Scanning Microscopy

Volume 5 | Number 1 Article 15

10-18-1990

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A. C. Groom
University of Western Ontario

E. E. Schmidt
University of Western Ontario

I. C. MacDonald
University of Western Ontario

Scanning Microscopy, Vol. 5, No. 1, 1991 (Pages 159-174) 0891-7035/91$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

MICROCIRCULATORY PATHWAYS AND BLOOD FLOW IN SPLEEN: NEW INSIGHTS FROM WASHOUT KINETICS, CORROSION CASTS, AND QUANTITATIVE INTRAVITAL VIDEOMICROSCOPY

A.C. Groom*, E.E. Schmidt, and I.C. MacDonald

Department of Medical Biophysics, Health Sciences Centre
University of Western Ontario
London, Ontario, Canada

(Received for publication June 7, 1990, and in revised form October 18, 1990)

Abstract

The dynamics of splenic microcirculatory blood flow and the role of the spleen with respect to red blood cells (RBCs) have been elucidated by means of several different experimental approaches. First, the organ was treated as a 'black box' and input/output relationships were studied, such as the kinetics of RBC and plasma washout during Ringer perfusion. Second, microcorrosion casts were prepared from minimal amounts of injected material, such that 'fast' pathways for flow were selectively identified. Third, high-resolution videomicroscopy was carried out in rat and mouse spleens transilluminated in vivo, with subsequent quantitative analysis of the data. New insights were obtained regarding fast and slow pathways for RBC flow; the origin of the high intrasplenic hematocrit; immature RBCs and the spleen; the pH, O_2 tension and glucose concentration within the reticular meshwork; microcirculatory pathways bordering the white pulp; entry of blood into venous sinuses, both by open ends in the marginal sinus/zone and via interendothelial slits in sinus walls; spontaneous cyclic contractions of capillary walls in vivo, impeding RBC flow (i.e. endothelial contractility); and leukocyte interactions with walls of venous vessels. The different experimental approaches have provided complementary information and have clarified a number of important issues about which uncertainty existed in the literature. Exploiting the quantitative analysis of high-resolution intravital videomicroscopic recordings has an exciting potential for gaining new insights into the workings of this complex and neglected organ.

Key Words: Spleen, microcirculation, washout kinetics, microcorrosion casts, in vivo microscopy, quantitative videomicroscopy, fast and slow pathways, open-ended venous sinuses, leukocyte adherence, origin of high splenic hematocrit.

**Address for correspondence:* A.C. Groom, Department of Medical Biophysics, Health Sciences Centre, University of Western Ontario, London, Ontario, Canada. N6A 5C1. *Phone No.:* (519) 661-3053.

Introduction

The complexity of the pathways for blood flow in the spleen makes the study of the microcirculation in this organ particularly challenging. Much has been learned about basic structure of the organ from examination of sections of splenic tissue, using light or transmission electron microscopy (e.g. Snook, 1950; Tischendorf, 1969; Rhodin, 1974; Weiss, 1977; Blue and Weiss, 1981). The greater depth of focus of the scanning electron microscope (SEM) has allowed three-dimensional views of microcirculatory pathways to be obtained (e.g. Fujita, 1974; Barnhart and Lusher, 1976; Suzuki et al., 1977). However, little information about the dynamics of flow can be obtained from the study of fixed tissue. In vivo microscopy of transilluminated spleens was begun over 50 years ago (Knisely, 1936; MacKenzie et al., 1941) but interpretation of viewed images was difficult and led to contradictory conclusions. This technique was later refined considerably (McCuskey and McCuskey, 1985), although quantitative analysis of the recordings was not employed.

The focus of our research has been the splenic microcirculation and the physiological role of the spleen with respect to red blood cells (RBCs). In order to elucidate the dynamics of microcirculatory flow, it has been necessary to employ several different experimental approaches. This paper focusses on new insights obtained by means of:

(1) Treating the organ as a 'black box' and studying input/output relationships, such as the kinetics of RBC and plasma washout during Ringer perfusion.

(2) Microcorrosion casts prepared from minimal amounts of injected material, such that 'fast' pathways for flow are selectively identified.

(3) High-resolution videomicroscopy of rat and mouse spleens transilluminated in vivo, with subsequent quantitative analysis of the data.

These three methods provide complementary information. For instance, RBC and plasma washout experiments provided the clue that in order to fill the fast microcirculatory pathways preferentially, by corrosion casting, one should inject only minimal quantities of casting material. In turn, these casts served

as a 'road map' for identification of flow pathways seen in vivo by videomicroscopy. This concerted approach has helped to clarify a number of important issues about which uncertainty existed in the literature.

Kinetics of RBC and Plasma Washout During Ringer Perfusion

A great deal can be learned about intrasplenic blood flow distribution by considering the organ as a 'black box' and studying input/output relationships. The storage and transit of RBCs in cat spleen has been studied by perfusing the isolated organ with cell-free Ringer solution, and sampling the outflow as a function of time (Song and Groom, 1971a). In this way, initial processing of the RBCs as with radiolabeling is avoided. The RBCs collected at the outflow are those that were contained within the spleen at the moment of cannulation, and are simply being washed out by Ringer perfusion. Since the system is non-recirculating, slow as well as fast clearance of RBCs from the organ may be quantitated. The cellular concentrations in successive samples of the outflow may be measured by means of an electronic cell counter. Washout of both RBCs and plasma may be studied simultaneously, if radioiodinated (^{125}I) serum albumin is injected into the general circulation and allowed to equilibrate intravascularly prior to isolating the spleen (Levesque and Groom, 1976). RBC and plasma concentrations are plotted semilogarithmically against the sequential volumes of perfusate per gram splenic weight.

The RBC washout curve (Fig. 1) may be resolved into the sum of three exponential components, using standard mathematical procedures (for explanation, see Song and Groom, 1971a). These components are shown in Figure 1 as solid lines, each being characterized by a very different volume of perfusate needed to reduce the concentration of RBCs by one-half. These $V_{\frac{1}{2}}$ values were 0.067, 4.7 and 97 ml/g, respectively, and on that basis the components were designated as fast, intermediate and slow, respectively.

By histological examination of spleens in which Ringer perfusion was ended after different volumes of fluid had passed through, the locations of RBCs were compared before perfusion and after cells comprising the fast and intermediate components, respectively, had been washed out. These experiments (Song and Groom, 1971b) showed that the fast component corresponded to RBCs in splenic vessels, the intermediate component to free RBCs within the reticular meshwork, and the slow component to RBCs adhering to the meshwork ('bound' RBCs).

Since the sum of three exponential terms can completely describe the RBC washout curve, a simple model consisting of three compartments in parallel is sufficient to approximate the washout processes from the spleen. Histological evidence, however, showed that the bound RBCs are sequestered from the blood flowing through the reticular meshwork (Song, 1972; Song and

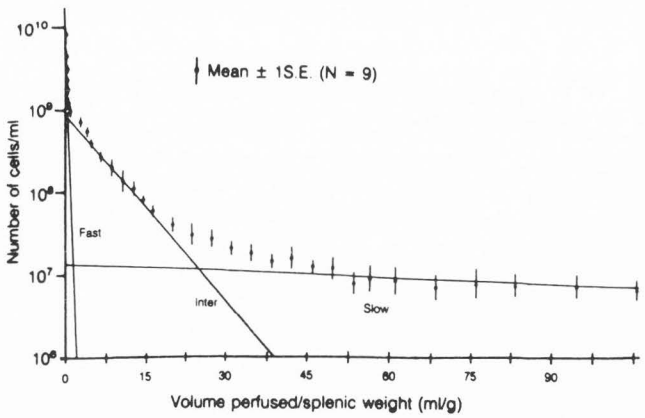

Fig. 1. Mean RBC washout curve from cat spleens during constant-flow perfusion with Ringer's solution. Cell concentration in outflow is plotted semi-logarithmically against cumulative volume of fluid perfused. Washout curve may be expressed as the sum of three exponential components, labeled FAST, INTER, and SLOW. (Reproduced with permission from Levesque and Groom, 1976.)

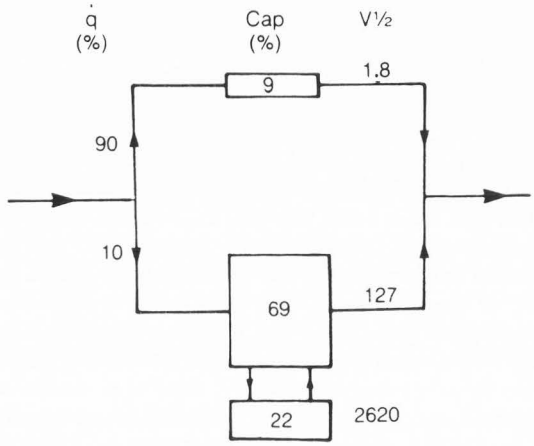

Fig. 2. Three-compartment model, derived from cell washout kinetics and morphological studies, for distribution of RBCs in cat spleen. Cap, capacity of compartment (% of total); q, flow to compartment (% of total flow); $V_{1/2}$, desaturation half-volume of compartment (ml perfusate). (Reproduced with permission from Groom, 1987.)

