Cell Tissue Res (1990) 261:231–237

Cell and Tissue Research

Intramural neurons in the urinary bladder of the guinea-pig

Giorgio Gabella

Department of Anatomy, University College London, London, United Kingdom

Accepted April 3, 1990

Summary. The urinary bladder of adult female guinea-pigs was stained histochemically to detect the presence of intramural ganglion neurons. Counts on whole-mount preparations of entire bladders revealed the presence of 2000–2500 neurons per bladder, either as individual nerve cells or, more often, as ganglia containing up to 40 neurons. Both ganglia and single neurons lie along nerve trunks and are interconnected to form a plexus. Ganglia occur in every part of the bladder; they are more numerous on the dorsal than on the ventral wall, and they are especially abundant in an area within a radius of 800 μm from the point of entry into the bladder wall of ureters and urinary arteries. The ganglia are located inside the muscle coat and close to muscle bundles; they usually lie nearer the mucosa than the serosa. Ultrastructurally, each ganglion is surrounded by a capsule; in addition to neurons and glial cells, the ganglia contain capillaries, collagen fibrils and fibroblasts; ganglion neurons are individually wrapped by glial cells and are separated from one another by connective tissue.

Key words: Urinary bladder – Autonomic ganglia – Intramural neurons – Cell counts – Autonomic nervous system – Guinea-pig

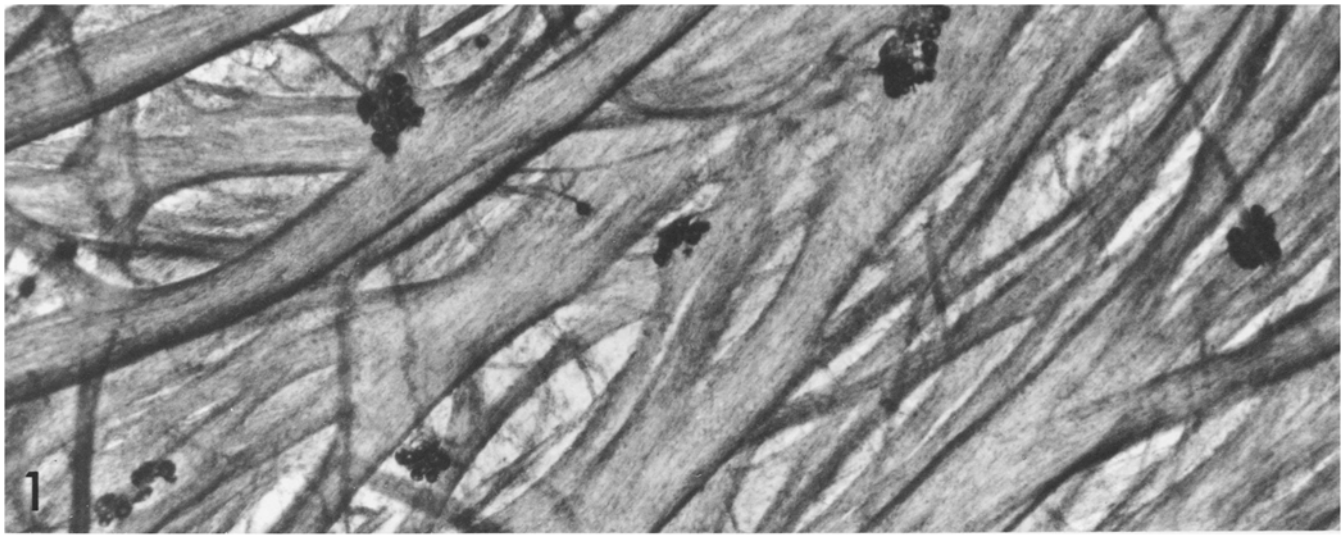
Although classically the efferent innervation of the mammalian bladder is described as originating in neurons within the pelvic ganglia (Langley and Anderson 1885; Wozniak and Skowronska 1967), these neurons being well characterized (e.g., Dail et al. 1975), the presence of ganglion neurons within the wall of the urinary bladder has been noted by several authors. Fehér et al. (1979) have observed them by electron microscopy in the bladder of the cat, and Gilpin et al. (1983) in the human bladder. Numerous ganglia, each including between 2 and 50 neurons, have been found in the bladder of newborn guinea-pigs by Crowe et al. (1986); many of them contain somatostatin, and a few contain substance P and vasoactive intestinal polypeptide. James and Burnstock (1988) have also found that many of the bladder neurons, both in situ and when cultured in vitro, contain neuropeptide Y and a few are positive for dopamine-beta-hydroxylase. Many other authors have not noted intramural neurons in the bladder, and the extent of the intrinsic neuronal apparatus of the bladder remains uncertain. Therefore, with this study on adult guinea-pigs an attempt has been made to establish: (i) to what extent there are neurons that are intramural; (ii) whether intramural neurons are concentrated in certain parts of the bladder; (iii) the position of ganglia with respect to the serosa, muscle coat and mucosa of the bladder wall; (iv) the basic ultrastructural characteristics of these ganglia, when they are compared with those of other autonomic ganglia, including the intramural neurons of the intestine. Lastly, the results in the guinea-pig have been compared with those in four other mammalian species.

