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AGE-RELATED CHANGES IN THE ELASTIC FIBER NETWORK OF THE HUMAN SPLENIC CAPSULE

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ABSTRACT

The structural arrangement of elastic fibers in the splenic capsule from 16 human cadavers ranging in age from 1 month to 76 years was studied by histologic sections stained with selective methods for elastin. In infants the elastic fibers of the splenic capsule were homogeneously intermingled with collagen fibers, an arrangement that stabilizes the capsule during spleen growth and enlargement. With aging, collagen fibers predominate in the outer capsular surface over elastic fibers with the latter more evident in the deep lamina of the splenic capsule. In elderly individuals, the elastic fibers shorten, fragment, and thicken. The progressive decrease in the amount of elastic fibers in the splenic capsule with aging may restrict splenic distention and contribute to involution of the spleen as one grows older.

Understanding of splenic capsular structure may help explain mechanical properties of the normal and diseased spleen. Thus far, little attention has been addressed to the capsular architecture and particular its elastic fiber makeup (1).

Elastic and collagen fibers in general participate in the regulation of organ distention, and elastin constitutes the primary element responsible for reversing distention of connective tissue. The microfilamentous component of the elastic fibers (oxytalan fibers) is capable of sustaining variations in organ distention and has notable anchoring and adhesive properties by providing intrinsic mechanical resistance (2-4).

To examine the arrangement and amount of the elastic fiber system of the human splenic capsule and to try to correlate the findings with aging, we studied splenic slices stained with selective techniques in human spleens from cadavers of a wide age range.

MATERIALS AND METHODS

Specimens of the splenic capsule were obtained from 16 human cadavers that ranged in age from 1 month to 76 years. They were arbitrarily divided into three groups according to chronologic age: young (1 month to 20 years), adult (21 to 60 years), and elderly (> 60 years). Each spleen was fixed with a 10% neutral formol saline for 24 hours. The splenic capsule was dissected under a stereomicroscope from each of three topographic regions: superior (SCS), inferior (ICS), and mid-portion (MPCS). The specimens were processed histologically after dehydration in ethanol, cleared in xylol, embedded in paraffin, and sectioned at 5 μm thickness.