Abbreviations

RBC	red blood cell
MS	marginal sinus
MZ	marginal zone
PALS	periarterial lymphatic sheath
IES	interendothelial slit(s)
PMN	polymorphonuclear leukocyte

Groom, 1972). When these cells are released from their bound state, they rejoin the RBCs flowing through. This suggests that there are only two compartments for whole blood within the spleen: the vessels constituting the fast pathway, and the reticular meshwork. The distinction between the intermediate and slow compartments is solely the result of a cellular factor. For this reason, the slow and intermediate RBC compartments are shown in Figure 2 in a mammillary arrangement rather than in parallel (Levesque and Groom, 1976).

The model (Fig. 2) shows that 90.3% of the RBC flow passes through a small (fast) compartment containing only 9% of the total RBCs in the organ, the remaining flow passing to the other two compartments. The intermediate compartment is perfused by 9.6% of the RBC inflow and contains 69% of all splenic RBCs, whereas the slow compartment is perfused by only 0.15% of the inflow and represents 22% of the total RBCs. Results qualitatively similar to the above have been obtained from RBC washout studies in rats (Cilento et al., 1980; Stock et al., 1983).

What was different about the 'bound' RBCs that formed the slow component of RBC washout? At different stages of the washout fairly pure (>85%) samples of RBCs from each compartment could be collected at the venous outflow. Determinations of cellular volume and specific gravity of RBCs from the three compartments showed that cells from the slow compartment were significantly larger and lighter than those of arterial blood (Groom et al., 1971). This suggested that these were younger cells (Piomelli et al, 1967), and supravital staining of blood smears confirmed that most of them were reticulocytes (Song and Groom, 1972). Further experiments showed that the reticulocyte washout curve was almost identical to that of RBCs from the slow compartment, and that these reticulocytes constituted 8% of all splenic RBCs, 1.5× the body's total daily production (Song and Groom, 1972).

These reticulocytes could not have arisen by splenic erythropoiesis, for counts of RBC precursors in histological sections revealed a complete absence of proerythroblasts; moreover, the ratio of reticulocytes to polychromatophilic normoblasts was 75 times greater than would be expected if the reticulocytes had been derived from RBC precursors within the spleen (Song and Groom, 1972). These results suggest that reticulocytes released from bone marrow are retained within the spleen for 1 to 2 days, after which they are returned to the circulation as mature RBCs (Song and Groom, 1971c, 1972). The spleen appears to function as a 'finishing school', with maturation of reticulocytes proceeding within the organ (Song, 1972). There is evidence that splenic macrophages serve as 'nurse' cells, assisting in the maturation of immature RBCs (Pictet et al., 1969).

Studies of the washout of both RBCs and plasma simultaneously have produced the first measurements of the hematocrit and total volume of blood in the fast versus the slower pathways (cat spleen: Levesque and

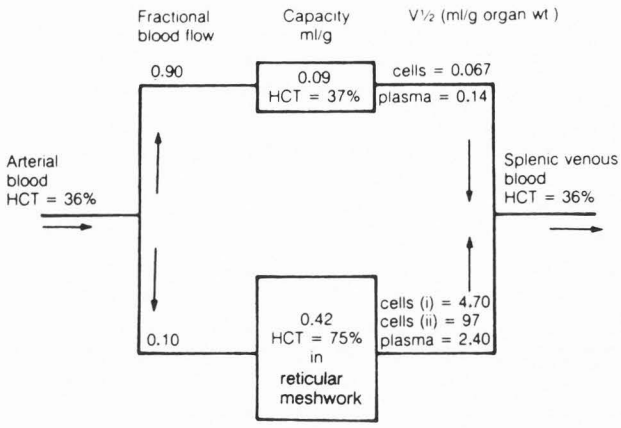

Fig. 3. Compartmental model for distribution of whole blood in cat spleen (see text). Nine-tenths of splenic arterial blood passes through the smaller compartment that contains blood of similar hematocrit (37%). One-tenth of total blood flow passes through the reticular meshwork (the major compartment) that contains blood of hematocrit 75%. (Reproduced with permission from Levesque and Groom, 1976.)

Groom, 1976). The plasma washout curve consisted of only two exponential components, the $V_{\frac{1}{2}}$ values corresponding closely to those of the fast and intermediate components of RBC washout. There existed no counterpart to the slow component of RBC washout; thus the latter must have resulted from a process peculiar to RBCs.

If one combines results from both RBC and plasma washout, a single model for the morphological distribution of blood within the spleen is obtained (Fig. 3). This is a two-compartment model, cells of the intermediate and slow RBC compartments (Figs. 1,2) being combined into a single blood compartment representing the reticular meshwork. The total blood volume was 0.51 ml/g splenic weight, of which more than 80% resided in the reticular meshwork. The 'hematocrits' of these two blood compartments (i.e. volume fraction of RBCs) were calculated to be 37% (fast pathways) and 75% (slow pathways). These hematocrit values were confirmed by direct measurements on sequential samples of blood drained from the organ, during contraction with the inflow occluded (Levesque and Groom, 1976). The hematocrit of the initial samples representing blood from fast pathways was 33.5%, whereas the hematocrit of the final samples representing slow pathways was 78.5%.

It has been known for many years that RBCs which have been exposed for long periods to the environment within the reticular meshwork develop increased mechanical and osmotic fragility (Emerson et al., 1956) and have a lower sodium-potassium ratio (Prankerd, 1960). It has been hypothesized that these changes are due to a 'hostile' metabolic environment for RBCs

within the spleen. It was thought that low intrasplenic pH, low O_2 tension and substrate deprivation lead to a loss of RBC deformability (Murphy, 1967; LaCelle, 1970; Weiss and Tavassoli, 1970) predisposing RBCs to destruction in the circulation. However, this hypothesis has been difficult to test experimentally.

By means of anaerobic collection of blood drained from cat spleen with the inflow occluded (see above) it was possible to obtain samples of blood from the reticular meshwork. The measurements showed that the pH was 7.20, the oxygen tension 54 Torr (approx.) and the glucose concentration 60% of that in venous blood (Groom et al., 1977). These were well above critical values, unless arterial inflow was occluded completely for 20 min or more. Similar conclusions have been drawn about conditions within the reticular meshwork of human and rat spleens, based on studies using microelectrodes in situ or aspiration of blood samples from the pulp (Vaupel et al., 1981, 1977). Thus, the hypothesis of a hostile environment for RBCs within the reticular meshwork simply due to very low values of pH, O_2 tension and glucose concentration is incorrect. Other explanations must be sought for deleterious changes observed in RBCs sequestered within the spleen.

Conclusions from 'black box' studies:

(i) There exist fast, intermediate and slow pathways for the transit of RBCs through the spleen.

(ii) The slow component of RBC washout consists largely of immature cells which are detained in the reticular meshwork. The spleen appears to function as a 'nursery' for reticulocytes after their release from bone marrow.

(iii) There exist two compartments for whole blood within the spleen. The fast compartment is comprised mainly of blood in the vasculature, whereas the slow compartment consists of blood in the reticular meshwork. The hematocrit of blood in the fast compartment is similar to that of arterial blood, whereas the hematocrit of blood in the reticular meshwork is twice that of arterial blood.

(iv) The levels of pH, O_2 tension and glucose concentration in the reticular meshwork are not as low as had been thought previously, and certainly not low enough to constitute a 'hostile' metabolic environment for RBCs within the spleen.

Microcorrosion Casts From Minimal Amounts of Injected Material

How can the morphological counterparts of the fast and slow pathways be studied in greater detail? The 'black box' studies of RBC and plasma washout gave us an important clue. Ninety percent of the inflowing blood travels via fast pathways which occupy only 18% of the total blood space in the relaxed spleen. This suggested that injection of only very small quantities of casting material into the splenic artery would yield a corrosion cast of the fast pathways, selectively. Extensive filling of the reticular meshwork would thereby be avoided, leaving an open view of blood vessels and their connections. Observation of the casts under the scanning electron microscope would enable such pathways to be traced in three dimensions over considerable distances.

Microcorrosion casts were prepared in this way from spleens of dogs, cats, rats, mice and humans (Schmidt et al., 1983a,b; 1985a,b; 1988). All showed, consistently, that the fast pathway consists primarily of arterial vessels, capillaries, marginal sinus (MS), portions of the marginal zone (MZ), venous sinuses (or pulp venules in nonsinusal spleens) and veins. Of particular interest to us were the pathways between capillary endings and venous vessels. It was immediately obvious from the casts that a major part of this intermediate region was comprised of the MS/MZ. This observation agrees with the findings of Herrlinger (Fig. 1, 1938) who perfused rat spleens with Ringer solution and showed that thorough clearance of blood from the marginal zone regions occurred before any clearance from the reticular meshwork of the red pulp could be detected.

A cast of the region bordering the periarterial lymphatic sheath (PALS) is shown in Figure 4. This gives a 3-dimensional view of the relationship between the PALS (white pulp has been corroded away), the central artery, the surrounding MS/MZ, and venous sinuses which have begun to fill with casting material. The reticular meshwork of the red pulp shows scarcely any filling in this cast. The artery bifurcates repeatedly, giving rise to arterioles which extend laterally beyond the PALS before ending as capillaries, either in the MS/MZ or in the red pulp. (Casts of many small vessels end blindly, the result of incomplete filling due to the injection of only very small amounts of material.)

