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# The Peripheral Adrenergic Innervation Apparatus

## I. Intraganglionic and Extraganglionic Adrenergic Ganglion Cells*

AHMAD EL-BADAWI**, and ERIC A. SCHENK

Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, New York, U.S.A.

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*Summary.* Adrenergic neurons, studied by the fluorescent method for norepinephrine, are widely distributed throughout the male urinary and seminal tract organs. They occur both within ganglia and outside of ganglia as isolated cells.

The extraganglionic cells are classified according to their location as paravascular, epineural, and terminal. The paravascular and epineural ganglion cells are morphologically similar to the cells found within ganglia, while the terminal ganglion cells differ mainly in being smaller and having multiple divergent axonal processes.

The existence of extraganglionic as well as ganglionic adrenergic cells within the innervated organs adds further support to, and extends the concept of the short adrenergic neuron.

According to the classical concept of peripheral autonomic innervation, peripheral parasympathetic ganglion cells are found in ganglia which lie very close to and within innervated organs (terminal autonomic ganglia), whereas peripheral sympathetic ganglion cells are confined to ganglia of the sympathetic chains (paravertebral ganglia) and ganglia of abdominal plexuses (prevertebral ganglia). As early as 1895 LANGLEY and ANDERSON came to the conclusion that sympathetic fibers to pelvic viscera relay partially in ganglion cells located in close proximity to the target organs. This concept gradually found support in several experimental observations, chief among which are the following: (1) Electrophysiologic studies on the guinea pig vas deferens (FERRY, 1963a, 1963b; KURIYAMA, 1963) have shown that the hypogastric nerve, which is the main pathway for sympathetic nerves to pelvic organs, contains two types of nerve fibers, suggesting that the nerve is composed of preganglionic as well as postganglionic fibers. (2) Male internal genital organs of several mammalian species have a high norepinephrine content which is not appreciably reduced after chronic hypogastric nerve section (SJÖSTRAND, 1962b, 1965). (3) Motor response of the guinea pig vas deferens to electrical stimulation of the hypogastric nerve is abolished (SJÖSTRAND, 1962a, 1965), and that of the cat urinary bladder and rabbit uterus reduced (CHESHER and THORP, 1965; SIGG and SIGG, 1964; VARAGIĆ, 1956) by the ganglionic blocking agent hexamethonium. More recently the existence of sympathetic ganglionic relays in pelvic organs has been confirmed in studies on the adrenergic innervation of these organs which have revealed the presence of adrenergic ganglion cells along the hypogastric nerve (FALCK et al., 1965), in male internal genitalia (EL-BADAWI and SCHENK, 1967b; FALCK et al., 1965; NORBERG and HAMBERGER, 1964; OWMAN

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** Lecturer in Urology, University of Alexandria, Alexandria, U. A. R. Presently on leave as Buswell Fellow in Urologic Research, University of Rochester, School of Medicine and Dentistry, Rochester, New York.

and SJÖSTRAND, 1965, 1966; SJÖSTRAND, 1965) and urinary bladder (EL-BADAWI, 1967; EL-BADAWI and SCHENK, 1966, 1967c; HAMBERGER and NORBERG, 1965a, 1965b) in several mammalian species.

In this report morphologic evidence for the occurrence of adrenergic ganglion cells within innervated organs, located both inside and outside of autonomic ganglia, will be presented.

## Material and Methods

Specimens from lower urinary organs (bladder, vesical end of ureter, proximal urethra), and seminal tract (epididymis, vas deferens, prostate) were obtained from ten cats. The tissues were excised under intravenous nembutal anesthesia and subsequently prepared for histochemical analysis of norepinephrine-containing elements by the method described by EL-BADAWI and SCHENK (1967b).