Staining Procedure

Three adjacent sections were submitted

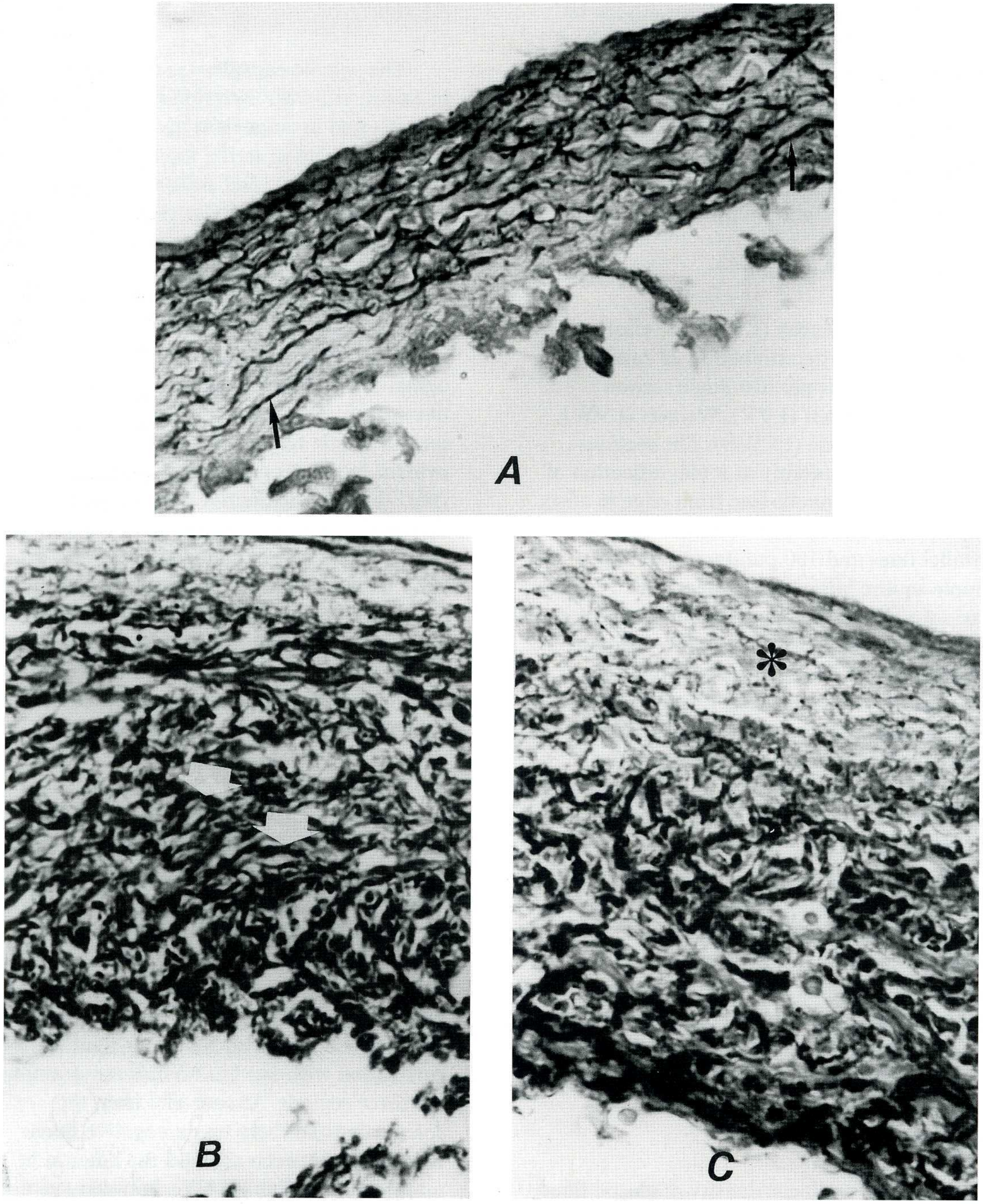

Fig. 1. a) Splenic capsule from a 7 month old infant. Elastic fibers (arrows) are thin, straight and organized in a uniform network among the collagen bundles.
b) Splenic capsule from adult aged 32 years showing partial breakdown of the network of elastic fibers, which are thicker and shorter (arrows).
c) Splenic capsule from man aged 76 years. There is a complete derangement of the network of elastic fibers, which are thick, short, tortuous, and closely wrapped around thick bundles of collagen fibers (arrows). There is a zonal differentiation into a superficial collagenous (*) lamina and a deep elastic lamina. a-c (Weigert-oxone, x600).

to one of the following selective methods for staining elastic fibers: Verhoeff's iodine-iron hematoxylin method (5), for mature elastic fibers; Weigert's resorcin-fuchsin (6), for mature and elaunin elastic fibers; Weigert's technique with previous oxidation using oxone, as previously described (3) for oxytalan, elaunin, and mature elastic fibers.

Morphometric Evaluation

The linear density (LV) of the elastic fiber system was determined in 25 random microscopic fields per histologic section stained by Verhoeff (LVV), Weigert (LVW), and Weigert-oxone (LVWO). The sections were scanned randomly at a magnification of 1,000x in a continuous line from edge to edge, employing a test eyepiece reticule with 10 parallel lines and 100 points, containing a simple square lattice test system with 10,500 μm^2 . An elastic fiber completely intersected by any of the test lines was counted. These intersections of fibers with a test reticule relate to the length of those fibers per unit of area as expressed by the formula: LV=2Na, where Na is the length of fiber per unit of area (7). The area of the tissue examined was determined by counting the number of points of intersection inside that tissue.

The thickness of the splenic capsule was determined by planimetric analysis at 100x magnification using a computerized system running Sigma-Scan™ Software (Jandel Scientific), attached to a Zeiss microscope using a camera Lucida and digitizing tablet. All three aspects of the splenic capsule were evaluated at three randomly different points.

Statistical Analysis

The data on linear density of elastic fiber concentration were analyzed using a one-way analysis of variance. $P \leq 0.05$ was considered statistically significant. The combined effects of age and linear density of elastic fibers and splenic capsule thickness were tested by linear regression analysis.

RESULTS

The splenic capsule was consistently rich in elastin but with variation in thickness, density, and arrangement according to age but not in relation to the topography. In infants, the elastic and collagen fibers in the splenic capsule had a relatively regular shape and distribution. Numerous strands of elastic fibers were seen dispersed among the collagen bundles. Thin elastic fibers lay parallel to the surface and formed a prominent network between collagen fascicles *(Fig. 1a)*. With aging, these features changed. More collagen developed in the outer surface with a relative lack of elastic fibers, which were now more evident in the deeper lamina of the capsule *(Fig. 1b)*. Two distinctive laminae now appeared, namely, a superficial lamina composed largely of collagen fibers and a deep lamina of elastic fibers. In the elderly, the elastic fibers were coiled into clusters, shortened, fragmented and thickened *(Fig. 1c)*.

