

The concentration of elastic fibres in the male urethra during human fetal development

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OBJECTIVE

To describe the distribution of elastic fibres in the developing male urethra and to provide stereological data of the concentration of elastic fibres in the human urethra.

MATERIALS AND METHODS

Urethras were obtained from 10 fresh normal human fetuses at 15–36 weeks of gestation. A place-matched spongy urethra of a 27-year-old normal adult man was also analysed. Samples were fixed in Bouin's solution, embedded in paraffin and histologically processed. The elastic system fibres were evaluated by light microscopy using Weigert's resorcin-fuchsin technique after oxidation.

Morphometric values were assessed by the point-counting method. The volumetric density (V_v) of elastic fibres was correlated with fetal age.

RESULTS

At 15 weeks the elastic fibres were sparse and homogeneously distributed. The size and thickness of elastic fibres increased with age, mainly in the third trimester of gestation. Elastic fibres formed a randomly orientated network in the trabeculae of the corpus spongiosum. The mean (SEM) V_v of elastic fibres in the spongy urethra was 5.2 (0.4)% in the fetus at 15 weeks and 14.8 (1.0)% at 36 weeks. In the urethra of the place-matched young man the V_v was 19.0 (1.3)%.

The concentration of elastic fibres in the spongy urethra increased significantly with age.

CONCLUSION

The high concentration of elastic fibres in the spongy urethra may partly explain its high extensibility. The progressive increase in elastic fibres during development implies functional adaptation of the fetal male urethra.

KEYWORDS

urethra, fetal development, elastic tissue, extracellular matrix

INTRODUCTION

The human anterior urethra is a unique organ formed by an almost central tube lined with epithelium and, beneath the basement membrane, by a layer of connective tissue that contains the vascular sinusoids of the corpus spongiosum and smooth muscle fibres [1]. The connective tissue includes cells, fibroblasts and an extracellular matrix containing abundant elastic fibres, proteoglycans, collagen and other glycoproteins [2–4]. The elastic system fibres are matrix components found in different organs, for which they provide elasticity and some biomechanical resistance [5]. Thus, elastic fibres are critical matrix components, mainly in organs that change shape under physiological conditions, e.g. the male urethra. Although the elastic fibres in the spongy urethra have been studied previously [4,6,7], to our knowledge there are no data quantifying or detailing the organization of elastic system fibres during urethral development in humans.

While the most common collagens are made essentially of inextensible structural

fibrils, elastin is characterized by a high degree of extensibility and elastic recoil. Thus, the fibroelastic elements (collagen and elastin) combine to form key structures in compliant tissues [5,8]. The human spongy urethra has a significant collagen content [2,3,9], and through the interaction of elastic fibres with collagen it was suggested that elastic fibres are important for urethral compliance, although this issue has still not been properly studied in the fetal urethra.

Thus we studied the elastic system fibres in the male urethra of the human fetus at different ages, to try to define the significance of this major extracellular matrix component in the development of the normal human urethra.

MATERIALS AND METHODS

The urethras were obtained from 10 fresh normal human fetuses which had died from causes unrelated to the urogenital tract. The fetuses were well preserved and none of them

had any kind of detectable congenital malformation. They were stored at 4 °C within 2 h of delivery. The local Committee on Human Research approved the investigation. Gestational age was estimated by the foot-length method [10], and was 15–36 menstrual weeks.

After dissection, the penis was removed, cross-sectioned at its mid-shaft and immediately immersed in Bouin's solution. The specimens were then processed by routine methods, and embedded in paraffin, from which 10 μ m thick sections were obtained. To verify the integrity of the specimens, from each studied penis two sections were stained with haematoxylin-eosin and two with Gomori's trichrome. This initial analysis showed that all penises were well preserved and fixed. The elastic system fibres were assessed using light microscopy after staining the material using Weigert's resorcin-fuchsin technique, with oxidation in 10% ozone. All specimens were prepared identically to minimize potential differences caused by processing.

