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Histology and fine structure of the muscularis mucosae of the human urinary bladder

J. S. DIXON AND J. A. GOSLING

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

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INTRODUCTION

In standard histological textbooks (Copenhaver, Kelly & Wood, 1978; Greep & Weiss, 1973; Ham & Cormack, 1979; Leeson & Leeson, 1981; Matthews & Martin, 1971; Reith & Ross, 1977; Windle, 1976) the mucosa of the urinary bladder is described as consisting of a layer of transitional epithelium and a supporting layer of loose fibro-elastic connective tissue, but no mention is made of a muscular is mucosae. Indeed, some texts state quite categorically that a muscularis mucosae is absent from the human urinary bladder (Cooper, 1948; Williams & Warwick, 1980). Current studies on the lower urinary tract have recently involved the examination of biopsies from a number of human urinary bladders, and, in most cases, a distinct muscularis mucosae was observed which was clearly separate from the detrusor muscle. The present paper describes some light and electron microscopic observations on the smooth muscle cells comprising this muscularis mucosae.

MATERIALS AND METHODS

Biopsies from the urinary bladder wall were obtained at cystoscopy in 25 patients (16 males, 9 females) being investigated for bladder instability. The age range of these patients was 22–69 years. In addition, tissue samples from four patients undergoing total cystectomy were also included in this study (age range 45–66 years). In each case, tissue was removed from the fundus, the anterior and lateral walls and the trigone, and was quickly processed either for light or electron microscopy. Biopsies showing any evidence of pathological change were excluded from this study.

Light microscopy

Each biopsy was quickly frozen in 2-methyl-butane cooled in liquid nitrogen and was subsequently transferred to a cryostat at −25 °C internal temperature. Serial sections 10–20 μm in thickness were prepared and either stained for routine histology (using Masson's trichrome technique) or processed to demonstrate tissue cholinesterases, using a modification of the method described by Gomori (1952).

Electron microscopy

Biopsies for electron microscopy were quickly cut into small (1 mm^3) pieces and placed in 2·5% glutaraldehyde, buffered at pH 7·3 with sodium cacodylate, for 2 hours at room temperature. Following a wash in buffer alone, the tissue was subsequently fixed in 1% osmium tetroxide in the same buffer for half an hour before

dehydration in ascending concentrations of ethyl alcohol and embedding in TAAB epoxy resin. Thin sections were cut, using a diamond knife and a Reichert OmU3 ultramicrotome, and mounted on uncoated copper grids. They were then double stained with alcoholic uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) prior to examination in a Philips EM300 electron microscope.

OBSERVATIONS

Light microscopy

Of the 100 biopsies examined 78 revealed a distinct muscularis mucosae, there being no difference in the frequency of its occurrence between the different regions of the bladder wall that were examined. The muscularis mucosae consisted of irregularly arranged bundles of smooth muscle cells lying approximately mid-way between the urothelium and the detrusor muscle (Fig. 1). Individual bundles varied considerably in diameter and formed a discontinuous layer which was frequently traversed by large blood vessels.

By use of a method to localise tissue cholinesterases, the muscle bundles of the muscularis mucosae were found to be rich in non-specific cholinesterase. They could thus be clearly distinguished from detrusor muscle bundles which are devoid of non-specific cholinesterase but rich in acetylcholinesterase. These enzyme studies also revealed a rich plexus of acetylcholinesterase-containing nerve fibres lying among the muscle bundles of the muscularis mucosae.