## Results

Peripheral adrenergic ganglion cells (AGC) are round or oval, of moderate size, and smaller than the corresponding cholinergic ganglion cells. Uniformly tiny granules of norepinephrine fluorochrome are distributed throughout the perikaryon and show a variable intensity of fluorescence in different cells (Figs. 1—9). Several processes of various lengths are present: the more common pattern is that of a single long axon and several short dendritic type processes; less frequently multipolar cells with a number of axonal and few or no dendritic processes are seen.

According to their site, peripheral AGC are classified into intraganglionic and extraganglionic types. Intraganglionic AGC are located within intrinsic autonomic ganglia[1] lying on the surface or in the depth of the innervated organ. Extraganglionic AGC lie outside ganglia, alongside nerve trunks (epineural cells), next to blood vessels (paravascular cells), or still more peripherally in close vicinity to the cells of the innervated tissue as an integral part of the adrenergic neuroterminal plexus[2] (terminal cells).

Peripheral AGC were encountered in all of the organs examined in this study. Their overall incidence as well as the relative frequency of each of the types vary in different organs and often also in different anatomical regions of the same organ. In general, intraganglionic, epineural and paravascular AGC are most frequently found in the urinary bladder, prostate and prostatic end of the vas deferens. On the other hand terminal AGC occur most commonly in the muscular plexus of the vesical end of the ureter and in the peritubular prostatic plexus.

### *Intraganglionic AGC*

These generally constitute the majority of peripheral AGC. They are found in mixed intrinsic ganglia, i.e., ganglia composed of both cholinergic and adrenergic cells (Fig. 1), and much less commonly in smaller and usually more deeply situated ganglia composed solely of adrenergic cells (Figs. 2, 3). Although cells within the same ganglion may show a different intensity of fluorescence, those which lie more deeply within the organ generally tend to contain brighter granules.

[1] These ganglia have usually been called "terminal". The term "intrinsic" is preferable to avoid confusion with "terminal AGC" which are even more peripheral and lie closer to the cells of the innervated tissue.

[2] By "adrenergic neuroterminal plexus" is meant the arborization of terminal adrenergic fibers which run in intimate relationship to the cells of the innervated tissue.