We analysed five randomized sections from each urethra, and in every section five fields, totalling 25 fields per urethra (fetus), and 250 fields for the final result. The sum of the five fields evaluated in each section is representative of the total area of each corpus spongiosum section [11]. The fields were digitized using a video camera coupled to a light microscope, with a final magnification of $\times 400$. Test-points were counted using the M42 test system and volumetric densities (V_v) of the elastic system fibres assessed as previously described [12].

We also analysed one spongy urethra, place-matched with the fetal samples, of a 27-year-old normal man who had died accidentally. As there are no quantitative data on the elastic system fibres in the male spongy urethra, neither in fetuses nor adults, the mean value obtained provided a final reference for the elastic fibre concentration in the adult urethra. The mean values of the elastic fibre concentration in the fetal urethra were correlated with fetal age by linear regression analysis; data are expressed as the mean (SEM) for each urethra, with $P < 0.05$ considered to indicate significant differences on statistical testing.

RESULTS

There was active mesenchymal proliferation around the urethral lumen in younger fetuses (15–18 weeks); at these ages elastic fibres were sparse and homogeneously distributed (Fig. 1A). At later fetal ages the spongiosum sinusoids became more evident, with a concomitant decrease in cellular density of the corpus spongiosum mesenchyme (Fig. 1B,C). The size and thickness of the elastic fibres increased with age, mainly in the third trimester. These fibres form a randomly orientated network in the trabeculae of the corpus spongiosum. The elastic fibres are not closely packed enough to form an identifiable layer (Fig. 1D,E). In adults the elastic fibres are long, numerous and have a characteristic tortuous appearance (Fig. 1F). Moreover, several fibres located beneath the epithelial basement membrane seem to follow the epithelial invaginations towards the urethral lumen.

The V_v of the elastic fibres in the spongy urethra was 5.2 (0.4)% in the fetus at 15 weeks and 14.8 (1.0)% at 36 weeks; in the urethra of the young man the V_v was

19.0 (1.3)%. The elastic fibre concentration increased roughly linearly and progressively from 15 to 36 weeks (Fig. 2).

DISCUSSION

The elastic system is formed by three types of fibres, i.e. oxytalan, elaunin and elastic. The oxytalan fibres are formed exclusively by microfibrils, the elaunin fibres by microfibrils and patches of amorphous material (elastin), and the elastic fibres by a large amount of elastin with microfibrils [5]. As the present purpose was quantitatively analyse the whole elastic fibre system we used Weigert's resorcin-fuchsin stain after oxidation, because it highlights all elastic components in the tissues. The intention was to describe the distribution of elastic system fibres in the developmental male urethra, and to characterize their conformational changes during development. Furthermore, the design provided stereological data on the concentration of elastic fibres in the human fetal urethra.

Although the elastic fibres in the spongy urethra were more apparent at the end of the fetal period there were scarce elastic fibres in the fetal urethra as early as 15 weeks (Fig. 1). As fetal growth progressed the number of elastic fibres increased roughly linearly. Early random orientation preceded a more orderly arrangement, maintained through later development and in the adult urethra.

The elastic system fibres are active in tissue compliance [5], mainly in organs that change shape under physiological conditions, e.g. the spongy human urethra. Mechanical force induces intense cellular and extracellular changes [13,14]. As shape and compliance are characteristics attributed to the extracellular matrix, mechanical forces may influence shape and tissue compliance. Furthermore, interestingly some congenital malformations of the male urethra are accompanied by changes in shape and compliance. The physical forces that are necessary for normal development may also lead to abnormal development. In addition to the amniotic pressure that uniformly acts on the fetal body [15], the development of the normal fetal male urethra occurs under two unique kinds of transitory but frequent biomechanical forces. The urethra can extend during erections and can dilate its lumen during voiding [16–18]. Certainly, matrix components

other than elastic fibres are also implicated in the final characteristics of the spongy urethra [2–4]. However, despite elastic fibres endowing urethral connective tissue with the critical properties of elasticity and resilience, no study has quantified elastic fibres in the human fetal urethra; thus to our knowledge we provide original data on this aspect during fetal development.

