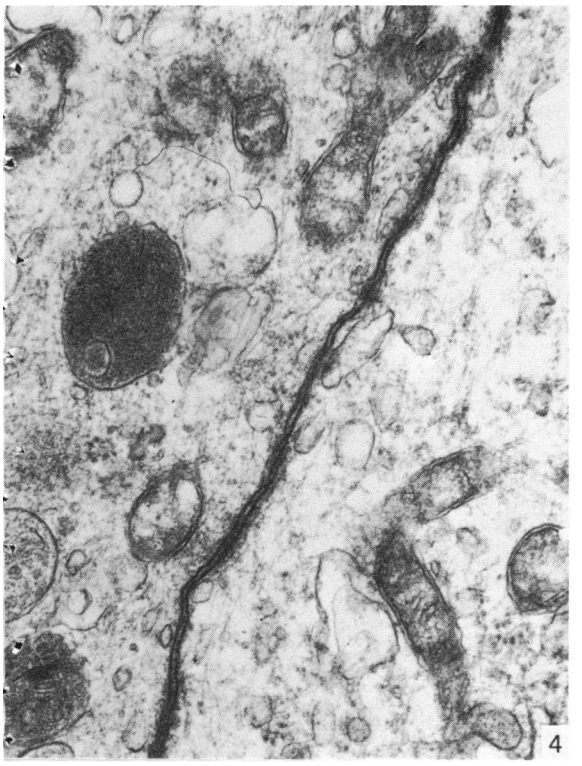
Fig. 4. In this electron micrograph, a region of close association between adjacent nerve cells is illustrated. The apposing membranes lie parallel to one another separated by an intercellular cleft of approximately 20 nm. × 35200.

Fig. 5. In this illustration of a junctional region between two intramural neurons, regularly spaced electron-dense bands bridge the intercellular space (arrow). × 112000.

Fig. 6. An electron micrograph of a presumptive cholinergic synapse within an intramural ganglion. The axon terminal contains mainly small agranular vesicles together with an occasional large granulated vesicle. × 45000.