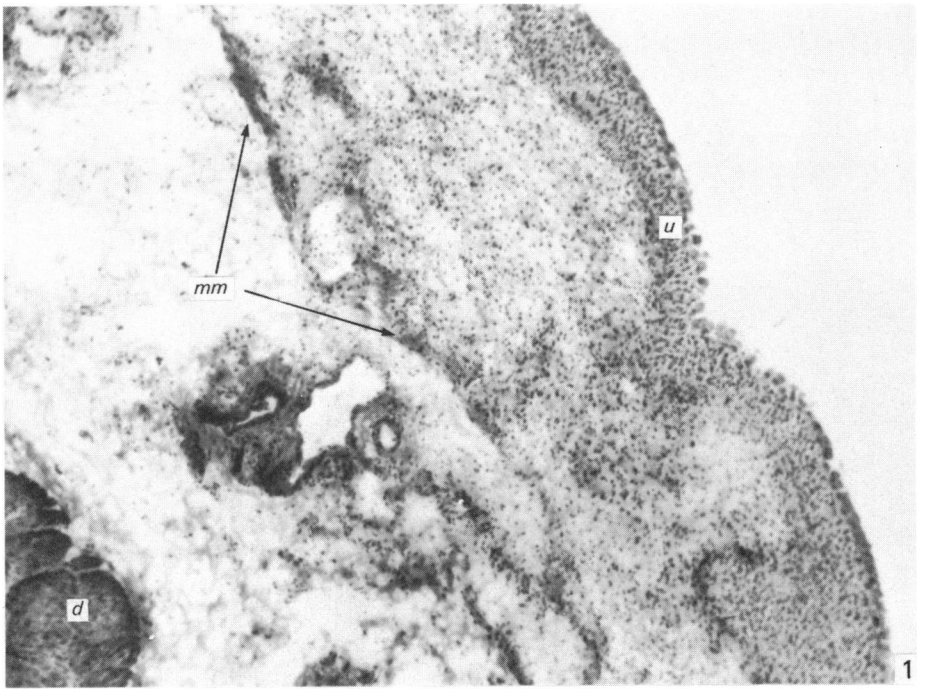
Electron microscopy

The smooth muscle cells comprising the muscularis mucosae had diameters of 3–5 μm at their widest part and most of them displayed a very irregular outline in cross section, caused by extensive folding of the plasmalemma (Fig. 2). In general, the myofilaments were confined to the central region of each cell while the peripheral sarcoplasm of many of the cells contained clusters of electron-dense glycogen granules (Fig. 2). In favourable planes of section, the smooth muscle cells were observed to possess an extensive sarcoplasmic reticulum (Fig. 3), consisting of flattened cisternae lying in close proximity to rows of caveolae attached to the cell membrane. Small oval mitochondria were frequently observed in association with the smooth reticulum (Fig. 3).

Each smooth muscle cell was surrounded by an electron-dense basal lamina. Frequently an intercellular junction was observed between two adjacent smooth muscle cells, where a gap of about 20 nm separated the apposed plasmalemmas (Fig. 4). At these junctions (or 'regions of close approach') the basal lamina of one cell was reflected back to become continuous with that of its neighbour. Small regions of the opposing membranes were sometimes associated with an increased electron density of the underlying sarcoplasm (Fig. 4).

Many of the smooth muscle cells were surrounded by a meshwork of electron-dense microfibrils (Fig. 5), some of which appeared to be in continuity with the cells' basal laminae. Amorphous electron-dense elastic fibres were also commonly observed in close association with the smooth muscle cells.

Numerous small groups of nerve axons and their associated Schwann cell cytoplasmic processes were observed among the smooth muscle cells of the muscularis mucosae. The varicose regions of these axons were either partly or completely denuded of Schwann cell cytoplasm and contained numerous small (50 nm diameter)



