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Review

Finding seconds count after contact with blood (and that is all I did)

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Abstract

On a surface, upon its contact with plasma at least five proteins in succession displace each other within 1 min. It took me about 30 years to discover that.

Keywords: Protein adsorption; Blood; Blood contacting biomaterials; Vroman effect; Plasma proteins

Contents

Do not think yourself as rare for studying interfaces; all humans, from birth to death, do the same thing. Even the blind who live by touch find nothing else to touch.

We cannot focus on thin air or sky
But for the passing grace of a bee or butterfly
Pinpointing space
To show us nothing by their insect/nothing interface.

More than by that knowledge, I try to humble myself by the grand mistakes I made as a means of reaching for the truth. Fortunately, I discovered my mistakes after publishing them, and my career was greatly helped by accidents and by my ability to accept them as gifts; without accidents no discoveries are made.

1. My mistakes

After escaping from Holland in 1940 when it surrendered to Germany, then traveling to Indonesia and winding up as a POW in Japan, I was sent to Holland via New York and there was told by Dr. Isidore Snapper, whom we called uncle because of his great intelligence, that there was no work in the Netherlands and I should stay here, and that my fiancée Georgine Sanders (Tineke) whom I had not seen for 6 years, could join me later. After failing to become rich quickly enough as an illustrator, the same uncle landed me a job with Dr. S.E. Moolten in New Brunswick, who believed to have found a hormone in the spleen that depressed platelet formation. I purified the substance from beef spleen for 9 years. No, I would not call that a mistake. One day, since we never could get clear results from rabbits, I took some of our mysterious ampoules by mouth, my platelet adhesiveness dropped and to my great fear and delight I wound up with a nosebleed. Meanwhile, Dr. Moolten being a very intelligent and adventurous thinker as well as a wonderful man, thought that perhaps platelets did not adhere to blood vessel walls because these walls were hydrophobic.

Mistake number one. I therefore injected some air into a mesenteric vein of a rabbit and noticed its meniscus was flat, and we published that as proof of non-wettability [1]. Well, any child over seven can tell you why the meniscus looked that way: because blood is opaque, any concavity would be invisible. And indeed much too late, I decided to inject some

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heparinized plasma of a rabbit into a vessel first, to move the blood out of the way, and then air into the area of clear plasma, and beheld its hollow meniscus: the blood vessel was wettable.

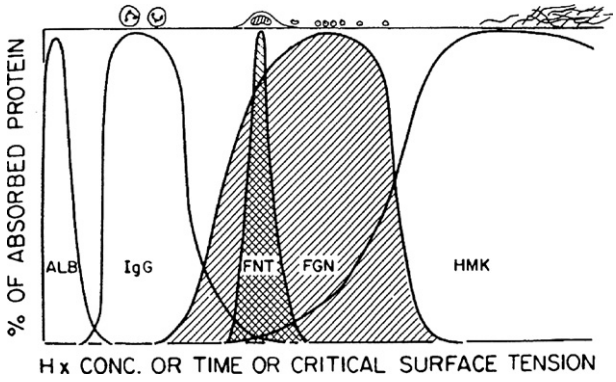
At that time, Tineke had joined us, and we developed a test for what we all thought was platelet adhesiveness and a more accurate bleeding time measurement, and she discovered that the few patients who had an abnormal bleeding time (stopping and briefly restarting) were the ones who used aspirin. Unfortunately, that did not fit our grant and we never published it.

Then, the same uncle saw to it that I got a job under Dr. L. Wasserman at Mount Sinai Hospital in Manhattan. Preferring my own thoughts to those of others, I wondered why people picked on barium sulfate to remove prothrombin and factor IX from plasma, and was it merely because its surface was wettable? I tested a bunch of wettable insoluble powders and found that all of them did the same thing to various degrees. Wasserman, strangely enough, was pleased to let me publish this in the Journal of the Mount Sinai Hospital, locally known as the Mount Sinai Gewalt [2].

Meanwhile, it took me years to realize that Dr. Sobotka, whose article in Medical Physics [3], a book I had bought many years earlier, had his lab on the floor right below me. I asked him for a hydrophobic powder and he gave me barium stearate. It removed no prothrombin and factor IX, but did remove factor V. I used that in my Ph.D. thesis. Another thing Dr. Sobotka had, was an ellipsometer, an instrument that measures the ellipticity of reflected light and that I was dying to use because it would show adsorption and removal of clotting factors at a reflecting interface. (Patience, I am slowly leading up to my next mistake.)