The MS around a lymphatic nodule (Fig. 5) is a distinct entity which fills by circumferential spreading of the injectate. It consists of anastomosing blood spaces lying between the white pulp and the MZ. In other casts where slightly more material was injected, filling of the MZ had occurred as well, by spreading of material radially outward from fenestrations in the outer wall of the MS. Since the white pulp within the nodule has been corroded away the central artery and its branches may be seen, together with follicular capillaries supplying the MS. Some arterioles pass intact through the MS/MZ and then curve back to terminate as capillaries in the MZ or the outer surface of the MS.

The outer aspect of the region bordering lymphatic nodules is shown in Figures 6 and 7. An arteriole ramifies over the convex surface of the MS (Fig. 6) and then terminates as capillaries supplying the MS. Hardly any filling of the MZ has occurred in this cast, and the MS appears as a thin (<10 μm) spherical annulus delineating the outer limits of the nodule but not enclosing it completely. The fact that filling of the MS occurred, by circumferential flow, ahead of any flow radially outward into the MZ indicates clearly that the MS constitutes a low resistance pathway. This ensures

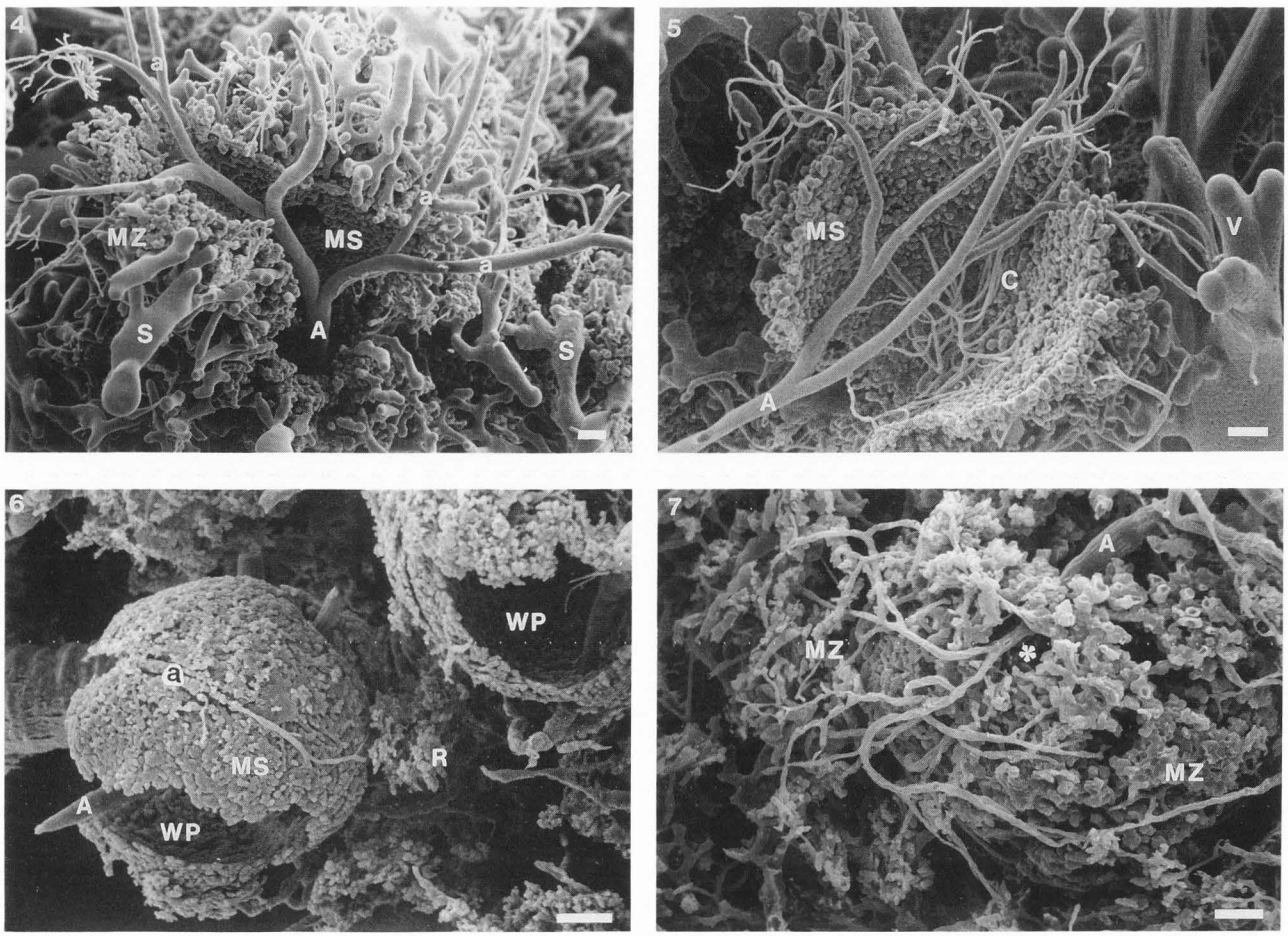

Fig. 4. Microcorrosion cast of dog spleen showing central artery (A) passing down the axis of periarterial lymphatic sheath (with white pulp corroded away). Many arteriolar branches (a) extend through marginal sinus (MS) and marginal zone (MZ) into red pulp (incompletely filled). Many venous sinuses (S) originating in MS/MZ have begun to fill. Bar = 100 μm. (Reproduced with permission from Schmidt et al., 1983a.)
Fig. 5. Microcorrosion cast of dog spleen. Central artery (A) bifurcates repeatedly within a lymphatic nodule, giving rise to follicular capillaries (C), ending in marginal sinus (MS), and to capillaries in red pulp (casts with 'blind' ends, due to incomplete filling). Collecting veins (V) partially filled. Bar = 100 μm. (Reproduced with permission from Schmidt et al., 1983a.)

Fig. 6. Microcorrosion cast of cat spleen showing marginal sinus (MS) surrounding lymphatic nodule (white pulp, WP, corroded away). Marginal sinus appears as a thin spherical annulus, due to circumferential spreading of injectate before outward radial spreading into marginal zone occurred. Arteriole (a) ramifies over convex surface of MS, before terminating there via capillaries. Branch of central artery (A) leaves the nodule. Reticular meshwork (R) has just begun to fill between the two nodules. Bar = 100 μm. (Reproduced with permission from Schmidt et al., 1983b.)
Fig. 7. Microcorrosion cast of normal human spleen. Central artery (A) passes to marginal zone (MZ) and, at this point (*), gives rise to numerous circumferentially directed arterioles and capillaries, most of which terminate there. Bar = 50 μm. (Reproduced with permission from Schmidt et al., 1988.)

that a large proportion of the blood flowing through the spleen via the fast pathway is distributed uniformly over the outer margin of the white pulp and into the MZ, thereby facilitating immune responses. In addition, numerous circumferentially directed arterioles and capillaries terminate in the MZ, as seen in casts from human spleen (Fig. 7).

