
Platelet-induced vitreous membrane formation*

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The role of extravascular coagulation in vitreous membrane formation was investigated in rabbits and owl monkeys. Eyes injected with platelet-poor plasma did not develop vitreous membranes. In contrast, the injection of platelet-rich plasma and gel-filtered platelet preparations into the vitreous cavity resulted in immediate membrane formation. These vitreous membranes were only slowly and incompletely removed by a low-grade histiocytic response. Membrane formation could be inhibited by prior incubation of the platelet preparations with acetylsalicylic acid or by homogenizing the platelets. Intact platelets with normal functional capacity appear to be important in the instantaneous formation of membranes when blood components escape into the vitreous cavity.

Key words: vitreous membranes, platelet-rich plasma, gel-filtered platelets, platelet aggregation, macrophage, electron microscopy, vitreous hemorrhage, ocular inflammation.

The serious clinical consequences of vitreous hemorrhage and intraocular inflammation have prompted a number of

studies of vitreous membrane formation in animals.¹⁻⁴ These studies have demonstrated that whole blood, erythrocytes, saccharated iron oxide, or leukocytes may stimulate membrane formation in the vitreous cavity of rabbits. These membranes only develop after a period of several weeks, and contain large numbers of proliferating fibroblasts.

However, clinical observations of the vitreous cavity after vitreous hemorrhage or during the course of severe intraocular inflammation suggest that membranes may also appear early. In both these situations, blood components are extravasating into the vitreous. Immediate vitreous membranes could develop because of plasma clotting, or aggregation of platelets, or both. These experiments document the effects of injecting platelets with or without plasma into the vitreous cavity.

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Materials and methods

Platelet preparations. Pooled arterial blood was collected from the ears of adult rabbits into plastic tubes containing an anticoagulant solution (1 part 3.8 per cent sodium citrate to 9 parts whole blood). Platelet-rich plasma (PRP) was prepared by centrifuging this fresh citrated blood at 50 g for 10 minutes at 23° C. and taking only the upper third of the supernatant. Platelet counts were performed on an automated Coulter counter, Model B; they varied from 500,000 to 1,500,000 per cubic millimeter. Platelet-poor plasma (PPP) was prepared from identical samples of fresh citrated blood by centrifugation at 10,000 g for 15 minutes at 23° C. PPP samples contained less than 20,000 platelets per cubic millimeter. One plasma sample containing 100,000 platelets per cubic millimeter was obtained by centrifugation at 1,000 g for 15 minutes at 23° C. Platelet preparations essentially free of plasma proteins (gel-filtered platelets) were obtained by gel filtration using a modification of the method of Tangen, Berman, and Marfey.⁵ A 4 by 2.5 cm. Sepharose 2B column was prepared and equilibrated with a buffer (0.02 M. Tris-HCl, 0.15 M. NaCl, and 0.001 M. MgCl₂, pH 7.4). After several washes with the buffer, 5 ml. of PRP was added to the top of the column. Two milliliter fractions of eluate were then collected from the column in separate plastic tubes. Visibly cloudy suspensions of platelets appeared after the void volume of the column and before the protein fractions. Absence of plasma proteins in the platelet samples was confirmed by low spectrophotometric absorbance at 280 nm. These protein-free, gel-filtered platelet preparations were suspended in Tris-NaCl buffer; they contained 200,000 to 750,000 platelets per cubic millimeter. Some PRP and gel-filtered platelet preparations were incubated with 2×10^{-4} M. acetylsalicylic acid. These preparations failed to aggregate *in vitro* when adenosine diphosphate (final concentration 1×10^{-6} M.) was added, as measured by the method of Born,⁶ using a chrono-log aggregometer. Additional platelet preparations were washed three times in the above Tris buffer and homogenized in a glass tube with a Teflon pestle which was rotated by motor at 1,700 r.p.m. for 5 minutes. The homogenate was centrifuged at 1,000 g for 15 minutes and the pellet discarded. The supernatant was used for vitreous injections.

Animal experiments. One tenth milliliter samples of fresh PRP, PPP, gel-filtered platelets, and preparations homogenized or incubated with acetylsalicylic acid were injected into the central vitreous region of a total of forty rabbit eyes, by puncturing the sclera 4 mm. posterior to the limbus with a 25-gauge needle. PRP and gel-filtered platelets prepared from rabbit blood were



Fig. 1. Vitreous membrane in a rabbit eye immediately after injection of 0.1 ml. of platelet-rich plasma.