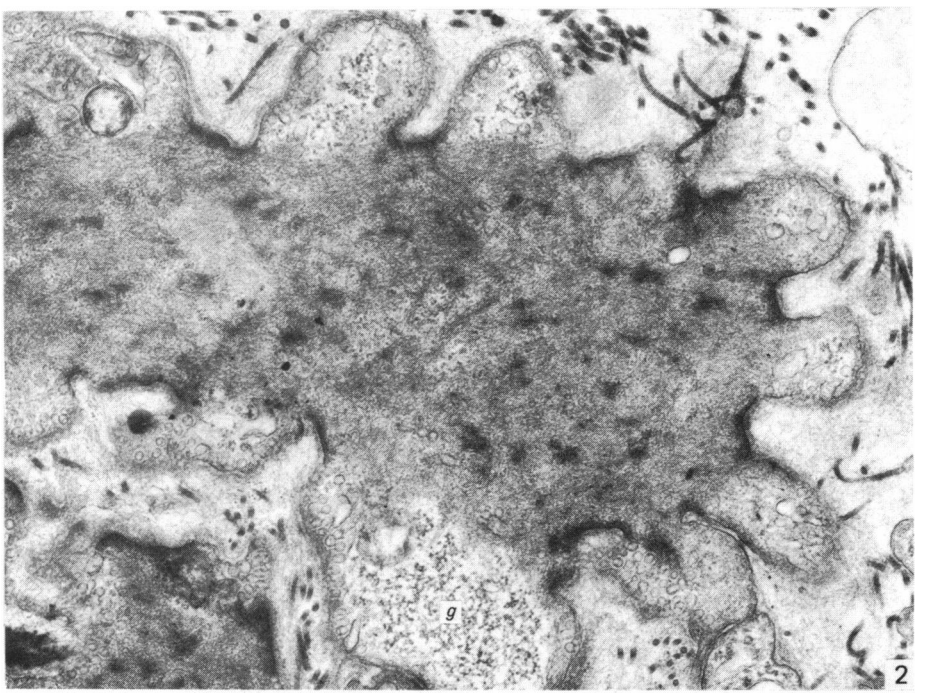


Fig. 1. Photomicrograph of a biopsy specimen from the fundus of the human urinary bladder. The muscularis mucosae (*mm*) lies approximately mid-way between the urothelium (*u*) and the detrusor muscle (*d*). Masson's trichrome. × 85.

Fig. 2. Sarcolemmal folds encompassing clusters of glycogen granules (*g*) are present in many of the smooth muscle cells forming the muscularis mucosae. × 21 600.