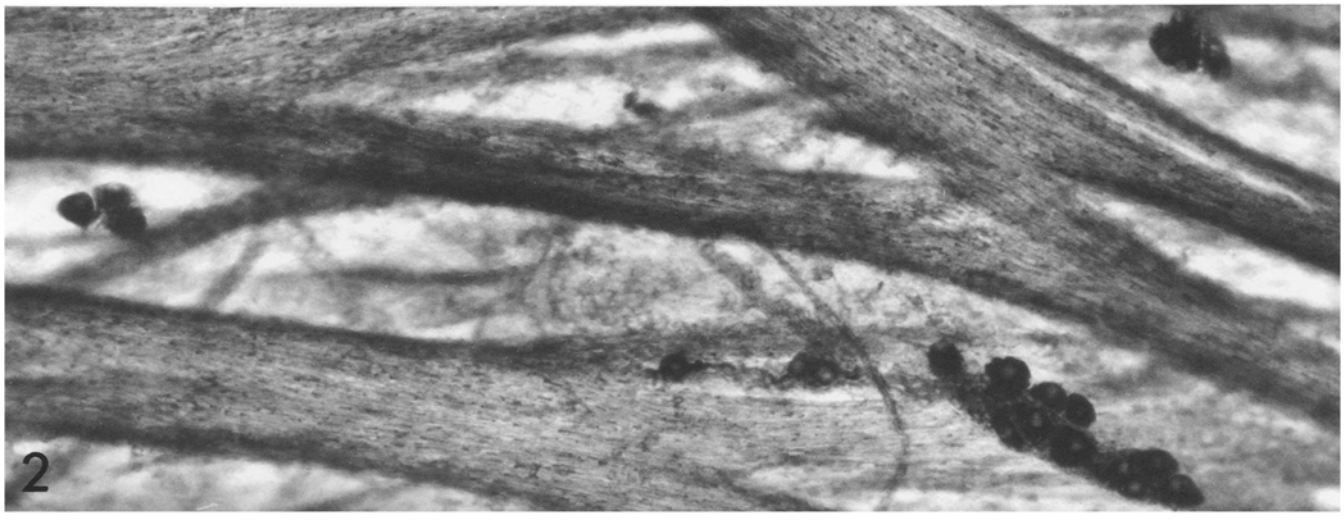
Send offprint requests to: Dr. Giorgio Gabella, Department of Anatomy, University College London, Gower Street, London, WC1E 6BT, UK

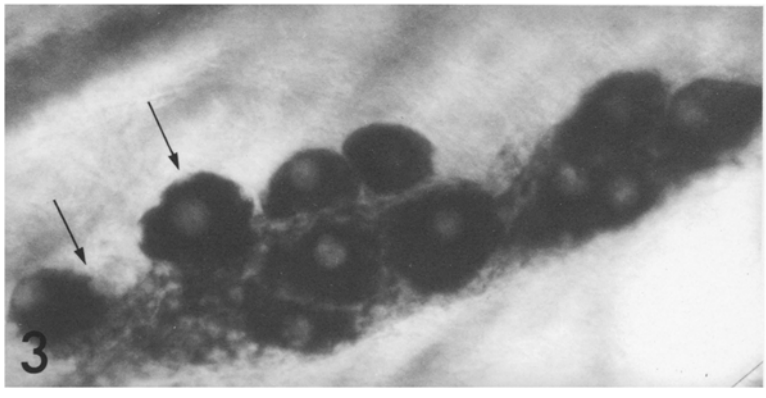
Materials and methods

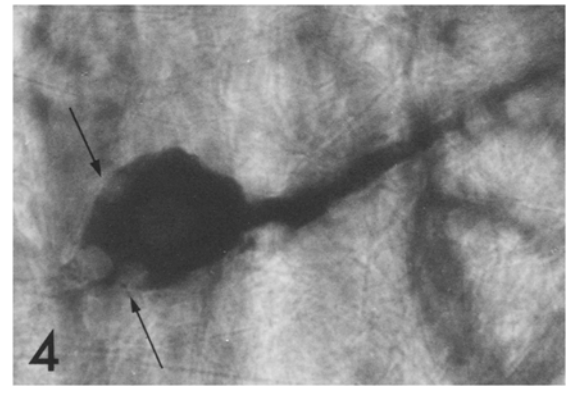
Female guinea-pigs weighing 250–500 g were used. They were killed with an intraperitoneal injection of sodium pentobarbitone (1 mg/kg body weight) or an intramuscular injection of Hypnorm (Janssen) (0.5 ml/kg), and bled through the carotid arteries. The abdomen was opened along the midline, and a cannula was inserted into the bladder via the urethra and held in position with a cotton-thread ligature. After cutting the pubic symphysis, the bladder and urethra were dissected out. The bladder was then injected with 2.5–3.5 ml of Krebs' solution to obtain full distension of its wall, and was processed according to one of the three following techniques.