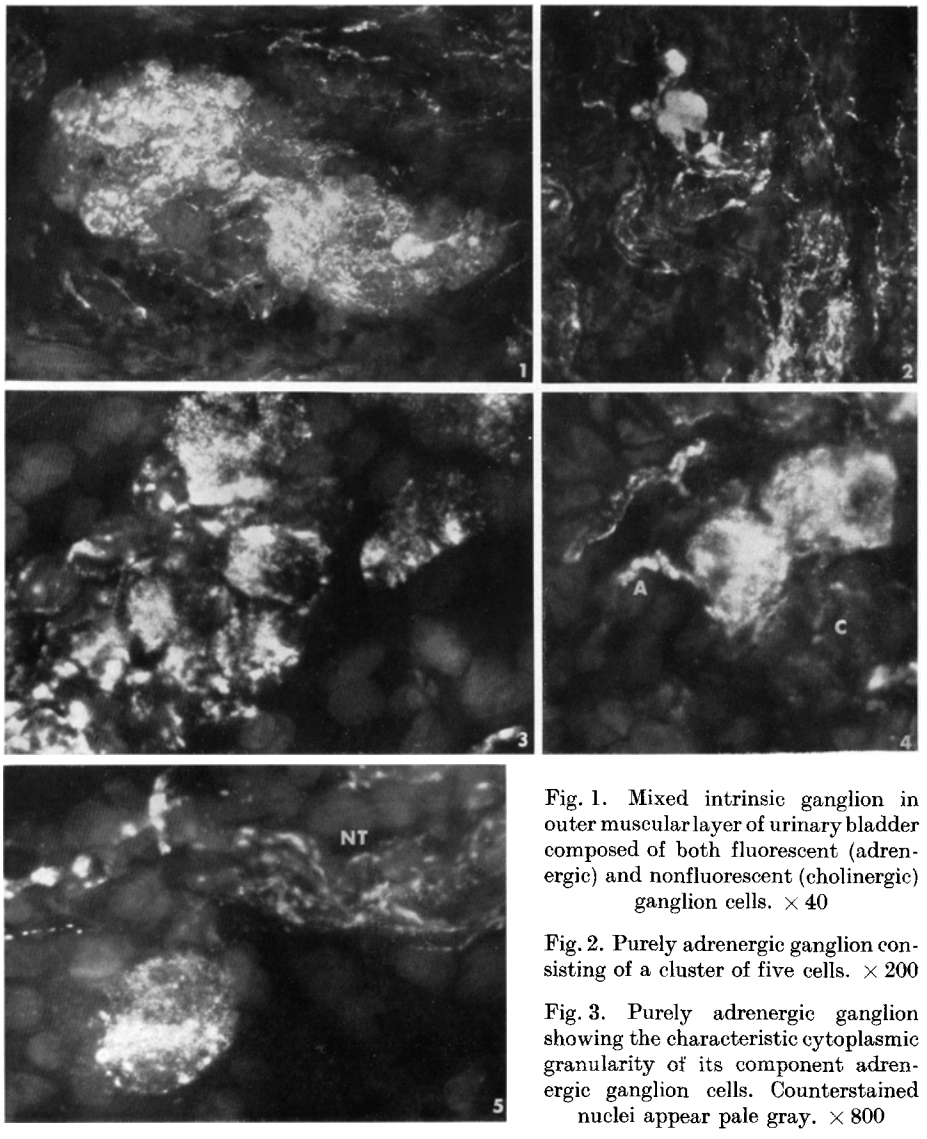


Fig. 1. Mixed intrinsic ganglion in outer muscular layer of urinary bladder composed of both fluorescent (adrenergic) and nonfluorescent (cholinergic) ganglion cells. × 40

Fig. 2. Purely adrenergic ganglion consisting of a cluster of five cells. × 200

Fig. 3. Purely adrenergic ganglion showing the characteristic cytoplasmic granularity of its component adrenergic ganglion cells. Counterstained nuclei appear pale gray. × 800

Fig. 4. Mixed intrinsic ganglion in prostatic capsule showing two adrenergic ganglion cells with interdigitating short dendritic processes and a third non-adrenergic cholinergic cell (*C*). An axon (*A*) is seen arising from one of the two adrenergic cells. × 800

Fig. 5. Epineural adrenergic ganglion cell showing the characteristic cytoplasmic granularity and lying next to a moderate-sized nerve trunk (*NT*) in the muscularis of urinary bladder. The cell is similar to intraganglionic cells (*cf.* Figs. 3, 4). × 800

The distribution of cells in mixed ganglia is not uniform; their relative abundance varies in different organs, different ganglia, and frequently also in the same ganglion at different levels of sectioning. Dendritic type processes are occasionally seen interdigitating with similar processes of contiguous ganglion cells (Fig. 4).

Axonal type processes, usually one from each cell (Fig. 4), course individually or less commonly as thin bundles within the ganglia. Occasionally, axons can be traced to where they leave the ganglion through an emergent nerve trunk.

### *Extraganglionic AGC*

These are identified by the characteristic appearance of their processes and cytoplasmic norepinephrine granules. These features serve to differentiate them from non-fluorescent Schwann cells and from fluorescent cells without processes such as mast and chromaffin cells.

*1. Epineural AGC.* These lie alongside sizable nerve trunks (Fig. 5) generally composed of both adrenergic and non-adrenergic fibers, coursing on or close to the surface of the organ, specially at a short distance from a related intrinsic ganglion. Occasionally they are also associated with thin nerve bundles, usually purely adrenergic, running in the depth of the organ.