The present data also suggest that during normal development of the fetal male urethra there is a progressive change from a resistant urethra, characterized by a wide urethral lumen, small vascular sinusoids and the presence of few elastic fibres, to a highly compliant urethra, with large spongiosum sinusoids and abundant elastic fibres. Moreover, there is an increase in elastic fibre concentration in the fetal male urethra during the developmental period when voiding function first occurs [17,18]. These observations suggest that pressure caused by fetal micturition may be a significant event in the normal development of the human male urethra, and could result in an increase in tissue compliance [19]. Interestingly, the V_v in the adult urethra was higher than in the urethra at the end of the fetal period. This suggests that other factors besides pressure, e.g. hormones and growth, may be important in the final remodelling of the extracellular matrix in the human male urethra.

The mechanism orchestrating the action of pressure on tissues is incompletely understood; some have suggested that pressure induces a decrease in cellular density, similar to that in the final stages of normal wound healing, where apoptosis is the mechanism through which vascular and fibroblastic cells are gradually eliminated [13,14]. Moreover, apoptosis is important in the normal development of the male anterior urethra [20]. The present results show elastic fibre concentration changes during the development of the spongy urethra and, although essentially quantitative, provides evidence for close relationships between biomechanical modifications (i.e. voiding, erection, and amniotic pressure) and the development of the urethral extracellular matrix.

In conclusion, elastic fibres are one of the major components of the extracellular matrix of the fetal urethra and, in addition to other matrix components and the spongy structure, they endow the anterior male urethra with a

FIG. 1. Distribution of the elastic component in the spongy urethra of human fetuses at various ages and in an adult urethra, as shown by Weigert's stain after oxidation. (A) Scarce and fine elastic fibres are visualized in the homogeneous and intense cellular mesenchyme of the corpus spongiosum in a fetus at 15 weeks of gestation. u, urethral lumen ($\times 400$). Trabeculae of the corpus spongiosum delimiting small vascular sinusoids in a fetus at 20 (B), 26 (C) and 31 weeks (D); *, spongiosum sinusoids ($\times 400$). (E) Trabeculae of the corpus spongiosum delimiting large vascular sinusoids; elastic fibres are abundant and organized in a fetus at 36 weeks; *, spongiosum sinusoid ($\times 400$). (F) High concentration of elastic fibres in the matrix of the spongy urethra. Fibres located beneath the epithelial basement membrane seemed to follow the epithelial invaginations towards the urethral lumen (adult urethra); u, urethral lumen ($\times 100$).