(i) For detecting and counting intramural neurons, the bladder from each of 10 guinea-pigs was stained with the nicotinamide-adenine dinucleotide (NADH) technique (Gabella 1987). The fresh organ was pre-incubated for 1–3 min with 0.3% Triton X-100 to enhance penetration of the medium, washed in Krebs' solution and immersed for 45–60 min in a medium for staining nicotinamine









Figs. 1–4. Whole-mount preparation of guinea-pig urinary bladder stained for NADH

Fig. 1. Low-magnification view, showing bundles of musculature and several interspersed ganglia. × 50

Fig. 2. An isolated neuron and neuron clusters of various sizes lie between bundles of smooth musculature. × 126

Fig. 3. A ganglion from the micrograph above, shown at higher magnification. Some neuronal profiles appear scalloped by the presence of unstained glial nuclei (*arrows*). × 320

Fig. 4. An isolated intramural neuron with a single large process. *Arrows* point to glial cell nuclei close to the perikaryon. × 500

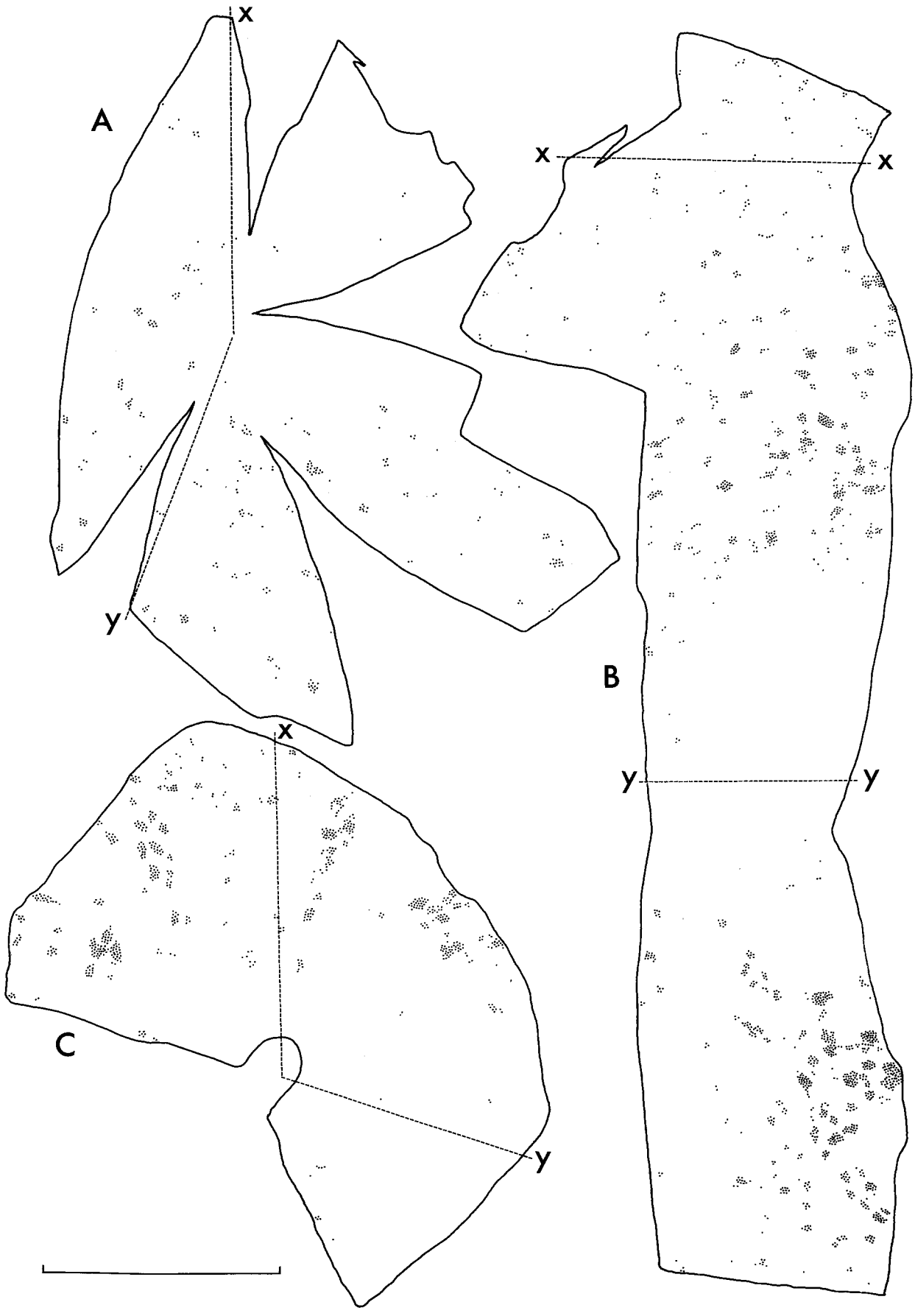


Fig. 5A–C. Tracings of the outline of three laminae from one entire urinary bladder. **A** cranial pole; **B** middle portion; **C** caudal pole, with the opening of the urethra. The *dashed lines* indicate where the mid-plane intersects the dorsal (*x*) and the ventral wall (*y*). Each *dot* represents a neuron. For graphic reasons clustered neurons are spaced a little more than in the original preparation. In this bladder, 2496 neurons are present. *Calibration bar*: 1 mm

