

Elastin VIII

A Correlative Study of the Aging Elastica of Arteries and Skin

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Summary. Nigrosin base in an acid alcohol solution and Gomori's aldehyde fuchsin gave excellent staining of the elastic fibers in the arteries and skin regardless of age. Neutral hydroalcohol solutions of alcohol soluble nigrosin stained the elastic fibers in the arteries and skin of humans above age 20. Clara's neutral hematoxylin stained the arterial elastica of children less than 10 years of age, but did not color the elastic fibers of the skin. By these staining procedures, it may be possible to obtain information about arterial elastica by a skin biopsy.

Key words: Elastin — Arteries — Skin — Nigrosin — Aging.

In 1969, Pizzolato and Lillie reported that Clara's neutral hematoxylin (1974b) could stain the arterial elastica of infants and children to about 10 years and that Schiff's reagent could stain these elastic fibers to about 20 years. We assumed that the staining mechanism was due to an aldehyde-quinonoid component.

More recently, Lillie, Pizzolato and Donaldson (1974a) found after a study of indulin nigrosin elastic stains that acid alcohol solutions of nigrosin base (C.I. 50415B, Solvent Black 7, SB7) stained the arterial elastica of all decades. We also observed that neutral hydroalcoholic solutions of alcohol soluble nigrosin (C.I. 50415, Solvent Black 5, SB5) stained the arterial elastica in humans above age 20. The suggestion was made at that time that there might be an acid lipoprotein complex developing in advancing age. A more extended study (Lillie et al., 1976) revealed that this SB5 staining of arterial elastica increased in frequency and density with advancing age and that it was always present when histologic lesions of arteriosclerosis were present in that artery.

In this report we want to make a correlative study of some histochemical characteristics of skin and arterial elastica in relation to the process of aging.

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Material and Methods

Previous observations have shown that, in the adult, the superior mesenteric and celiac arteries exhibited the least arteriosclerotic changes and were used in this study. The upper abdominal aorta from infants and young children was examined. Arteries with moderate or severe arteriosclerosis or calcification were excluded from this investigation because of the frequent involvement of the arterial elastic lamina. A 1 × 2 cm piece of skin was removed from the abdominal wall near the umbilicus and from the scalp just behind the ear. The skins and blood vessels were fixed together in neutral, phosphate buffered 4% formaldehyde (Pizzolato, 1976) 48 to 100 h and processed in the usual manner through alcohol, high octane gasoline and paraffin. Paraffin sections were cut at 5 to 7 μ , dewaxed in xylene brought to the appropriate stains as previously described (Lillie et al., 1974a). About 10 cases from each decade were examined.

Comparisons were made of the following stains:

1. Nigrosin base (SB7, Matheson, Coleman, Bell, NX365-B365) in 1% hydrochloric acid in 70% ethanol, 4 h, counterstained by the Van Gieson method (SB7-VG).
2. Nigrosin, alcohol soluble (Matheson, Coleman, Bell, NX385-B365) in 60% isopropanol, 30 min, and mounted in Apáthy's gum syrup.
3. Gomori's aldehyde fuchsin, aged 10–30 days, (stored at 23 °C) 20 min (Gomori, 1950).
4. Schiff's reagent, 1 h (Lillie, 1965)
5. Clara's hematoxylin in pH 6.5 phosphate buffer, 40 h (Lillie et al., 1974b)
6. Phosphotungstic acid, gallein (PTAG) (Lillie et al., 1974c)
7. Routine hematoxylin (Lillie) and eosin (Lillie, 1965)
8. Orcein in acid alcohol, Taenzer-Unna (Lillie, 1965)

Results

Nigrosin base in acid alcohol and properly aged Gomori's aldehyde fuchsin gave excellent staining of elastic fibers in the skin and arteries in all decades and they are recommended as superior stains for the recognition of elastic fibers. SB7-VG gave gray black to black almost continuous fibers in the elastic and muscular arteries. Immediately adjacent to the elastic fibers and appearing attached to them are delicate red fibrils similar in color to the dense collagenous fibers of the adventitia. Between the red and black fibers are gray yellow fibers exhibiting a greater predominance in the muscular arteries. In the larger arteries, the red and yellow fibers are very close to each other suggesting a mosaic pattern, with varying numbers of black fibrils among them. When no counterstain is used, the elastic fibers are black and the intervening areas are gray with some clear spaces.

The skins from the scalp and abdomen reveal elastic fibers as fine to coarse among the large dense red collagenous bundles. Although most elastic fibers are in the middle portion of the dermis, few almost inconspicuous black fibrils extend upward and appear to touch the stratum germinativum. The smooth muscles stain pale grey yellow. With SB7 alone, the elastic fibers and fibrils are in the middle portion of the dermis, few almost inconspicuous black fibrils

For the first two decades, SB5 gives no color to the elastic fibers in the aorta and muscular arteries. The absence of staining is conspicuous by the fact that a clear zone is noted among the gray staining smooth muscles with barely visible nuclei and pale gray fibrous connective tissue. In the adults, this stain reveals dark gray blue elastic fibers on a light gray background. The skin shows no obvious elastic fibers among the gray staining collagenous

bundles in the young. Staining of the skin elastica becomes apparent when the arterial elastica is encountered and with the same gray blue coloration. In the adults, this stain reveals dark gray blue elastic fibers on a light gray background. The skin shows no obvious elastic fibers among the gray staining collagenous bundles in the young. Staining of the skin elastica becomes apparent when the arterial elastica is encountered and with the same gray blue coloration.

Gomori's aldehyde fuchsin colors the elastica of the skin and arteries of all ages a bright magenta red with a pale pink to colorless background. Of additional interest, the mast cells of the skin were observed to be more prominent and more numerous in comparison with our controls with toluidine blue at pH 2-3.

Schiff's reagent yields pink elastic fibers on a pale pink background of collagen in the arteries and skin to about age 20, similar to our previous studies (Pizzolato and Lillie, 1967). Clara's hematoxylin reveals blue gray arterial elastica only up to age 10 and the skin elastica is not conspicuous at any age. Since the quality of hematoxylin may vary, the observation of keratohyalin granules and the elastica of the very small arteries served as controls similar to earlier presentation (Pizzolato and Lillie, 1967).

PTAG stains the arterial elastica as a continuous red violet. The spaces between them are colored a pale brown with occasional red violet fibrils and pale blue nuclei. The collagen of the adventitia is brown. The dermis reveals collagenous bundles and barely visible red violet elastic fibers. Age has no influence on staining.

Orcein colors the elastica of skin and artery a dark brown to a red brown for all ages. The background is pale brown with nuclei staining brown.

Discussion

Nigrosin base and Gomori's aldehyde fuchsin give excellent staining of the elastic fibers in the arteries and skin regardless of age and they are recommended for the identification of elastic tissue in normal and pathologic sites. There is an excellent correlation with the staining features of the skin and arterial elastica, hematoxylin being the only exception.

Using these staining techniques a skin biopsy may possibly reveal sufficient information about the elastic tissue to interpolate pathologic changes in elastica of arteries and perhaps lung. Hornebeck et al. (1977) have suggested that elastolytic enzymes play an important role in the fragmentation of the elastic lamella occurring during arteriosclerosis. Perhaps these enzymes may simultaneously alter the skin elastica which can be demonstrated by histochemical procedures. These workers have also observed an increase elastic content of breast neoplasms and suggest that breast carcinoma elastin is closely related to newly synthesized aorta elastin. These findings could be demonstrated with positive staining, with the Clara's hematoxylin and with Schiff's reagent; SB5 should be negative. Such histochemical procedures are now in progress.

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