Observations of the Fenestrated Membrane of the Human Arterial Wall

By

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Summary: The difference in the resistance to alkali or acid between collagenous and elastic fibers has been applied to differentiation of the two fibers. However, it is often difficult to keep the arterial elastic membrane intact and to recognize the direction of the artery correctly since the arterial tissue block has been hitherto simply scraped or teased with needles or pincettes in alkaline or acid solution. A newly devised method using heated potassium hydroxide (KOH) solution provides a simple technique for isolation of a large elastic fenestrated membrane in the muscular arterial wall. The characteristic of this method is handling of the isolated membrane in gradually diluted alkaline solutions after maceration in a strong alkaline medium. Internal elastic membrane with fenestrations has been found in all human arteries examined, although the size and form of the fenestrations have varied according to the age and arterial site. The detailed procedure and significance of this method have been discussed, suggesting its applicability to gerontological or pathological studies on the elastica of the arteries.

In human muscular arteries, substantial lamina of elastin is present at the outer surface of the tunica intima, i.e., the internal elastic membrane is an elastic septum which separates the tunica intima from the tunica media. This elastic membrane generally contains many openings or windows of varying size at points where the elastic tissue is deficient. This is the origin of the name, fenestrated membrane or Membrana fenestrata, in the arterial wall.

A technique for demonstrating the fenestrated membrane from prestained arterial tissue blocks has been described elsewhere (Mitsui and Hosoda, 1970, Hosoda and Mitsui, 1969, 1970). However, if the arterial tissue block is simply scraped and teased as described in these reports, the isolated fenestrated membrane is generally irregular in shape and smaller in size, and the relationship between the axis of the artery and that of windows of the fenestrated membrane frequently becomes obscure.

The present study dealt with isolation of the fenestrated membrane as a whole using heated potassium hydroxide solution after arterial tissue blocks were stained with orcein. A characteristic technique for mounting a dehydrated, isolated fenestrated membrane in synthetic resin or Canada balsam has been emphasized in the present study. These simple methods are believed to demonstrate a clear, large area of the fenestrated membrane and to provide new knowledge concerning the structures of the arterial wall.

Materials and Methods

Human radial, ulnar, and cerebral arteries were removed from embalmed cadavers.
It is interesting to note that even in such materials the elastic tissue was well preserved and amenable to orcein staining as already indicated by Lansing (1959). Paraffin sections of these arteries thinner than 10 μm were prepared to observe the distribution of elastic fibers in the arterial wall on the one hand, and stained tissue blocks of the same arteries were used to isolate fenestrated membranes on the other hand. However, in paraffin sections, the endothelium was frequently damaged, and exfoliated from the tunica intima due to postmortem changes in the cadaver.

Procedures for isolating fenestrated membranes are as follows:

1) The tissue block of the arterial wall is trimmed to clarify the longitudinal axis of the artery. For this, a rectangular piece of the block roughly 8 mm long and 4 mm broad is appropriate.

2) This piece is immersed in the following orcein solution for 3 h at 40°C or overnight at room temperature.

Orcein, natural (Sigma) ....... 0.1 g
70% ethanol ................. 100 ml
35—37% hydrochloric acid ...... 2 ml

This mixture is filtered. It is not necessary to add the surface active agent described previously (Mitsui and Hosoda, 1970).

In this way, the tissue block is stained deep brown.

3) The block is washed in 50% ethanol for 2 min.

4) It is then washed in distilled water for 2 min.

5) It is immersed in 30% aqueous solution of potassium hydroxide (KOH) in a shallow container (Fig. 1) at 45°C for 1.5—2.0 h. The temperature is not allowed to rise above 45°C. The brownish stained tissue block of the artery promptly turns blue in this solution.

6) The tunica intima plus fenestrated membrane is separated from the tunica media using a fine needle and pincette under the dissecting microscope. It is necessary to remove the muscular tissue adherent to the fenestrated membrane as much as possible. However, in this case, it is convenient to keep a small piece of the muscle tissue at the end of the tunica intima since this muscular piece indicates the side of the fenestrated membrane (Figs. 5 and 7), the opposite side being the endothelium of the tunica intima. This procedure must be carefully performed in 30% KOH in a shallow container at room temperature.

7) Next, the fenestrated membrane thus prepared is carefully placed in 20% KOH, and after a few min. transferred to 13% KOH and kept for 1 min., then transferred to

8) 9% KOH for 1 min.

9) 6% KOH for 1 min.

10) 4% KOH for 1 min.

11) 2.5% KOH for 1 min.

12) 1.5% KOH for 1 min.

13) Finally the membrane is placed in 1% KOH in a Petri dish, and immediately floated onto a slide glass using a needle (Fig. 3). It is recommended to spread albumin glycerol on the slide glass beforehand. In this case, it is important to place the fenestrated membrane upwards on the slide glass; otherwise the membrane is covered with the tunica intima and the membrane becomes entirely invisible. Again, it is necessary to spread out the membrane sufficiently on the slide glass under the dissecting microscope.