Epineural cells are morphologically similar to intraganglionic cells. However, they generally fluoresce more brightly particularly when compared to cells of superficially situated ganglia. They are usually solitary, but sometimes alongside a large nerve trunk occur in small groups of two to five arranged as a cluster or short file. Occasionally adrenergic and non-adrenergic ganglion cells are seen lying alternately along the nerve trunk. Interdigitation of dendritic type processes of contiguous adrenergic cells is not uncommon. After a short independent course, the axonal processes join a related nerve trunk or bundle, or terminate directly in a nearby adrenergic neuroterminal plexus in the musculature or lamina propria of the organ.

*2. Paravascular AGC.* Solitary adrenergic cell bodies not associated with nerve trunks or bundles, are occasionally seen at a short distance from large or medium-sized arteries and veins which lie in the pedicle of the organ, on its surface, or along its principal connective tissue planes. Such cells are constantly found in association with blood vessels in the lamina propria of the urinary bladder (Fig. 6), in the periprostatic connective tissue (Fig. 7) and in the adventitia of the prostatic end of the vas deferens.

Paravascular cells closely resemble intraganglionic and epineural cells and contain moderately to intensely bright granules, especially when associated with vessels which are deeply situated in the organ. Axons of paravascular cell bodies for the most part join the perivascular neuroterminal plexus; some axons anastomose with the fibers of the plexus in the smooth muscle or lamina propria.

*3. Terminal AGC.* These lie in close vicinity to cells of the innervated tissue, and form an integral part of the adrenergic neuroterminal plexus. They are sparsely scattered within the meshwork of the plexus in smooth muscle, around blood vessels, and in the lamina propria, in this order of frequency.

Compared to the three types of AGC already described, terminal AGC are smaller and usually contain more disperse and coarser cytoplasmic granules of moderate or intense fluorescence (Figs. 8, 9). They usually have no dentritic processes. One or more axonal processes, after short widely divergent courses and occasionally after dichotomous branching, join the fibers of the adrenergic neuroterminal plexus.