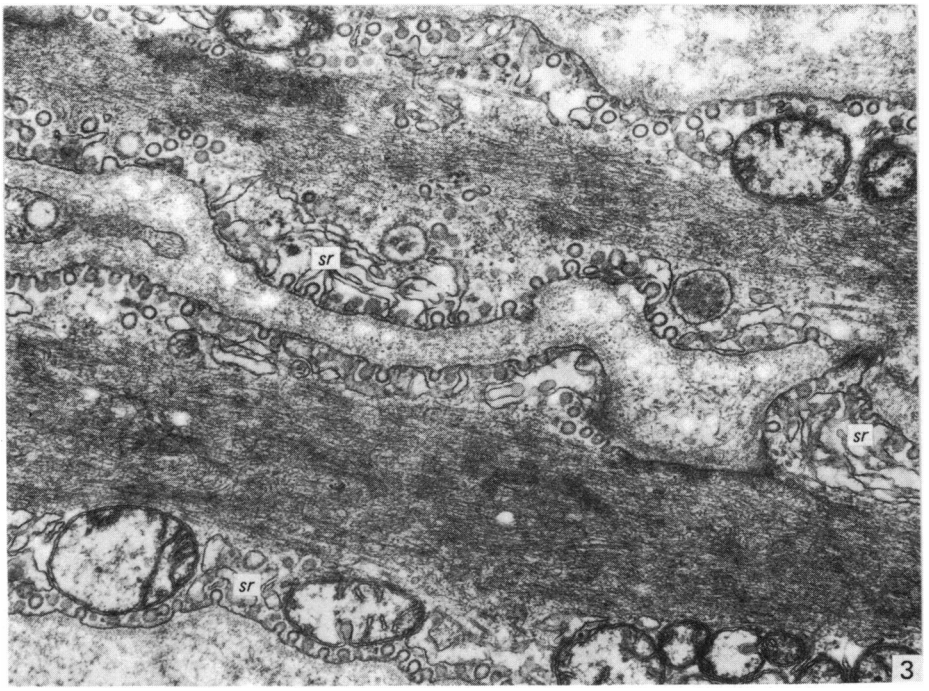
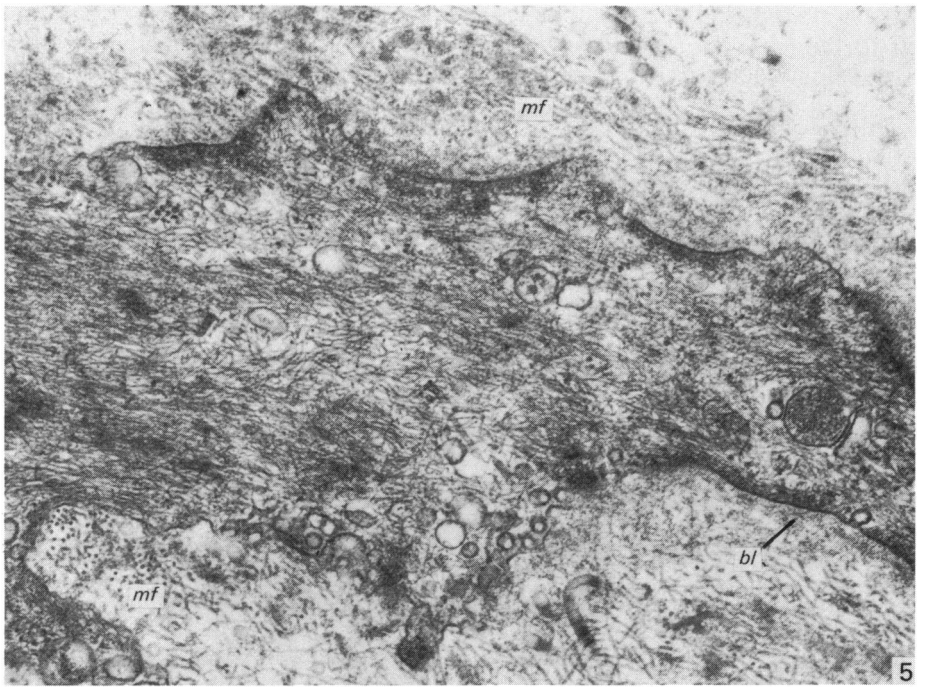


Fig. 3. An extensive sarcoplasmic reticulum (*sr*) is observed in these smooth muscle cells from the muscularis mucosae. × 22400.

Fig. 4. An intercellular junction (region of close approach) between two muscle cells of the muscularis mucosae. × 36000.



