Arterial intimal-medial permeability and coevolving structural responses to defined shear-stress exposures

DONALD L. FRY
Laboratory of Experimental Atherosclerosis, Departments of Internal Medicine and Pathology, College of Medicine, Ohio State University, Columbus, Ohio 43210
Received 12 March 2001; accepted in final form 31 July 2002

Fry, Donald L. Arterial intimal-medial permeability and coevolving structural responses to defined shear-stress exposures. Am J Physiol Heart Circ Physiol 283: H2341–H2355, 2002. First published August 22, 2002; 10.1152/ajpheart.00219.2001.—The purpose of this research was to examine the evolution of arterial shear-stress-induced intimal albumin permeability and coevolving structural responses in swine arteries. Uniform laminar shear-stress responses were compared with those of a simulated “flow separation” stress field. These fields were created using specially designed flow-configuring devices in an experimentally controlled, metabolically supported, ex vivo thoracoabdominal aorta preparation. The Evans blue dye-albumin complex (EBD-alb) permeability patterns that evolved were measured by a reflectometric method. The corresponding tissue structural responses were evaluated by histological, immunostaining, and ultrastructural microscopic techniques. It was shown that when a previously in vivo-adapted artery is challenged by a new mechanochemical environment, it undergoes a sequence of adaptive processes over the ensuing 95 h. Intimal regions of laminar shear-stress exposure (−16 dyn/cm²) responded initially (23 h) with an increase in permeability. With continued stress exposure, intimal-medial structural changes ensued that restored the artery to a physiologically normal permeability. Over this same period, adjacent endothelial regions exposed to simulated flow separation stress fields (−0.03–0.27 dyn/cm²) developed early and progressively increasing permeability. This was associated with formation of local intimal edema, loss of intimal matrix material, and development of distinctly raised, gelatinous-appearing intimal lesions having a potentially preatheromatous architecture.

arterial stress adaptation; apoptosis; atheroma; atherosclerosis; endothelial barrier function; flow separation and reattachment phenomenon; porcine artery; gelatinous lesion; laminar shear stress; transendothelial mass transport

SINCE THE 1960S, there has been an increasing interest in the effects of various hemodynamic shear-stress exposures on endothelial-intimal permeability, structure (17, 21, 22), and susceptibility to atherogenesis (4, 23, 24). Atheroma are slowly developing, raised intimal lesions that are the leading cause of morbidity and death in modern societies. Early studies suggest that hemodynamic factors could influence the endothelial cell surface barrier in an adverse manner to initiate the chain of aberrant interstitial mass transport and cellular events of atherogenesis (2, 5, 23, 27, 30). Because one of the initiating events appears to be endothelial barrier failure (3, 29, 30, 42, 45), most subsequent work has focused on endothelial cell biology. To do this, special tissue culture systems were developed in which controlled shear stresses could be applied to cultured monolayers of endothelial cells. Endothelial “responses” to these controlled shear-stress exposures were based initially on observations of endothelial cell integrity, shape, population density, and apparent rheological changes. With the use of such cultured endothelial monolayers, it was found that an increase in shear stress was associated with increased endothelial pinocytotic activity and restructuring of the endothelial cell cytoskeleton. Elongation and realignment of the endothelial cells in the direction of the imposed shear stress (9, 13) was also observed, confirming earlier in vivo observations (17).

More recently, there have been improvements in methodologies for estimation of genetic expression, e.g., measurements of changes in the patterns of cellular proteins, mRNA, transcription factors, etc. Accordingly, it is possible to use such measurements to identify the underlying intracellular processes associated with endothelial shear-dependent observations. This important field of research is relatively new and growing rapidly (16). A few references of relevance to the present work are noted among the following: it has been shown that laminar shear-stress exposure tends to upregulate the various genes that are thought to maintain the integrity of the endothelial surface; examples are the upregulation of the genes for production of superoxide dismutase (15), nitric oxide (15), prostacyclin (18), integrin (6, 49), plasminogen (14), etc.

In addition to studies of steady laminar flow fields, turbulent and temporally as well as spatially, varying flow fields also have been studied in cell culture systems and reported to activate various other unique patterns of genes (6, 10, 11, 31, 37). Some endothelial genes appear to be activated by particular aspects of the flow fields. For example, endothelial phenotypic expression has been reported to vary with location in endothelial spatial shear-stress gradients (12, 41).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Moreover, the expression of certain other genes appears to depend on various temporal gradients of shear-stress exposure such as those generated by impulse, pulsatile, and ramp flows (1, 19). Studies of such spatial and temporal shear-stress gradients are of considerable interest and importance because they can provide new insights into the cascades of stress-dependent signaling mechanisms in endothelial cells.

Thus previous studies have provided a large body of important new information from endothelial cell monolayers. It is important to place such observations in the context of the intimal-medial tissue system. To this end, there have been efforts by a few workers to study the effect of flow on arterial mass transport in the in vivo state (2, 22, 42) or in perfused vessels (38). In addition, perfused vessel segments (5, 32, 35) or surgical preparations (7, 40) have been used for studies of stress-induced phenotypic expressions of endothelial and smooth muscle cells in the intact arterial structure. The observations from these kinds of tissue studies have an added physiological significance because the cells are interacting in the native arterial structural environment. However, these kinds of approaches can only provide limited experimental control for studies of certain more complex questions. For example, such control is essential for studies of processes that initiate and orchestrate the coevolution of cellular interactions that lead to the development of atheroma and preatheromatous intimal structures.

Thus, to study these more complex tissue choreographies, an experimentally controlled, metabolically supported, ex vivo artery preparation was developed in an effort to provide the requisite control in an arterial architectural arena. This tissue-oriented methodology consists of an ex vivo metabolically supported swine artery preparation and specially designed perfusion and flow-configuring devices. With the use of this methodology, new observations are presented that explore coevolving, shear-stress-dependent, arterial responses as they appeared during 0- to 95-h periods of arterial shear-stress adaptation. For the present protocols, the following types of simulated hemodynamic shear-stress exposures were studied: 1) uniform laminar flow, 2) flow “separation-reattachment phenomena,” 3) multidirectional diverging-converging flows, and 4) stagnant flows.