14) Excess 1% KOH on the slide glass is drained and carefully blotted away.

15) The slide glass is let stand in air for 2 days to affix the fenestrated membrane to the slide glass. This slide glass must not be kept in a 37°C oven since the fenestrated membrane is frequently torn.

16) The membrane is stained with the
orcein solution as usual and differentiation is made in two changes of absolute methanol for 30 seconds each.

17) The membrane is cleared in xylene and mounted in Permount or Canada balsam (Fig. 4).

Results and Discussion

The fenestrated membrane isolated with the above-mentioned technique is illustrated in Figures 5–10. The windows of the fenestrated membrane are clearly recognizable against the brownish background under the microscope. This membrane in the cross section of the artery generally looks wavy (Figs. 13, 15 and 17), while it was flat when isolated and spread on the slide glass by the above-mentioned technique. The windows of the membrane were round, oval or elliptical in shape and showed a variety in sizes even in the same artery. The long axis of the oval or elongated windows generally ran parallel to the longitudinal axis of the artery. It might be considered that the data of the fenestrated membrane thus prepared are unreliable since the influence of heated 30% potassium hydroxide solution should be very strong on the elastic membrane. This is experimentally true if maceration with the potassium hydroxide solution is performed for more than 3 hours. In other words, the maceration should be performed within a definite time to obtain accurate results. Excessive maceration impairs the elastic tissue, and as a result the stained tissue block of the arterial wall is decolorized and becomes extremely soft, and isolation of the fenestrated membrane becomes technically difficult. However, Mitsui, one of the present authors, has confirmed in unreported experiments that the purified elastin (produced by Sigma, U.S.A.) was not dissolved completely in the above-mentioned potassium hydroxide solution after a week or longer. The essential nature of this phenomenon will need further investigation.

A certain difference in the structure of the fenestrated membrane seems to exist between the cerebral artery and other medium-sized arteries as shown in Figures 6, 8, 10, 16 and 18. The windows in the membrane of the cerebral artery appear to be smaller in size than those of other muscular arteries in the human body. However, the significance of this difference in the windows is not clear. This difference was already reported by Hassler (1962) who observed paraffin sections cut tangentially to the surface of the arterial wall. Age changes in the fenestrated membrane were also suggested by the present study. The windows of the fenestrated membrane in old people were extremely large (Fig. 14).

In our method, it is important to separate smooth muscular tissue adequately from the tunica intima after maceration with potassium hydroxide; otherwise the fenestrated membrane is hidden under the muscular tissue and becomes undiscernible. The smooth muscle fibers in this case become very soft and swell intensely although they retain the color of orcein very clearly. Smooth muscle fibers of the tunica media are thought to adhere to the internal elastic membrane (Lang, 1966) or bridge the space between adjacent elastic laminae in the elastic arteries (Kawase, 1973).

One of the characteristics of our method is the way in which the fenestrated membrane was treated after isolation with strong alkaline solution. If the membrane thus isolated is immediately placed in distilled water, the membrane curls up completely due to its elasticity (Fig. 2), making it extremely difficult to spread and attach the membrane to the slide glass. For this reason the membrane isolated with
strong alkaline solution was transferred stepwise to gradually diluted alkaline solutions in shallow containers (Figs. 1, 2 and 3).

In areas of intimal thickening which may occur in pathological arteries, a layered plexus of elastic fibers frequently exists (Figs. 11 and 12), and the defective internal elastic membrane may permit passage of smooth muscle fibers from the tunica media to the tunica intima. Consequently, it becomes difficult in these areas to isolate a single fenestrated membrane distinctly. It has been reported that, in atherosclerotic plaques, the amino acid composition of elastin is altered and elastic fibers are split and fragmented (Kohn, 1977). In smaller arteries the isolation of the fenestrated membrane is generally difficult since their walls are too thin. Again, arterioles in the human retina lack an internal elastic membrane (Hamai, 1971).

Usefulness of other chemicals for maceration of the arterial wall has been examined. However, better results than those with potassium hydroxide, could not be obtained with other chemicals such as sodium hydroxide, acetic acid, formic acid or EDTA. Elastic fibers are resistant to boiling water, acids and alkalis. In the present study fenestrated membrane consisting of elastic fibers was isolated by taking advantage of its resistance to alkali under conditions that destroy other tissue constituents. The elastic fibers are also resistant to digestion by collagenase. Treatment with the enzyme, collagenase, will digest collagenous fibers from tissue sections and leave elastic fibers intact. Gessner (1953) and Lang (1965) demonstrated a fenestrated membrane of the human posterior tibial artery after digestion with collagenase for 36 hours. By this method a large membrane was isolated and many windows, both large and small, could be observed under the microscope. Suwa (1976) tried to isolate elastic membranes by immersing frozen sections of arterial wall in 2% caustic soda (NaOH) for 1 to several days. With this method, however, it is not always easy to decide the immersing time in caustic soda for maceration. Lansing (1959) pointed out that the breakdown of elastic tissue might be a factor in age changes, i.e., the deterioration of the elastica of arteries is age dependent. Karrer (1961) described in his electron microscopic study that interruptions and rarefactions of the elastic membranes might occur in the arteries in age changes. Dees (1923) emphasized that the usually consulted descriptions of the elastic lamina of arteries showed considerable variations and that the lamina is not a single plate consisting of elastic fibers. It was suggested by the present study that the above-mentioned windows varied in shape according to the sort of artery and that fibrous components in varying amounts were found to adhere to the fenestrated membrane. It is well known that the internal elastic membrane folds in empty or contracted arteries, changing the shapes of the windows in the membranes (Arey, 1968). Suwa (1970) noted that the shape of the windows might be different between fresh arteries and formalin-fixed ones. In other words, shortening of the length of the artery by fixation might decrease the number of elongated windows with long axes running parallel with the longitudinal axis of the artery. Conversely, the round windows might increase in number.