Whereas there was a positive linear correlation between age and the splenic capsule thickness *(Fig. 2)*, there was no difference of thickness among the three topographic aspects of the splenic capsule. The equation curves were: ICS = 54.58+1.586 x age (R^2=0.59, $p<0.001$), SCS=49.95+1.159 x age (R^2 =0.74,$p<0.001$), MPCS=49.77+1.880 x age (R^2 =0.63, $p<0.001$).

The amount of elastic fiber system obtained through the linear density (LV) from sections of the superior, inferior and mild-point aspects of the splenic capsule stained by selective methods also showed a significant correlation with age but no difference among the three regions. At each site from the splenic capsule there was a negative linear correlation between age and the amount of mature elastic fibers (LVV), between age and mature and elaunin elastic fibers (LVW) and between age and mature, elaunin and oxytalan elastic fibers (LVWO). *Figs. 3a-c* represent, respectively, the regression curves of the LVV, LVW and LVWO from the inferior aspect of the splenic capsule.

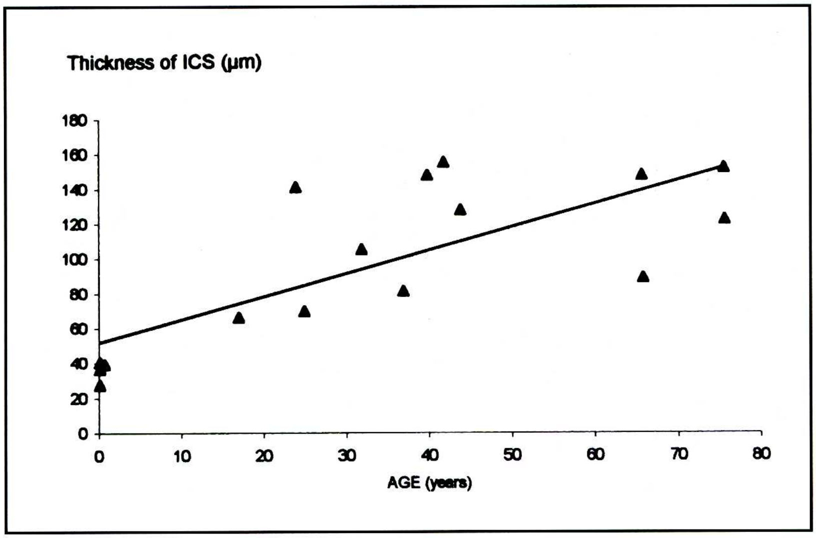

Fig. 2. Thickness of the inferior aspect of the capsule of the spleen (ICS) as a function of age. The best fit linear regression line is shown.

DISCUSSION

The splenic capsule helps regulate intrasplenic pressure, and the amount of elastic fibers in the splenic capsule correlates with the function of the splenic reticulo-endothelial system. The arrangement of these two systems is special, and elastic fibers course within the spleen according to the distribution of the intrasplenic vasculature. This architectural arrangement enables distention and contraction of the splenic capsule which facilitates and regulates intrasplenic blood flow (8-10).

Alteration in the shape of elastic fibers is associated with the deposit of excess collagen bundles, and a rearrangement of the three-dimensional organization of the elastic fibers occurs with aging. Synthesis of collagen fibers induces stretching of the elastic fibers, which appear to fracture and wrap around the newly deposited collagen bundles (11-12). With aging in general, there is diminution of the oxytalan fibers and reduction in the elaunin and elastic fibers in skin, trans-versalis fascia, and muscular fascia. Tissue looseness and poor recoil with stretching and fine wrinkling is the natural consequence (14,15).

Intermingling of elastic and collagen fibers helps prevent excessive distortion of the splenic capsule and spleen shape. The capsule stretches during expansion and its elastic fiber component helps return the spleen to its resting condition. With advanced age, the splenic capsular elastic fibers decrease and the collagen content increases. This derangement accounts for decreased compliance of the spleen as one ages with limitation of splenic expansion and gradual reduction in spleen size as blood flow progressively decreases with reduced compliance of the spleen.