**METHODS**

*Vessel harvest.* All procedures used in this study were carried out in accordance with institutional guidelines regarding the care and use of experimental animals and were done under aseptic conditions. All animals were fully anesthetized with telazol (6 mg/kg body wt)-xylazine (2 mg/kg body wt) followed by 2.5 mg/kg pentobarbital. Thereafter, pentobarbital was given as necessary to maintain a surgical level of anesthesia. A left lateral thoracotomy was performed, and heparin (3 U/ml blood) was then given and circulated 4 min before vessel excision. (This was to inhibit activation of platelets.) The descending thoracoabdominal aorta was then excised from young adult, ~70-kg, mixed sex, Hampshire-Durak, normocholesterolemic swine \(n = 17\). Swine are generally accepted models for studies of human atherogene-

Figure 1. A longitudinally opened, physiologically stretched artery (light yellow) with endothelial surface facing up and secured in the tissue-holding device (THD). The tissue is captured between the two parts of a “well assembly” device, a top well assembly (TWA) on the endothelial surface and a matching bottom well assembly (BWA) on the adventitial surface. When applied to the arterial surface, the device isolated the arterial sheet of tissue into 18 consecutive matching endothelial and adventitial chambers, respectively, along the vessel (as noted by the numbers on the side of the TWA in Fig. 1). As shown to the left in Fig. 1, the BWA chambers were equipped with pressure and adventitial perfusion lines. In the present study, the BWA chambers were also equipped with screen-covered, longitudinally grooved, adventitial support devices. The purpose of these was 1) to prevent downward deformation of the pressurized vessel surfaces so that the endothelial surface in each well was maintained in a flat configuration and 2) to direct a flow of fresh nutrient media along each adventitial surface to facilitate nutrient and metabolite diffusion at this surface.
After these tissue preparative procedures, the THD, containing the tissue and the attached TWA and BWA, was transferred into an environmentally controlled chamber, called an “organ support system” (OSS), for selected durations of exposure to various defined endothelial shear-stress distributions, as described subsequently. The gas environment was continuously monitored and refreshed by flowing fresh filtered (USP) air, O₂, and CO₂ through the chamber at 37°C (29). All wells were pressurized at 100 mmHg (transmural). In accordance with the particular experimental goal, the specially designed flow-configuring devices were then inserted into each well and secured by fitted mounting devices that capped the opening of each well. In the present protocols, contiguous quartets of wells in the TWA were chosen to examine four adjacent types of stress fields at four replicate sites along the vessel to minimize location dependence in the data (e.g., see wells 2–5 in Fig. 1 for one such quartet).

Before the details and application of the above flow-configuring devices are described, it is appropriate first to describe the OSS and the manner in which the devices were used. The overall experimental system to accomplish the above objectives is diagrammed in Fig. 2. The top portion of Fig. 2 represents the exterior environment of the OSS, whereas the bottom portion (under the horizontal, hatched OSS wall) represents the controlled interior environment of the OSS. Referring first to the top region of Fig. 2, each circle represents a four-channel bank of peristaltic pumps with 0.25-inch inner diameter (ID) tubing adjusted to full occlusion. Referring to Fig. 3, these pumps supplied pulsatile flows (72 cycles/min (cpm, i.e., pulse rate), peak flow of 87 ml/min, a minimum flow of −12 ml/min, and a mean flow of 52 ml/min) to each quartet of the flow-configuring devices shown in Fig. 1. Referring to Fig. 2, these devices are diagrammed in the midline elevation view of the TWA. (For simplicity, the applicator mounts have been deleted in the diagram.) In this particular protocol, flow was supplied directly to each FA (or F) flow-configuring device as indicated by the solid lines and arrows in Fig. 2. The supply pumps to these lines operated steadily (“STEADY OP”) throughout the experimental duration.

In contrast, the two circles on the right in Fig. 2 (WASTE and REFRESH) each represent four-channel sets of pumps that operated periodically to refresh and simultaneously remove old nutrient reagent from the TWA (see dashed lines with arrows). These pumps were programmed to be activated every 2 h for 20-s durations. This was to supply fresh media (REFRESH pump) to the FA/R wells, thereby displacing the 2-h old media in the wells, pumps, and tubings of the FA, R, and F wells into the W well. This overflow went from well to well into the W well via rectangular apertures (12 mm wide × 4 mm deep) across the top of each well partition (note clear area at the top of the hatched region in Fig. 2). This displaced media was simultaneously pumped (WASTE pump) from the W well into a waste beaker (OLD MEDIA), as indicated by the dashed lines. These REFRESH and WASTE pumps were set at higher mean flow rates than the STEADY OP pumps on the left in Fig. 2. The larger flow rate was to ensure adequate replacement of all (2 h) old media. The volume provided during the 20 s of refresh flow was threefold greater than the volumes of each well quartet, pumps, and lines. The chemistries of both refresh and waste media in the system were measured at the beginning and end of each 2-h period throughout the study. A Radiometer ABL30 was used for measurement of blood gases (PCO₂, PO₂, pH, and HCO₃⁻), and an EML105 was used for Na⁺, K⁺, Ca²⁺, Cl⁻, lactate, and glucose measurements at 37°C. These 2-h chemistries did not change significantly throughout the entire duration of a study. Accordingly, the chemical composition of the milieu on the vessel was maintained as virtually constant by the periodic refreshment scheme noted above.