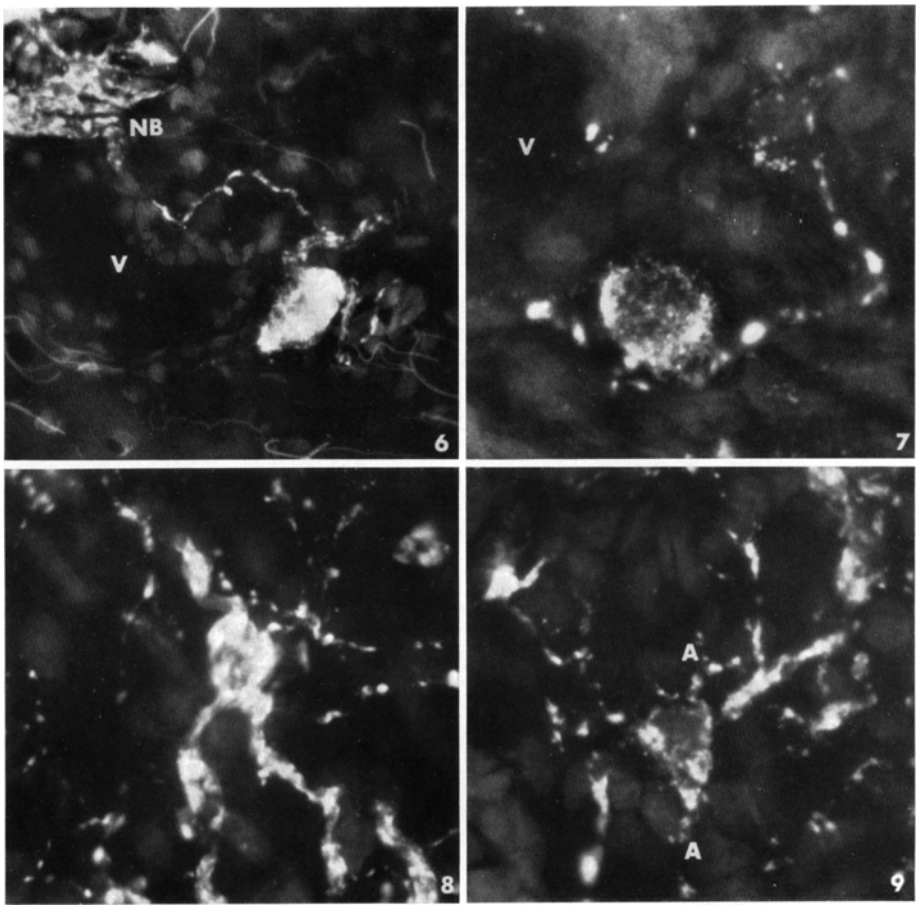


Fig. 6. Paravascular adrenergic ganglion cell lying next to a vein (*V*) in the lamina propria of urinary bladder. An axon is seen derived from the cell and gives off a branch fiber to the vein. An adrenergic nerve bundle (*NB*) lies on the other side of the vein. × 200

Fig. 7. A paravascular adrenergic ganglion cell adjacent to a vein (*V*) in the prostatic intertubular tissue. The cell has a single axon and few dendritic processes, and is similar to intraganglionic and epineural cells (*cf.* Figs. 3—5). × 800

Fig. 8. A multipolar terminal adrenergic ganglion cell in the prostatic peritubular musculature. Several axonal processes arise from the cell, diverge widely and join ramifications of the muscular neural plexus. × 800

Fig. 9. A multipolar terminal adrenergic ganglion cell in the muscular neuroterminal plexus of the vesical end of the ureter. The cell has the characteristic features of a terminal ganglion cell (*cf.* Fig. 8), and gives rise to several divergent axons (*A*). × 800

## Discussion

Our findings corroborate the presence of "intrinsic" adrenergic ganglia located within the innervated organs and provide evidence for a system of even more peripheral adrenergic ganglion cells. These cells have been arbitrarily classified on the basis of their location as paravascular, epineural, and terminal. They are

most frequently found as isolated cells and since they are not associated with intrinsic ganglia, have been designated extraganglionic cells. Whereas the paravascular and epineural ganglion cells are structurally similar to those adrenergic ganglion cells found within intrinsic ganglia insofar as they are of comparable size, contain fine densely crowded granules of fluorochrome, and possess both multiplic dendritic and usually solitary axonal processes, the terminal adrenergic ganglion cells differ in having a smaller size, coarser and more disperse granules, no dentritic processes, and one or more divergent axonal processes.

It is of interest that OWMAN and SJÖBERG (1966) have depicted a cell in the myometrium (Fig. 8) — referred to subsequently by OWMAN and SJÖSTRAND (1966) as a chromaffin cell — which has structural features similar to terminal adrenergic ganglion cells which we have described. The presence of norepinephrine containing axonal processes issuing directly from a cell body rich in norepinephrine, suggests to us that terminal cells are more reasonably interpreted as ganglionic rather than chromaffin. We suspect that norepinephrine containing cell bodies frequently described as chromaffin cells (OWMAN and SJÖSTRAND, 1965; 1966; SJÖSTRAND, 1965) may indeed have been terminal ganglion cells, the axonal processes of which did not appear in the plane of the section.

Under optical conditions the fluorochrome demonstrated in intrinsic peripheral adrenergic ganglion cells by our procedure was always identified as sharply outlined uniform tiny granules, especially in sections less than 10 microns in thickness. The occurrence of granules in sympathetic ganglion cells was also reported in the superior cervical ganglion of the rat (ERÄNKÖ and HÄRKÖNEN, 1963), but is believed by NORBERG and HAMBERGER (1964) to be the exception and not the rule, the usual picture being a diffuse fluorescence of the norepinephrine-containing cell. We believe, however, that diffuse fluorescence may in fact be the result of diffusion of norepinephrine or of its fluorochrome, especially since the method usually used (FALCK, 1962) entails exposure of the section to formaldehyde vapor at 80° C for one hour or longer. In a previous study we had found that granular fluorescence of adrenergic cells is very rarely obtained if the sections are treated in this manner instead of being exposed at 55° C for an average of 30 min as routinely adopted in our present procedure (EL-BADAWI and SCHENK, 1967b).