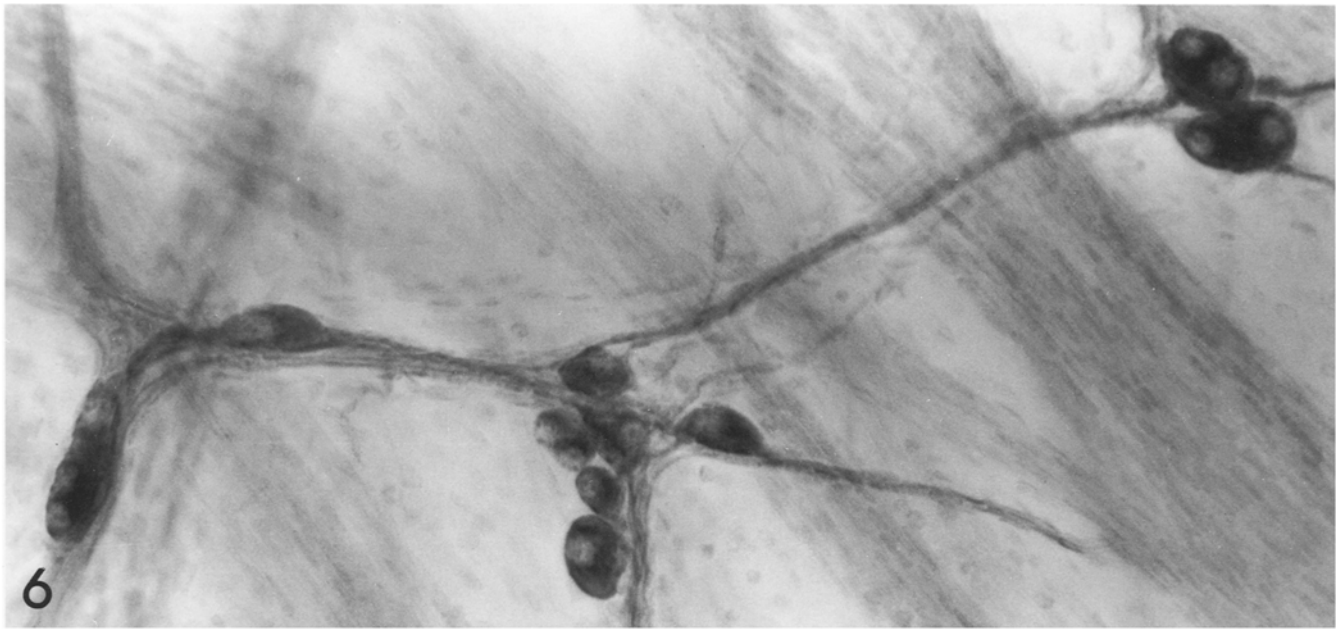


Fig. 6. Whole-mount preparation stained for acetylcholinesterase, showing nerve trunks and clusters of intramural neurons. ×320