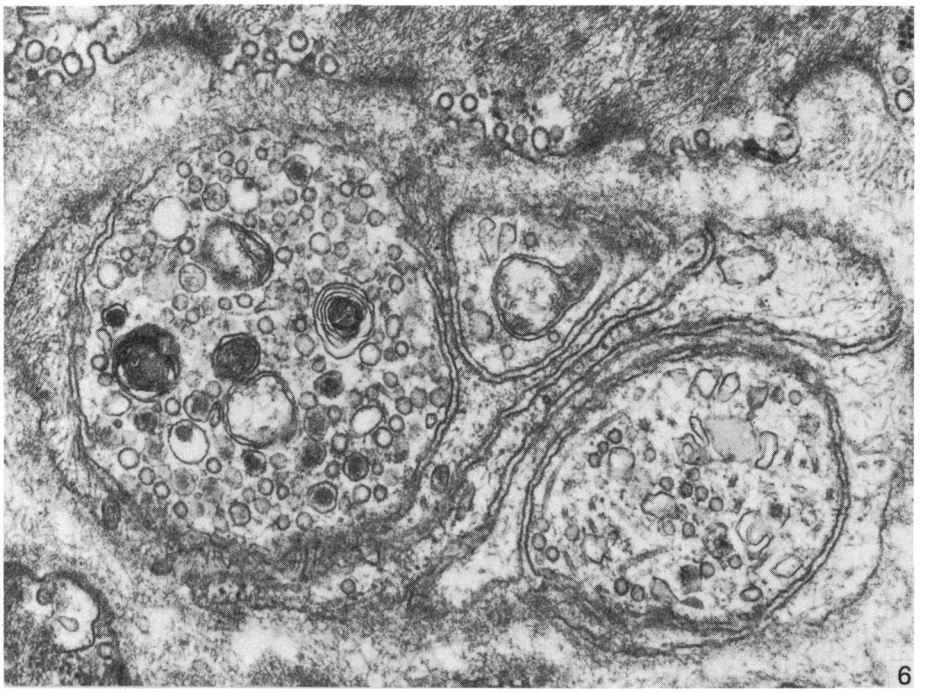


Fig. 5. Electron-dense microfibrils (*mf*) from a meshwork around this smooth muscle cell from the muscularis mucosae. Some of the microfibrils appear to be in continuity with the cell's basal lamina (*bl*) × 33600.

Fig. 6. An axon terminal packed with small agranular and large dense-cored vesicles lies adjacent to a smooth muscle cell from the muscularis mucosae. × 33600.

agranular and large (100 nm diameter) granulated vesicles together with mitochondria (Fig. 6). The closest observed approach of an axonal varicosity to the surface of a smooth muscle cell was about 100 nm.

DISCUSSION

The results of the present study have demonstrated that, contrary to popular belief, the mucosa of the human urinary bladder possesses a muscularis mucosae. The constituent smooth muscle bundles are irregularly arranged and form a discontinuous layer, which might explain why the presence of a muscularis mucosae has not been widely recognised previously. While the majority of specimens examined in our study were obtained from patients with possible detrusor instability, a muscularis mucosae was observed with similar frequency in specimens obtained at total cystectomy. The latter tissue samples were selected from non-pathological regions of the bladder in each case and were considered to represent control specimens. Thus, the presence of a muscularis mucosae can in no way be attributed to detrusor instability and is considered to be a normal feature of the human urinary bladder wall although it is much less well-defined than that of the intestinal wall.

Concerning the possible functional significance of a muscularis mucosae in the human urinary bladder one can only speculate. Like that of the gut it could possibly be involved in causing localised movements of the mucosal lining, although this seems rather unlikely in view of the discontinuous nature of the muscularis mucosae in the bladder. Furthermore, it is difficult to appreciate the need for such a mechanism in the bladder, since absorption is not thought to occur to any significant extent.

The presence of a muscularis mucosae in the bladder wall may have an embryological explanation, because both the bladder and intestines are hindgut derivatives. However, it is curious that a similar muscularis mucosae does not occur in the bladders of other mammals such as the rat, rabbit, cat or dog (personal observations).

The electron microscopic observation of large numbers of elastic microfibrils in close association with the smooth muscle cells of the muscularis mucosae could be interpreted as indicating that such cells are actively involved in the synthesis and secretion of connective tissue components. A similar mechanism is known to occur for the smooth muscle cells of the aorta, for example (Gerrity, Adams & Cliff, 1975).

Whatever the precise function of the muscularis mucosae may be, it is evident that descriptions of the human bladder wall which appear in many textbooks of human histology require revision in the light of the findings now reported.

SUMMARY

Study of biopsy specimens has revealed the presence of a muscularis mucosae in all regions of the human urinary bladder. The muscularis mucosae is discontinuous and consists of irregularly-arranged muscle bundles composed of relatively small-diameter smooth muscle cells. These cells are both morphologically and histochemically distinct from those forming the detrusor muscle, being rich in non-specific cholinesterase and glycogen. However, like detrusor muscle, the muscularis mucosae is richly supplied with acetylcholinesterase-positive nerve fibres. In the electron microscope, the constituent smooth muscle cells possess an extensive sarcoplasmic reticulum and large, peripheral clusters of dense glycogen granules; the myofilaments are confined to the central regions of the cells. Numerous intercellular junctions

occur between adjacent cells while presumptive cholinergic nerve terminals containing small agranular and large granulated vesicles lie in close proximity to the muscle cells' surface.

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