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Immunohistochemical study of the corpora cavernosa of the human clitoris

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ABSTRACT

The microarchitecture of the corpora cavernosa of the human clitoris was investigated by immunohistochemistry. The distribution pattern of the nerve network was demonstrated by S-100 and neuron specific enolase immunoreactivity. Vascular and nonvascular muscle cells were identified by desmin and/or vimentin expression, and fibroblasts and endothelial cells by vimentin immunoreactivity. The findings show that tissue organisation in the corpora cavernosa of the clitoris is essentially similar to that of the penis except for the absence of a subalbugineal layer interposed between the tunica albuginea and erectile tissue. This has functional implications, suggesting that the clitoral erection cycle differs from that of the penis.

Key words: Clitoris; corpora cavernosa.

INTRODUCTION

Although it is accepted that the human clitoris plays a functional role during sexual arousal (Gillan & Brindley, 1979; Levin, 1980; Ottesen, 1983; Ottesen et al. 1987), its structure is still not completely known (Williams-Ashman, 1990; Williams-Ashman & Reddi, 1991). A detailed histological description of corpora cavernosa of the human clitoris has not been performed, probably due to its close structural resemblance to the penis (Blandau, 1983; Sevely, 1988; Williams-Ashman & Reddi, 1991) or to the fact that it has been considered a rudimentary organ without apparent function (Stefani et al. 1988). As the erectile tissue of the clitoris becomes engorged with blood and tumescent during sexual excitement (Levin, 1980; Williams-Ashman & Reddi, 1991), we studied the tissue architecture and cell types that are responsible. The expression of desmin, a marker for myogenic cells (Lazarides, 1982; Steinert & Roop, 1988), vimentin, a marker for cells of mesenchymal origin (Lazarides, 1982; Steinert & Roop, 1988), neuron specific enolase (NSE), a marker for neurons (Marangos et al. 1982) and S-100 protein, a marker for glial cells and certain nonneural cell types (Cocchia, 1981; Cocchia & Michetti, 1982; Donato, 1991), has been investigated by immunohistochemistry at the light microscope level.

MATERIALS AND METHODS

Specimens of the human clitoris were obtained during surgery from 2 women, aged 50 and 55 y, operated for squamous cell carcinoma of the glans clitoris. Tissue specimens were also taken at autopsy from 3 women aged between 40 and 55 y.

Tissue samples were fixed, immediately after removal, in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 12 h. After rinsing in phosphate-buffered saline (PBS), the samples were dehydrated in ethanol and embedded in paraffin wax via xylene. The light microscope study was carried out on 5 μm transverse sections taken at different levels from the proximal to distal ends of the corpora cavernosa. Serial sections from each specimen were stained with haematoxylin and eosin or processed for immunohistochemistry using the biotin-streptavidin method (Amersham, UK). Endogenous peroxidase activity was quenched with 0.5% H_2O_2 in absolute methanol for 30 min. Nonspecific binding was blocked with 1%

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bovine serum albumin (BSA) (Sigma) in PBS for 30 min at room temperature. Polyclonal antibodies against NSE and S-100 protein and monoclonal antibodies against desmin and vimentin were obtained commercially from Dakopatts a/s, Denmark. Sections were then incubated overnight at 4 °C with primary antibodies diluted in PBS containing 1% BSA as follows: S-100 antiserum 1:5000, NSE antiserum 1:2000, antidesmin 1:200 and antivimentin 1:100. Rabbit and mouse immunoglobulins at the same dilutions as the primary antibodies were used as controls. After washing with PBS, biotinylated secondary antibodies diluted 1:100 were applied to slides for 1 h at room temperature. The slides were washed again with PBS then incubated with biotin-streptavidin-peroxidase-complex diluted 1:400 for 1 h at room temperature and, after rinsing with PBS, developed with 3,3′diaminobenzidine tetrahydrochloride (Sigma) for 5 min at room temperature. The specificity of the antibodies was tested and confirmed by immunoreactivity on other kinds of tissues. Sections were examined under a light microscope (Zeiss, Axiophot).

RESULTS

Sections from each specimen stained with haematoxylin and eosin revealed no pathological changes and exhibited a similar tissue architecture. Each clitoris consisted of 2 corpora cavernosa composed of erectile tissue separated by a connective tissue septum and surrounded by a fibrous tunica albuginea (Fig. 1). The erectile tissue was composed of fibromuscular trabeculae pervaded by irregular communicating sinusoids. Bundles of nerve fibres were evident superficial to the tunica albuginea. Vessels of differing calibre were present deep and superficial to the tunica albuginea, in the septum and in the erectile tissue. The subalbugineal connective tissue layer, with a rich venous plexus, the presence of which between the tunica albuginea and the erectile tissue has been described in the penis (Fournier et al. 1987), was not observed in the clitoris (Fig. 2).