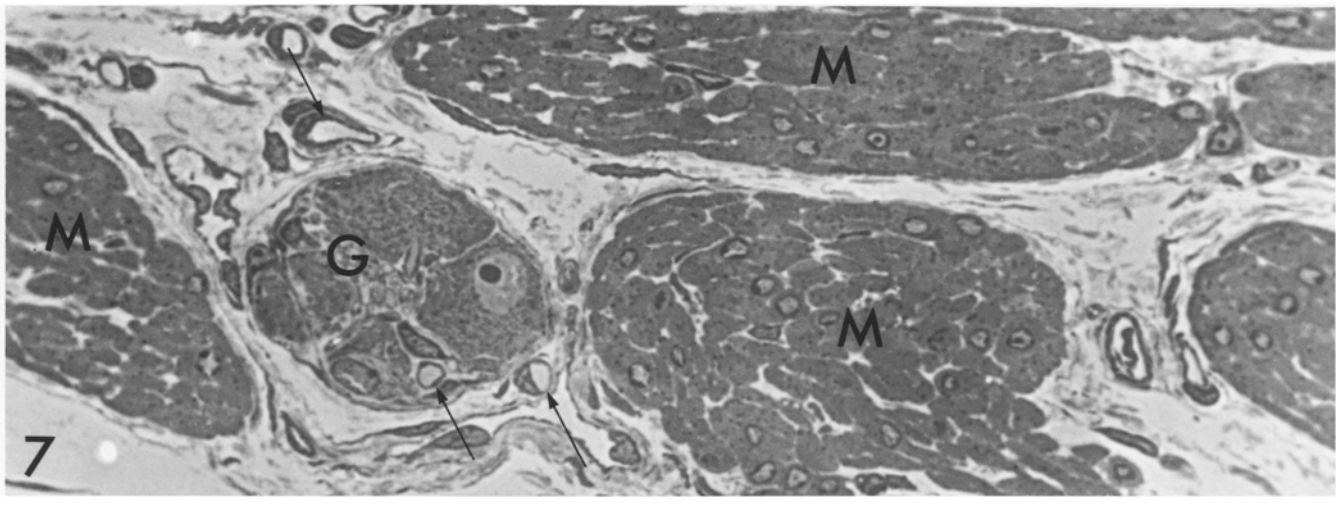


Fig. 7. Plastic section of bladder wall (mucosal side at top), showing muscle bundles (*M*) and a ganglion (*G*) surrounded by a capsule. The *arrows* point to intraganglionic and periganglionic blood vessels. ×800

dihydrogenase. The bladder was then fixed in 10% neutral formalin, and after 1–7 days was cut open, divided into three segments (cranial pole, equatorial band, caudal pole) and mounted in 70% glycerol.

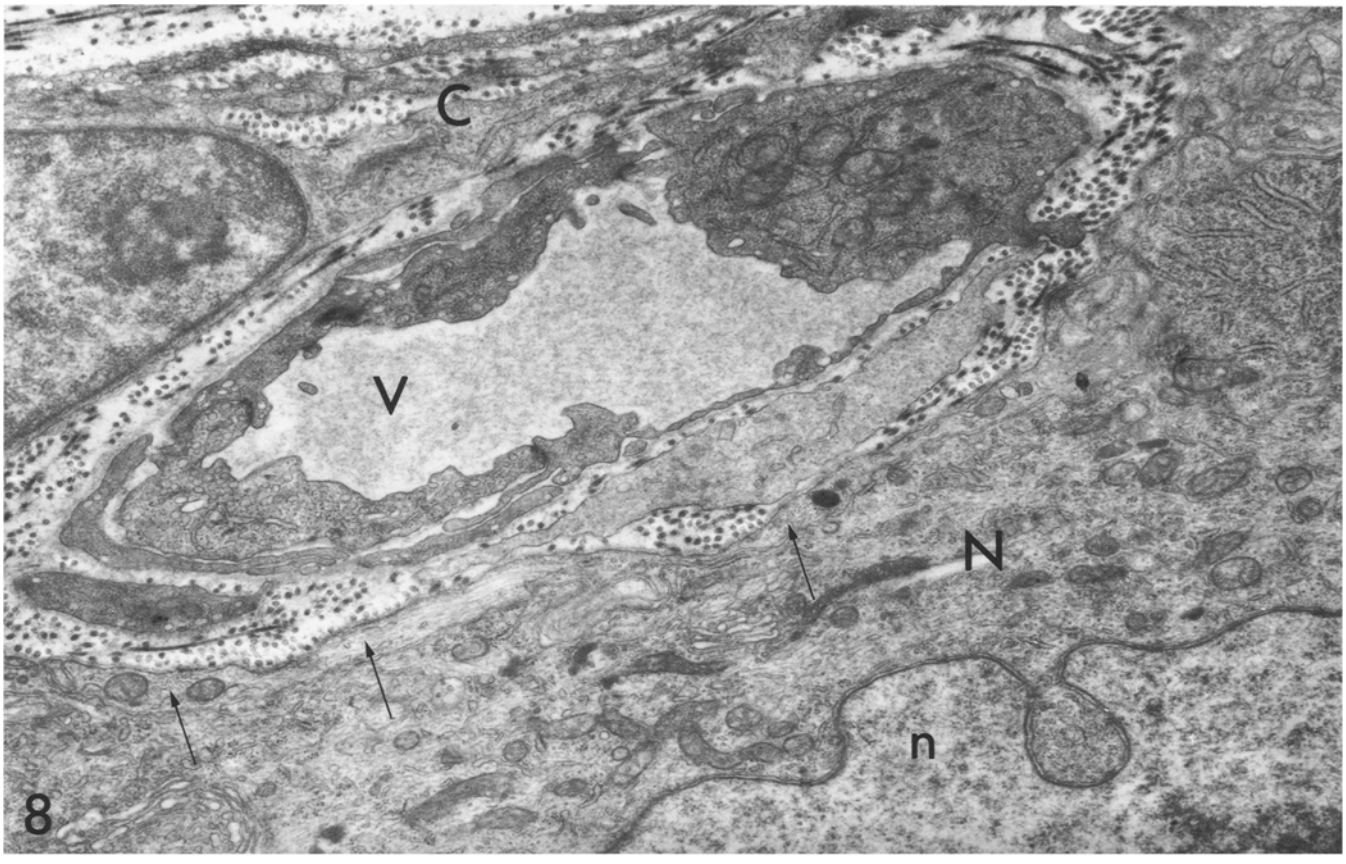
(ii) For the detection of acetylcholinesterase activity, the bladder from each of 3 guinea-pigs was processed according to the variation of the Karnovsky and Roots (1964) method proposed by Baker et al. (1986).