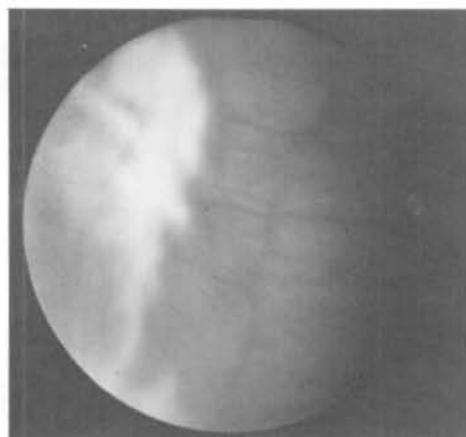


Fig. 2. Vitreous membrane in an owl monkey eye immediately after injection of 0.1 ml. of rabbit platelet-rich plasma. The membranous aggregate is loose in appearance and mobile in the fluid vitreous.

also injected into the fluid central vitreous of the eight owl monkey eyes. The injections were performed under indirect ophthalmoscopic control in order to observe immediate vitreous changes. Photographs were taken of any membranes formed in the vitreous cavity with a portable Kowa fundus camera. The injected eyes were studied clinically by slit lamp biomicroscopy with a contact lens and indirect ophthalmoscopy for periods up to six months.

Light and electron microscopy. Eyes in which membranes formed were enucleated at 5, 10, and 60 minutes, at two weeks, and at four months

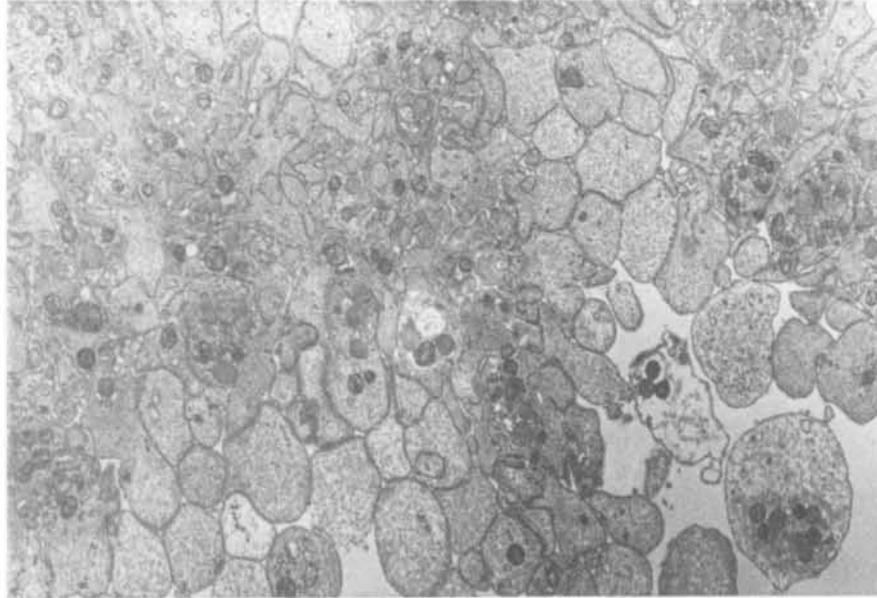


Fig. 3. Vitreous membrane five minutes after platelet-rich plasma injection. Individual aggregated platelets are degranulated, and show varying degrees of degeneration. Few intracellular organelles remain ($\times 15,000$).

after vitreous injection. By means of an operating microscope, individual newly formed membranes were removed from the opened vitreous cavity. Membrane samples for light and electron microscopy were fixed by 4 per cent glutaraldehyde, washed in phosphate buffer containing 0.4 M. sucrose, and postfixed by 1 per cent osmium tetroxide for one hour. Samples were dehydrated and then embedded in epon. Sections cut for light microscopy were stained with toluidine blue. Thin sections were cut with an LKB ultramicrotome, and were examined and photographed with a Phillips EM200 electron microscope.

Results

Clinical observations. Platelet-rich plasma caused discrete membranous strands to form immediately in the vitreous cavity of all rabbit eyes injected (Fig. 1). The membranes had a dense white gelatinous appearance, with edges fanning out into the vitreous gel. In contrast, platelet-poor plasma caused no visible membranous reaction when injected into the vitreous cavity. Plasma containing 100,000 platelets per cubic millimeter caused fine vitreous strands which were barely visible.

Gel-filtered platelet preparations (without plasma proteins) also resulted in the instantaneous formation of discrete, white membranes, when injected into the vitreous cavity of rabbit eyes. These membranes were similar in appearance to those induced by platelet-rich plasma (Fig. 1).