Muscle cells in the trabeculae were identified by their desmin expression. These muscle cells showed two orders of orientation with respect to the long axis of the clitoris. Muscle cells located within the trabeculae were tightly packed and oriented in a transverse plane, while those surrounding the sinusoids were disposed longitudinally and appeared to be more numerous than the transverse ones (Fig. 3). No desmin-positive muscle cells were observed in the septum or tunica albuginea.

Muscle cells around blood vessels were characterised by the presence of desmin and/or vimentin immunoreactivity. In the tunica media of all arteries,

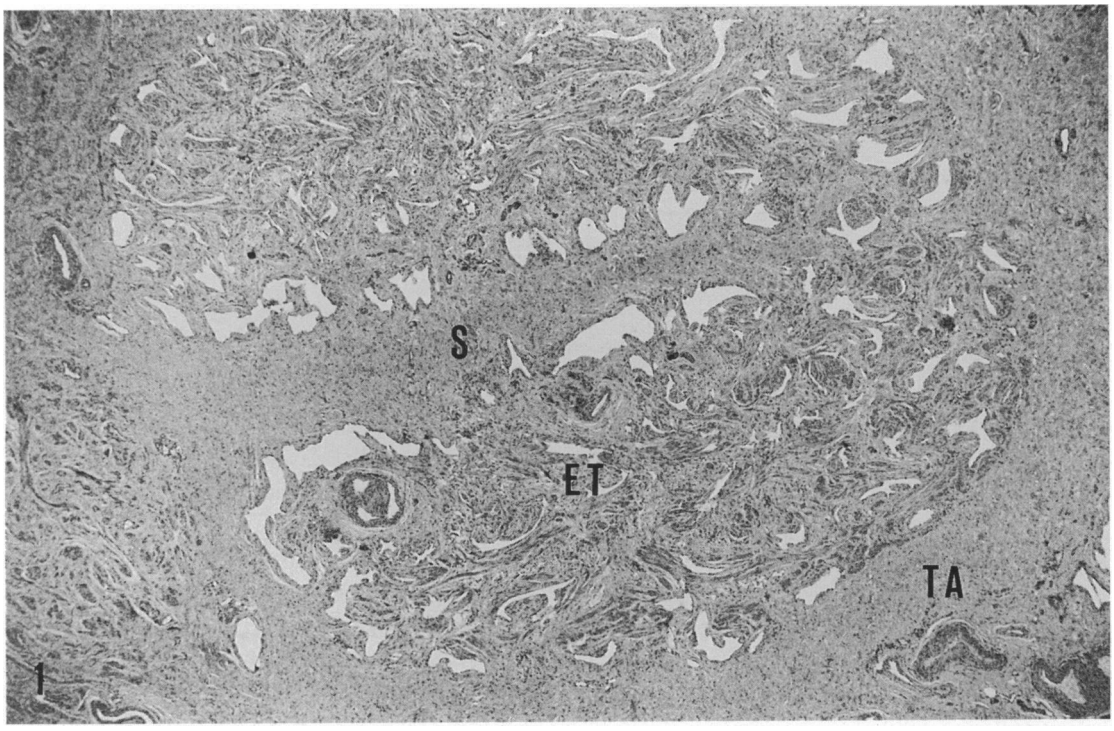

Fig. 1. Transverse section of clitoris. TA, tunica albuginea; ET, erectile tissue; S, septum. Haematoxylin and eosin, (×30).

