Effects of the Metal Salts on the Staining of Elastic Fibers with Hematein

By

Masumi AKITA, Seong Ho LEE* and Katsuji KANEKO

First Department of Anatomy, Saitama Medical School, Saitama 350-04, Japan

*Kongju National Junior College, Korea

—Received for Publication, July 12, 1987—

Key words: Hematein, Elastic fibers, Elastin, Metal salts, Stain

Summary: The effects of various metal salts on the staining of elastic fibers with hematein were tested at light and electron microscopic levels.

Potassium tungstate and sodium molybdate were effective at light and electron microscopic levels. Ammonium metavanadate, stannous chloride, potassium tellurate and uranyl acetate moderately stained elastic fibers at light microscopic level. However, these metal salts, except stannous chloride, did not show the marked enhancement of the electron density of elastin. Stannous chloride moderately enhanced the electron density of elastin.

It was suggested that hematein is capable of binding to elastin and subsequently or simultaneously chelating metal ions which render the elastin electron opaque.

It has been known that hematoxylin has an affinity for elastic fibers (Pizzolato and Lillie, 1969. Lillie et al., 1974), and hematoxylin enhances the electron density of elastic fibers (Brissie et al., 1974).

We found that hematein, which is an oxidation product of hematoxylin, exhibited stronger affinity for elastic fibers than hematoxylin, at pHs near neutrality. Furthermore, the affinity of hematein for elastic fibers was enhanced by metal salts such as tungstate and molybdate (Kaneko and Akita, 1977a, 1977b, 1978).

In this study, the effects of various metal salts on the staining of elastic fibers with hematein were tested at light and electron microscopic levels.

Materials and Methods

Specimens from rabbit aorta were fixed in 10% formalin, Zenker-formalin or Bouin's fluid and cut in paraffin at a thickness of 5 μ m.

Hematein was prepared according to the method of Arshid et al. (1954). Fifty milligram of hematein were dissolved in 50 ml of 0.1 M phosphate buffer (pH 7.0), and then added 50 mg of metal salts. The metal salts used in this study were listed in Table 1, according to Pizzolato and Lillie (1967).

Deparaffinized sections were stained with the staining solutions for 30 minutes.

For electron microscopy, specimens from rabbit aorta were fixed in 2% glutaraldehyde and post-fixed in OsO_4 . Specimens were

Table 1. Effects of metal salts on staining of elastic fibers with hematein

Group	Metal salt	Staining effect
I-A	Lithium carbonate, Li_2CO_3	—
	Cesium chloride, CsCl	—
I-B	Cupric sulfate, CuSO_4	—
	Cupric chloride, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	—
	Silver nitrate, AgNO_3	—
	Chloroauric acid, $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$	—
II-A	Beryllium nitrate, $\text{Be}(\text{NO}_3)_2$	—
	Magnesium chloride, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	—
	Strontium nitrate, $\text{Sr}(\text{NO}_3)_2$	—
	Barium nitrate, $\text{Ba}(\text{NO}_3)_2$	—
II-B	Zinc acetate, $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$	—
	Cadmium nitrate, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	—
	Mercuric chloride, HgCl_2	—
III-A	Aluminum chloride, $\text{AlCl}_3 \cdot 6 \cdot \text{H}_2\text{O}$	—
	Gallium nitrate, $\text{Ga}(\text{NO}_3)_3 \cdot 8\text{H}_2\text{O}$	—
	Thallous nitrate, TlNO_3	—
III-B	Yttrium nitrate, $\text{Y}(\text{NO}_3)_3 \cdot x \text{H}_2\text{O}$	—
IV-A	Potassium silicofluoride, K_2SiF_6	—
	Germanium dioxide, GeO_2	—
	Stannous chloride, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$	+
V-A	Bismuth nitrate, $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$	—
V-B	Ammonium metavanadate, NH_4VO_3	++
VI-A	Sodium selenate, Na_2SeO_4	—
	Potassium tellurate, $\text{K}_2\text{TeO}_4 \cdot 3\text{H}_2\text{O}$	+
VI-B	Chromium trioxide, CrO_3	—
	Potassium dichromate, $\text{K}_2\text{Cr}_2\text{O}_7$	—
	Sodium molybdate, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	+++
	Potassium tungstate, K_2WO_4	+++
VII-B	Manganese dioxide, MnO_2	—
	Potassium permanganate, KMnO_4	—
VIII	Iron (II) sulfate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	—
	Cobalt chloride, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	—
	Nickel sulfate, $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$	—
	Ruthenium trichloride, RuCl_3	—
	Palladium chloride, PdCl_2	—
	Osmium tetroxide, OsO_4	—
Lanthanides	Lanthanum nitrate, $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$	—
	Neodymium nitrate, $\text{Nd}(\text{NO}_3)_3 \cdot x\text{H}_2\text{O}$	—
Actinides	Uranyl acetate, $\text{UO}_2(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$	+

—, no change, Increasing of staining was indicated as +(slight) and +++ (extreme).

dehydrated through graded ethanol and embedded in epoxy resin. Ultrathin sections mounted on copper grids were stained for 5 to 10 minutes with the staining solutions, which exhibited positive staining reaction at light microscopic level, and followed by uranyl acetate and lead citrate.