With moderately larger volumes of material

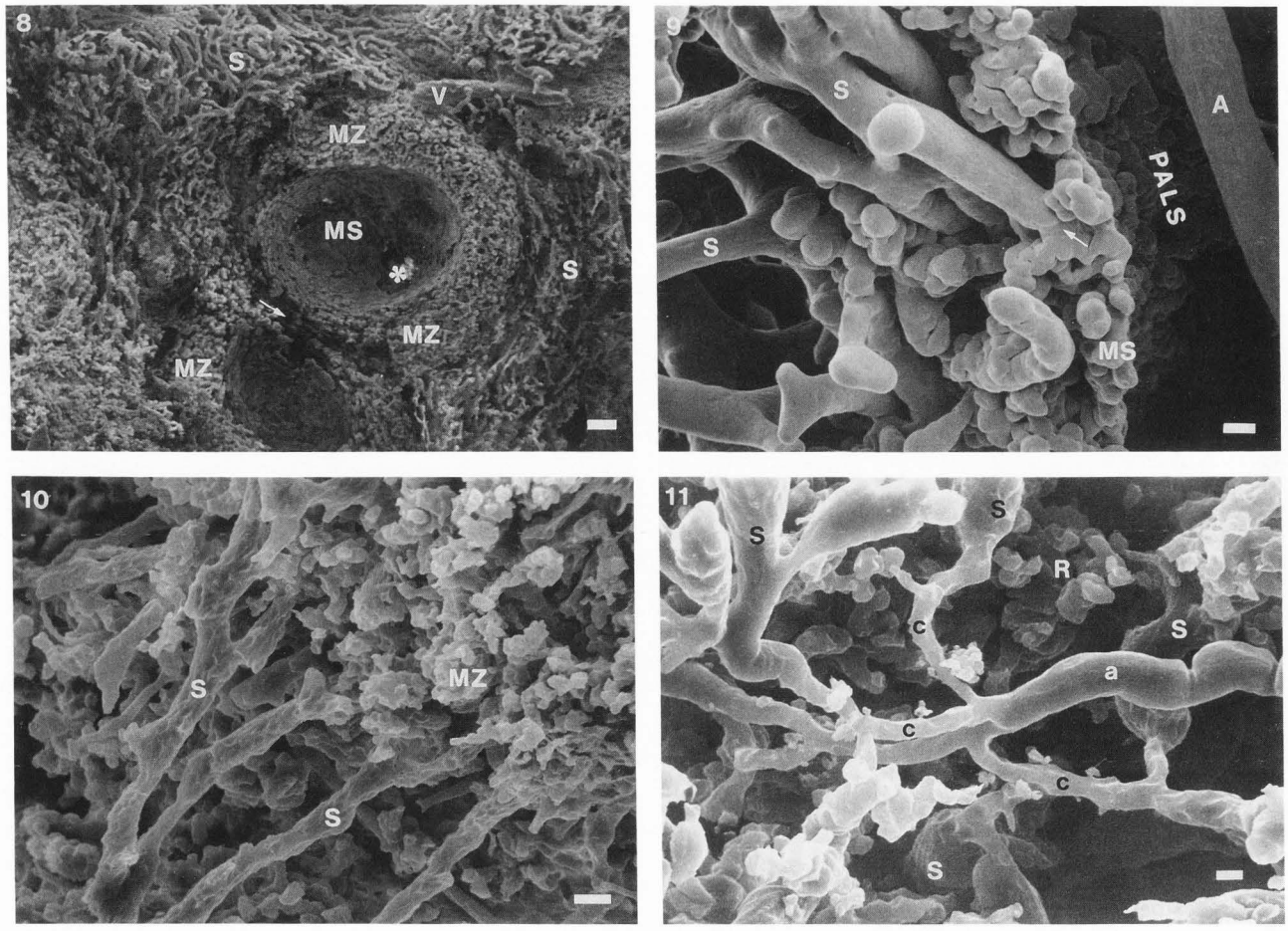

Fig. 8. Microcorrosion cast of normal human spleen showing relationship between lymphatic nodules (white pulp corroded away), marginal sinus (MS), marginal zone (MZ), and venous sinus network (S) in red pulp. Opening in MS (*) is site where central artery (cast accidentally broken off) entered nodule. In region where two nodules are close together, MZ is narrow (→). A collecting vein (V) has begun to fill. Bar = 100 μm. (Reproduced with permission from Schmidt et al., 1988.)

Fig. 9. Microcorrosion cast of dog spleen showing interconnecting venous sinuses (S) originating in marginal sinus (MS) bordering periarterial lymphatic sheath (PALS). Note continuity (→) between MS and lumen of venous sinus, i.e. the sinus begins as an open-ended tube which has filled before any filling of the reticular meshwork occurred. A, central artery. Bar = 25 μm. (Reproduced with permission from Schmidt et al., 1983a.)

Fig. 10. Microcorrosion cast of normal human spleen showing many venous sinuses (S) originating via open ends in marginal zone (MZ). Bar = 20 μm.

Fig. 11. Microcorrosion cast of dog spleen showing direct connections of arterial capillaries (c) to venous sinuses (S). Arteriole (a) enters from right and bifurcates repeatedly in quick succession, giving rise to many short capillaries, each of which leads to one end of a venous sinus. Sinuses are only partially filled because of very small amount of casting material injected; nevertheless, 10 venous sinuses are fed from this one terminal arteriole. Between sinuses, areas show start of reticular meshwork filling (R). Bar = 10 μm. (Reproduced with permission from Goresky and Groom, 1984.)

injected, the interrelationship between lymphatic nodules, MS, MZ, and the venous sinus network in the red pulp may be seen (Fig. 8). This cast from normal human spleen demonstrates the appearance of the MS as a flattened, almost continuous, system of anastomosing blood spaces lining the outer margin of the white pulp. Previous investigators have described the MS in humans as a poorly delimited component of the MZ (Barnhart and Lusher, 1979) or else as being totally absent (Van Krieken et al., 1985). However, we find consistently that the MS is present in normal human spleens obtained from organ transplant donors (Schmidt et al., 1988), but that it is absent in certain diseases such as idiopathic thrombocytopenic purpura (unpublished observations). Considerable filling of the MZ and the network of venous sinuses is seen (Fig. 8) even though

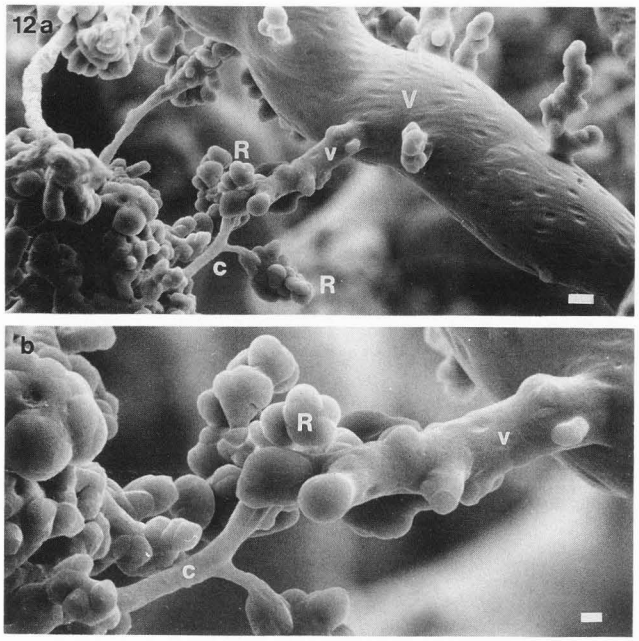

Fig. 12. Microcorrosion cast of cat spleen (nonsinusal). (a) Arterial capillary (c) terminates in reticular meshwork (R), closely adjacent to pulp venule (v) which drains into collecting vein (V). Because of retrograde flow, material is beginning to emerge from fenestrations in pulp venule into reticular meshwork. Bar = 25 μm. (b) Close-up of area from (a) shows shortest route found between capillary ending and pulp venule. The forward (capillary, c) and retrograde (venular, v) flows have reached adjacent reticular spaces (R) but have not quite merged. Bar = 10 μm. (Reproduced with permission from Schmidt et al., 1983b.)

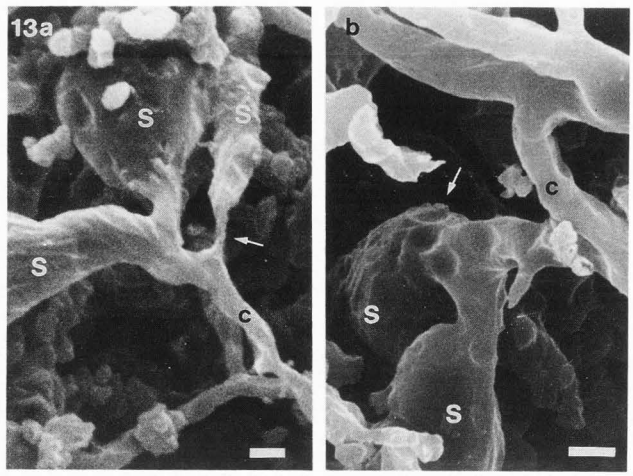

Fig. 13. Microcorrosion casts of dog spleen showing possible sites for control of flow from arterial capillaries (c) directly into venous sinuses (S). (a) Extreme narrowing of the 'neck' (→) leading to one venous sinus is seen, whereas the entrances to two adjacent sinuses are more widely open. Bar = 10 μm. (b) A ring of impressions (→), possibly made by endothelial cell nuclei, is seen around the entrance to a venous sinus (i.e. where the localized constriction was found in (a)). Bar = 10 μm. (Reproduced with permission from Schmidt et al., 1982.)

the reticular meshwork of the red pulp remains unfilled. This was surprising since it is generally considered that venous sinuses originate as blind-ended sacs, and that entry of blood into sinuses occurs from the reticular meshwork exclusively via interendothelial slits (IES) in sinus walls (Cho and DeBruyn, 1975; Barnhart and Lusher, 1976; Weiss et al., 1985; Fujita et al., 1985).

How did so much material reach the venous sinuses while the surrounding reticular meshwork remained unfilled? Figure 9 shows venous sinuses beginning as open-ended tubes, continuous with the MS bordering the PALS. The diameter of the region of continuity is 25 μm (approx.), the same caliber as the venous sinus and much larger than would be expected if flow into the sinus were limited to channels the size of IES. The abundance of such open-ended origins of venous sinuses is shown in Figure 10 (human spleen), in this case draining the MZ. In casts prepared by the minimal injection technique there is no filling of reticular meshwork around most venous sinuses and their surfaces are quite bare and exposed. These sinuses could not possibly have filled through the IES in their walls. The quantity of material which has passed into the venous system indicates that the route provided by open-ended venous sinuses must be one of very low resistance to flow, and represents a major part of the fast pathway through the spleen.

Another part of the fast pathway in spleens of some species (e.g. dog) is provided by direct connections of arterial capillaries to venous sinuses. Typically the terminal arteriole bifurcates repeatedly to give rise to many short capillaries, each of which leads to one end of a venous sinus (Fig. 11). Such connections are abundant in dog spleen (Schmidt et al., 1982, 1983a) but sparse in rat and human (Schmidt et al., 1985a, 1988). In the nonsinusal spleens of cat and mouse we have not found any direct connections of capillaries to pulp venules (Schmidt et al., 1983b, 1985b). The shortest route that we have found in a nonsinusal spleen (Fig. 12) still involved a distance of 15 to 25 μm through the reticular meshwork, between the arterial capillary ending and the pulp venule.