**Designs for the flow-configuring devices.** As mentioned, the quartet format of the wells (Fig. 2) was chosen in the present study so that at least 4 types of flow fields could be replicated at 4 separate regions along the 18-well preparation to average out site variability (30). For reference purposes, the sketched curved arrows in Fig. 2 in the FA, R, and F well chambers represent the general directions of the STEADY OP velocity fields. The corresponding directions of the endothelial surface shear-stress fields are suggested by the second
set of arrows sketched in each of the squares below each well in Fig. 2. Because the boundaries, entrance conditions, and fluid constitutive properties of the flow were known, the velocity fields and corresponding endothelial shear-stress fields were evaluated by computational fluid dynamics (CFD). Because the requisite boundary conditions for such computations are determined by the geometries of the flow applicators in the wells, these designs will be described next.

One flow-configuring device called an “FA” (see Fig. 2) was designed to simulate ordered in vivo laminar flow fields. When the FA device was installed in a well, a flow channel with a rectangular cross section was formed for flow between the smooth, flat bottom surface of the FA device and the endothelial surface of the artery. Thus the cross section of the flow channel was rectangular, being formed on the top by this device and on the bottom by the flat surface of the interstitial endothelium. The adjustable separation (δ in mm) of these two parallel surfaces formed the small dimension of the rectangle. The transverse dimension (w; in mm) of the channel was formed by the distance (12 mm) between the lateral well walls of the TWA. The length of the FA well between its parent and daughter regions was also 12 mm; however, the length of the uniform part of the FA flow channel was formed by the length (10 mm) of the flat bottom surface of the FA device, as discussed below.

Referring again to the sketched arrows in Fig. 2, the STEADY OP efflux flow from the FA endothelial surface exited, as indicated by the curved arrow, through the aforementioned opening across the top portion of the right partition, into the adjacent flow return well (R well). Simultaneously, the R well media were continuously drawn directly back to the FA pump from the R well through the return pipe. (The 3-mm-diameter pipe was situated 3 mm above the midline of the endothelial surface at the right partition of the R well.) Thus the FA and R flow systems comprise a single two-well flow system.

In contrast to the FA well, the designs of the R well, and remaining flow-configuring devices, were developed to simulate more complicated, nonuniform types of flow fields of relevance to atherosclerosis. The flow configuration of the R well was designed to approximate the essential features of the flow separation-reattachment phenomena that occur in entrance regions of arterial daughter branch vessels. The relationship of such branching configurations to the design of the R well simulation is diagrammed in Fig. 4. The top diagram in Fig. 4 is a schematized representation of the in vivo flow patterns in such regions of vessel branching; the bottom diagram represents an effort to simulate the essential features of this flow situation on the endothelial surface of the R well.

Referring first to the top diagram in Fig. 4, a vertical parent artery is shown with a horizontal daughter branch extending to the right. The corresponding flow fields are approximated by the streamlines shown as lightly sketched lines with arrows representing velocity directions in the flow. Note that the right streamline in the (vertical) parent vessel must bend sharply to the right to enter the (horizontal) daughter branch. The momentum of the vertical parent flow prevents the streamline from immediately reattaching to the horizontal daughter wall, thereby creating a region of disordered, slower moving (low momentum) fluid. This region is called the region of flow “separation” (S) and extends from the “point of separation” (S1) to the “point of flow reattachment” (S2) (Fig. 4).

The bottom diagram in Fig. 4 represents the flow configuration in the R well designed to simulate the essential features of the above separation-reattachment flow field. It can be seen that the endothelial surface of the R well is exposed upstream to an area of flow separation originating at the point where the vertically directed velocity from the FA device effluent must be directed horizontally into the R well. In contrast to this region of simulated separation, the contiguous downstream region of the R well surface sees a more-ordered elevated shear-stress field below the R well flow-return pipe (located 3 mm above the midline endothelial surface at the right partition). This region experiences a reasonably ordered, radially converging field of increasing velocity and shear stress, as suggested by the converging arrows on the enface view of the R well floor in Fig. 2. This shear-stress field is quantified subsequently in RESULTS by the computed R well shear-stress field (see Fig. 5). Thus, like the above daughter branch, the endothelial surface of a R well sees a disordered low shear-stress flow on its upstream region (flow separation), immediately downstream from which it sees a more ordered shear-stress field (flow reattachment), as occurs in the in vivo flow situation.

**F well configuration.** The flow configuration of the F well was designed to examine the effect of ordered radiating shear-stress fields and gradients. To do this, a two-pipe flow-configuring device was designed, as shown for the F well in Fig 2. This configuration had proximal (left) inflow and distal (right) efflux pipe orifices located 3 mm above the endothelium in the midline of the well, as shown in Fig. 2. Unlike the R well, the surface of the F well experienced two, nonuniform, shear-stress fields, as sketched in Fig 2 and quantified in RESULTS (Fig. 5). The input pipe on the left created a diverging laminar stress field emanating from a
central stagnation point below the pipe orifice. The efflux flow into the right pipe created a similar but converging stress field.

*W well configuration*. The last well (W well) in the quartet was a “zero shear-stress exposure” well, in which the efflux pipe was located remotely at the fluid surface 29 mm above the endothelial surface. The periodic 2-h waste flow from the other wells into the W well occurred through the aforementioned rectangular aperture of the left partition and then to the “waste” efflux pipe on the right. Moreover, this only occurred during the 20-s refreshment of the media in the quartet every 2 h. Accordingly, the shear stress on the W surface was virtually zero, even during the brief (20 s) refreshment periods. (A removable rectangular silicone rubber dam was inserted in the rectangular aperture across the top of the right partition to isolate the two quartets.) The design of the W well flow configuration was to examine the tissue responses to various durations of flow cessation temporarily in vessel branches during severe reapporportionment of flow distribution or after (temporary or permanent) surgical occlusion of a vessel.