Fig. 7. An axonal varicosity containing a heterogeneous population of small agranular and large granulated vesicles, together with multivesicular bodies, myelin figures and mitochondria. The profile lies in close proximity to the surface of an intramural ganglion cell (*G*). × 28800.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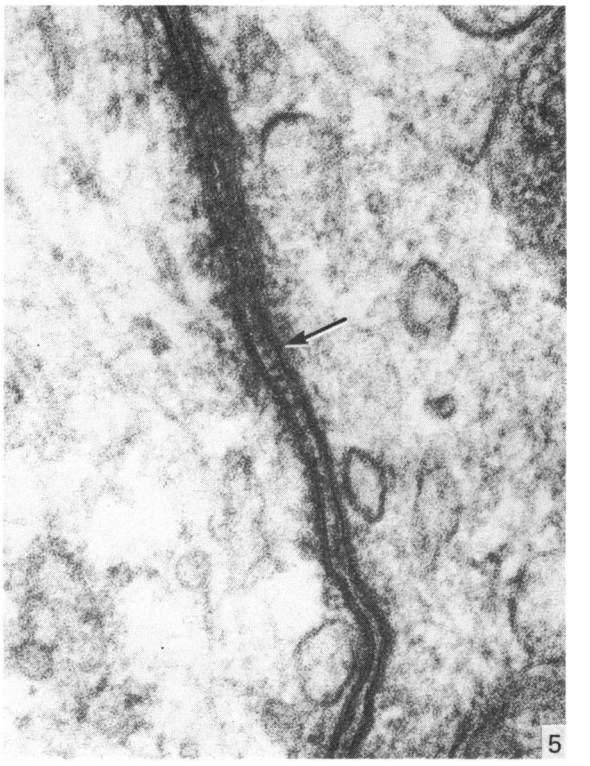
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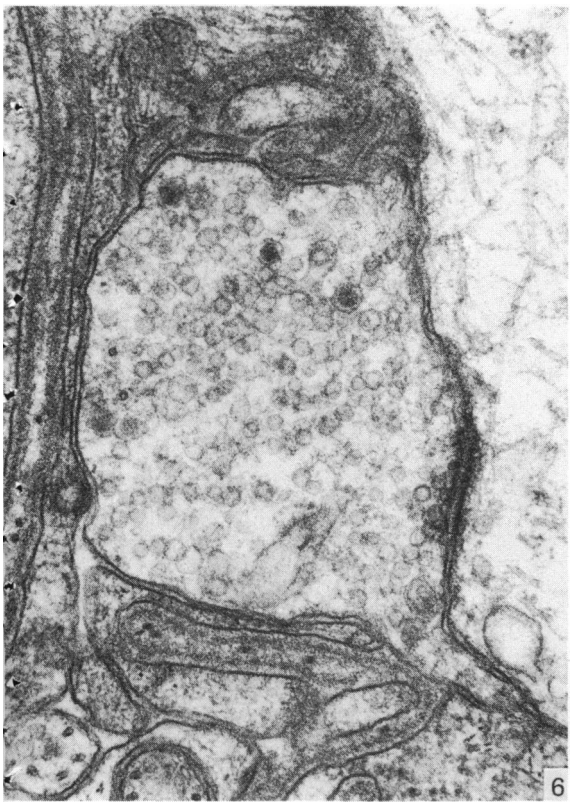
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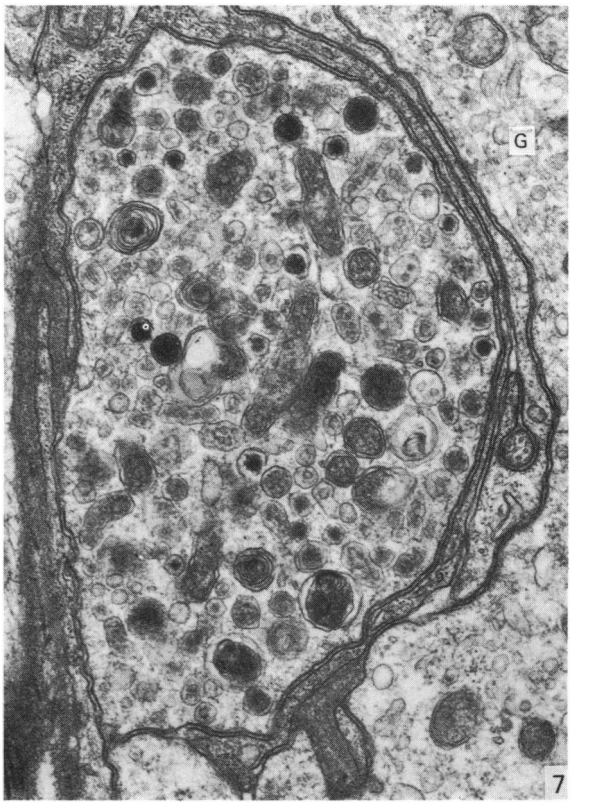
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ganglion cell, although this may well be due to the relatively small number of ganglia examined. While nerve profiles of this type could be interpreted as representing degenerating axons (Cook & Burnstock, 1976), it is interesting to note that similar axonal varicosities are described in the enteric plexuses to which an inhibitory function is assigned (Gabella, 1972).

SUMMARY

Using fresh biopsy specimens, intramural ganglia of the human urinary bladder have been examined by electron microscopy. The fine structural features of these neurons were compatible with their classification as parasympathetic in type. In several ganglia, groups of neurons were observed lying in close apposition to one another without any intervening satellite cell cytoplasm. Apart from occasional septate junctions the apposing membranes lacked any specialisation.

The majority of terminals occurring in association with these intramural neurons were axosomatic in location. The terminals contained numerous small agranular and occasional large granulated vesicles and structurally were presumptive cholinergic in type. Occasional axon varicosities packed wtih a heterogeneous population of small agranular vesicles, large vesicles of variable density and multivesicular bodies lay in close association with the ganglion cells. The functional significance of these morphological features in terms of ganglionic transmission has been considered.

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