Vitreous membranes also formed immediately when the same rabbit platelet preparations, with or without plasma proteins, were injected into the fluid central vitreous of owl monkeys (Fig. 2). In monkeys, the injected samples diffused further within the vitreous before condensing, and the membranes formed were fibrillar and less compact than those formed in rabbits.

Postoperatively, all eyes manifested minimal signs of inflammation. A mild flare was evident for two to three days only at slit lamp examination, whether vitreous membranes had formed or not. Later, occasional cells and pigmented opacities were seen floating in the vitreous around the newly formed membranes. By two weeks, the membranes appeared thinned and less

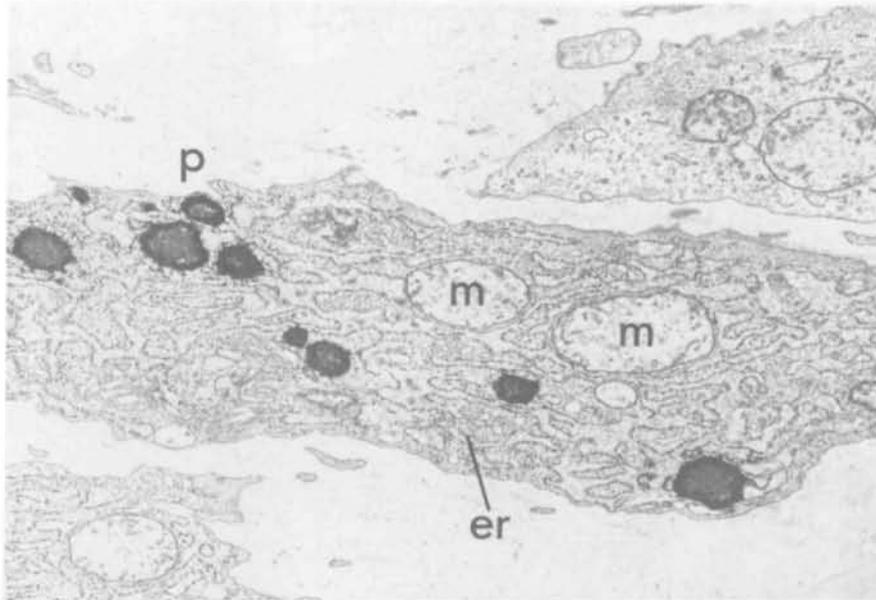


Fig. 4. Macrophages in vitreous membrane induced by platelet-rich plasma after two weeks. Endoplasmic reticulum (er), mitochondria (m), pigment granules (p) ($\times 25,000$).

gelatinous, but they remained mobile in the central vitreous. Membranes induced by PRP slowly decreased in size, but never disappeared entirely. In all eyes followed, fine discrete membranous strands were still present four to six months after injections. In contrast, membranes induced by gel-filtered platelets were usually no longer visible by six weeks. However, during this period only a slight inflammatory reaction was clinically visible. No eyes showed any signs of vitreous traction on the retina at any stage during the period of clinical observation.

When homogenized platelet preparations were injected into the vitreous cavity, no visible strands formed. Injection of acetylsalicylic acid-treated platelet preparations caused either faint strands or no visible membranes.

Light and electron microscopy. Microscopic sections of a PRP-induced membrane which was removed from the eye within five minutes showed the typical mosaic pattern of aggregated platelets

(Fig. 3).^{7,8} The characteristic dense granules had already undergone the release reaction. Most intracellular organelles were already disrupted by this time, although scattered mitochondria and lysosomal granules persisted. The platelet cell membranes were intact, with a 200 to 300 Å gap between the individual cells, and characteristic pseudopod formation was evident. No electron-dense fibrin nor collagen was found within the membranes examined. One hour after injection, the individual platelets showed signs of disintegration of their limiting plasma membranes. At two weeks, light microscopy showed an amorphous strand or tissue containing a few scattered cells. Electron microscopy showed these cells to have an abundant rough endoplasmic reticulum and prominent pigment granules (Fig. 4). These cells were probably macrophages rather than fibroblasts. There was no evidence of collagen formation. At four months, no cells were found in the white, mobile strands (Fig. 5). Pigment granules were scattered along the edges of