Possible sites for control of flow from arterial capillaries directly into venous sinuses are shown in Figure 13. An extreme narrowing of the 'neck' leading to a venous sinus is sometimes seen (Fig. 13a), and a ring of impressions, probably made by endothelial cell nuclei, often occurs around the entrance to a sinus (Fig. 13b). We know of no histological evidence to indicate that smooth muscle cells are present at this location. However, a possible explanation for these observations could be contraction of specialized

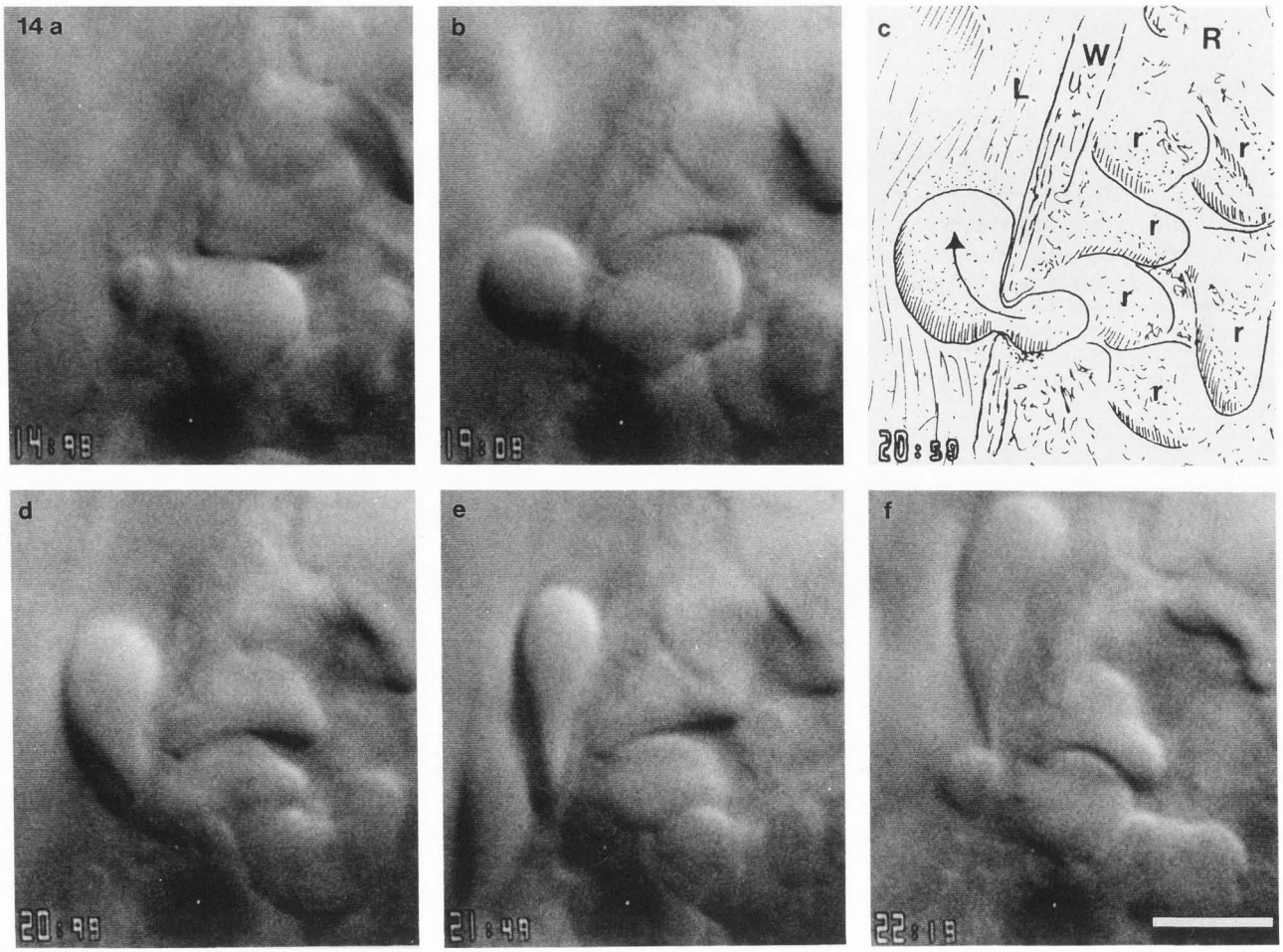

Fig. 14. Photomicrographs from videomicroscopy of transilluminated rat spleen in vivo. Sequential images of a red cell squeezing through an interendothelial slit in the wall of a venous sinus. Numbers in lower left corners give time in seconds and hundredths of a second. The venous sinus (diameter 17 μm) runs vertically at left of photos and is only partially shown. The schematic shows the locations of the sinus lumen (L), sinus wall (W), and numerous red cells (r) within the reticular spaces of the red pulp (R). Bar = 5 μm. (Reproduced with permission from MacDonald et al., 1987.)

endothelial cells, as seen in other tissues (Lübbers et al., 1979; Weigelt, 1982) as well as in spleen (Drenckhahn and Wagner, 1986).

Conclusions from microcorrosion casting studies:

(i) There are indeed morphological counterparts to the fast and slow pathways for blood flow through the spleen, revealed by 'black box' studies (section 1). The fast pathway consists primarily of arterial vessels, capillaries, marginal sinus, portions of the marginal zone, short routes through the reticular meshwork, and the venous vessels. The slow pathway involves longer distances through the labyrinthine reticular meshwork of the red pulp.

(ii) The marginal sinus is a distinct entity, consisting of anastomosing blood spaces lying between the white pulp and the marginal zone. It forms a low resistance pathway through which blood passes circumferentially before spreading outward radially into the marginal zone.

(iii) Open-ended venous sinuses draining the marginal sinus/zone form a major part of the fast pathway in sinusal spleens (e.g. human).

(iv) Direct connections of arterial capillaries to venous sinuses are abundant in some species (e.g. dog) but sparse in others (e.g. human, rat). In nonsinusal spleens, evidence of direct connections between capillaries and pulp venules was not found.

High Resolution Videomicroscopy of the Splenic Microcirculation in vivo

Microcorrosion casts enabled us to identify the morphological counterparts to the fast and slow pathways for blood flow through the spleen. In vivo microscopy made it possible to visualize and describe

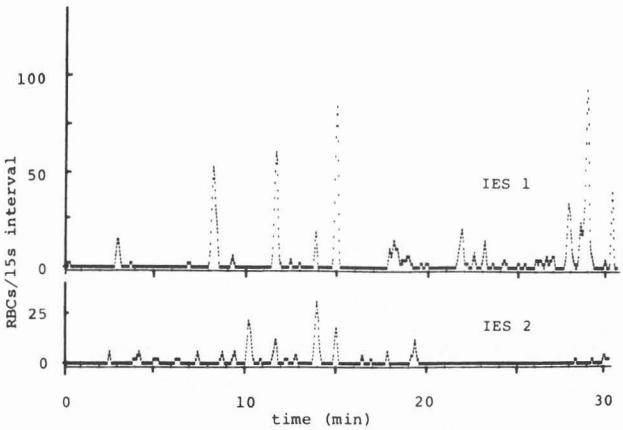

Fig. 15. Rate of RBC flow (cells/15 sec) through two closely adjacent interendothelial slits (IES) in a venous sinus in rat spleen, measured simultaneously over a 30-minute period by in vivo microscopy (see Figure 14). Flow consisted of a series of brief, discontinuous bursts of RBCs, separated by periods of zero, or near zero, flow (see text). (Reproduced with permission from MacDonald et al., 1987.)

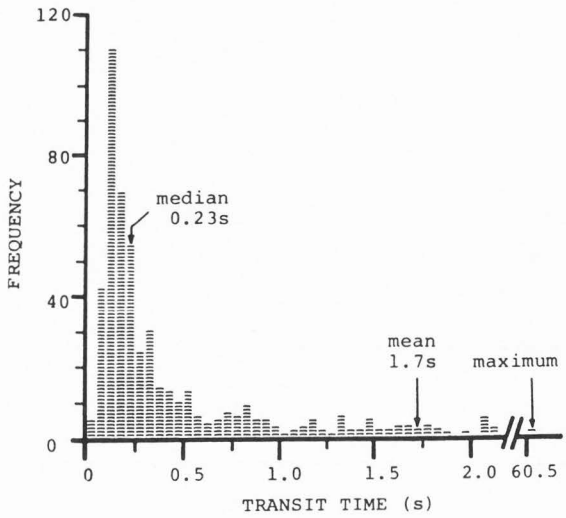

Fig. 16. Frequency distribution of transit times for RBCs passing through an interendothelial slit. Note that the abscissa is broken to show the maximum transit time of 60.5 sec. (Reproduced with permission from MacDonald et al., 1987.)

quantitatively the movement of blood through the various pathways elucidated by the other techniques. However, in vivo microscopy is restricted to animal spleens that are sufficiently thin to be transilluminated (e.g. rat, mouse). With the aid of microcorrosion casts of spleens from these same species (Schmidt et al., 1985a,b), positive identification could be made of the channels through which blood was seen to flow.

In order to obtain high resolution video images of the splenic microcirculation we used an inverted microscope and oblique lighting (MacDonald et al., 1987). The advantages provided by the inverted microscope are a stationary field of view and the ability to use objective lenses of high magnification and short working distance (e.g. 100×, oil immersion). Use of oblique lighting, by means of a fiber optic source positioned at about 45° to the optical axis of the microscope, enhances image contrast considerably. More light is reflected from one side of each structure than the other, and this shadowing effect imparts a 3-dimensional quality to the image. Slow-motion video playback facilitates quantitative analysis of the movements of rapidly flowing cells.