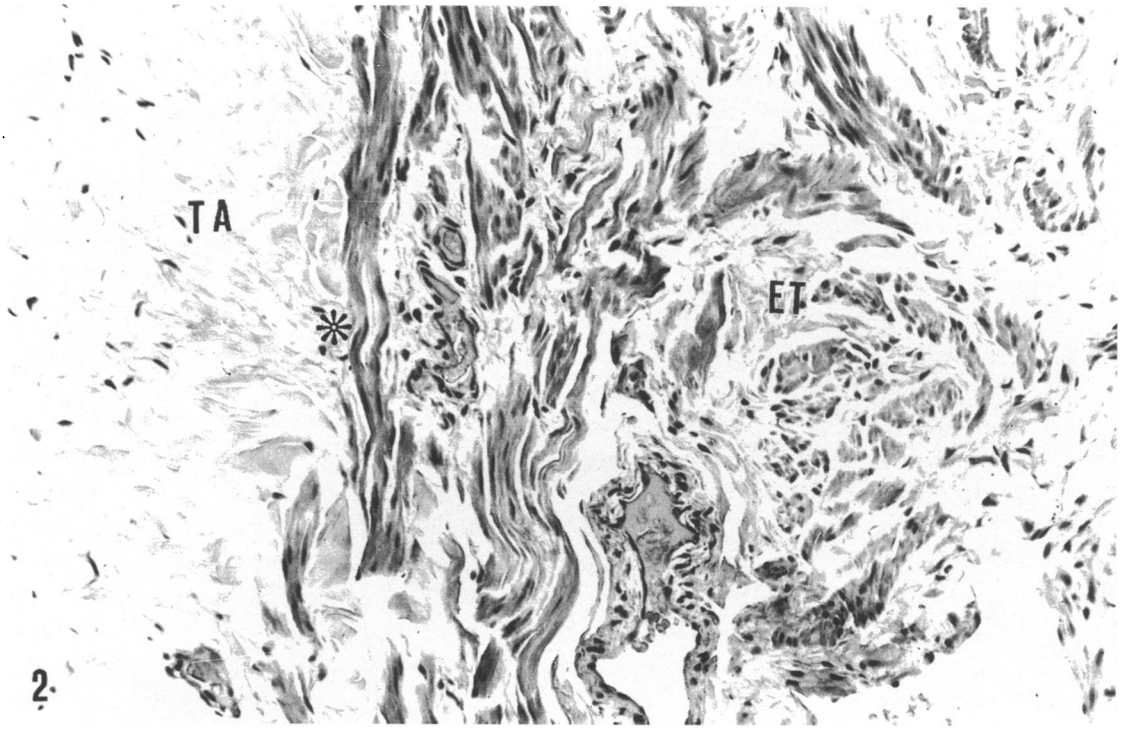

Fig. 2. Corpus cavernosum of clitoris. Note absence of the subalbugineal loose connective layer (asterisk) between the erectile tissue (ET) and the tunica albuginea (TA). Haematoxylin and eosin, (× 230).

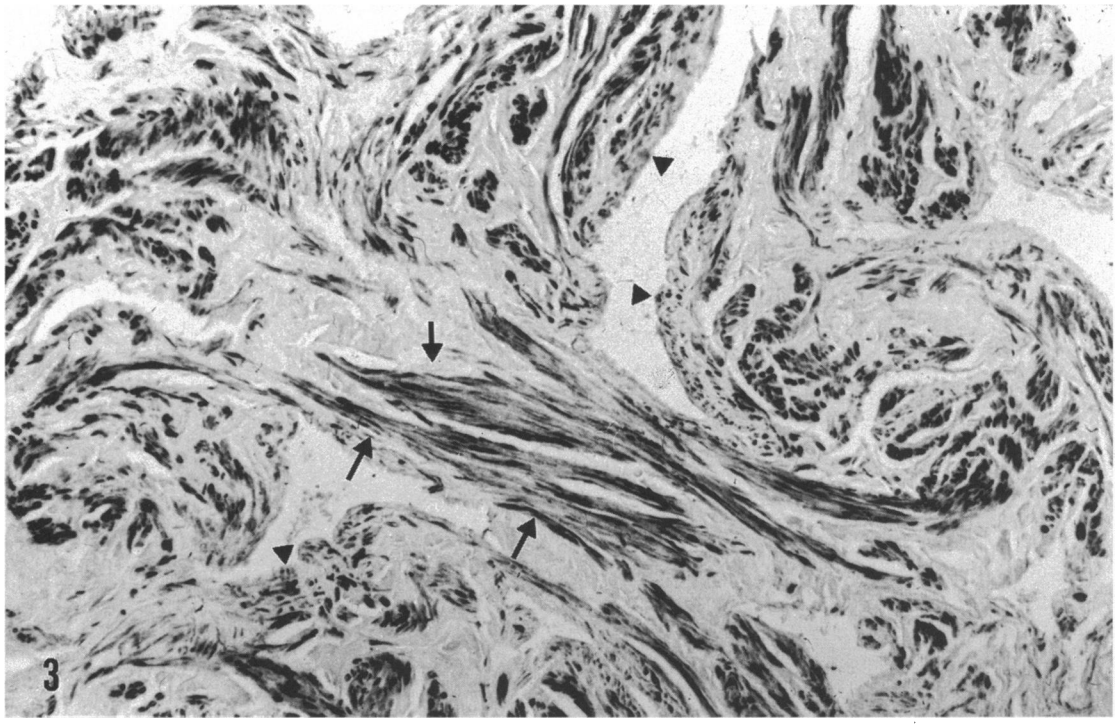

Fig. 3. Trabecular muscle cells showing desmin immunoreactivity are oriented both transversely (arrows) and longitudinally (arrowheads). (× 140).

almost all muscle cells appeared to display vimentin immunolabelling (Fig. 4), and the number of desmin-positive muscle cells appeared clearly related to vessel diameter. The amount of cells labelled by desmin immunoreactivity was high in large arteries and decreased gradually until disappearing in the smallest