Computations of endothelial surface shear-stress distributions. Methodology for direct measurements of endothelial surface shear stresses in the small flow geometries of the present study is currently unavailable. Moreover, analytic mathematical formulas for the complex flow geometries of the study also do not exist. Accordingly, such shear-stress estimates must rely on approximations provided indirectly from calculations of the relevant flow velocity and corresponding shear-stress fields using a computer. Performing such approximations is referred to as CFD. Software for such complex calculations is commercially available. Doing such computations is essentially a two-step process. In the first, the enclosed geometry of the flow environment is defined and filled with a “finite-element computational mesh” that divides the entire volume of the flow system into a three-
dimensional array of contiguous mechanically interacting finite fluid elements. The preceding geometries of the FA, R, F, and W wells define the requisite enclosed flow environment for the mesh. This mesh and the sizes of the finite elements in the mesh are detailed subsequently in the Appendix.

Evans blue dye-albumin complex permeability measurements. At the end of each experimental stress exposure, all flow devices were removed from the wells, cultures for bacteria and fungi were taken, and the nutrient media were then replaced with 100% sterile serum containing a known concentration (c₀, in nmol/cm³) of Evans blue dye-albumin complex (EBD-alb). This EBD-alb-containing serum medium was stirred continuously throughout this period (~1 h) to avoid concentration polarization of EBD-alb. This EBD-alb-containing serum medium was replaced with 100% sterile serum containing a known concentration of EBD-alb in the serum, and this was replaced with 100% sterile serum containing a known concentration of EBD-alb in the serum, and then transferred to the buffered sucrose solution. After these fixation periods, the tissues were prepared for light microscopy by acetone and alcohol dehydration followed by glycolmethacrylate resin (GMA) infiltration. The tissues were then multiply embedded in GMA as a single block for comparative light microscopic study of the stress-dependent structural changes along contiguous wells. Each multiply embedded GMA block, which contained the four types of stress field-exposed tissues from a given quartet, was serially sectioned at 2 µm to obtain longitudinal arterial histological sections from the central axial region of all wells. These were stained with methylene blue-basic fuchsin. In addition, using a modified resin etching technique (47), some of the formalin-fixed tissue sections were also immunostained for endothelial cell nitric oxide synthase (ecNOS; Serotec No. MCA1746).

In an effort to characterize significant ultrastructural features associated with the initiation of structural changes in the flow separation stress exposures, several glutaraldehyde-fixed tissues from the 23-h simulated flow separation regions (dark region of interest (R_d)) were prepared for scanning (SEM) and transmission electron microscopy (TEM). Standard alcohol dehydration, critical point drying, and gold sputter procedures were used for the SEM preparations. Preparative procedures for the TEM were standard alcohol dehydration, resin embedding, sectioning, and osmium staining techniques.

RESULTS

The results of the computed endothelial shear stress fields are presented in Fig. 5 for the FA, R, and F flow configurations as noted at the top of each column. (The W shear-stress field was zero and thus does not appear.) Referring to the bottom row in Fig. 5, note that the computed endothelial shear-stress fields are represented by the magnitudes and directions of the vector field plots for each well. The small, horizontal bar at the bottom in Fig. 5 is the scale factor for that vector field. (Note also that the endothelial stress vectors are equal in magnitude to, but 180° from, the corresponding fluid shear-stress vectors.) The circles in each field represent the location of the flow supply pipes for reference purposes. Those for the R and F wells represent the actual flow applicator portion of the unit 3 mm above and facing the endothelial surface directly. The circle for the FA well does not communicate directly to the endothelial surface, but feeds the left vertical flow manifold, which was designed to produce the uniform flow field on the FA endothelial surface. The stress vector fields corresponding to the R well and the F well are seen to be nonuniform. These nonuniformities are related to the converging or diverging flow fields around the pipe orifices. The corresponding three-dimensional velocity vector fields were too complex to depict in two dimensions, but followed the general paths that were indicated in the flow logic diagrams in Fig. 2 or 6.
More importantly, the regional magnitudes of the associated shear-stress fields on the endothelial surfaces are represented in Fig. 5, top, by the numbered (1–12), color-coded regions in the figure. Each color-coded region represents the corresponding area-averaged shear-stress vector magnitudes between selected shear-stress isopleths. The corresponding numbered keys to this color coding appear in Fig. 5, middle, as the mean shear stress (±SD) for each of the numbered colored regions in Fig. 5, top.

Referring to the magnitudes of the FA endothelial surface shear-stress field, it can be seen that the shear stress was uniform at ~16 dyn/cm² over most of the FA flow channel. More specifically, stress was sharply elevated to ~26 dyn/cm² along the first 2 mm of the channel (the “entrance effect”), ~16 dyn/cm² over the next 7 mm, and ~3 dyn/cm² over the last 1 mm of the flow channel. Thus properties of the shear-stress field over the 7-mm-long, uniform shear-stress region of the FA regions were shown to be laminar and uniform except very near the well walls (Fig. 5, see FA vector field). The properties of the R and F stress fields were much more nonuniform and interesting than those of the FA fields. However, it is more appropriate to consider these more complex stress patterns in the DISCUSSION, after presentation of the corresponding tissue stress-response data.

The corresponding stress associated evolution of permeability changes in the FA-R and F wells are shown in the photographs of Fig. 6, bottom. These EBD-alb uptake data appear as the blue patterns in each color-coded region of wells under the OSS flow logic diagram (Fig. 6, top). The various durations of shear-stress exposure are noted at the left of each row of photographs. Referring to the final row of well tissues, it can be seen that a 1-h exposure to the FA, F, and R types of shear-stress fields was not accompanied by a significant change in the intimal EBD-alb uptake responses. However, referring to the next row (23 h), it can be seen that the uptake increased during the initial 23 h of exposure to each of the different stress fields. With the exception of the F wells, such patterns appear as uniformly mottled blue with little relationship to the type of imposed stress field. However, over the ensuing stress exposure durations, these patterns appear to change progressively with time to converge toward the virtually stable new set of EBD-alb uptake patterns shown for the 75- and 95-h stress exposures (Fig. 6).