The variable intensity of adrenergic cell fluorescence often noted in AGC in our material, most likely reflects heterogeneity of the cells with respect to their norepinephrine content. There is evidence that differences in the norepinephrine content of neurons may be related to their site (MALMFORS, 1963) and to the rate of norepinephrine formation in their cell bodies (DAHLSTRÖM, 1964).

Our observations support the concept that the vas deferens, male internal genital glands, and lower urinary organs are innervated by short adrenergic neurons, namely, neurons derived from intrinsic adrenergic ganglia. The finding of adrenergic cell bodies not associated with intrinsic ganglia indicates that the population of short adrenergic neurons includes not only neurons derived from ganglia, but also neurons with extraganglionic cell bodies. One may then propose a system of peripheral adrenergic short neurons derived from intrinsic ganglia, shorter neurons with epineural and paravascular cell bodies, and still shorter neurons represented by terminal adrenergic ganglion cells.

The functional significance of short adrenergic neurons has been discussed in part by SJÖSTRAND (1965). The existence of extraganglionic as well as intraganglionic adrenergic cells has further implications:

(1) An isolated *in vitro* organ or portion of an organ, even though it may not contain intrinsic ganglia, may still contain intact extraganglionic cells and thus retain some degree of autonomous sympathetic activity.

(2) Peripheral AGC are expectedly responsible for a considerable fraction of the norepinephrine content of an organ, or region of an organ, in which they abound. This must be given due consideration in any study of the norepinephrine content of an intact or denervated organ, especially where this content is compared to the distribution and relative abundance of adrenergic nerve fibers.

(3) In an isolated preparation or *in vivo*, an adrenergic response to, or release of norepinephrine by, cholinomimetic agents (FERRY, 1966) could be explained by the excitation of intrinsic extra- as well as intraganglionic cells effected through stimulation of their preganglionic fibers which, in the absence of any evidence to the contrary, must be assumed to be entirely cholinergic. This ganglionic type of cholinergic link in peripheral adrenergic innervation offers an alternative to the nonganglionic terminal link proposed in the Burn-Rand hypothesis (BURN and RAND, 1965). It is obvious that a search for morphologic evidence for the existence of a ganglionic type cholinergic link in organs other than the pelvic organs must take into consideration the extraganglionic epineural, paravascular, and terminal adrenergic cell bodies, as well as intrinsic adrenergic ganglia.

## References

BURN, J. H., and M. J. RAND: Acetylcholine in adrenergic transmission. Ann. Rev. Pharmacol. **5**, 163—182 (1965).

CHESHER, G. B., and R. H. THORP: The atropine-resistance of the response to intrinsic nerve stimulation of the guinea-pig bladder. Brit. J. Pharmacol. **25**, 288—294 (1965).

DAHLSTRÖM, A.: Observations on the accumulation of noradrenaline in the proximal and distal parts of peripheral adrenergic nerves after compression. J. Anat. (Lond.) **99**, 677—689 (1965).

EL-BADAWI, A.: Histochemical studies of the organization of the peripheral autonomic nervous system. Fed. Proc. **26**, 794 (1967).

—, and E. A. SCHENK: Dual innervation of the mammalian urinary bladder. A histochemical study of the distribution of cholinergic and adrenergic nerves. Amer. J. Anat. **119**, 405—428 (1966).

— — The distribution of cholinergic and adrenergic nerves in the mammalian epididymis. A comparative histochemical study. Amer. J. Anat. **121**, 1—14 (1967a).

— — Histochemical methods for the separate, consecutive and simultaneous demonstration of acetylcholinesterase and norepinephrine in cryostat sections. J. Histochem. Cytochem. **15**, 580—588 (1967b).

— — A new theory of the innervation of the urinary bladder musculature. I. Morphology of intrinsic vesical innervation apparatus. J. Urol. (Baltimore). (In press) (1967c).