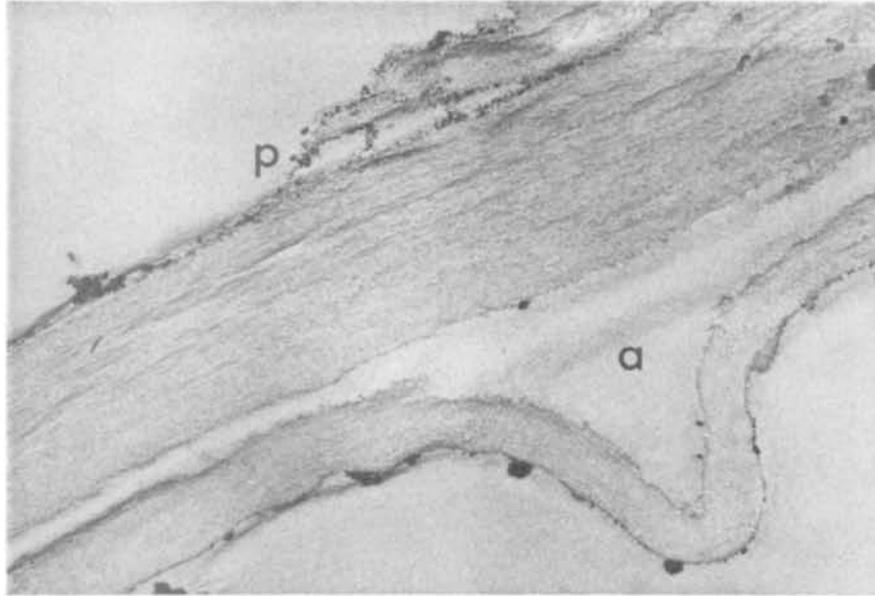


Fig. 5. Vitreous membrane induced by platelet-rich plasma at four months. Note pigment granules (p) along the edges and the absence of cells. Matrix is not identifiable by electron microscopy alone. Cleavage (a) is an artifact ($\times 46,000$).

the central amorphous material. This material showed none of the electron microscopic characteristics of collagen or fibrin, and its composition remained undetermined.

Membranes induced by gel-filtered platelets were also found to be composed of aggregated platelets upon electron microscopic examination. At five minutes, the cell borders of the aggregated platelets were less distinct than those of PRP membranes. All intracellular organelles had already degenerated. Insufficient membranous material remained for microscopic processing two weeks after gel-filtered platelet injections.

Discussion

These experiments demonstrate that visible membranous structures can form immediately when blood components come into contact with the vitreous. Fresh citrated plasma does not cause this reaction, unless it also contains platelets. Furthermore, platelet suspensions can form vitreous membranes in the absence of plasma

proteins. If the platelets are homogenized or platelet function is inhibited with acetylsalicylic acid, vitreous membranes are not formed. Clearly, intact functional platelets are necessary for this reaction.

The immediate electron microscopic appearance of these vitreous membranes is identical to that seen when platelet preparations are aggregated *in vitro* by collagen suspensions, adenosine diphosphate, or latex particles.^{7, 8} In addition, in experiments which will be reported elsewhere,⁹ we have found that vitreous collagen fractions will aggregate platelets *in vitro*. Thus, the immediate formation of these membranes *in vivo* is probably due to collagen-stimulated platelet adhesion and aggregation.^{10, 11}

Vitreous membranes induced by platelet preparations cause minimal ocular inflammation and are inefficiently removed. As the aggregated platelets degenerate, a mild macrophage response is generated as seen microscopically at two weeks (Fig. 4). Why removal of the deposited material tends to be incomplete is not clear. From this aspect, platelet membranes resemble

vitreous floaters seen clinically since they may also be incompletely removed. Presumably, degenerated material in the vitreous can cease to attract macrophages.

The ocular reaction to these membranes is very different from that when red or white blood cells are injected into the rabbit vitreous cavity in large numbers.¹⁻⁴ Following the injection of erythrocytes or leukocytes, continued inflammation is usually associated with a proliferation of fibroblasts. Contraction of these fibroblastic membranes later may lead to retinal detachment. Platelet-induced vitreous membranes do not result in fibroblastic proliferation, and the membranes show no clinical signs of contraction, even after six months.

However, possible clinical examples of platelet-induced vitreous membranes are seen. Fine, white, mobile strands are sometimes visible in the vitreous cavity immediately after penetrating injuries, in eyes subject to small hemorrhages, such as those with signs of early proliferative diabetic retinopathy, and in eyes with intraocular inflammation.

The present experiments shed no light on the relative importance of platelets and fibrin deposits in the formation of early vitreous membranes. Citrated blood was used here, and it prevents coagulation of plasma proteins, at least partly, by binding calcium.¹² Platelet adhesion to collagen and the subsequent release of adenosine diphosphate triggers the enzymatic cascade of intravascular coagulation.¹⁰ Platelets may well be important also in the process of fibrin deposition in the vitreous cavity.

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