In addition to these visual assessments, the magnitudes of these EBD-alb permeability patterns were estimated by the photometric scanning and analytic procedures described in METHODS. The individual area-averaged permeability values (μ × 10⁶ cm²/s) for each major region of interest in the wells appear in the four scatter plots of Fig. 7. In Fig. 7, A–D, u is on the ordinate and the duration of the respective stress exposure (t; in h) is on the abscissa. Each data point represents the mean (n = 4) value of the u data for the particular shear-stress field at its four locations along the vessel to minimize location dependence in the data (30). Referring to Fig. 7A, the FA (circles) and the F (triangles) permeability responses were similar despite the radically different directions and magnitudes of the stress vectors on these endothelial surfaces. A single smooth “trend” curve for these FA and F stress-dependent permeability responses was sketched through these data points for subsequent comparisons to corresponding trends for the other types of stress field exposures (Fig. 7, B–D).

The FA/F temporal trend curve suggests that after the abrupt transition from the in vivo stress-adapted state to the new ex vivo stress-stressed state, the permeability rose, after a brief 3- to 4-h latent period, to a maximum at ~23 h. The curve then declined gradually over the ensuing hours (75–95 h) toward the former in
vivo-adapted values. Unlike the permeability patterns in the FA/F regions of interest, which were reasonably uniformly distributed over the well surface, the R well permeability patterns tended to approach a bimodal distribution (see the R well column of the photographs in Fig. 6). This can be seen most clearly for the R wells for the 75- and 95-h exposures in Fig. 6. Accordingly, the permeability estimates for each mode of the R well regions of interest were analyzed somewhat arbitrarily as from predominantly uniform regions of "light blue" or "dark blue." This division was admittedly subjective and ignored the regions of transitional uptake values between the two "uniform" regions of the R wells. The permeability data for the "light blue" region of interest in the R well are presented in Fig. 7B, labeled as RL. As noted, this predominantly lightly stained region is around the flow return pipe (simulated flow reattachment region). The temporal data for the "dark blue" region of interest in the R well appear in Fig. 7C, labeled as RD, the simulated flow separation region in the imposed flow field.

The temporal evolution of permeability in the reattachment region (RL) rose to a maximum at 23 h and then decreased briefly with a trend resembling that of the FA/F curve in Fig. 7A; however, this decline reversed around the 40-h point and thereafter rose slowly to higher values. Referring to the RD trend curve in Fig. 7C, this curve appears to have a shape that is similar to that of the RL curve but has a greatly increased magnitude (note ordinate scale change in Fig. 7, C and D). Moreover, comparing these RD response data with those in Fig. 7D for the flow "stagnation" simulation (W well), the permeability responses appear to be indistinguishable. Thus only the FA and F shear-stress fields appear to be associated with a normal permeability adaptation. Not surprisingly, all of the above stress-dependent permeability response data will be shown to be associated with interesting coevolving intimal-medial structural changes including raised intimal lesions, as described below.

**Gross visual changes.** Visual examination of the endothelial surface textures of unstained and only slightly stained EBD-alb uptake regions in all wells consistently revealed a smooth glistening surface that was flat except for the normal, slightly elevated patterns of the underlying bands of intimal connective tissue. In contrast, most darkly stained (high permeability) areas of the RD (and W) endothelial surfaces developed elevated, edematous-appearing intimal regions covered with slightly gray fields of small, rounded elevations. These "dark blue" regions presented a pebbly, gelatinous appearing surface texture resembling fields of small blisters. An en face photograph of such a surface is shown in Fig. 8 for a 23-h R well with the RD (flow separation) region on the left and the RL (flow reattachment) region on the right. In some of the edematous RD regions, a grayish, opalescent, mucoid-appearing surface layer tended to obscure the blistered texture. The corresponding microstructural characteristics of these lesions, as well as normal regions, are detailed below.

**Histological changes.** The evolution of histological changes associated with the evolution of the permeability responses in Fig. 7 are represented by the photomicrographs in Fig. 9. The top photomicrograph represents a "control" tissue section from a nonstress-
In contrast, the intimal structural responses for the Rd exposures appear to be a progressive endothelial degeneration with intimal edema that begins to form during the first 23 h of exposure. By 75 and 95 h of exposure, these changes have advanced to total destruction of the normal endothelial-intimal architecture and the development of raised, edematous, and gelatinous-appearing intimal lesions. These lesions were found only in the high permeability (dark blue) regions (e.g., see Figs. 8 and 9). The histological components of these raised lesions appear to consist of partially digested cellular and structural debris suspended in a proteinaceous liquid milieu that is frequently covered with a single layer of abnormal (endothelial?) cells. The content of the larger, more mature, intimal lesions in the 75- and 95-h Rd tissues appear to have more of this proteinaceous-appearing, homogenous phase containing even larger aggregates of coalesced and flocculent material with poorly defined structure. Adjacent “endothelial cells” appear to have migrated and extended cytoplasmic projections over and, to a much lesser extent, under the proteinaceous region of suspended debris.

It is of considerable interest to compare the subjacent medial remodeling responses of the Rd tissues with those in the Fa tissues. Despite the extensive differences in their intimal responses, the corresponding medial remodeling of the Rp and Fa tissues were virtually the same. For example, by 75 h, and particularly by 95 h of exposure, both types of intimal-medial interfacial layers appeared to have remodeled into virtually identical compact tissue barriers of increased smooth muscle cells and elastin plates embedded in greatly increased matrix material (light grayish lavender).