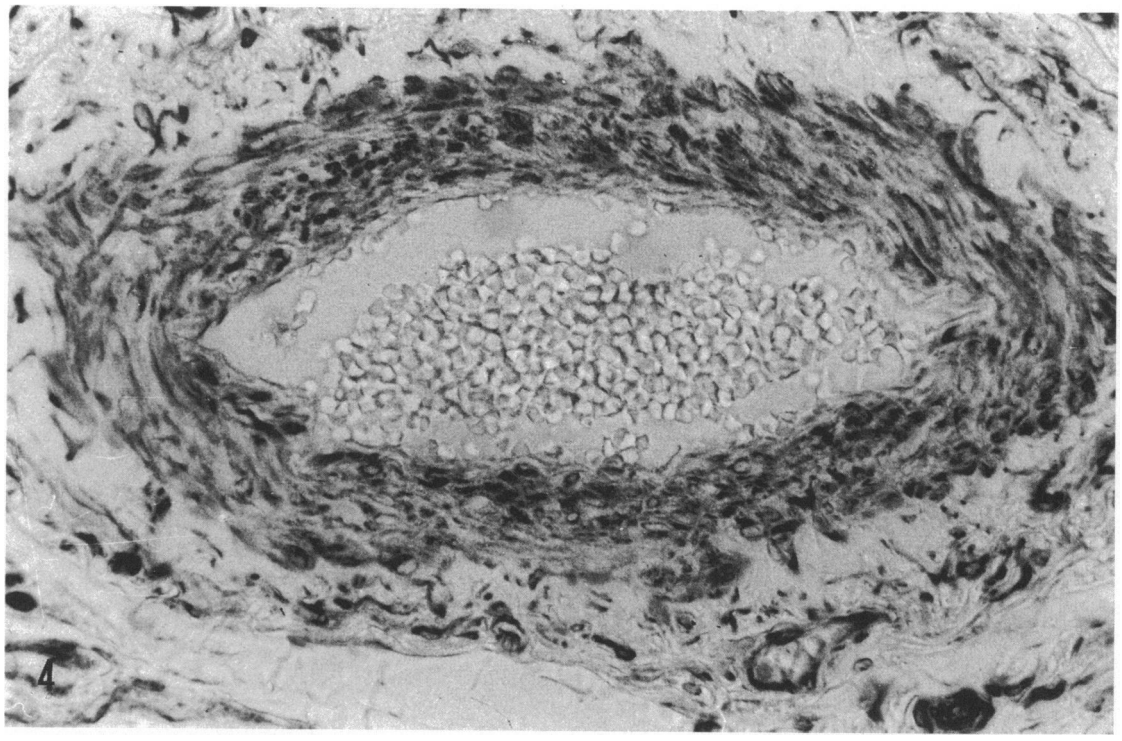

Fig. 4. Muscle cells of a large artery showing vimentin immunoreactivity. Cell types of mesenchymal origin located around the artery are also vimentin positive. (× 460).

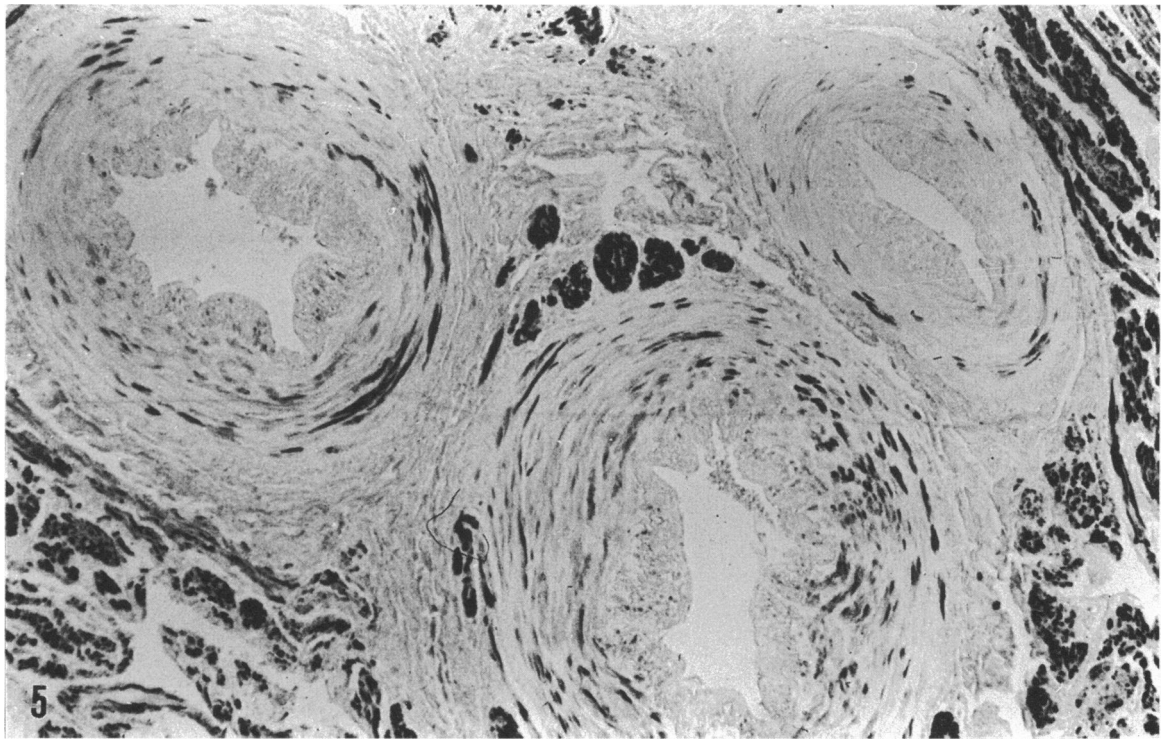

Fig. 5. Muscle cells of arteries of differing calibre showing desmin immunoreactivity. (× 100).