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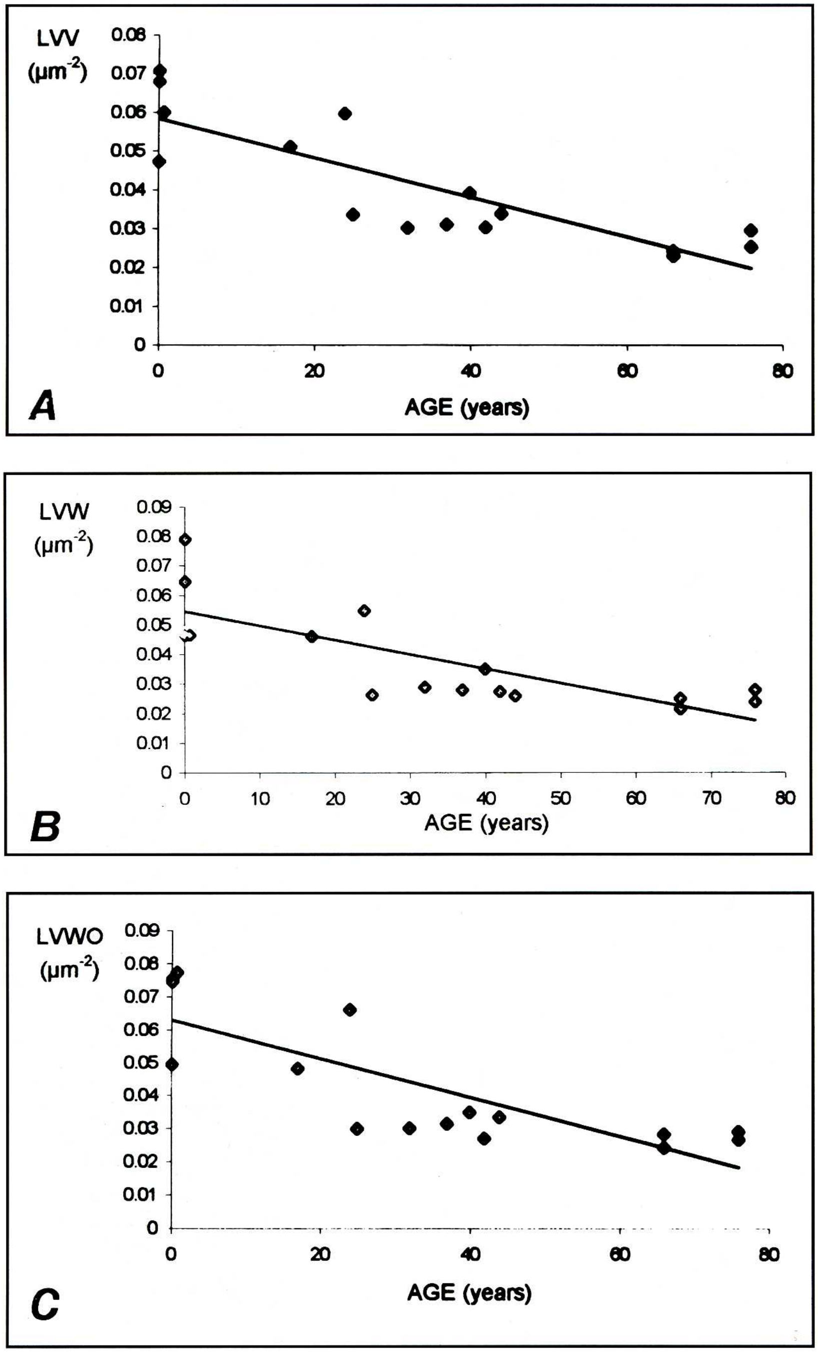

Fig. 3. a) Linear density of mature elastic fibers (LVV) of the inferior aspect of the splenic capsule as a function of age. The best fit linear regression line is shown (0.54-0.0004 x age).
b) Linear density of mature and elaunin elastic fibers (LVW) of the inferior aspect of the splenic capsule as a function of age. The best fit linear regression line is shown (0.048-0.0004 x age).
c) Linear density of mature, elaunin and oxytalan elastic fibers (LVWO) of the inferior aspect of the splenic capsule as a function of age. The best fit linear regression line is shown (0.058-0.00005 x age).

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