

# This Week's Citation Classic®

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**Bull B S, Rubenberg M L, Dacie J V & Brain M C.** Microangiopathic haemolytic anaemia: mechanisms of red-cell fragmentation: *in vitro* studies. *Brit. J. Haematol.* 14:643-52, 1968.

(Medical Research Council Group for the Study of Haemolytic Mechanisms, Department of Haematology, Royal Postgraduate Medical School, London, England)

During disseminated clotting, the fibrin microclots that form throughout the vasculature are harmless to red cells while they float freely. However, when the microclots arrest, the onrushing red cells drape over and around fibrin strands or get snared in a fibrin noose. Red-cell fragments are then produced wherever the blood flow is sufficiently rapid, as in the arterioles of lung and kidney. [The SCI® indicates that this paper has been cited in over 140 publications since 1968.]

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Why are fragmented red cells such a characteristic feature of diseases associated with disseminated intravascular clotting? In the early 1960s, this question was under study in the laboratories of J.V. Dacie (now Sir John Dacie) at the Royal Postgraduate Medical School, London. Michael Brain, together with Dacie and Hourihane, had already emphasized the role of vascular lesions in the pathogenesis of micro-angiopathic hemolytic anemia (MAHA). In a landmark paper published in 1962,<sup>1</sup> they underscored the strong association between MAHA and the presence of damaged, fibrin-lined arterioles. Martin Rubenberg and I, both postdoctoral fellows from the US, arrived in 1967 to find Dacie and Brain still at work on the mechanism whereby intravascular clotting caused red-cell fragmentation.

Prior to my arrival, an animal model of intravascular clotting had been developed. Rabbits injected with precisely measured

amounts of Malayan Pit Viper venom would sometimes show MAHA. The model was very unpredictable. All too often the animals died or, conversely, failed to show any ill effects at all. In frustration, and hoping to be able to elucidate the pathophysiology, I generated a "mechanical rabbit." It was the simplest imaginable kinetic model: a peristaltic pump served as the "heart," two injection ports as the "ears," and a stainless steel perforated disk as the microcirculation.

The model was activated by filling it with blood, starting the pump, and then injecting the venom. I naively expected the clots to be strained out on the upstream side of the perforated filter disk. If that had occurred, the disk would have become occluded, and the circuit would have exploded. Serendipitously, the holes in the disk had been acid-etched. Microprotrusions snagged the fibrin microclots and allowed them to billow out, parachute-like, downstream. The first few clots attached to the disk were sticky. Their strands caught other microclots *ad infinitum*. Hundreds of square microns of adhesive fibrin strands accumulated. The result was a relatively stable interaction between strands and red cells that could be fixed, photographed, and analyzed at leisure.

The paper has probably been cited because the pictures say it all. Maybe we are fascinated by violence on a micro (and therefore manageable) scale? Strangely enough, despite the fact that all of the pictures of the fragmentation process came from the "mechanical rabbit," no other investigator in the intervening years attempted to recapture the instant of fragmentation in human material. The requirements are, of course, daunting, for the vessel must not only contain a developing clot of precisely the right kind, but it must also be fixed under full-flow conditions. If this is not achieved, the flow-distorted red cells relax and resume a more normal shape. Last year, we rectified this research omission and successfully perfused, then fixed, tissue from a patient who had died with MAHA.<sup>2</sup> The distorted and fragmented red cells from the human tissue were identical to those that, 17 years earlier, had been produced in the "mechanical rabbit."

1. Brain M C, Dacie J V & Hourihane D O'B. Microangiopathic haemolytic anaemia: the possible role of vascular lesions in pathogenesis. *Brit. J. Haematol.* 8:358-74, 1962. (See also: Brain M C. Citation Classic. *Current Contents/Clinical Practice* 7(18):12, 30 April 1979.)
2. Young T W, Keeney G L & Bull B S. Red cell fragmentation in human disease (a light and scanning electron microscope study). *Blood Cells* 10:493-502, 1984.