More than 10 years later will find us, Tineke and me and our daughters, in Brooklyn and me with a position at the Veterans Hospital there. I got a grant approved that gave me a great assistant, Ann Adams, and an ellipsometer that was far superior to the one in Mt. Sinai. Very early on, we found that adding plasma to buffer, rapid adsorption of something onto the reflecting slide occurred. Then, if the plasma had not previously been in contact with glass, some of the adsorbate was being desorbed, unless the plasma lacked factor XII.

Mistake number two. I must have been hysterical or at least young at that time, and wrote a poem entitled “De reactie van plasma thromboplastin antecendent (PTA) met Hageman factor (HF)”, stating I had discovered clear evidence that factor XII is adsorbed and then removed in part by factor XI. It was published, and then republished in the collected poems [4]. What a stupid and arrogant mistake! At the same time, I wrote a fortunately much more cautious little article stating that the phenomenon of adsorption and then removal may possibly be related to the activation of blood clotting. I got that published in Nature [5]. Later, giving a talk about all this in Wurzburg, Dr. S. Witte pointed out that I had not proved the identity of the adsorbate. What else could it be, I asked. How about fibrinogen, he answered. Why fibrinogen, I asked. Well, he answered, it is a funny molecule. Much later I heard that he thought many things were fibrinogen. Meanwhile, I rushed home, ordered antiserum to fibrinogen and we found fibrinogen was indeed, though only briefly, adsorbed before it was replaced by high molecular weight kininogen (HMK) [6]. Of course we eventually found several proteins preceding fibrinogen and all replacing each other, their sequence being: albumin followed by IgG followed by fibrinogen and fibronectin followed by HMK thus [7]:



(Reproduced with permission.) The figure shows the conditions affecting the composition of proteins adsorbed out of normal intact plasma; specifically the relative concentrations of proteins expected to remain adsorbed under a choice of conditions shown along the abscissa. H × CONC: plasma film thickness times concentration. (Above each relevant protein peak, consequences to later events are shown (from left to right): adhesion of granulocytes, adhesion and spreading of cells (e.g. monocytes), adhesion of platelets and formation of fibrin.)

Those were only the proteins we studied with the help of their antisera. I assume many more are interlaced among them, and all following each other within a minute, if the plasma touched glass or some other high surface energy negatively charged material.

2. And two accidents

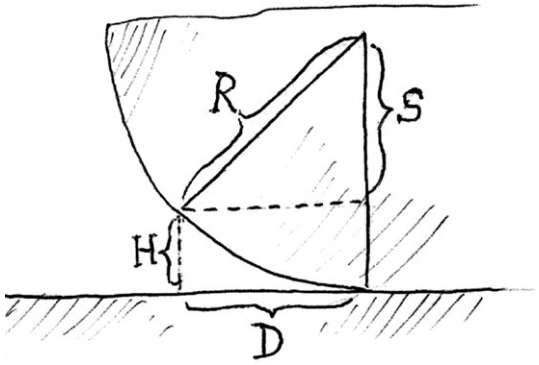
Accident number one. The work done by Sobotka et al. with their ellipsometer required a reflecting surface with a preformed thin layer of material to create interference colors. This layer was made by dipping a metal slide 15 times into a film of barium stearate formed and compressed in a surface film balance.

The film was very delicate and the preparation could only be used once for each experiment. By the time I was ready to use our own ellipsometer, I visited a cousin of ours who was working on semiconductors, and did so by anodizing tantalum sputtered glass slides to create oxide films of proper thickness and intense interference color. He gave me some of these and they worked perfectly and were indestructible unless dropped on a hard floor. Much later I asked for a few more, but no such intense interference colors could any longer be reproduced, and, the manufacturer said, they must have been caused by some unknown contaminant of the tantalum source.

Accident number two. Prof. E.F. Leonard, already a long-time friend, wondered how flow would affect the sequential protein adsorption we had found. He gave us a couple of stagnation point flow chambers consisting of a round space to be covered by a glass cover slip supported by four tiny tips whose height determined the width of the chamber space. Blood or plasma would enter at the center of it, hitting the glass at right angles at the "stagnation point" where theoretically no flow along the glass surface occurs, and from there would flow out in all directions to the chamber's periphery. The results were entirely unexpected to us: the stagnation point did not distinguish itself, but fibrinogen remained longest in the disturbed flow forming a wake from the tiny support points to the periphery, and in the narrow spaces right around these four points [8]. Narrow spaces, I probably exclaimed, and wondered how I could create these in a more controlled way. Perhaps between a large ball bearing ball and a glass slide, I wondered, and I indeed went to a store where I was given two of them by the owner obviously regarding me insane.

