Studies on Solute Transfer in the Vascular Endothelium*

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ABSTRACT

Barium chromate, Prussian blue, and cobalt-cobalticyanide can be precipitated \textit{in vivo} in the endothelium of mesenteric vessels by injection of the appropriate anions into the blood stream, and topical application of the precipitating cations to the exposed mesenteries of mice and frogs. Precipitation in the endothelium occurs in the form of a diffuse fine punctate precipitate, and also as continuous lines demarcating endothelial cell outlines. A striking feature is the frequent occurrence of this type of precipitation in a tapering zone downstream from mural thrombi in the veins.

INTRODUCTION

Several possibilities exist whereby substances can pass through the endothelial cell sheet. These include (1) through the plasma membranes of the endothelial cells; (2) through sites in the endothelium where the plasma membrane of the endothelial cell is lacking, such as the interendothelial cell junctions or intercellular gaps, and perforations or canals in the endothelial cells; (3) by way of phagocytic or pinocytic activity of the endothelial cells; (4) combinations of the preceding possibilities.

Methods are described in this paper for the \textit{in vivo} study of sites of transfer in the endothelium of the mesenteric blood vessels of frogs and mice.

Experimental Methods

The approach decided upon was to cause the precipitation of a substance in the course of its passage across the endothelial cell barrier. This was done by injecting a solution containing a precipitable component into the blood stream, and applying a solution containing its precipitant to the outside of a blood vessel. This procedure is feasible only for thin tissues, such as the mesentery, where the precipitating agent can penetrate rapidly through extracellular spaces to the walls of the blood vessels.

Three combinations were used. In the first, sodium ferricyanide was injected intravascularly or intramuscularly and ferrous sulfate was applied locally with the formation of Prussian blue where they came in contact. The second combination was intravascular potassium cobalticyanide and extravascular CoCl$_2$, forming cobalt-cobalticyanide, which after fixation of the tissue was converted into CoS with yellow ammonium sulfide. The third was intravascular sodium chromate and topical barium chloride. The intestinal mesenteries of anesthetized 70 to 100 gm. frogs \textit{(Rana catesbiana)} and 30 to 40 gm. mice were exteriorized for observation as described by Chambers and Zweifach (1).

In most experiments normally fed mice were used. However, observations were also made on mice starved for 3 days. The starvation facilitated \textit{in vivo} visualization of the intestinal vessels by decreasing fat deposits surrounding the blood vessels.

The precipitation of Prussian blue in the endothelium was brought about as follows: For the frog 0.2 to 0.3 ml. of a 1.7 per cent solution of sodium ferricyanide, isotonic with amphibian Ringer, was injected intravascularly; while for the mouse, 1 ml. of a 2.0 per cent solution, isotonic with mammalian Ringer, was injected intramuscularly into the four limbs. Starting immediately after the ferricyanide had been injected, a freshly prepared solution of ferrous sulfate at pH 4.5-5.0 was applied topically for 2 to 8 minutes. For the frog, a 2.8 per cent solution of FeSO$_4$·7H$_2$O, isotonic with amphibian Ringer was applied, while for the mouse, a 4.2 per cent solution, isotonic with mammalian Ringer at 37°C., was used.

In another series of experiments, cobalt-cobalti-
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cyanide was precipitated in the endothelium as follows: immediately after exposure of the mesentery, a solution of potassium cobalticyanide prepared according to Vanino (2) was injected. For the frog, 0.5 ml of a solution containing equal parts of amphibian Ringer and 2 per cent potassium cobalticyanide was injected intravascularly; while for the mouse, 1 ml of the 2 per cent potassium cobalticyanide was injected intramuscularly into the limb musculature. Immediately after the cobalticyanide had been injected, a solution containing one part 2.5 per cent CoCl₂·6H₂O and two parts of the appropriate Ringer's solution was applied topically. Application of the CoCl₂ Ringer solution at 37°C to the mouse mesentery was continued for periods of 5 to 10 minutes, since an initial temporary arrest of flow occurs in all the small blood vessels.

Precipitation of BaCrO₄ in the endothelium was brought about by injecting 0.5 ml of 1.2 per cent Na₂CrO₄ intravascularly into the frog, and 0.5 ml of 1.4 per cent Na₂CrO₄ intravascularly into the mouse. A 1.5 or 1.8 per cent solution of BaCl₂, for the frog and the mouse respectively, was immediately applied topically for ~ 2 minutes. Since the barium ion causes contraction of the vascular and intestinal smooth muscle, the circulation in the mesenteric vessels is rapidly impaired, especially in the mouse.