ones (Fig. 5). All muscle cells of veins exhibited desmin (Fig. 6) but not vimentin immunoreactivity, regardless of vessel diameter. Fibroblasts and endothelial cells lining vessels and sinusoids were labelled exclusively by vimentin immunostaining.

The topographic distribution of the nerve network

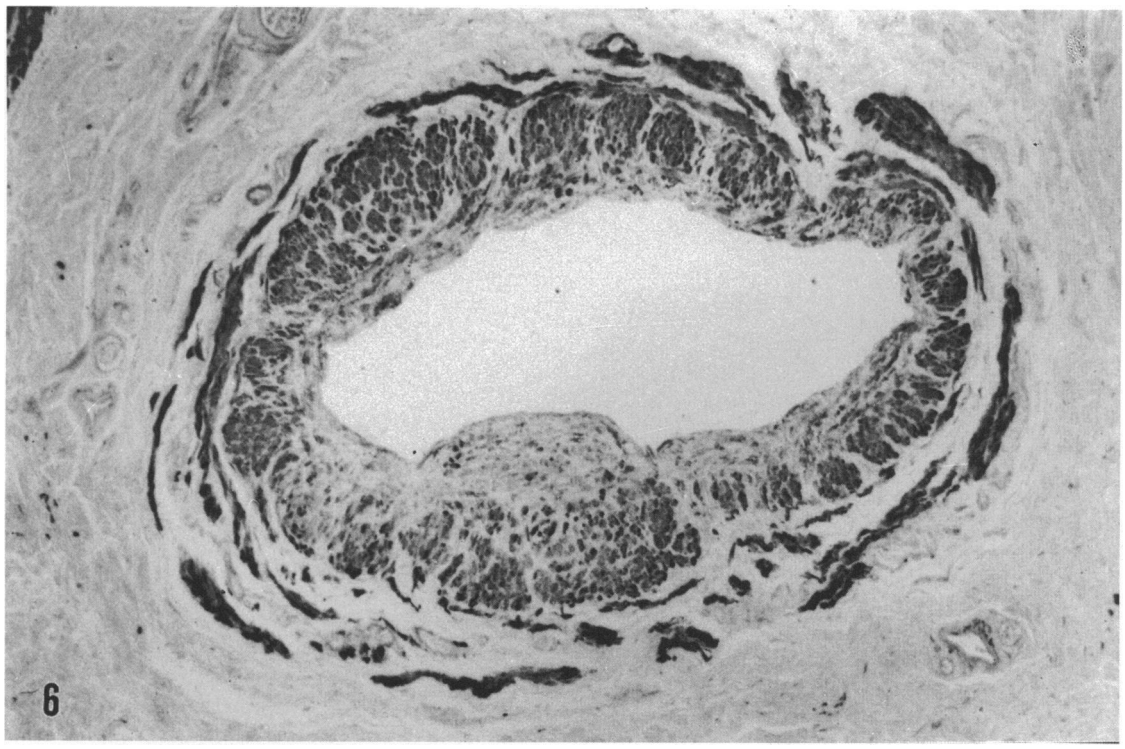

Fig. 6. Muscle cells of a large vein, arranged in transverse and longitudinal layers, expressing desmin immunoreactivity. (× 230).