Placing one of them on a glass slide and injecting intact plasma (plasma not yet activated by its contact with glass), we found only a tiny spot of fibrinogen around the center of contact: the radius of curvature of these balls was still too small. Dreading to return to that man and ask for a 1 ft diameter ball bearing ball, I spent nights wondering what to use that had a perfect roundness and finally realized that lenses were the perfect object, being light and having a known radius of curvature. In the Convex Lens On Slide Experiments (CLOSE), we found a whole series of proteins in concentric rings being left between the two surfaces. (Rather than adding more bulk to my self-references, I would prefer to have you google me or google the "Vroman effect", so generously named by Brash and Horbett.) Eager to add some mathematics, I am supplying a tiny TrueBasic program to calculate the height of liquid column at the outer edge of the protein deposit:

```
! CLOSE
PRINT "LENS RADIUS OF CURVATURE, IN mm";
INPUT R
PRINT "RADIUS OF PROTEIN DEPOSIT AT OUTER
EDGE";
INPUT D
LET H = R-(R^2-D^2)^.5
PRINT "H = ";H*1000;"MICRONS"
END
```



The rings of proteins left by intact plasma between lens and slide were, from inside to outside, albumin, IgG, fibrinogen and fibronectin, HMK, in a sequence matching their concentration.

And that is about all I did, in the course of about 50 years, for which my total income must have been about a million dollars. If I had gone around this country and Canada asking every person, including every baby, to give me one penny and I will promise to tell them what happens to their fibrinogen when their blood touches glass, I don't think my study would have gotten anywhere. So thank you, United States Government.

3. Some consequences

Platelets adhere to a surface where they find fibrinogen [9], and specific white blood cells will adhere where they find a protein matching their receptors (see above figure). For example, we often did the following simple experiment.

Place a drop of intact plasma on a glass slide. After about 2 min, tilt the slide to let the drop run down, follow immediately with a saline rinse, then cover the slide with platelet rich plasma. Gently rinse a few minutes later and fix. You will find large numbers of platelets adhering only to the run, where contact was too short to remove the adsorbed fibrinogen, and none to the original drop site, where fibrinogen has been removed by HMK. On hydrophobic surfaces, the sequence of proteins

adsorbed stops usually at fibrinogen, so that human platelet rich plasma, depending on flow conditions, will deposit many more platelets on various plastics than on glass. In practice, what these results stress is the need for introducing time to in vitro blood compatibility studies. Any article that claims to have done a compatibility study by exposing a material surface to plasma or blood for only one length of time is worthless.

4. The future

No oversight is complete without a majestic view of the future.

I have noticed that the least advance in the development of blood compatible materials is made in the application of relatively narrow artificial arteries.

I suggest that, considering the very rapid initial events following contact of blood with material will depend on minute details, with each event being a function of the previous one. To express that a bit mathematically, y sub $n+1$ will be a function of y sub n, and this dependence on its own history will lead to a nonlinear (or so-called chaotic) continuum which is known to be totally unpredictable. Perhaps something like a pre-adsorption of albumin may help for a while, but at any time the patient containing the device may make a gesture that bends it or twists it fatally.

And now I will describe a mystery that someone may solve in a nearer future. We found that injecting a clear solution of IgG between a lens (curvature about 140 mm) and a slide caused it in about 1 min to form a thick ring; a small one at about 8 mg/ml, a larger one with more diluted solutions. Its formation can be watched as interference colors when one of our anodized tantalum slides is used (but an oxide coated silicon wafer may serve as well). The bright sky blue ring on a bronze interference color background after rinsing and drying is spectacular. My explanation is that above the ring site under the lens, the column of solution contains exactly enough IgG molecules to form first a monolayer deposit, then a second layer on top, after which the supernatant is depleted of IgG. Outside the ring, such a dimer is re-dissolved by excess IgG. John Brash witnessed my demonstration of this phenomenon in Ed Leonard's lab, but even using all of our ingredients (IgG, Veronal buffer, lens and slide) he could not reproduce it in Hamilton. One difference in conditions is our age, so I hope in the future some 80-year-old scientist will be able and willing to reproduce this before I die. I am now 92.

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