(iii) For thin-section work, the content was extracted from the lumen of the bladder of 8 guinea-pigs via the urethral cannula and was replaced with fixative; the whole distended bladder was then immersed in fixative, viz. 5% glutaraldehyde, buffered to pH 7.4 in 100 mM Na cacodylate at room temperature. After 10–15 min in fixative, the bladder was cut open and divided into strips of known orientation and position. The fixation lasted for 2 h, and was followed by a thorough wash in buffer and osmication for 60 min in 2% osmium tetroxide in the same buffer. The specimens were then washed in buffer, treated for 30 min in a saturated aqueous solution of uranyl acetate, dehydrated in graded ethanols, and infiltrated with epoxypropane and Araldite resin. Semi-thin sections, 1–3 μm thick, were cut with glass knives, stained with toluidine blue and examined in a light microscope. Thin sections, cut with glass knives, were stained with uranyl acetate and lead citrate and examined using an electron microscope.

The procedure for NADH staining of the whole bladder was applied to 6 rats, 2 rabbits, 2 ferrets and 2 mice, all being adult females.

Results

In the whole-mount preparations of guinea-pig bladders stained for diaphorase, numerous ganglion neurons were found (Fig. 1). They were heavily stained; their distribution and number could be studied in detail. The lightly stained muscle bundles did not obscure the ganglia (Fig. 2); the epithelium was unstained, although it was



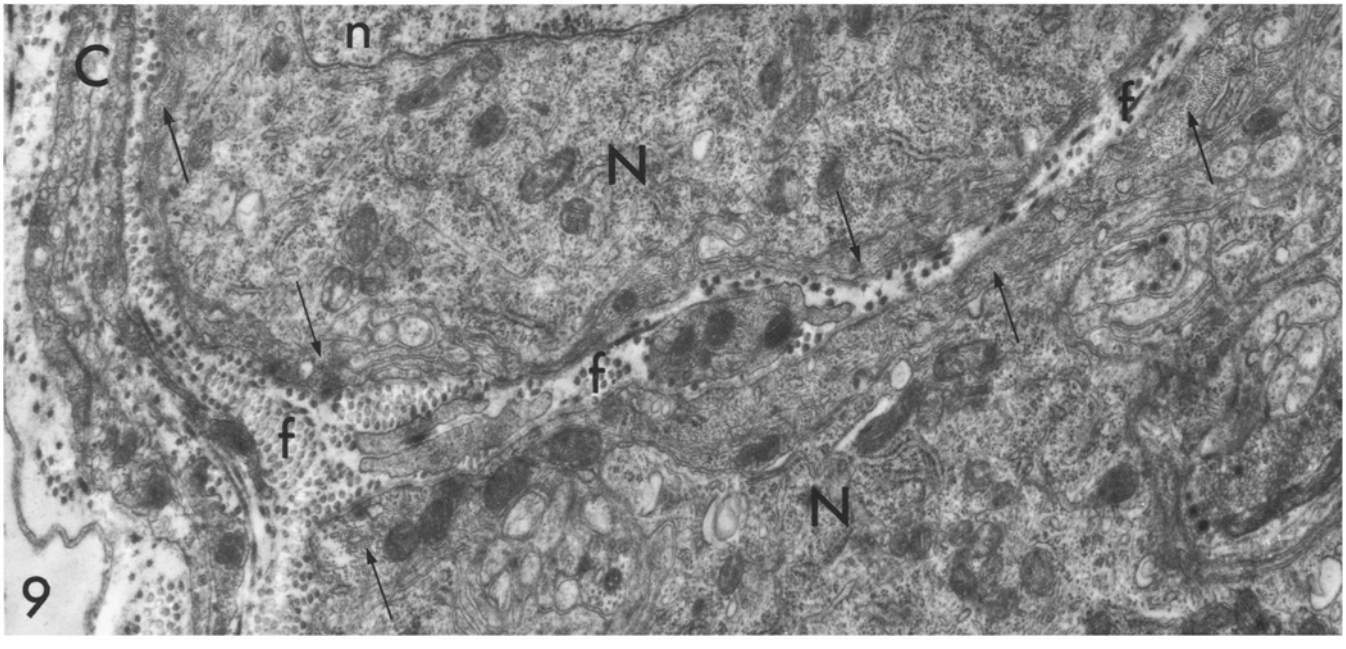


Fig. 8. Electron micrograph of an intramural ganglion. *C* capsule of the ganglion; *V* capillary, with fenestrations of the endothelium; *N* ganglion neuron, with its nucleus (*n*) and part of the nucleolus at the *bottom right corner*. *Arrows* point to the glial wrapping. ×14000