The biological significance of the windows of the internal elastic membrane is difficult to determine. However, there is no doubt that the structure of the internal elastic membrane differs with the caliber and thickness of the arteries. It is thus desirable for a clearer understanding of morphology of the fenestrated membrane
to be achieved in the future through detailed histological studies for which the present paper may provide a basis.

References

Explanation of Figures

Plate I

Fig. 1. Tissue blocks of a muscular artery stained with orcein are placed in a 30% KOH solution in a shallow container. Close-up view. X 2.5

Fig. 2. The tissue blocks in Figure 1 start curling up due to their elasticity (arrow) immediately after water is added to the KOH solution. This quality of the elastic tissue makes it difficult to obtain a sufficiently spread specimen of the internal elastic membrane as described in the text. Close-up view. X 2.5

Fig. 3. Diagram illustrating how to float an isolated fenestrated membrane (F) onto a slide glass using a needle. One per cent KOH solution is in this Petri dish.

Fig. 4. Isolated fenestrated membranes mounted in Permount or Canada balsam.
Plate II

Fig. 5. An isolated fenestrated membrane of the right radial artery of a 42-year-old man who died by hanging himself. M (Smooth muscular fibers of the tunica media), Arrow (Longitudinal axis of the artery). Close-up view. X 16

Fig. 6. A high magnification micrograph of the same specimen as in Figure 5. Arrow (Longitudinal axis of the artery). X 330

Fig. 7. An isolated fenestrated membrane of the right radial artery of a 41-year-old man who died of a cerebral hemorrhage. M (Smooth muscular fibers of the tunica media), Arrow (Longitudinal axis of the artery). *(Artificial fissure). Close-up view. X 37

Fig. 8. A high magnification micrograph of the same specimen as in Figure 7. Arrow (Longitudinal axis of the artery). X 330

Fig. 9. A spread fenestrated membrane of the left radial artery of an 11-year-old boy who died from suffocation. Arrow (Longitudinal axis of the artery). X 330

Fig. 10. A spread fenestrated membrane of the left radial artery of a 19-year-old woman who died from suffocation. Arrow (Longitudinal axis of the artery). X 330

Fig. 11. Cross section of the right middle cerebral artery of a 52-year-old man who froze to death. Paraffin section stained with orcein. L (Lumen of the artery), I (Intimal thickening indicating a pathological process), Arrow (Split internal elastic membrane). In such cases as this artery, isolation of the fenestrated membrane is difficult due to the complexity of the layered plexus of elastic fibers. X 165

Fig. 12. A spread fenestrated membrane (F) and elastic fiber plexus in the tunica intima (P) of the right radial artery of a 44-year-old woman whose cause of death was unknown. P (Elastic fiber plexus in the tunica intima). *(Artificial fissure). A finding like this is rarely observed in normal muscular arteries. Arrow (Longitudinal axis of the artery). X 330
Plate III

Fig. 13. Cross section of the left radial artery of a 102-year-old woman who died of senility. H-E stain. L (Lumen of the artery), E (Internal elastic membrane = fenestrated membrane). X 200

Fig. 14. A spread fenestrated membrane of the same artery as in Figure 13. Orcein stain. Extremely large windows can be seen. It is shown in this case that the long axis of the windows is not always in parallel with the longitudinal axis of the artery (arrow). X 330

Fig. 15. Cross section of the left radial artery of a 12-year-old boy who died from suffocation. H-E stain. L (Lumen of the artery), E (Internal elastic membrane = fenestrated membrane). X 660

Fig. 16. A spread fenestrated membrane of the same artery as in Figure 15. Many elastic fibers from the tunica media adhere to the fenestrated membrane. Orcein stain. Arrow (Longitudinal axis of the artery). X 330

Fig. 17. Cross section of a branch of the right middle cerebral artery of a 52-year-old man who froze to death. Orcein stain. L (Lumen of the artery), E (Internal elastic membrane = fenestrated membrane). X 60

Fig. 18. A spread fenestrated membrane of a branch of the left middle cerebral artery of a 55-year-old man who died of cardiac insufficiency. Many small windows can be seen. Orcein stain. Arrow (Longitudinal axis of the artery). X 330