**Immunohistology for ecNOS.** In view of the intimal devastation, cellular migration, and apparent sequestration or engulfment activity (see below) associated with the “surface” cells in the Rd intimal lesions, the question of their phenotypic identity arose. In many ways, these “surface” cells acted more like macrophages or migrating “modified” smooth muscle cells than endothelial cells. In an effort to clarify their identity, a formalin-fixed, 75-h pair of Fa and Rp well tissues were immunostained for ecNOS. These sections appear in the photomicrographs in Fig. 9, bottom. Referring to the Fa tissue on the left, it can be seen that the endothelial surface cells are strongly ecNOS positive (stained red). Referring to the corresponding Rp tissue section on the right, these “endothelial” cells also appeared to be ecNOS positive, although somewhat less so. Unlike the Fa tissue, the Rp tissue also developed discrete ecNOS positive extracellular “granules.” These appear to be scattered throughout the matrix of the subjacent intimal-medial tissues but not in or on the smooth muscle cells of this layer. (Negative control slides indicated no nonspecific binding.) This suggests that the “normal” Fa and “abnormal” Rp intimal surface cells are of endothelial origin and are not macrophages or smooth muscle cells.

Exposed but otherwise methodologically identically prepared (including 1-h EBD-alb exposure) ex vivo tissue preparation. This “control” histology provides a reference for evaluating structural differences due to preparative and EBD-alb exposure procedures from the structural differences induced solely by the duration and type of shear-stress exposures. Examples of salient stress-induced changes are represented by the paired Fa and Rd tissue sections shown for 23-, 75-, and 95-h shear-stress exposures, respectively.

Comparison of the control photomicrograph (Fig. 9, top) with the tissues for 23-, 75-, and 95-h shear-stress exposures demonstrates a temporal progression of stress-dependent structural changes. The nature of these structural progressions is clearly different for the Fa (left) and Rp (right) stress field exposures. The sequence of intimal structural changes for both exposures appears to be a complex progression of intimal-medial cellular and structural remodeling. Referring to the Fa stressed tissues, it appears that subtle endothelial nuclear inclusions and/or fragmentation (apoptosis?) begin to appear at 23 h. These changes become more evident in the 75-h tissues. By 95 h, however, the endothelial cells appear to have become more ordered with less nuclear fragmentation. Throughout these ongoing Fa remodeling processes, the structural integrity of the luminal interfacial endothelial cell layer appeared to have been preserved. The apparent goal of this Fa intimal-medial tissue remodeling response appears to be restoration to a functionally normal, albeit hypercellular, endothelial surface and intima. This Fa well endothelial-intimal layer has become undergirded by a remodeled, densely compact, nonedematous tissue layer of increased smooth muscle cells, connective tissue, and tissue matrix. The matrix appears as the irregular grayish lavender structures occupying the space between the musculoelastic lamellar layers.

---

**Fig. 8.** Gross appearance of the endothelial surface after a 23-h exposure to a R well shear-stress field. On the left, observe the edematous field of small blister-like elevated lesions in the darkly EBD-stained Rd (simulated flow separation) region. On the right, note the virtual absence of these lesions in the lighter-stained Rl region (simulated flow reattachment).
Early lesion ultrastructure. The endothelial ultrastructural features of the initial 23-h R\textsubscript{D} stress-dependent responses are also of considerable interest. Typical changes are represented in the scanning and transmission electron micrographs of Fig. 10. The scanning electron micrographs (Fig. 10A) show a variety of elevated (light gray) structures lying on the (dark gray) endothelial surface. Looking more closely, this darker surface contains scattered small openings (black) to the subendothelial tissues. There also appear to be at least three other surface structures of note. The first are elevated, blebbed spheres with radiating cytoplasmic tethers to or from the subjacent surface. The second are two large, relatively flat structures resembling migrating cellular lamellapodia, one of which seems to be engaging the upper region of a blebbed sphere. The third are irregular patches of particulate debris scattered on the endothelial surface.

The transmission electron micrograph in Fig. 10B demonstrates phagocytic activity of an intimal (endothelial?) surface cell at the luminal-plate interface. The cell has a very large phagosome (middle right) containing aggregates of flocculent tissue debris and (middle left) lamellapodia extending to the left in an apparent effort to engulf a typically apoptotic-appearing cell. (For example, note peripheral clumping of nuclear chromatin, a small rupture in the nuclear membrane, preserved organelles, and dense cytoplasmic particles resembling “apoptotic” bodies.)

**DISCUSSION**

The methodology detailed in this report represents an effort to develop an experimentally accessible arterial intimal-medial tissue system in which controlled, simultaneous observations of induced pathophysiological processes can be studied. In its present state, the methodological simulation of an in vivo artery has obvious imperfections, as implied in the detailed descriptions in METHODS. Those imperfections most relevant to interpretation of the present observations are as follows: 1) cell-free serum and modified MCDB-131 nutrient media instead of blood, 2) new planar instead of cylindrical mural strain fields, 3) metabolic dependency of the outer medial lamellar layers on diffusion from the adjacent adventitial flow of nutrient media instead of from the vasoasor, 4) potential sequella of the initial tissue harvest and preparatory procedures, and 5) shear-stress estimation in the FA system is very sensitive to the value of $\delta$. Further discussion of these “imperfections” exceeds the scope of this report and must be the subject of continuing studies.

Despite its imperfections, this methodology provided previously unavailable flexibility for experimental design and control of many experimental variables in arterial tissue preparations from swine (and ultimately from human donor arteries). The present study provided a well-controlled set of new data regarding temporal sequences of intimal-medial functional and structural changes associated with arterial adaptation to defined flow fields of interest. It was shown that when a previously in vivo-adapted artery is challenged by a new mechanochemical environment, it reacts first with an increased permeability. This probably represents a physiological dismantling of the endothelial barrier in preparation for adaptation to its new laminar or disturbed flow environment.

After the initial 23-h exposure to the new experimentally controlled environment, the subsequent sequences of permeability and structural changes appeared to become more shear-stress field dependent. Two distinctly different temporal paths of adaptive dynamics were observed. One path was shown to be a constructive adaptive sequence with a return to normal intimal barrier function. This was associated with the locally ordered laminar shear-stress field exposures of the FA and F wells. In contrast, the other path was shown to be a progressively destructive sequence of increasing intimal permeability, edema, loss of matrix, structural degradation, and raised intimal lesion formation. This path was associated with the disordered, low shear-stress fields of the R\textsubscript{D} and W regions of the artery. These data are of interest regarding in vivo atherogenesis.