Immediately after completion of the topical administration (for each of the three combinations of ions used), the blood was washed out of the mesenteric vessels by injecting Ringer's solution into the aorta. The mesentery was then stretched over a grooved stainless steel ring and tied on with thread. The preparations were washed in Ringer's, fixed in Camoy's fluid, dehydrated, and mounted in cedar oil for immediate observation. Application of the CoCl₂ Ringer solution at 37°C to the mouse mesentery was continued for periods of 5 to 10 minutes, since an initial temporary arrest of flow occurs in all the small blood vessels.

Permanent mounts were made by cutting the stretched membranes off the steel rings under xylene, floating them onto slides and mounting in balsam.

RESULTS

The formation of the precipitates in the endothelium can be observed directly in the living preparation while the blood circulation still continues, although the cobalt-cobalticyanide precipitate can be visualized only by phase microscopy. Fixation preserves all structural details.

BaCrO₄.—In both frog and mouse mesenteries widespread precipitation occurs in the endothelium almost instantaneously after application of the reagents. In all vessels with continuing flow, the endothelial cells are outlined by a continuous line of precipitate (Figs. 1 to 4). In addition, a crystalline precipitate forms in the cytoplasm of occasional individual cells, leaving the nuclear region free of precipitate (Figs. 5 and 6).

The Prussian Blue and Cobalt-Cobalticyanide Methods.—In contrast to the generalized nature of the barium chromate precipitation, the complex-ion methods result in a spotty reaction. At variable periods after topical application of the reacting cations to the mouse mesentery, small thrombi appear and become attached to the walls of the larger veins. These frequently originate at sites where the smaller tributary veins empty into the larger vessels. Precipitate was deposited in the endothelium in the region of the thrombi and downstream from the thrombi for a considerable distance, as gradually tapering triangular-shaped areas (Fig. 7). The deposition of precipitate in the endothelium downstream from a thrombus follows the flow pattern. This is shown in Fig. 12, in which a thrombus is located in the tributary vein (see arrow). Examination of the stained zones under high magnification reveals that the precipitate is finely punctate and the outlines of endothelial cells are sharply delineated (Figs. 10 and 11).

In the frog, the Prussian blue reaction is essentially similar, except that thrombi of the type seen in the mouse are infrequent. Instead, at certain regions a single layer of white cells and thrombocytes become attached to the endothelium of the larger veins. Precipitate is deposited between the neighboring white cells, resulting in the formation of plaques. Regions, extending a considerable distance downstream from a plaque, are occasionally observed in the endothelium which contain a punctate precipitate and cell outlines delineated with Prussian blue (Fig. 14). The picture is identical to that observed in the mouse in the trail of a thrombus. Application of the cobalt-cobalticyanide method in the frog does not induce plaque formation.

In the small vessels and capillaries of the frog and mouse, using both complex-ion methods, endothelial cell outlines appear occasionally, and in many regions a dense punctate precipitate covers the
luminal surface of the endothelium (Figs. 8, 9, 13, and 15). Where endothelial nuclei are present, the precipitate covers the luminal surface of the nuclei. Frequently, precipitation occurs in the endothelium at sites where the minute vessels branch, forming a band encircling one of the branching vessels at its origin (Fig. 8). The distribution of the precipitate in these areas is occasionally diffuse and not in discrete particles.

In order to determine the injurious effects of the three combinations of reagents used, 0.5 to 1 ml. of 1 per cent trypan blue in Ringer's was injected intravascularly, and the preparations observed in vivo. Nuclear staining by this method indicates cell damage (3). The precipitating reagents when injected, or applied separately, did not induce nuclear staining. However, when the reagents were injected and applied in combination, the endothelial nuclei were invariably stained in all cells which contained, or were outlined by precipitate. Beyond the margins of areas containing precipitate, nuclear staining ceased abruptly.

DISCUSSION

In all three methods, the endothelial cells were outlined by the precipitate. Initial deposition of precipitate must have occurred while the cells were still living, since application of the reagents alone did not induce nuclear staining with trypan blue. Cell damage occurred only after the precipitate had formed. These results indicate that passage of ions can occur through the intercellular regions, as previously proposed (4, 5). The evidence is most convincing in the barium chromate method, due to the practically instantaneous appearance of the precipitate and the generalized nature of the reaction.

Whether or not the punctate precipitate which occurs within or covering the endothelial cells indicates sites of transfer cannot be finally decided at this stage. The punctate precipitate in the BaCrO₄ method occurs frequently in isolated cells. Since this precipitate occurs concomitantly with the intercellular precipitation, the possibility cannot be ruled out that the cells containing punctate precipitate had been injured as the result of the intercellular precipitation.