Fig. 9. Two neurons (*N*) with their glial wrapping (*arrows*) are separated from one another and from the capsule (*C*) by collagen fibrils (*f*). ×18000

clear that the medium had penetrated through the full thickness of the musculature and the mucosal tunica propria. The neurons occurred singly or, more commonly, in clusters that included up to 40 neurons (Figs. 3, 4). They were found in all parts of the bladder, including the cranial pole (Fig. 5). Their distribution, however, was not uniform. Neurons were more abundant on the posterior than on the anterior wall, and were more abundant near the equatorial region than near either pole of the organ. The spatial density of ganglion neurons

was highest in the regions that were near the entry of the ureter, and these are also the regions of entry of the major blood vessels into the bladder; it was lowest in the central portion of the ventral wall (Fig. 5). The total number of neurons was counted in the bladder of 3 guinea-pigs, and the results were 1721, 2496 and 2699 neurons per bladder. The distribution of neurons was similar in all animals.

Ganglia were readily detected in whole-mount preparations stained for acetylcholinesterase (Fig. 6). Nerve bundles were also stained and formed a network; all ganglion neurons were situated along their paths.

In semithin sections, all the ganglia were located between muscle bundles, and usually lay nearer the mucosa than the serosa. Ganglion neurons had a large, round, pale nucleus with a prominent nucleolus, and were easily recognized. The nuclei of the supporting glial cells were smaller, elongated and richer in chromatin. Every ganglion was surrounded by a capsule, and there were small blood vessels inside the capsule and immediately outside (Fig. 7).

In the electron microscope, the entire ganglion was enveloped by a capsule, 1–4 cells thick and often reduced to thin laminar processes (Fig. 8). Both surfaces of these cells were covered by a basal lamina, the laminar processes (0.1–0.3 μm thick) being studded with numerous caveolae. Collagen fibrils lay inside and outside the capsule. The neuronal perikarya were surrounded by glial cells rich in gliofilaments. The glial wrapping was very attenuated at some points but no discontinuities were observed; a basal lamina lay over its external surface. A glial wrapping surrounded each individual neuron, so that neurons were separated from one another by glial processes and by collagen fibrils (Fig. 9). The thicker portions of the glial wrapping contained short perikaryal processes and incoming nerve fibres synapsing on these processes.

Intraganglionic vessels were blood capillaries. They intervened between perikarya or between a perikaryon and the capsule. They were commonly fenestrated, although the number of fenestrations per vessel profile was small (2–5). In addition to collagen, blood vessels, fibroblasts, capsule cells, glial cells and neurons, the ganglia contained bundles of nerve fibres; whereas some fibres were myelinated, the majority were unmyelinated.

Bladders from mice, ferrets, rats and rabbits were stained for NADH. No neurons were found in the rat and the mouse bladders, whereas rare ganglia or individual neurons were found in ferret and rabbit: the number of neurons ranged between 8 and 35, almost all of them occurring within a radius of 250 μm from the point of entry of the main arteries.

Discussion

The first conclusion of this work is that intramural ganglion neurons are indeed present in the guinea-pig bladder, and in large numbers. This situation is characteristic of the guinea-pig. In contrast, intramural ganglion neurons were not observed in the rat and mouse, and in the ferret and rabbit they amounted to at most a few tens of neurons. As in the guinea pig (Crowe et al. 1986), intramural neurons are found in man (Kulkin 1961; Gilpin et al. 1983) and cat (Fehér et al. 1979), although their number in these species is unknown.

The number of neurons within the guinea-pig bladder is high, being of the order of 2000 or more. The numerical difference in the three animals in which total counts were made is large, but not larger than the inter-individual variability found in other autonomic ganglia or plexuses, e.g. the ciliary ganglion of the rabbit (Johnson and Purves 1981) or the tracheal plexus of the mouse (Chiang and Gabella 1986).

Ganglion neurons are present in every area of the bladder, including the cranial pole or dome. Their density, however, is not uniform. Ganglion neurons are more abundant in two regions of the bladder within a radius of a mm or so from the points of entry of the ureters. Whether this arrangement has a physiological significance, one cannot tell. However, it seems relevant to consider that these areas of the bladder are also the points of entry of the two major urinary arteries; one may assume that the neuronal precursors that colonize the bladder in embryonic life penetrate into the organ migrating along blood vessels. If this assumption is correct, then the uneven distribution of intramural neurons reflects aspects of the migratory process. This migration probably takes place in early embryonic life. In newborn guinea-pigs, the intramural innervation of the bladder is similar to that of the adult animal (data not presented). The uneven distribution of bladder neurons contrasts with the highly regular and uniform distribution of intramural neurons in the small intestine, along both circumference and length of the gut. Even in the stomach and the colon of the guinea-pig, where there are gradients of neuronal density around the circumference, the arrangement of ganglia and nerve strands is far more regular than in the bladder (Gabella 1987). In both the gut and the bladder, the ganglia lie along nerve trunks, and the result is a meshwork with ganglia at the knots and with nerve strands making up the meshes.