Atherosclerosis normally occurs in vivo at particular localized sites of “physiologically” increased permeability in the chronically stress-adapted arterial tree (23, 30, 45). In the present work, the stress-induced permeability patterns of the 75- and 95-h stress exposures appear to be approaching the end stages of such stress adaptation. Therefore, these temporally averaged, ex vivo response data should be reasonable approximations to that of an in vivo, physiologically stress-adapted vessel, were it exposed to the same type of chronic laminar or low shear-stress field patterns used in the present study. Accordingly, it is of interest to compare the shapes of these 75- to 95-h simulated “physiologically adapted” EBD-alb permeability patterns to the patterns and values of the corresponding “chronic” shear-stress exposures shown in Fig. 5. This comparison allows one to examine the relationships of regional stress patterns to the corresponding regional physiologically increased permeability in the 75- to 95-h “stress-adapted” tissues shown in Fig. 6. Referring to Fig. 5, top and middle, as well as Figs. 6 and 9 together, simple inspection indicates that increased EBD-alb permeability and raised intimal lesions cor-

---

Fig. 9. Photomicrographs of 2-µm-thick, methylene blue-basic fuchsin-stained resin-embedded tissue sections demonstrating the evolution of the shear-stress-induced intimal-medial histological responses: control tissue (top), FA tissues (left), and matching R\textsubscript{D} tissues (right). Durations of stress exposure are noted in the center of each set of paired FA and R\textsubscript{D} photomicrographs. Staining characteristics for methylene blue-basic fuchsin are as follows: endothelial nuclei, deep purplish-blue; cytoplasm, red; smooth muscle cell nuclei, dark blue; elastic lamella, white; tissue matrix, irregular grayish lavender material in the interlamellar spaces; and collagen, light pink. Bottom: resin-embedded, formalin-fixed FA and R\textsubscript{D} sections from a 75-h study immunostained for endothelial cell nitric oxide synthase (red) and counterstained with hematoxylin (blue). Solid bar = 50 µm.
relate negatively with the intensity of the shear-stress exposure. Referring to Fig. 5, these comparisons suggest that stresses between 0.38 (regions 5–8) and 0 dyn/cm² (W well) were unable to sustain survival of the endothelial barrier and associated intimal structure. Thus the integrity of the endothelial surfaces and subjacent tissues depended on maintenance of organized, temporally averaged shear-stress fields having magnitudes of at least 1.43 dyn/cm², i.e., region 12 of the F well. Referring to the R1 well stress field, it appears that shear stresses as low as 0.15 dyn/cm² appear to maintain some initial but temporary protection for the endothelial barrier. However, by 75–95 h of exposure, the barrier in all regions of the R well appeared to be failing, with raised lesions continuing to develop, particularly in R0 shear-stress region 8 (0.03 dyn/cm²).

As mentioned in RESULTS, comparisons of the FA and R0 medial structural responses in Fig. 9 indicated that the subjacent medial tissues also play an active and significant role in the arterial adaptive remodeling processes. This role appears to be one of cellular, connective tissue and matrix hyperplasia to strengthen or preserve the intimal-medial junctional tissue barrier. The similarity of this medial hyperplastic remodeling in both the FA and R0 tissues suggests that the driving forces for these medial structural responses were not related directly to the detailed types of stress field exposures. Instead, this remodeling appeared to be driven by other related forces such as increased interstitial chemical and hydration gradients, as well as the generation of various metabolites and stress-induced paracrine substances associated with the antecedent permeability response trends. In any case, the sequence and nature of the medial adaptive processes for FA and R0 environments appeared to be indistinguishable. Such adaptive processes are of interest and importance in understanding the pathophysiological mechanisms of atheroma formation.

Intimal pathophysiological implications. Were some of the hemodynamically dependent lesions that have been described to occur in a blood-filled artery, then the exposed intimal collagen obviously would be thrombogenic. Moreover, with or without thrombus deposition, the detailed patterns of transmural mass transport of water and macromolecules would be greatly distorted from that in the normal permeability case. In the normal case, the endothelial surface is essential for the continued maintenance of an optimized cellular, structural, and molecular environment in the arterial interstitium. Among other things, this normal interstitial state is maintained by the structure-dependent diffusive and convective paths of mass transport across the pressurized endothelial cell layer and its subjacent, gelled matrix system. The normal unpressurized endothelial cell surface and basement membrane act as a diffusive barrier that, when pressurized, also acts as a macromolecular sieving barrier (28, 29). In this case, the macromolecular flux becomes “sieved” or retarded with respect to the transmural water flux at the plasma-endothelial interface (27, 29). Accordingly, the chemically active concentration of plasma macromolecules at the normal endothelial (or at the basement membrane) interface would become progressively higher than that in the plasma were it not continuously diffused or swept away by the adjacent blood flow (27–29). Moreover, the associated transendothelial pressure drop related to this sieving is essential for maintenance of an optimal hydration of the compact (gelled) state of the subjacent endothelial-intimal matrix. With failure of the endothelial surface barrier, such as occurred in the R1 and W tissues, the transendothelial pressure gradient is lost, allowing the intimal gel phase to swell and ultimately dissolve. The previous, normally protein-poor, intimal plasma filtrate no longer can be maintained. Instead, this dilute filtrate is replaced by virtually pure plasma molecules mixed with soluble matrix molecules from the now unconstrained dissolution of the intimal gel-phase matrix (gel ↔ sol) (8, 34, 44). The overall transmural pressure gradient will now drive a convective flux of this unsieved mixture of macromolecules further into the deeper tissue layers. When this flux encounters a new macromolecular barrier, e.g., the aforementioned compact subjacent intimal-medial layer (Fig. 9), a new sieving site will be created. Because this site is seques-tered from the blood flow, the resulting accumulation of rejected solutes can rise to abnormally high (chemically active) interstitial concentrations (27–29). These regions finally reach new but abnormally high steady-state transmural concentration distributions. This new steady-state mass transport occurs at the point where diffusive fluxes (mostly retrograde to the lumen) from the sieving site become equal to the rates of convective solute rejection at the site (26, 27). Unlike the associated accumulation of soluble matrix molecules, the associated interstitial accumulations of plasma-borne atherogens at the site, e.g., oxidized free and bound LDL (46), only can be removed by inflammatory phagocytic transport and degradation (thus initiating an atheromatous atheroma?). The R0 intimal-medial structural responses shown in Fig. 9, right, provide the necessary architectural elements for such pathological mass transport scenarios.

