

LOCALIZATION OF SUBSTANCE P-IMMUNOREACTIVITY IN THE DEVELOPING HUMAN URINARY BLADDER

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Development of substance P-containing nerve fibers was studied in eight human fetal urinary bladders obtained from fetuses of 12 to 26 weeks gestation using monoclonal antibodies against substance P. At 12 weeks occasional fibers were seen in the trigonal submucosa and bladder wall while fine fiber plexuses, in fewer amount, were present along the blood vessels entering the bladder. Increasing proportion of thick substance P fibers were seen in 15, 19 and 26 week trigonal submucosal blood vessels and muscle layers. Occasional immunopositive fibers were also observed to enter the epithelium.

Relatively little study has been devoted to innervation of the developing human urinary bladder. Ultrastructural observations of Hoyes, Ramus and Martin (6) on the innervation of vesical musculature in early human fetuses suggested a predominant sympathetic supply on the basis of simultaneous occurrence of both small and large dense cored vesicles in the axonal varicosities in large proportion. Our previous studies (11, 12) in fetal human urinary bladder, using silver impregnation and acetylcholinesterase localization have demonstrated the presence of submucosal nerves between 11 and 13 weeks of gestation. The acetylcholinesterase positive nerves in the submucosa of adult mammalian bladders (2) have been said to be sensory in nature and are associated with profiles having 40–50 nm agranular and large 100 nm granulated vesicles. In our studies (12) cholinergic terminal plexuses on muscle fibers and adrenergic nerves as demonstrated by histochemical methods became apparent subsequently and the latter nerves were relatively less in proportion. Ultrastructurally nerve profiles with predominant granulated vesicles, clear vesicles and mixed vesicle types were seen. Since there is increasing evidence that substance P is a sensory neurotransmitter conveying noxious afferent impulses to the spinal cord from the soma and visceral organs and is involved in certain slow reflexes in the neonatal spinal cord (8, 9), the present study was undertaken to localize substance P in the developing human bladder.

MATERIAL AND METHODS

Eight human fetuses (4×2) of 12, 15, 19 and 26 weeks gestation (CRL 55 mm to

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215 mm) were obtained by hysterectomy and autopsy with prior consent from the Department of Gynaecology and Obstetrics of our hospital. The age of the fetuses was assessed by crown rump length measurement (3). The urinary bladders were immediately dissected, split open and immersion fixed in cold Zamboni's fixative composed of 4% paraformaldehyde, 0.05% glutaraldehyde and 0.2% picric acid in 0.1 M sodium phosphate buffer (pH 7.4). The time period elapsed between fetal death and histological processing ranged from 30 to 45 min in the case of hysterectomy and 90 min in the case of autopsy specimens. The content of substance P in postmortem animal and human material and its immunohistochemical appearance has been found not to be affected by autopsy delay nor mode of fixation (4).

The tissues were fixed overnight at 4°C, washed in 0.1 M phosphate buffer and subsequently stored in 0.1 M phosphate buffer with 30% sucrose. After freezing in liquid nitrogen and subsequent thawing in 0.1 M phosphate buffer, cryostat sections of 30–40 μm were cut and washed with 0.1 M phosphate buffer saline (PBS) containing 0.1% Triton-X for one hr and treated for 10 min with 1:9 mixture of 3% H_2O_2 in methanol. Incubation of sections for immunocytochemistry was carried out as follows: 45 min in 10% normal rabbit serum, 15 min wash in PBS with 0.1% Triton-X, overnight at 4°C in monoclonal rat-mouse hybrid antisubstance P IgG fraction