As to the question of the position of the ganglia within the bladder wall, they are truly intramural, indeed intramuscular, and usually lie nearer the mucosa than the serosa. The ganglia lie therefore close to, and usually surrounded by, muscle bundles, and in this respect, they resemble the myenteric ganglia of the gut. It is known that enteric ganglia are affected by the mechanical activity of the muscle, the shape of ganglia and constituent cells changing with contraction and relaxation (Gabella 1981). One would expect the same to happen in the intramural ganglia of the bladder. There are more subtle questions related to the effect of wall stress, at various degrees of distension, on the position and orientation of ganglia, which, however, cannot be discussed until more is known of the distribution of stresses through various components of the bladder wall.

The ultrastructure of the ganglia was not studied in detail. The main question that was pursued was whether the structural plan of these ganglia is similar to that

of enteric ganglia or not. It has been reasoned that the peculiar structural features of the enteric ganglia (no connective tissue capsule, absence of intraganglionic vessels, compactness of structure with exclusion of true extracellular space and connective tissue, special distribution of glial cells) are related to their closeness to smooth muscle, and it has been expected that enteric and bladder ganglia would be rather similar in structure, since both sets of ganglia are truly intramural. This expectation has proved to be wrong; the intramural ganglia of the urinary bladder have turned out to be quite different in structure from those of the gut. They resemble rather those of the trachea (Coburn 1987; Baluk et al. 1985) or the heart (Ellison and Hibbs 1976), in several respects. Indeed, the ganglia have a distinct capsule, which is uninterrupted except at the points of entry or exit of nerve trunks. Inside the ganglia, there are blood vessels, collagen fibrils and fibroblasts, in addition to neurons and glial cells. Neurons are individually wrapped by glial cells and form discrete units that are separated from one another by a thin layer of connective tissue. The bundles of nerve fibres within ganglia and in nerve trunks have a structure similar to that of peripheral nerve in general rather than to that of enteric connecting strands.

Acknowledgements. This work was was supported by a grant from the Medical Research Council (UK). Peter Trigg provided excellent technical assistance and Peter Baluk stained the cholinesterase preparations.

References

Baker DG, McDonald DM, Basbaum CB, Mitchell RA (1986) The architecture of nerves and ganglia of the ferret trachea as revealed by acetyl-cholinesterase histochemistry. J Comp Neurol 246:513–526

Baluk P, Fujiwara T, Matsuda S (1985) The fine structure of the ganglia of the guinea-pig trachea. Cell Tissue Res 239:51–60

Chiang C-H, Gabella G (1986) Quantitative study of the ganglion neurons of the mouse trachea. Cell Tissue Res 246:243–252

Coburn RF (1987) Peripheral airway ganglia. Annu Rev Physiol 49:573–582

Crowe R, Haven AJ, Burnstock G (1986) Intramural neurones of the guinea-pig urinary bladder: histochemical localization of putative neurotransmitters in cultures and newborn animals. J Auton Nerv Syst 15:319–339

Dail DW Jr, Evan AP Jr, Eason HR (1975) The major ganglion in the pelvic plexus of the male rat. Cell Tissue Res 159:49–62

Ellison JP, Hibbs RG (1976) An ultrastructural study of mammalian cardiac ganglia. J Mol Cell Cardiol 8:89–101

Fehér E, Csányi K, Vajda J (1979) Ultrastructure of the nerve cells and fibres in the urinary bladder wall of the cat. Acta Anat 103:109–118

Gabella G (1981) Ultrastructure of the nerve plexuses of the mammalian intestine: the enteric glial cells. Neuroscience 6:425–436

Gabella G (1987) The number of neurons in the small intestine of mice, guinea-pigs and sheep. Neuroscience 22:737–752

Gilpin CJ, Dixon JS, Gilpin SA, Gosling JA (1983) The fine structure of autonomic neurons in the wall of the human urinary bladder. J Anat 137:705–713

James S, Burnstock G (1988) Neuropeptide Y-like immunoreactivity in intramural ganglia of the newborn guinea pig urinary bladder. Regul Pept 23:237–245

Johnson DA, Purves D (1981) Post-natal reduction of neural unit size in the rabbit ciliary ganglion. J Physiol (Lond) 318:143–159

Karnovsky MJ, Roots LJ (1964) A "direct-coloring" thiocholine method for cholinesterase. J Histochem Cytochem 12:219–221

Kulkin SG (1961) Über die Dogielschen Zellen (II. Typ) in den intramuralen Nervengeflechten der Harnblase des Menschen. Anat Anz 110:127–139

Langley JN, Anderson HK (1885) The innervation of the pelvic and adjoining viscera. II. The bladder. J Physiol (Lond) 19:71–84

Wozniak W, Skowronska U (1967) Comparative anatomy of pelvic plexus in cat, dog, rabbit, macaque and man. Anat Anz 120:457–473