To the Editor:

A recent exchange of letters appearing in Blood was of particular interest to our group. Lemons et al. had published a report in Blood that stimulated a letter from Dr. Morgenstern, to which one of the authors responded. Dr. Morgenstern had no objections to the biochemical results of the article. Rather, he was concerned that the authors chose to discuss their molecular findings in the context of a morphological design with which Dr. Morgenstern disagreed. In the introduction to their article the authors stated: “In one model of platelet activation, these stimuli (collagen, thrombin, and ADP) trigger morphological changes in the platelet resulting in the apparent movement of the secretory granules to the center of the cell and their subsequent fusion with the surface connected canalicular system (SCCS).”

Dr. Morgenstern objected to this model. His letter stated, “The disadvantage with this model is that the fusion of membranes of secretory organelles with membranes of the SCCS (i.e., open canalicular system, OCS) has never been demonstrated. In contrast, it was clearly

Platelet Secretory Process

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Dr. Morgenstern objected to this model. His letter stated, “The disadvantage with this model is that the fusion of membranes of secretory organelles with membranes of the SCCS (i.e., open canalicular system, OCS) has never been demonstrated. In contrast, it was clearly
shown in studies using rapid freezing with a time resolution in the range of milliseconds to capture fusion events, the secretory organelles (granules and dense cored bodies) fuse with the plasma membranes when the platelets were stimulated before. He bolsters his argument with several citations of his own work and that of others, including one of our publications showing that α granules in bovine platelets that lack the OCS of human cells do fuse with the plasma membrane to secrete products to the exterior following activation in suspension.

The first paragraph of the responding letter by Whiteheart answers the concerns of Dr Morgenstern very well. He points out: "It is well established that, subsequent to stimulation, human platelets undergo a series of morphological rearrangements that include the formation of pseudopods and centralization of secretory granules. The process culminates in the release of granule contents into the extra platelet space. . . ."

What Whiteheart is saying, in essence, is that it doesn’t matter whether secretory organelles fuse directly with the plasma membrane or channels of the OCS because they are the same membranes. The network of surface membrane invaginations making up the OCS is unique. It is not found in any other type of blood cell. Behnke’s early studies and the work of other investigators have shown that OCS channels are not only continuous with the cell surface but are identical morphologically. That the platelet surface and linings of the OCS are identical has also been shown by ultrastructural immunocytochemistry. Monoclonal and polyclonal antibodies, together with immunogold techniques, have shown that the exposed surface and OCS channels in frozen thin sections are uniformly covered by GPIIb/IIIa and GPIb/IX/V receptor complexes.

Morgenstern did not appear concerned about whether the SCCS and exposed surface are identical membranes. He points out: "It was concluded (ie, from his studies) that the fate of the SCCS during stimulation is to become evaginated within seconds to allow the surface enlargement necessary for formation of pseudopodia or spreading. Fully stimulated platelets do not show SCCS, but do show the membranes of degranulating organelles." His point seems to be that OCS channels return to the exposed surface in the earliest stages of shape change after activation and are unavailable for fusion with α granules and dense bodies to facilitate secretion. These observations are incorrect.

The SCCS is evaginated during platelet spreading on surfaces, but the process takes minutes, not microseconds, and elements of the SCCS remain in over 20% of fully spread platelets. Activation in suspension does not cause evagination of a significant number of OCS channels. Channels of the OCS do become dilated in platelets activated in suspension, and may appear to be part of the exposed surface, but are not evaginated. Had Morgenstern carefully read the articles from our laboratory cited in his letter, and others referred to in those citations, he would not have made the statements he has on this subject.

Nor would Morgenstern have made the comment that fusion of membranes of secretory organelles with membranes of the OCS has never been shown. The reports from our laboratory that he cited, and others, answer this concern quite well. In one of the experiments, platelets in suspension were combined with 20-nm colloidal gold particles coated with fibrinogen (Fgn/Au), then exposed to thrombin for 1, 3, and 5 minutes without stirring. At these intervals the platelets were fixed, frozen, and frozen thin sections prepared. The sections were stained with a polyclonal antifibrinogen antibody and then staph protein A bound to 10-nm gold particles. Thrombin stimulated uptake of 20 nm Fgn/Au gold particles into OCS channels and their transfer to α granules in the process of discharge into the OCS (Fig 1). Protein A 10-nm gold particles demonstrated fibrinogen in nonlabilized α gran-

![Fig 1](https://example.com/figure1.png)
ules and those in the process of discharge into the OCS. The work showed that the OCS was truly a final common pathway; hence, the name of the article.\textsuperscript{19}

The second experiment also involved human platelets.\textsuperscript{18,20} Cells in suspension were combined with thrombin for 15-second to 5-minute intervals and fixed in solutions containing tannic acid. Tannic acid combines with the surface membrane glycoproteins to form a mordant dye that binds and converts osmic acid to osmium black, an electron dense stain. Tannic acid under these conditions binds only to those membranes exposed to the exterior, or membranes continuous with the outside. Thus, it stains the platelet surfaces and membranes lining channels of the OCS. It has one other desirable feature. Tannic acid also selectively stains fibrinogen and fibrin strands in the same manner as the glycocalyx.\textsuperscript{20}

The surface and OCS membranes are the only structures stained by osmium black in resting platelets. After exposure to thrombin, the dense stain also enters \( \alpha \) granules that have become labeled and communicate with the OCS.\textsuperscript{20} Fibrinogen and fibrin in the process of extrusion from \( \alpha \) granules fill channels of the OCS. During this process the OCS becomes dilated, as do the labeled \( \alpha \) granules, yielding the appearance of swollen vacuoles (Fig 2). In experiments where the platelets were combined with fibrinogen-coated gold particles before exposure to thrombin and fixation through the tannic acid staining procedure, Fgn/Au was present in OCS channels and in swollen \( \alpha \) granules. The findings showed that the OCS is a two-way street, and \( \alpha \) granules communicate with channels of the OCS to secrete their contents.

As far as Lemons et al\textsuperscript{3} are concerned, it may matter little whether \( \alpha \) granules fuse with the exposed surface or channels of the OCS, because their membranes are identical. However, the morphological background serving as a palette for their work on the molecular machinery should be as accurate as possible. I hope this letter will help to clarify the background.

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Web Access to the American Society of Hematology Slide Bank

To the Editor:

The third edition of the American Society of Hematology (ASH) Slide Bank contains well over 5,000 hematopathologic images of great use to hematology educators and trainees. The University of Washington Health Sciences Center for Educational Resources (http://cer.hs.washington.edu/hscer) serves as the Slide Bank archivist and agent. From them, images and image sets can be purchased in various forms. The images originally were available only as 35-mm slides, but subsequently were made available on laser videodisc1 and CD-ROM media. When used in a program serving many trainees, these products have several drawbacks, including: (1) the requirement for the user to physically possess the media to see the images, and (2) the risk of loss of, or damage to, the media through repeated handling by various users. (Loss is the most common.) As director of my institution’s hematology training program, I was interested in improving faculty and trainee access to the Slide Bank while reducing the risk for media damage or loss. Other collections of medical images have been made available through the web successfully for several years now.2-4 I sought to construct a web interface to the Slide Bank CD-ROM.

With materials and funding support from Microsoft and Ortho Biotech, respectively, in January 1998 I purchased for my train-
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