Results

Light microscopy

Elastic fibers were strongly stained with potassium tungstate and sodium molybdate (Fig. 1, 2). Ammonium metavanadate and uranyl acetate showed moderate affinity for elastic fibers (Fig. 3, 4). Elastic fibers were slightly stained with stannous chloride and potassium tellurate. These results were summarized in Table 1.

Electron microscopy

Elastic fibers consist of central amorphous components (elastin) and microfibrils. Elastin was electron-microscopically translucent in the tissue sections treated with uranyl acetate and lead citrate. The electron density of elastin was slightly increased by hematein, tungstate or molybdate, respectively. It was markedly enhanced by molybdate-hematein and tungstate-hematein followed by uranyl acetate and lead citrate (Fig. 5, 6). Stannous chloride moderately enhanced the density of elastin, and nuclei and cytoplasm were strongly enhanced the electron density (Fig. 7). Potassium tellurate, ammonium metavanadate and uranyl acetate slightly enhanced the electron density of elastin.

Discussion

Heavy metals were used for increasing contrast of tissue sections in the electron microscopy (Watson, 1958a, b). Heavy metals tested in his works were as follows;

uranyl acetate, phosphotungstic and phosphomolybdic acids, sodium uranate, lead acetate, barium hydroxide and lead hydroxide. Among several heavy metals tested including lead monoxide (Karnovsky, 1961), lead citrate (Raynolds, 1963), potassium permanganate (Lawn, 1960. Sutton, 1968), chromyl chloride (Bullivant and Hotchin, 1960), uranyl acetate and lead citrate have been widely used. However, elastin, which was an amorphous component of elastic fibers, showed no affinity for cationic stains such as uranyl acetate or lead citrate applied to tissues that have been routinely prepared for electron microscopy as described by Raynolds (1963). Elastin had a marked affinity to phosphotungstic acid (Pearse and Molinari, 1960. Paule, 1963. Greenlee et al., 1966). Silicotungstic acid also enhanced the electron density of elastin (Yoshihama et al., 1986).

In this study, tungstate and molybdate were effective at light and electron microscopic levels. Ammonium metavanadate, stannous chloride, potassium tellurate and uranyl acetate moderately stained elastic fibers at light microscopic level. However, these metal salts, except stannous chloride, did not show the marked enhancement of the electron density of elastin. Palladium chloride and hematein did not stain elastic fibers at light microscopic level. However, Morris et al. (1978) reported that palladium chloride followed by lead citrate was useful for electron stain for elastin. Vanadyl sulfate and vanadyl sulfate-ammonium hepta-molybdate mixture were used for electron stain, but no mention was made of elastin (Callaham and Horner, 1964).

At light microscopic level, we reported that hematein showed stronger affinity to elastic fibers than hematoxylin and the affinity was markedly enhanced by adding metal salts (Kaneko and Akita, 1977a, 1977b, 1978). The mode of elastic fibers staining by unmordanted hematoxylin and

hematein remains obscure. Lillie *et al.* (1974) suggested that hematoxylin binds to tyrosine or possible desmosine content of elastin on a speculative basis, from the blocking by nitrations. Horobin and James (1970) proposed that elastin staining, generally, occurred by large dye molecule adhesion by van der Waals forces. Brissie *et al.* (1974) reported that hydrogen bonding assumed to be responsible for staining of elastin. In contrast with these findings, there is no single mechanism covering all elastin staining (Lillie and Hullmer, 1976). We previously reported that hematein and metal salt-complex binds to elastin by both hydrogen and ionic bonds (Kaneko and Akita, 1977b). It was suggested that complex organic molecules, such as hematoxylin and hematein, are capable of binding to elastin and subsequently or simultaneously chelating metal ions which render the elastin electron opaque. The nature of the group in elastin binding with hematein and metal salt-complex has not been determined. Further studies are needed to determine the nature of the group might account for this staining.

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Explanation of Figures

Plate I

Fig. 1. Potassium tungstate and hematein stain. Rabbit aorta. X 200

Fig. 2. Sodium molybdate and hematein stain. Rabbit aorta. X 200

Fig. 3. Ammonium metavanadate and hematein stain. Rabbit aorta X 200

Fig. 4. Uranyl acetate and hematein stain. Rabbit aorta. X 200

Plate II

- Fig. 5. Sodium molybdate and hematein stain, followed by uranyl acetate and lead citrate. Rabbit aorta. Elastin = asterisks, $\times 12,000$
- Fig. 6. Potassium tungstate and hematein stain, followed by uranyl acetate and lead citrate. Rabbit aorta. Elastin = asterisks, $\times 12,000$
- Fig. 7. Stannous chloride and hematein stain, followed by uranyl acetate and lead citrate. Rabbit aorta. Elastin = asterisks, $\times 21,000$