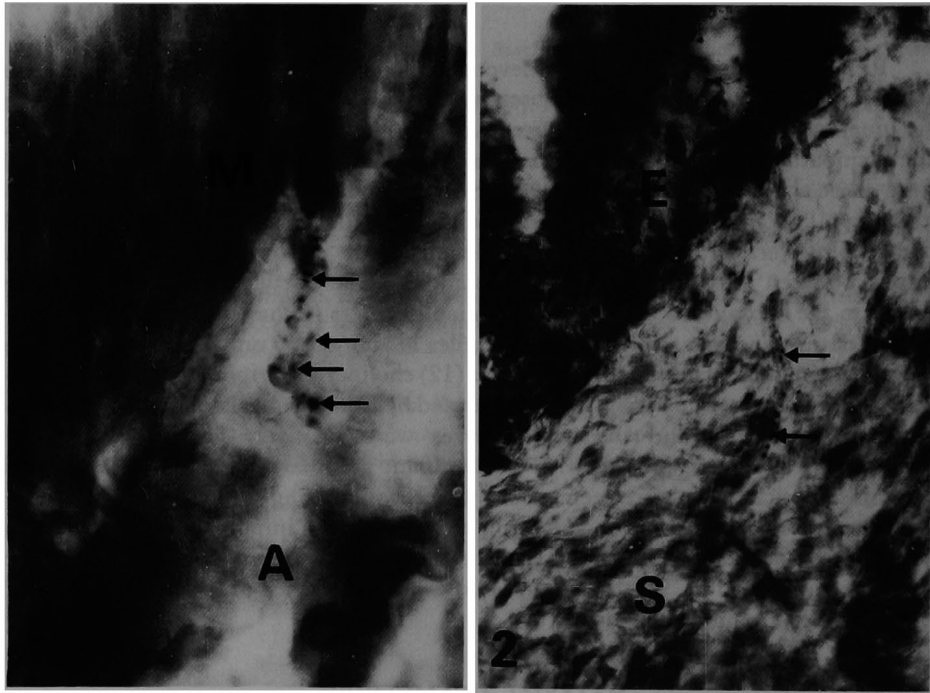


FIG. 1. Varicose substance P-immunoreactive fibers (\uparrow) on a trigonal adventitial blood vessel at 12 weeks. M: muscle, A: adventitia. $\times 310$

FIG. 2. Immunopositive fine varicose fiber (\uparrow) on a submucosal vessel of trigonal region at 12 weeks. E: epithelium, S: submucosa. $\times 310$

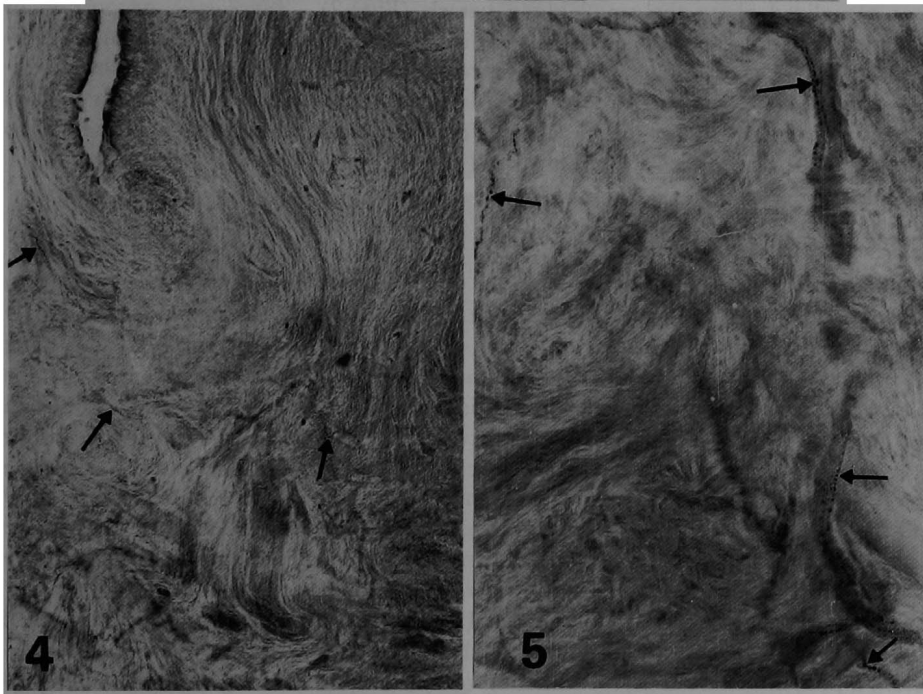
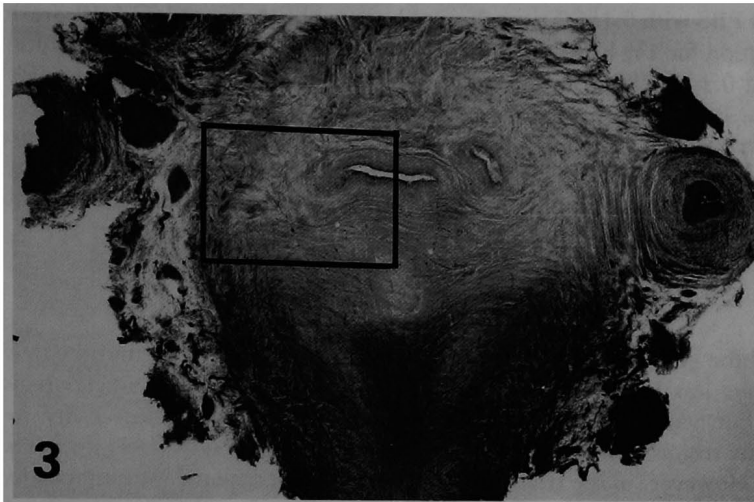