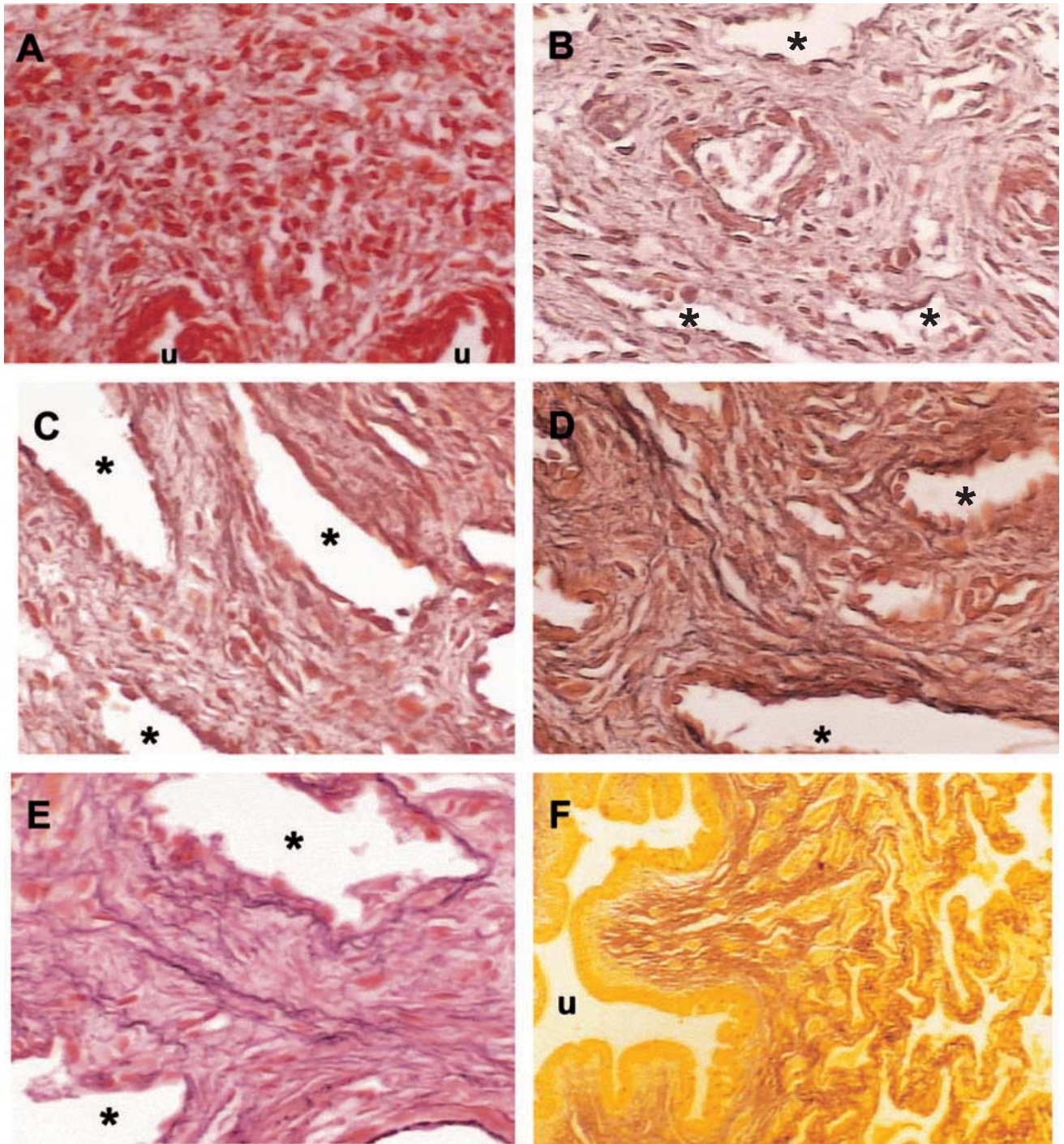
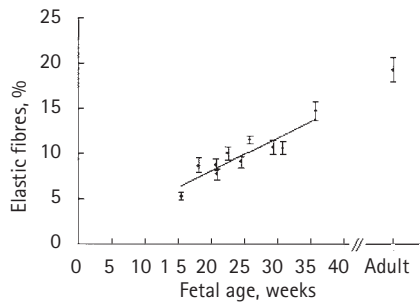


FIG. 2. Elastic fibres in the human fetal urethra. Correlation between the volumetric density of elastic fibres and the fetal age (in menstrual weeks). The relative concentration (in percentage) of elastic fibres increased with fetal age ($r = 0.903$; $P < 0.001$). Error bars indicate one SEM of the mean.



large capacity to expand under tension [21]. The progressive increase in elastic fibre concentration during development implies functional adaptation in the fetal male urethra by 'in utero' biomechanical forces. Furthermore, this analysis of the elastic fibres of the corpus spongiosum in human fetuses provides original data on normal components of the male urethra during development.

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CONFLICT OF INTEREST

None declared.

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