The kinetics of RBC passage through interendothelial slits (IES) into venous sinuses have been analyzed in rat spleen (MacDonald et al., 1987). The direction of RBC flow was invariably from the reticular meshwork into venous sinuses, not the reverse as has been suggested (Pictet et al., 1969). Sequential photomicrographs of an RBC squeezing through an IES are shown in Figure 14. The venous sinus runs vertically at the left of each micrograph and is only partially shown (see schematic: Fig. 14c). To the right of the sinus wall is the reticular meshwork where numerous RBCs are visible. The IES itself cannot be seen, but the position and shape of the RBC within it reveal its location. One RBC has begun to emerge into the lumen of the venous sinus in Figure 14a, and it is shown at the half-way point in its passage through the IES in Figure 14b. In the subsequent micrographs the RBC passes further into the lumen until it is tethered by a long 'tail' which remains momentarily trapped by the IES (Fig. 14f). Finally, the RBC escapes completely (not shown) and is swept away by the rapid flow within the lumen.

A noteworthy characteristic of RBC flow through IES is its discontinuous nature. Flow occurs as short bursts separated by periods of zero, or near zero, flow (Fig. 15). Such a pattern could be caused by: (i) changes in pressure difference across the sinus wall, (ii) changes in RBC supply to the reticular meshwork at the IES, (iii) changes in flow resistance at the IES itself. Factors (i) and (ii) would produce synchronous bursts of RBCs through closely adjacent IES, whereas (iii) would generally produce asynchronous bursts. Analysis of RBC flow through two such IES simultaneously, for 30 min, demonstrated that most of the bursts were asynchronous (Fig. 15). Therefore, the factor primarily responsible for the observed pattern of RBC flow was changes in resistance at the IES.

What could have caused these changes in flow resistance of the IES? Temporary obstruction of IES by leukocytes or platelet aggregates was observed occasionally, but was not a major cause. Thus, RBC bursts must have been caused by changes in caliber of the IES with time. Drenckhahn and Wagner (1986) have demonstrated the contractility of microfilamentous

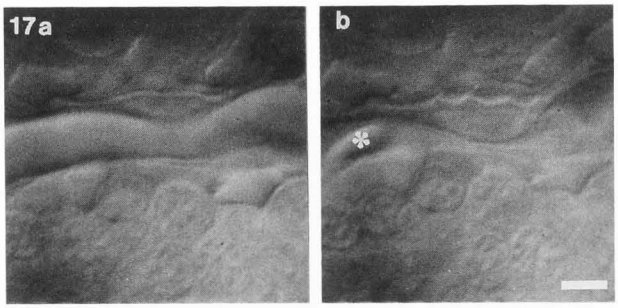

Fig. 17. Photomicrographs of a capillary wall contracting spontaneously in mouse spleen, in vivo. A bulging endothelial cell is seen on one side of the lumen. (a) Capillary relaxed with rapid flow and, hence, blurring of RBC images. (b) endothelial cell has bulged markedly into the lumen, reducing luminal width almost to zero and slowing blood flow sufficiently that an individual RBC (*) can be seen. Bar = 5 μm. (Reproduced with permission from Ragan et al., 1988.)

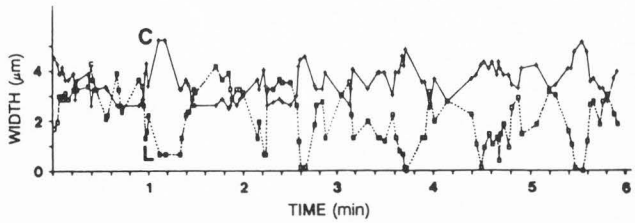

Fig. 18. Sequential measurements, from a capillary contracting spontaneously in rat spleen, of lumen width (L) and endothelial cell width (C), plotted versus time. Numerous cycles of contraction/relaxation are seen, with lumen width often being reduced to zero. Note that when L decreased C increased, and vice versa. (Reproduced with permission from Ragan et al., 1988.)

bundles which run along either side of the IES within the endothelial cells of the sinus wall. They suggested that such contractile activity might control the width of the IES. Our results are consistent with this view. Figure 15 shows that IES can be held closed for considerable periods of time. In fact, only 20% of the total number of IES present allowed any RBCs to pass during any 5 minute period (MacDonald et al., 1987). The mean RBC flow rate per IES was 15 cells/min for six active IES analyzed, whereas the maximum instantaneous flow rate was 10 cells/s. The number of IES per area of sinus wall, through which RBCs were seen to pass in a 5 minute period, was found to be approximately 1.5 IES/100 μm^2. Therefore, the mean rate of passage of RBCs across sinus walls was 23 RBCs/100 μm^2/min.

Transit times of RBCs through IES covered an enormous range, representing more than 3 orders of magnitude even for a single IES (Fig. 16). The distribution was very skewed, as reflected in the great difference between the median (0.23s) and the mean (1.7s). When these data were considered in the form of a cumulative frequency distribution, it became apparent that the fastest 10% of the RBCs passed through the IES in <0.09s whereas the slowest 10% took from 2.2 to 60.5s. One might speculate that some of the longer transit times were associated with abnormal RBCs (e.g., Heinz body cytoplasmic inclusions). While this may have been true, we found no evidence to confirm it from visual examination of the videotapes during slow-motion playback. Our conclusion is that in normal animals the range of RBC transit times through IES is governed less by the properties of the RBCs than by changes in caliber of the IES with time.

The possibility that capillary walls may be contractile, and thus participate in the local regulation of microvascular blood flow, has long been a matter of controversy. However, recent evidence from in vivo microscopy has established that, on stimulation, certain endothelial cells in capillaries bulge into the lumen, causing RBC flow to slow or even to stop (McCuskey and Chapman, 1969; Reilly and McCuskey, 1977; Lübbers et al., 1979; Weigelt, 1982; Dietrich et al., 1984). What has remained uncertain is whether such endothelial cell contraction actually occurs in vivo during normal physiological conditions. In spleens of rats and mice we have videorecorded spontaneous cyclic contractions of capillary walls, often reducing the flow of RBCs to zero. At high magnification it was possible to distinguish clearly the boundaries of cells composing the vessel walls, and we were able to measure the widths of endothelial cells and capillary lumens, throughout many cycles of contraction and relaxation (Ragan et al., 1988).

Photomicrographs of a capillary wall contracting spontaneously are shown in Figure 17. This capillary branched from an arteriole 125 μm to the left of the field of view and drained into the marginal sinus 40 μm to the right. On one side of the lumen, an endothelial cell bulged markedly into the lumen causing the abluminal side of the cell to become wrinkled (Fig. 17b). Thirteen contractions were observed during an 11 minute period, and 11 of these contractions reduced the lumen width to <1 μm. This stopped RBC flow completely except for a few occasions when an RBC was able to squeeze very slowly past the constriction.

Measurements were made of lumen width and endothelial cell width at frequent intervals throughout the period of observation (11-12 min) for 3 different capillaries where spontaneous contractions occurred. One example of such data plotted as a function of time is shown in Figure 18 (a 6 minute excerpt only). This demonstrates the complementary relationship between lumen width (L) and endothelial cell thickness (C). Almost invariably, when L decreased C increased, and vice versa. The contractions did not occur at regular intervals, and the periods of closure lasted from less than 1s to 20s.

The strong correlation between L and C, for all 3 capillaries analyzed, was revealed more readily by plots of L versus C. Each plot appeared to be a straight line

(albeit with a large standard error of estimate), the mean value of the slope being approximately -1 (Ragan et al., 1988). In 2 capillaries a pericyte was associated with the abluminal surface of the bulging endothelial cell. Measurements of the pericyte width (P) during spontaneous cyclic contractions of the capillary wall provided an opportunity to assess the relative contributions of pericyte and endothelial cell to the luminal narrowing. Multiple linear regression analysis showed that changes in P contributed to luminal narrowing by only one-seventh to one-half as much as changes in C. (When no pericyte was present, as in Figure 17, the contractility was due to the endothelial cell alone.) We conclude that the capillary contractility we observed in spleen was due primarily to cyclic contractions of an endothelial cell.

What could be the functional significance of such contractions? It is known that in spleen a switching of flow paths can occur between the fast pathway and the reticular meshwork (Levesque and Groom, 1981; Schmidt et al., 1982); this is probably related to the RBC filtration mechanism of the organ. The endothelial contractions we have observed in arterial capillaries may well contribute to this redistribution of intrasplenic blood flow.

A major unsolved problem is how the spleen concentrates RBCs to a volume fraction equivalent to twice the arterial hematocrit. Of the various possibilities suggested, the most well known is that hemoconcentration is carried out within venous sinuses which are under the control of efferent and afferent sphincters (Knisely, 1936). However, other investigators have not been able to confirm such a mechanism. Furthermore, nonsinusal spleens concentrate RBCs to an equally high hematocrit within the reticular meshwork. Thus, the primary mechanism of RBC concentration must be independent of the presence of venous sinuses. What is common to spleens of all species is the presence of a reticular meshwork interposed between the capillary endings and the start of the venous channels. This arrangement is unique to the spleen, and it is in the slow pathways of the reticular meshwork that filtration of abnormal RBCs, and the concentration of normal RBCs to a hematocrit of approximately 80%, take place. Recently, we have hypothesized that if shear rates in the reticular meshwork are low enough for abnormal RBCs to be culled from the circulating blood, then this will also result in an increased concentration of normal RBCs (Groom et al., 1988; Groom and Schmidt, 1990).