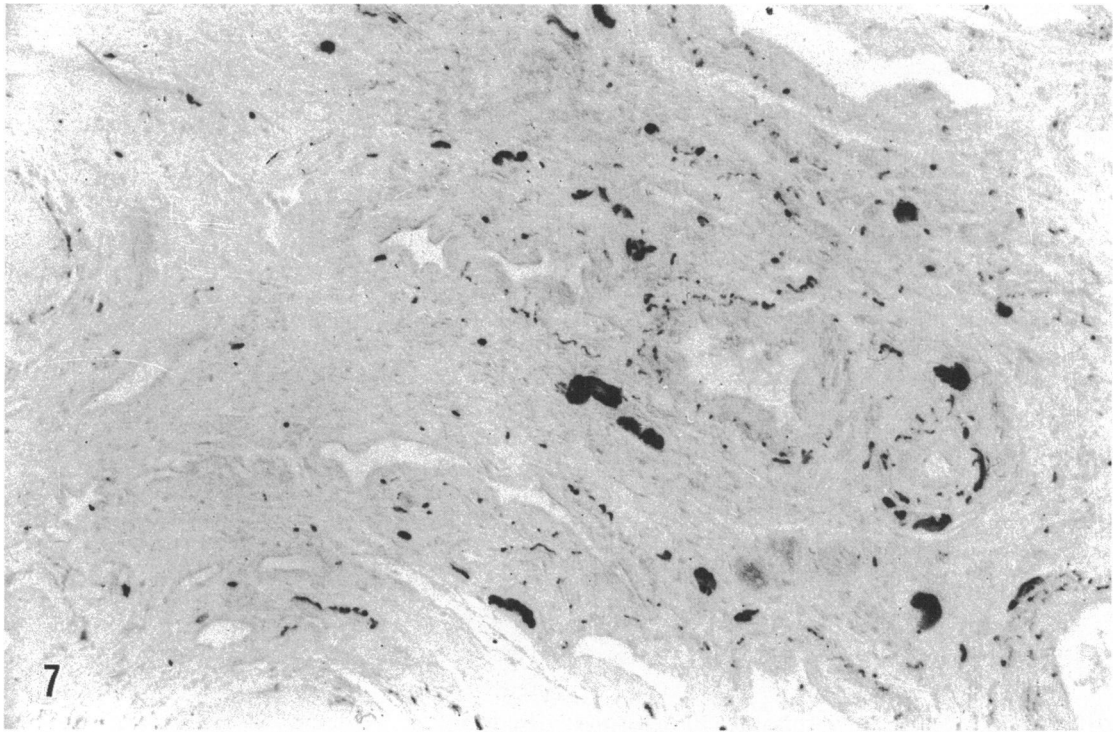

Fig. 7. Bundles of S-100 immunoreactive nerve fibres are scattered throughout the erectile tissue. (× 140).

supplying the clitoris corpora cavernosa was detected both by S-100 and NSE immunoreactivity (Figs 7, 8). The labelling was confined to nerve structures: S-100 was present in Schwann cells, whereas NSE was found in axons. Many thick nerve bundles were observed superficial to the tunica albuginea, whereas deep to

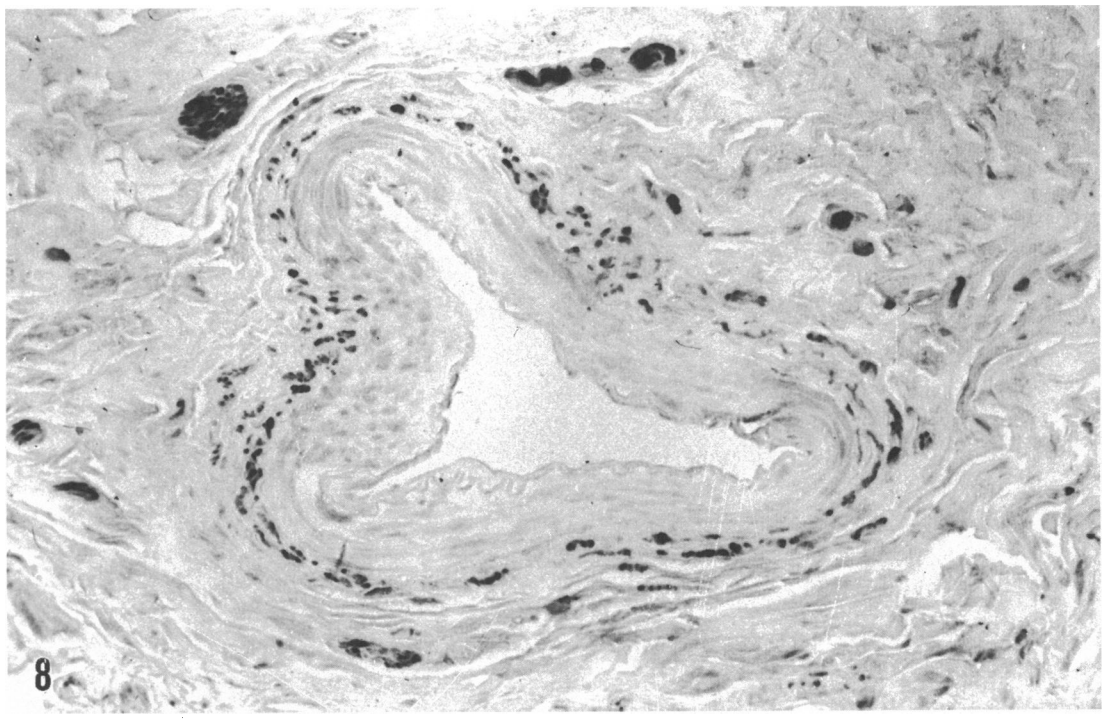

Fig. 8. Erectile tissue of clitoris. NSE immunoreactive nerve fibres are present in the tunica adventitia of an artery and throughout the erectile tissue. (× 300).

the tunica albuginea and the septum few and thin nerve fibres were present. Several thin nerve fibres, singly and in small bundles, were found throughout the erectile tissue and appeared in close relation to muscle cells of the trabeculae. A rich nerve network was also present in and around the tunica adventitia of vessels, especially in those of larger diameter. Nerve fibres related to arteries seemed to outnumber those associated with veins.