ERÄNKÖ, O., and M. HÄRKÖNEN: Histochemical demonstration of fluorogenic amines in the cytoplasm of sympathetic ganglion cells of the rat. Acta physiol. scand. **58**, 285—286 (1963).

FALCK, B.: Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. Acta physiol. scand. **56**, Suppl. 197, 1—25 (1962).

—, C. OWMAN, and N. O. SJÖSTRAND: Peripherally located adrenergic neurons innervating the vas deferens and the seminal vesicle of the guinea pig. Experientia (Basel) **21**, 98—100 (1965).

FERRY, C. B.: The innervation of the vas deferens of the guinea-pig. J. Physiol. (Lond.) **166**, 16 P (1963a).
— The post-ganglionic fibers of the vas deferens of the guinea-pig. J. Physiol. (Lond.) **169**, 72 P (1963b).
— Cholinergic link hypothesis in adrenergic neuroeffector transmission. Physiol. Rev. **46**, 420—456 (1966).
HAMBERGER, B., and K.-A. NORBERG: Adrenergic synaptic terminals and nerve cells in bladder ganglia of the cat. Int. J. Neuropharmacol. **4**, 41—45 (1965a).
— — Studies on some systems of adrenergic synaptic terminals in the abdominal ganglia of the cat. Acta physiol. scand. **65**, 235—242 (1965b).
KURIYAMA, H.: Electrophysiological observations on the motor innervation of the smooth muscle cells in the guinea-pig vas deferens. J. Physiol. (Lond.) **169**, 213—228 (1963).
LANGLEY, J. N., and H. K. ANDERSON: The innervation of pelvic and adjoining viscera. V. Position of the nerve cells on the course of the efferent nerve fibers. J. Physiol. (Lond.) **18**, 131—139 (1895).
MALMFORS, T.: Evidence of adrenergic neurons with synaptic terminals in the retina of rats demonstrated with fluorescence and electron microscopy. Acta physiol. scand. **58**, 99—100 (1963).
NORBERG, K.-A., and B. HAMBERGER: The sympathetic adrenergic neuron. Acta physiol. scand. **63**, Suppl. 238, 1—42 (1964).
OWMAN, C., and N.-O. SJÖBERG: Adrenergic nerves in the female genital tract of the rabbit with remarks on cholinesterase-containing structures. Z. Zellforsch. **74**, 182—197 (1966).
—, and N. O. SJÖSTRAND: Short adrenergic neurons and catecholamine-containing cells in vas deferens and accessory male genital glands of different mammals. Z. Zellforsch. **66**, 300—320 (1965).
— — On short adrenergic neurons in the accessory male genital organs of the bull. Experientia (Basel) **22**, 759—761 (1966).
SIGG, E. B., and T. D. SIGG: Sympathetic stimulation and blockade of the urinary bladder in cat. Int. J. Neuropharmacol. **3**, 241—251 (1964).
SJÖSTRAND, N. O.: Inhibition by ganglionic blocking agents of the motor response of the isolated guinea-pig vas deferens to hypogastric nerve stimulation. Acta physiol. scand. **54**, 306—315 (1962a).
— Effect of reserpine and hypogastric denervation on the noradrenaline content of the vas deferens and the seminal vesicle of the guinea pig. Acta physiol. scand. **56**, 376-380 (1962b).
— The adrenergic innervation of the vas deferens and the accessory male genital glands. Acta physiol. scand. **65**, Suppl. 257, 1—82 (1965).
VARAGIĆ, V.: An isolated rabbit hypogastric nerve preparation with observations on the hypogastric transmitter. J. Physiol. (Lond.) **132**, 92—99 (1956).

Dr. A. EL-BADAWI
Dr. ERIC A. SCHENK
Dept. of Pathology, University of Rochester
School of Medicine and Dentistry
260 Crittenden Boulevard
Rochester, New York 14620, U.S.A.