The punctate precipitates obtained in the ferroferricyanide and cobalt-cobalticyanide methods occur frequently without intercellular precipitation and show a definite relationship to thrombi, formed largely of platelets. In this respect, these methods differ from the BaCrO₄ method, in which thrombus formation occurs to only a slight degree. One possible explanation for the occurrence of punctate precipitate downstream from the thrombi is that platelet debris is carried downstream and becomes attached to the endothelial surface, providing a protective matrix in which precipitate can build up without being carried away. In this case, the precipitate would reveal transfer sites in the endothelium. The granules, however, having been formed by accretion, would not necessarily reveal any underlying structural dimensions.

Another possible alternative is that a diffusible factor is liberated from a thrombus, causing increased permeability of the endothelial cells downstream. However, attempts to increase permeability by applying histamine did not promote a similar reaction.

Since the thickness of the endothelial cell lies at the limits of the resolving power of the light microscope, a final decision about the relationship between the precipitation in the endothelium and the morphological structures involved awaits investigation with the electron microscope.

BIBLIOGRAPHY

EXPLANATION OF PLATES

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Fig. 1. Frog mesentery. Endothelium of vein, showing deposition of BaCrO₄ in the intercellular regions. Dark-field illumination. × 84.

Fig. 2. Same as Fig. 1. Note the long endothelial nuclei. Phase contrast. × 354.

Fig. 3. Frog mesentery. Small venule with deposition of BaCrO₄ in the intercellular regions of the endothelium, and as a punctate precipitate in the endothelial cells. Phase contrast. × 354.

Fig. 4. Mouse mesentery. Endothelium of vein, showing deposition of BaCrO₄ in intercellular regions. Phase contrast. × 354.

Fig. 5. Mouse mesentery. Individual endothelial cell in endothelium of vein, containing precipitate of BaCrO₄. Nuclear area free of precipitate. Phase contrast. × 354.

Fig. 6. Same as Fig. 5. The nucleus is clearly outlined. Phase contrast. × 780.
FIG. 7. Mouse mesentery, showing Prussian blue reaction in large vein. Two thrombi ($T_1$ and $T_2$) are to right, attached to walls of main branches. Blood flow had been from right to left (horizontal arrow). Amorphous precipitate in matrix of thrombi. Gradually tapering trails of punctate precipitate in endothelium downstream from thromb. For thrombus $T_1$, trail extends almost to left border, and for thrombus $T_2$, trail extends across field, and beyond the border. × 42.

FIG. 8. Mouse mesentery. Surface view of small vein which traverses field. Punctate precipitation of Prussian blue in endothelium; no cell outlines. Also visible is a band-shaped deposit of Prussian blue in endothelium of capillary (see arrow). These band-shaped deposits characteristically occur immediately beyond the point where a capillary branches. × 459.

FIG. 9. Same as Fig. 8, but showing the small vein in optical section, with punctate precipitate located in endothelium. Note particularly region marked by arrows, where the edge of the vessel is accurately in focus. × 459.

FIG. 10. Mouse mesentery, showing Prussian blue reaction in endothelium of large vein, at border of a stained region. Punctate precipitate in upper half of figure. Cell outlines delineated by continuous lines of Prussian blue precipitate in middle portion, enclosing punctate precipitate. Toward lower side of figure, only pale endothelial cell outlines visible. × 459.

FIG. 11. Mouse mesentery. Surface view of small vein, showing deposition of Prussian blue in endothelium as punctate precipitate, also as continuous lines outlining borders of endothelial cells. The diameter of the vein indicated by vertical arrow is shown at left. × 459.
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Fig. 12. Mouse mesentery, showing precipitate of cobalt sulfide in large vein. Thrombus formation in tributary vein (arrow), causing an extensive tapering trail of precipitate in large vessel downstream from mouth of tributary. X 128.

Fig. 13. Mouse mesentery, showing granular precipitate of cobalt sulfide in endothelium of venule and capillaries. X 275.

Fig. 14. Frog mesentery, showing punctate precipitate of Prussian blue and deposition of precipitate in intercellular lines in endothelium of a large vein. X 615.

Fig. 15. Frog mesentery, surface of a capillary, showing deposit of CoS granules in endothelium. Note absence of granules inside endothelial nuclei (E₁ and E₂), and the presence of granules over the luminal surface of endothelial nucleus. The lower third of the capillary surface is out of focus. Fixed in osmic acid solution (6). X 1000.
(Mende and Chambers: Solute transfer in vascular endothelium)