FIG. 3. Low power photomicrograph of an immunostained tangential section through the trigone of the bladder at 19 weeks. $\times 310$

FIG. 4. Photomicrograph of the region demarcated in Fig. 3 at a higher magnification showing substance P fibers (\uparrow) in the submucosal and muscular layers. $\times 75$

FIG. 5. High power photomicrograph from an area in Fig. 4 showing the varicose (black) immunostained substance P fibers (\uparrow) along the blood vessels in the trigonal musculature. $\times 310$

clone NCL/34 HL (Seralab, U.K.) diluted to 1:100, wash for one hr with several changes of PBS with 0.1% Triton-X, one hr in rabbit Anti-rat IgG 1:10, wash for one hr, incubation for 1½ hr in monoclonal rat PAP at 1:30 dilution. All dilutions were made with 0.1 M PBS with 0.1% Triton-X. The above procedure except for antistubstance P incubation was carried out at room temperature. Peroxidase activity was localized with 0.06% 3-3' diaminobenzidine HCl in 0.01% H₂O₂. Sections were then washed in 0.1 M phosphate buffer (pH 7.4) and treated with 2% osmium tetroxide dissolved in phosphate buffer for half an hour and then dehydrated and mounted in araldite. Control sections were not treated with antistubstance P.

RESULTS

In all the gestational age periods studied the substance P-immunoreactive fibers were always less in the detrusor muscle as compared to the trigonal region. At 12 weeks thin varicose fiber plexuses with substance P-immunoreactivity were seen entering the trigonal bladder wall along the adventitial blood vessels, in fair amounts (Fig. 1). However, in the trigonal submucosa only occasional immunopositive fibers were visualized along the small blood vessels (Fig. 2). Increasing proportion of substance P fibers were present at 15, 19 (Figs. 3-5) and 26 weeks (Figs. 6-9) of gestation. At 26 weeks, the fibers were seen to traverse along the blood vessels in the adventitia, muscular layers and submucosa. Isolated individual immunopositive fibers were found running on the muscle fibers (Fig. 8). Occasional fibers were observed to enter the epithelium from the submucosa (Fig. 9).

DISCUSSION

The study demonstrates the presence of substance P immunoreactive nerves in the developing human urinary bladder as early as 12 weeks of gestation with a subsequent increase in proportion and intensity of immunopositive nerves with increasing gestational age. The localization of substance P in the submucosal nerves at the age of 12 weeks confirms our previous observation of early appearance of sensory nerves in the bladder. There is considerable evidence in support of substance P being a sensory neurotransmitter in the peripheral somatic or visceral afferent nerves (8, 9). In the present study, isolated occasional fibers were demonstrable on the muscle fibers at 19 and 26 weeks of gestation. It has been suggested that substance P may be responsible for exerting a contractant effect on the urinary bladder (7). However, its excitatory contractile function in rabbit, guinea pig and rat (10) has not found much support.