The immunohistochemical analysis carried out with our panel of antibodies showed that the erectile tissue was closely adjacent to the tunica albuginea, confirming the absence of the subalbugineal venous plexus, as shown by sections stained with haematoxylin and eosin. No immunoreactivity was detectable in control sections treated with normal rabbit and mouse immunoglobulins instead of specific antibodies.

DISCUSSION

This study provides the first morphological description of the corpora cavernosa of the human clitoris based on immunohistochemistry. Our findings, besides confirming previous histological observations (Blandau, 1983), add new information about the characterisation and distribution of tissues and different cell types.

The longitudinal and transverse orientation of muscle cells of erectile tissue, better displayed by desmin immunoreactivity than by staining with haematoxylin and eosin, may be significant in mediating the blood flow into sinusoids during tumescence and detumescence. Our hypothesis is supported by the previously demonstrated role of trabecular muscle cells in modulating intracavernous blood flow during the erection cycle in the human penis (Goldstein & Padma-Nathan, 1990; Bosch et al. 1991; Saenz de Tejada et al. 1991).

The presence of desmin and vimentin immunoreactivity in arterial muscle cells in the clitoris is in agreement with that previously reported for arteries of other human tissues (Frank & Warren, 1981; Gabbiani et al. 1981; Osborn et al. 1981, 1987), whereas the finding that only desmin is expressed in muscle cells of veins is in contrast to previous observations on other tissues which indicated that veins display both desmin and vimentin immunoreactivity (Gabbiani et al. 1981; Osborn et al. 1981). In addition, we demonstrated that in the arteries the number of desmin-positive muscle cells gradually decreases and finally disappears as vessel diameter is reduced. This suggests that desmin could play different roles in muscle contractility during blood flow regulation at different levels along vessels.

The present study has shown the presence of a rich

network of nerve bundles and terminal branches as detected by immunoreactivity for NSE and S-100 in relation to the corpora cavernosa of the clitoris. Previous studies, based on VIP (Lundberg et al. 1980; Ottesen, 1983) and NPY (Cocchia et al. 1990) immunostaining, revealed a restricted distribution of nerve fibres in the clitoris, compared with that observed in our present investigation. This discrepancy raises the possibility that a number of clitoral nerve fibres are non-VIP and non-NPY immunoreactive.

The tissue architecture of the corpora cavernosa of the clitoris has classically been conceived as being similar to that of the penis (Sevely, 1988; Williams-Ashman & Reddi, 1991). Our findings, although generally confirming this view, reveal that an important difference consists in the absence in the clitoris of the subalbugineal layer between the erectile tissue and the tunica albuginea, the presence of which has been described in the penis and which posseses a rich venous plexus (Benoit et al. 1987; Fournier et al. 1987; Aboseif & Lue, 1988). In the male corpora cavernosa the systemic blood pressure expands the dilated sinusoids against the tunica albuginea; this compresses the subalbugineal plexus and reduces venous outflow, making the penis rigid (Benoit et al. 1987; Fournier et al. 1987; Aboseif & Lue, 1988). Thus, taking in mind the 4 haemodynamic events during the penile erection cycle (flaccidity, tumescence, rigidity, detumescence) (Tudoriu & Bourmer, 1983; Wespes & Schulman, 1986; Aboseif & Lue, 1988) and that the rigidity is due to the compression of the venous plexus, as described above, the absence of the venous plexus in the clitoris suggests that this organ achieves tumescence but not rigidity during sexual arousal. However, this aspect needs further investigation to establish whether the clitoris displays an erection cycle similar to that of the penis.

REFERENCES

Aboseif SR, Lue TF (1988) Hemodynamics of penile erection. *Urologic Clinics of North America* **15**, 1–7.

Benoit G, Delmas V, Gillot C, Jardin A (1987) The anatomy of erection. *Surgical and Radiologic Anatomy* **9**, 263–272.

Blandau RJ (1983) The female reproductive system. In *Histology*, 5th edn (ed. L. Weiss), p. 942. London, Basingstoke: Macmillan.

Bosch RJLH, Benard F, Aboseif SR, Stief CG, Lue TF, Tanagho EA (1991) Penile detumescence: characterization of three phases. *Journal of Urology* **146**, 867–871.

Cocchia D (1981) Immunocytochemical localization of S-100 protein in the brain of adult rat. An ultrastructural study. *Cell and Tissue Research* **214**, 529–540.

Cocchia D, Michetti F (1982) The S-100 antigen in non-neural tissues. In *Protides of the Biological Fluids*, vol. 30 (ed. H. Peeters), pp. 43–46. Oxford: Pergamon Press.