Our hypothesis, based on morphological and kinetic studies, is that the primary RBC filtration mechanism depends on adhesion in the reticular meshwork, rather than on mechanical filtration at interendothelial slits (IES). Thus, the RBC filtration process is based primarily on membrane surface properties, rather than on cellular deformability. The diameters of channels within the reticular meshwork are generally too large to impede RBC flow mechanically. However, the

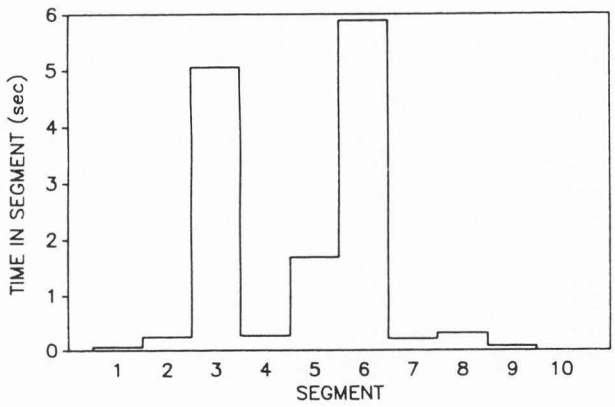

Fig. 19. Histogram showing variations in the amount of time spent by an RBC in successive 7 μm segments of a path through the reticular meshwork. Interactions between the RBC and the meshwork caused intermittent slowing down or stoppage of the RBC, while the 'plasma' continued flowing.

meshwork presents an enormous contact surface area for blood cells, and its large total cross-sectional area for flow results in very low blood velocities and, consequently, low shear rates.

At high shear rates blood cells flow as a monodisperse suspension, whereas at the low shear rates in the reticular meshwork even RBCs are likely to adhere to each other and to other surfaces in the meshwork. Abnormal RBCs will adhere strongly and become trapped, while normal RBCs will adhere transiently but repeatedly as they pass through the meshwork. Since plasma flows on freely, its velocity will be higher than that of RBCs. This is the opposite of the conditions responsible for the 'Fahraeus effect' in small blood vessels where the mean velocity of RBCs exceeds that of plasma, resulting in a tube hematocrit lower than that of the arterial supply. In the reticular meshwork it is this 'inverse' Fahraeus effect which produces the increased hematocrit.

Confirmation of this hypothesis was obtained by quantitative analysis of in vivo videomicroscopic recordings of blood flow in spleens of rats and mice. Mean RBC velocity in arterial capillaries was 242 μm/s ± 133 (SD) whereas that in the reticular meshwork was merely 8 μm/s ± 19 (SD), lower by 1 to 2 orders of magnitude. This confirms that low shear rates are to be expected in the reticular meshwork, creating conditions favorable for RBC adhesion. Moreover, direct evidence for the slowing of individual RBCs with respect to the plasma phase was obtained by the use of isolated, Ringer-perfused spleens. In blood-perfused spleens the hematocrit in the reticular meshwork is so high that it is difficult to follow the motions of individual RBCs over distances greater than a few cell diameters. Therefore, we washed out most of the RBCs by Ringer-perfusion to give a clearer view of the motions of individual cells.

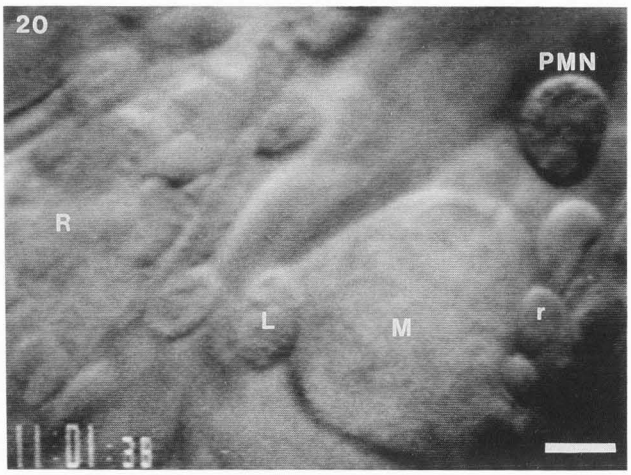

Fig. 20. Large macrophage (M) migrating along wall of venous sinus in rat spleen. A PMN has just become detached from it, whereas the lymphocyte (L) remains attached. Note the differences in size and appearance of PMN, L, and M, allowing them to be distinguished in vivo. Three RBCs (r) are entering the lumen via interendothelial slits in sinus wall. R, reticular meshwork. Bar = 5 μm. (Reproduced with permission from Schmidt et al., 1990.)

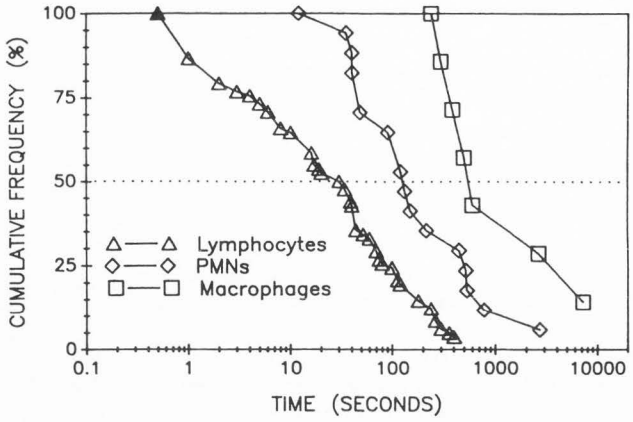

Fig. 21. Cumulative frequency distributions for adherence times of leukocytes to venular walls in spleens of rats and mice in vivo. For lymphocytes, filled triangle at 0.5 sec signifies adherence times <1 sec. Many adherence times represent underestimates, where cells were already adhering when observations began. Median times 30 (n = 83), 130 (n = 17), and 560 sec (n = 7) for lymphocytes, PMNs, and macrophages, respectively. (Reproduced with permission from Schmidt et al., 1990.)

Visual observations confirmed that interactions occur between RBCs and the reticular meshwork, causing intermittent slowing down or stoppage of the cells. This was quantitated for 18 RBCs which travelled for up to 70 μm along the same path through the meshwork. The path was divided into successive 7 μm segments, and the time spent by individual cells in each segment was determined from slow-motion replay of the videotape. The example shown in Figure 19 for one of the RBCs illustrates that the residence times in various segments along the path varied dramatically, ranging from 0.07s to 5.89s. There were 3 segments in which delayed passage of this RBC was especially pronounced. Similar measurements for each of the 18 cells studied showed that some RBCs were repeatedly detained and took 10-15 times as long as others to cover the same distance along the path. In addition, certain positions on the path were more likely than others to detain RBCs. Careful examination of the RBC interactions with the meshwork at such positions was made from slow-motion replay of the videorecordings. This showed that slowing of RBC movement could be due to adhesion and/or mechanical obstruction (e.g. sandbagging of an RBC over a reticulum fiber crossing the path).

The velocity of 'plasma' (the suspending phase: in this case, Ringer solution) was considered to be at least that of the fastest RBCs within each segment. These were freely flowing cells, whose velocities closely matched those of platelets or carbon particles suspended in the perfusing fluid. In this way, the mean plasma velocity over the whole path was found to be 76 μm/s. In contrast, the mean RBC velocity was only 27 μm/s. This means that RBCs traveled through this portion of the meshwork with an average velocity only 36% that of the plasma. Assuming this to be representative of flow through the meshwork in general, it follows from the law of conservation of mass that an arterial hematocrit of 40% will generate a hematocrit of 64.5% within the reticular meshwork.

In summary, we have demonstrated that the passage of RBCs through the reticular meshwork is slowed considerably with respect to that of the suspending plasma. A necessary consequence of this is that intrasplenic hematocrit must rise above that of the arterial supply. This is not to deny that interendothelial slits in walls of venous sinuses also play a role in concentrating RBCs. The squeezing of RBCs through IES will presumably slow the flow of RBCs with respect to plasma, and will also provide additional resistance to flow through the reticular meshwork. This will lower shear rates yet further and enhance RBC adhesion within the meshwork. However, since nonsinusal spleens concentrate RBCs equally well as sinusal spleens, the reticular meshwork must provide the primary mechanism for generating the high intrasplenic hematocrit.

It appears, therefore, that the high intrasplenic hematocrit is merely a side-effect of the flow conditions necessary for the filtration of abnormal RBCs from the blood. The intermediate circulation, consisting of a reticular meshwork, provides for shear rates low enough for immature, abnormal, and possibly senescent RBCs to

adhere to the meshwork and be removed from the circulation. Even normal RBCs will adhere transiently but repeatedly, as we have demonstrated, lowering the mean RBC velocity below that of plasma. As a biophysical consequence, intrasplenic hematocrit rises above that of the inflowing blood.

High resolution videomicroscopy of spleen also has great potential for studying leukocyte behaviour in vivo. Using this technique it is possible to differentiate between lymphocytes, polymorphonuclear leukocytes (PMNs) and macrophages, based on their size, shape and appearance (Schmidt et al., 1990). A large macrophage migrating along the wall of a venous sinus is shown in Figure 20, together with a PMN and several lymphocytes. The PMN had just become detached from the macrophage, whereas a lymphocyte was still adhering to it. This particular macrophage was observed for more than 2 hours as it moved slowly (1 μm/min) along the wall of a venous sinus in which RBC velocity was 365 μm/s ± 79 (SD). During this period it underwent many changes of shape, including the development of a trailing pseudopod.