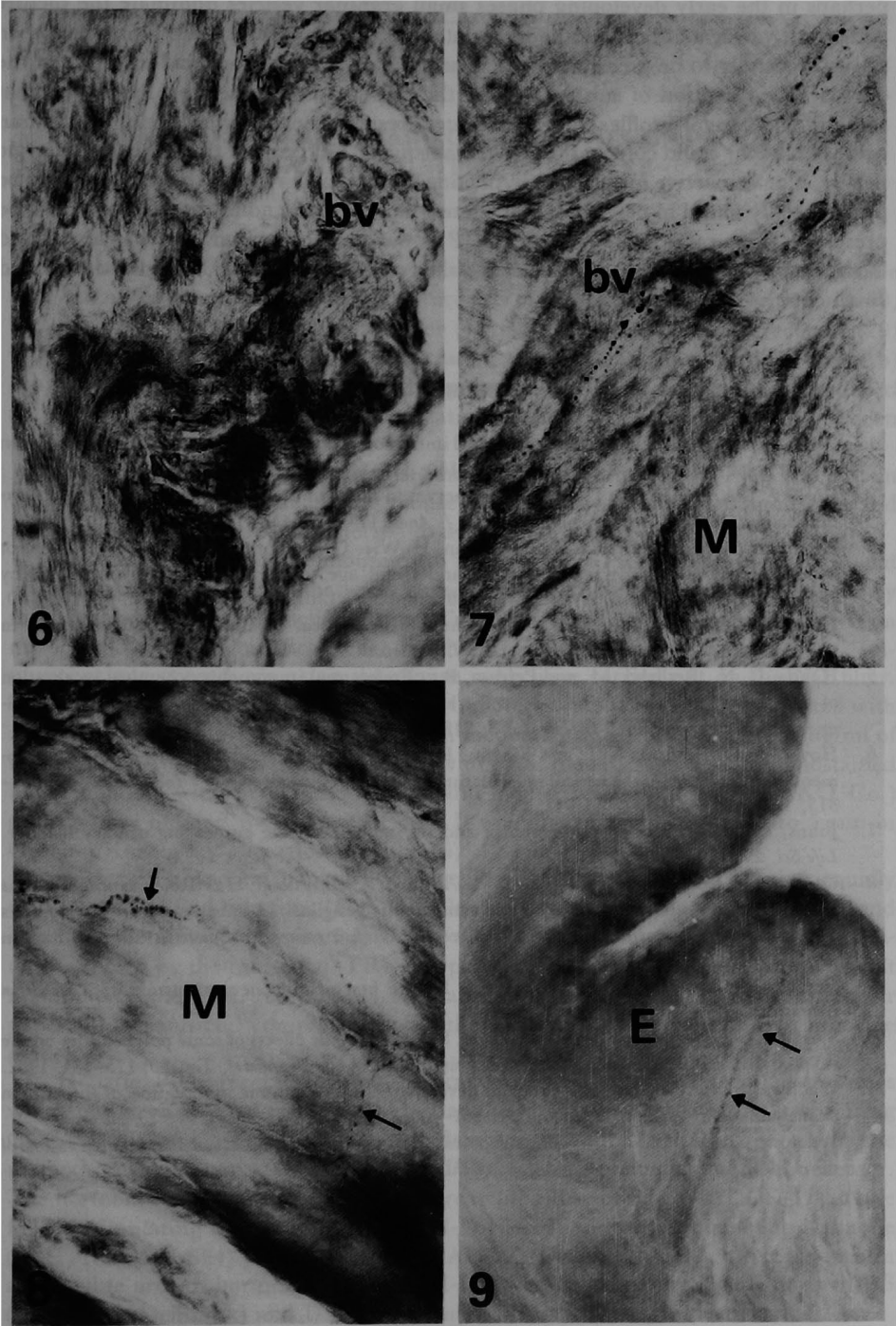
The nerve profiles in early human fetal bladders both in our study (12) and that of Hoyes *et al.* (6) show dense granular vesicles in large proportion. Hoyes *et al.* suggested a predominant adrenergic function on this basis. Although various other profiles were occasionally seen in our electron microscopic observations it was felt that

FIG. 6. Substance P immunoreactive (black) fibers along an adventitial blood vessel (bv) at 26 weeks. ×310

FIG. 7. Immunopositive substance P fibers along the blood vessel (bv) in the trigonal muscle (M) layer at 26 weeks. ×310

FIG. 8. Isolated immunoreactive fiber on the muscle (M) at 26 weeks. ×310

FIG. 9. A fine fiber along a blood vessel entering the epithelium (E) from submucosa. ×310



perhaps in the early developing phases small granular vesicles dominate the picture with adult variegated profiles appearing at a later phase. Substance P has been seen ultrastructurally to be present in the dense core granules in the small intestine of the cat (1). Localization of substance P in the present study, therefore, indicates that some of the axonal profiles with granulated vesicles seen in the developing human urinary bladder could convey this neurotransmitter. It is likely that other peptidergic nerves, e.g. vasoactive intestinal polypeptide (VIP) which recently has been seen to be preferentially found in pelvic visceral afferent nerves of the adult cat (5), could also be present in these early fetal bladders. VIP in the adult cat spinal cord was also found in the dense core vesicles within axonal enlargements containing both dense core and small clear round vesicles.

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