Cocchia D, Rende M., Toesca A, Viola R, Stolfi VM (1990) Immunohistochemical study of neuropeptide Y-containing nerve fibers in the human clitoris and penis. *Cell Biology International Reports* **14**, 865–875.

Donato R (1991) Perspectives in S-100 protein biology. *Cell Calcium* **12**, 713–726.

Fournier Jr GR, Juenemann K-P, Lue TF, Tanagho EA (1987) Mechanisms of venous occulusion during canine penile erection: an anatomic demonstration. *Journal of Urology* **137**, 163–167.

Frank ED, Warren L (1981) Aortic smooth muscle cells contain vimentin instead of desmin. *Proceedings of the National Academy of Science of the USA* **78**, 3020–3024.

Gabbiani G, Schmid E, Winter S, Chaponnier C, de Chastonay C, Vandekerckhove J, Weber K, et al. (1981) Vascular smooth muscle cells differ from other smooth muscle cells: predominance of vimentin filaments and a specific a-type actin. *Proceedings of the National Academy of Science of the USA* **78**, 298–302.

Gillan P, Brindley GS (1979) Vaginal and pelvic floor responses to sexual stimulation. *Psychophysiology* **16**, 471–481.

Goldstein AMB, Padma-Nathan H (1990) The microarchitecture of the intracavernosal smooth muscle and the cavernosal fibrous skeleton. *Journal of Urology* **144**, 1144–1146.

Lazarides E (1982) Intermediate filaments: a chemically heterogeneous, developmentally regulated class of proteins. *Annual Review of Biochemistry* **51**, 219–250.

Levin RJ (1980) The physiology of sexual function in women. *Clinical Obstetrics and Gynecology* **7**, 213–252.

Lundberg JM, Hökfelt T, Änggård A, Unväs-Wallensten K, Brimijoin S, Brodin E et al. (1980) Peripheral peptide neurons: distribution, axonal transport and some aspects on possible function. In *Neural Peptides and Neuronal Communication* (ed E. Costa & M. Trabucchi), pp. 25–35. New York: Raven Press.

Marangos PJ, Polak JM, Pearse AGE (1982) Neuron specific enolase, a probe for peptide-secreting cells of the diffuse neuroendocrine systems. In *Systemic Role of Regulatory Peptides* (ed. S. R. Bloom, J. M. Polak & E. Lindenlaub), pp. 447–473. Stuttgart and New York: F. K. Schattauer Verlag.

Osborn M, Caselitz J, Weber K (1981) Heterogeneity of intermediate filament expression in vascular smooth muscle: a gradient in desmin positive cells from the rat aortic arch to the level of the arteria iliaca communis. *Differentiation* **20**, 196–202.

Osborn M, Caselitz J, Püschel K, Weber K (1987) Intermediate filament expression in human vascular smooth muscle and in arteriosclerotic plaques. *Virchows Archiv A (Pathological Anatomy and Histopathology)* **411**, 449–458.

Ottesen B (1983). Vasoactive intestinal polypeptide as a neurotransmitter in the female genital tract. *American Journal of Obstetrics and Gynecology* **147**, 208–224.

Ottesen B, Pedersen B, Nielsen J, Dalgaard D, Wagner G, Fahrenkrug J (1987) Vasoactive intestinal polypeptide (VIP) provokes vaginal lubrification in normal women. *Peptides* **8**, 797–800.

Saenz de Tejada I, Maroukian P, Tessier J, Kim JJ, Goldstein I, Frohrib D (1991) Trabecular smooth muscle modulates the capacitor function of the penis. Studies on a rabbit model. *American Journal of Physiology* **260**, H1590–H1595.

Sevely JL (1988) The male clitoris. *American Journal of Obstetrics and Gynecology* **159**, 533–534.

Stefani R, Serra GP, Loffredo F, Spiga S (1988) Sulla struttura e sull'origine del glande clitorideo. *Archivo Italiano di Anatomia ed Embriologia* **93**, 277–295.

Steinert PM, Roop DR (1988) Molecular and cellular biology of intermediate filaments. *Annual Review of Biochemistry* **57**, 593–625.

Tudoriu T, Bourmer H (1983) The hemodynamics of erection at the level of the penis and its local deterioration. *Journal of Urology* **129**, 741–745.

WESPES E, SCHULMAN CC (1986) Role hémodynamique de l'albuginée dans l'erection. *Acta Urologica Belgica* **54**, 114–122.

WILLIAMS-ASHMAN HG (1990) Enigmatic features of penile development and functions. *Perspectives in Biology and Medicine* **33**, 335–374.

WILLIAMS-ASHMAN HG, REDDI AH (1991) Differentiation of mesenchymal tissues during phallic morphogenesis with emphasis on the os penis: roles of androgens and other regulatory agents. *Journal of Steroid Biochemistry and Molecular Biology* **39**, 873–881.