In addition to adherence to macrophages, all three types of cells adhered to walls of venous vessels. Lymphocytes adhered for times ranging from <1s to >400s, PMNs for 1 min to >45 min, and macrophages for 4 min to 2 hours. (Many of these adherence times represent underestimates, where cells were already adhering when observations began.) Cumulative frequency distributions of adherence times (Fig. 21) show that the median times were 30s, 130s, and 560s, for lymphocytes, PMNs and macrophages, respectively. This indicates that the adhesive forces between cell and endothelium are considerably greater for macrophages than PMNs, and for PMNs than lymphocytes. The use of in vivo videomicroscopy of spleen, in combination with experimental 'perturbations' to modify leukocyte adherence to endothelium, to stimulate phagocytosis, or to induce an immune response, offers exciting possibilities for studying the kinetics of leukocyte interactions in vivo.

Conclusions from in vivo videomicroscopic studies:

(i) RBC passage through interendothelial slits (IES) in venous sinus walls is always in a direction from the reticular meshwork into the sinuses. It is characteristically discontinuous in nature, i.e., short bursts of RBCs separated by periods of zero flow. Even for closely adjacent IES the bursts are asynchronous, the result of changes in caliber of individual IES with time. The mean rate of passage of RBCs across sinus walls is 23 RBCs/100 μm^2/min.

(ii) Some arterial capillaries demonstrate spontaneous cyclic changes in luminal caliber, the bulging of an endothelial cell often reducing the flow to zero. Quantitative analysis showed that these changes were caused by endothelial contractility. Such contractions may contribute to a redistribution of intrasplenic blood flow, between the fast pathway and the reticular meshwork.

(iii) The high intrasplenic hematocrit is primarily due to the flow conditions in the reticular meshwork. The shear rates are low enough that immature and abnormal RBCs adhere to the meshwork and are removed from circulation. Normal RBCs adhere to the meshwork transiently but repeatedly, lowering the mean RBC velocity below that of plasma. As a biophysical consequence, intrasplenic hematocrit rises above that of the inflowing blood.

(iv) Lymphocytes, PMNs and macrophages may be distinguished in vivo based on their size, shape and appearance. Cell-cell and cell-vessel wall interactions may be studied quantitatively (e.g., adherence times and speeds of migration). The large differences between median adherence times for the three types of cells indicate that the adhesive forces between cell and endothelium are considerably greater for macrophages than PMNs, and for PMNs than lymphocytes. The method has great potential for studying leukocyte behaviour in vivo, such as the homing and migration of lymphocytes, and immunologically-related interactions of macrophages and lymphocytes.

Concluding Remarks

The study of splenic blood flow and microcirculatory pathways through the spleen, by means of several different but complementary approaches, has yielded a number of important new insights which have been summarized at the end of each section of this paper. Until now, our research has focussed largely on the dynamics of blood flow in the normal spleen. To study further the mechanisms which regulate flow and retention of blood cells, we plan in future to perturb the system by neural or pharmacological stimulation, or by injection of abnormal cells. Exploiting the quantitative analysis of high-resolution intravital videomicroscopic recordings has an exciting potential for gaining new insights into the workings of this complex and neglected organ.

Acknowledgements

Many people contributed to various phases of the research reported in this paper. Particular mention should be made of the work of Drs. S.H. Song, M.J. Levesque and D.M. Ragan. We thank Mrs. Barbara Anderson for typing this manuscript. This research was supported by a grant to ACG from the Medical Research Council of Canada.

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Discussion with Reviewers

R.S. McCuskey: You have stated that 'since nonsinusal spleens concentrate RBCs equally well as sinusal spleens, the reticular meshwork must provide the primary mechanism for generating the high intrasplenic hematocrit'. Should not this be modified to reflect that non-sinusal spleens, e.g. mouse, also have interendothelial slits through which RBCs leave the reticular meshwork (McCuskey, McCuskey, 1985: text reference)?

Authors: The structure of the walls of *pulp venules* in non-sinusal spleens is quite different from that of *venous sinuses* in sinusal spleens. Whereas venous sinuses have characteristic "rod cells" aligned parallel to the long axis of the vessel (Fujita, 1974: text reference) pulp venules are lined with flattened, irregularly shaped endothelial cells and have relatively few and irregularly distributed "fenestrations" in their walls (Hataba Y, Kirino Y, Suzuki T, J. Electron Microsc. 30: 46-56, 1981). The sizes of these fenestrations are mostly large enough to

allow unimpeded entry of RBCs into the venule (Blue J, Weiss L. Am. J. Anat. 161: 135-168, 1981). In contrast, the narrow interendothelial slits in walls of venous sinuses provide a greater test of RBC deformability (Blue, Weiss, 1981; Groom, Schmidt, 1990: text references). To reflect this difference we have consistently referred to openings in venous sinus walls as "interendothelial slits" (IES) and to openings in pulp venule walls as "fenestrations". Since IES will presumably slow down RBCs with respect to plasma more than fenestrations would, they must contribute to some extent to the generation of a high hematocrit. However, the similarity of intrasplenic hematocrit values in sinusal and nonsinusal spleens suggests that the primary mechanism for hemoconcentration is provided by the reticular meshwork.

T. Fujita: Why do you use the term "marginal sinuses" for the spaces between the white pulp and marginal zone? They are merely the circumferentially extended spaces in the innermost layer of the marginal zone. To avoid confusion, the term "sinus" should be restricted to the splenic sinus with its specialized wall structure.
Authors: The existence of a distinct vascular space lying between the white pulp and the marginal zone was first described by Andrew (Am. J. Anat. 79: 1-73, 1946), Altschul and Hummason (Anat. Rec. 97: 259-264, 1947), and Snook (1950: text reference). The term 'marginal sinusoid' was coined by Baillif (Am. J. Anat. 92: 55-115, 1953) and the term 'marginal sinus' adopted by Snook (Anat. Rec. 148: 149-159, 1964) and subsequent investigators. Our casts show that the MS fills preferentially (Figs. 5,6) before filling of the marginal zone and surrounding red pulp occurs. Thus, the MS is quite distinct from the MZ, in terms of both morphology and pattern of filling. Since the term 'sinus' is a generic term used for many different structures throughout the body, it must be used with an appropriate descriptor. In the spleen, the term is commonly applied to three structures: venous sinus, marginal sinus and, in humans, perimarginal cavernous sinus.

T. Fujita: The casts shown in Figures 11 and 13 seem to represent the terminal portions of penicillar arteries. The swollen structures labeled "S" are presumed to be saccular terminals of the arteries. What are the bases of your view that the swollen parts are sinuses?
Authors: The casts in Figures 11 and 13 show, in a limited field of view, the connecting portions of arterial capillaries to what we believe are venous sinuses, and only a short length of each sinus can be seen. In other pictures showing a larger field of view (e.g. Figure 4b in Schmidt et al., 1983a, and Figures 1-5 in Schmidt et al., 1982: text references) the length of venous sinuses that may be seen is up to 150 μm, or as great as 250 μm in other unpublished micrographs. (As indicated in the legend of Figure 11 of the present paper, most sinuses are only partially filled due to injection of minimal amounts of casting material.) The diameters of these sinuses are 40-50 μm. These dimensions are much larger than those of 'saccular terminals' (ampullary dilatations) of arterial capillaries, which are on the order of 25 μm in length and 10-15 μm in diameter (Fujita et al., 1985: text reference).

A.J. Bowdler: While the microscopic evidence of the capillary connections of the direct pathway appear convincing in Figures 11 and 13, is there good evidence that they are in fact lined with endothelium?
Authors: Continuity of endothelium between arterial capillaries and venous sinuses has been demonstrated in chicken spleen, by means of light and transmission electron microscopy (Miyamoto H, Seguchi H, Ogawa K. J. Electron Microsc. 29: 158-172, 1980; Olah I, Glick B. Am. J. Anat. 165: 445-480, 1982).

A.J. Bowdler: Given that it might be possible for adequate nutritional support to be given to the cellular structures of the spleen without a dedicated endothelium-lined vasculature connecting arterial capillaries to venous channels, would the authors like to suggest a functional value for a defined structural pathway? Also, given that the authors found no direct connections between capillaries and pulp venules in the nonsinusal spleen, is there a functional difference between sinusal and nonsinusal spleens which would account for the presence or absence of such direct connections?
Authors: There appear to be two ways of providing a fast pathway for blood flow through the spleen, thereby bypassing the filtration bed in the reticular meshwork. Sinusal spleens employ very short pathways through the reticular meshwork (anatomically open but functionally closed pathways) as well as vascular direct connections (endothelium-lined pathways which are thus anatomically closed). Nonsinusal spleens appear to have only the former. We know of no functional value that would favor one type of pathway over the other. Similarly, we know of no functional difference between sinusal and nonsinusal spleens that would account for their different microvascular arrangements.

A.J. Bowdler: The evidence that the transit time for RBCs through the sinus wall can be extremely variable is of considerable interest. Do the authors feel that this may be a mechanism whereby the sinusal filter can be adapted to particles of differing sizes (e.g. micro-organisms), or does this operate almost exclusively at a level of filtration applicable to RBCs?
Authors: It is difficult to answer this question. Although interendothelial slits can become narrowed to the point where RBCs no longer pass through, we do not know the minimum slit width. We strongly suspect that microorganisms are removed primarily by leukocytes in the reticular meshwork, the underlying physical determinant being surface properties rather than size and deformability.