Relationship to collateral fields of research. Most of the present set of observations are unique but have relevance to a number of previous important observations. First, assuming that the present gelatinous lesions are related to subsequent atheromatous changes, this finding would be in agreement with earlier correlative data, suggesting that human atheroma appear to develop at presumed sites of low or disturbed shear-stress fields (33). Second, the gross and microscopic architectures of the induced intimal lesions of this report are very similar to the human “gelatinous lesions” described in the older histopathological literature (36, 50) as potential precursors of atheromas. Third, it has been shown that human gelatinous lesions occurred most frequently in regions near or on the edges of mature, raised atheroma (36). In the case of elongated raised human atheromas, it was noted that gelatinous lesions tended to localize frequently at the upstream and particularly at the downstream end.
of the raised atheroma. These are sites where locally disturbed, low shear-stress fields, similar to the present $R_D$ fields, might be expected. Fourth, in this regard, it has been shown that such sites also have a significantly higher incidence of apoptotic endothelial cells than other regions (48). Taken together with the observations in Ref. 36, one could infer colocalization of gelatinous lesions with apoptotic activity as also might be inferred from the electron micrographs in Fig. 10 of the present study. Fifth, this inference would be consistent with recent tissue culture studies in which sustained exposure to laminar shear stress was shown to prevent chemically induced activation of cellular apoptotic enzyme systems (15). In contrast, disturbed flow has been shown to induce apoptosis in the endothelial cell monolayer (20). Finally, partial ligations of the left carotid artery in immature (3 wk old) rabbits have been shown to be associated with greater apoptotic degradation of the arterial endothelial and smooth muscle cells in ligated than in nonligated control arteries (7).

The present research has demonstrated, for the first time, a chain of evidence directly linking vascular regions of disturbed, low shear-stress exposure (<1.43 dyn/cm²) to colocalized evolution of decreased endothelial barrier function in a porcine arterial preparation. These regions of decreased endothelial barrier function were shown to develop a proteinaceous-appearing intimal edema, loss of subendothelial ground substance, and the subsequent development of raised, partially covered intimal lesions resting on a remodeled, hyperplastic intimal-medial interface. As discussed before, this intimal-medial structural configuration has the essential features of a preatheromatous architecture. This work would seem to provide new experimental points of departure for interdisciplinary studies of endothelial, smooth muscle, blood cellular, macromolecular, and stress-generated messenger interactions as they evolve in the complex intimal-medial architecture where atherogenic (and antiatherogenic) processes normally reside.

APPENDIX

Finite-element computations of endothelial surface shear-stress distributions. As mentioned in Methods, shear-stress computations must rely on approximations provided indirectly from calculations of the relevant flow velocity fields using a computer. Performing such approximations is referred to as CFD. Software for this complex calculation is commercially available. Doing these computations is essentially a two-step process. In the first, the entire enclosed geometry of the flow environment is defined by the flow-configuring devices and the TWA boundaries of the flow. This flow system is then filled with a finite-element computational mesh that divides the entire volume of the flow into a three-dimensional array of contiguous mechanically interacting finite fluid elements defined by the nodes (knots) in the mesh.

The larger the number of nodes in each cubic millimeter of the mesh, the better will be the approximation to the actual flow field. Thus the accuracy of the approximation depends on the size of the available computer memory to store an enormous number of such elements. To determine the upper limit of this mesh resolution, the computed shear-stress values (in the FA flow field at the endothelial surface) were computed as a function of various node densities. The linear regression of these inert computed FA stress data versus node densities had an intercept of $-16 \text{ dyn/cm}^2$ (400 nodes/mm³) and a slope of $0.00264 \text{ dyn/cm}^2/\text{nodes/mm}^3$, defining a dependency of the computed shear stress on node density. These data indicated that the computed shear-stress values decreased slightly (~5%) over the range of 400–100 nodes/mm³. Accordingly, these data revealed a very modest dependency of computed shear stress on mesh element density.

The second step was to supply the maximum available node density (400 nodes/mm³), boundary conditions, fluid constitutive parameters, and the entrance velocity field of the flow system to the CFD solver. This solver then calculated the requisite velocity fields and associated endothelial surface shear-stress distributions for the study. The software to deal with the boundary geometry, mesh generation, and fluid dynamic calculations was run on an Origin 200 (Silicon Graphics) computer. Gambit (version 1.3, FLUENT) was used to generate the geometry and create its computational mesh. FIDAP (version 8.6, FLUENT) was used to compute numerical solutions to the combined Navier-Stokes and continuity equations assuming a Newtonian fluid with no-slip (zero) flow on the boundaries and a blunt velocity profile for
the inlet flow (52 ml/min). This entrance flow condition was located at the top of the FA inlet pipe to the manifold channel on the left of the FA flow applicator. The constitutive fluid parameters were as follows: viscosity ($\mu$) = 0.0144 poise (50% serum and MCDB solution) and fluid density ($\rho$) = 1.035 g/ml.

The specific boundary conditions for the present work corresponded to the smooth wetted inner surface geometries of the various flow-configuring devices, the well walls in the TWA, and the endothelial surfaces in the well bottoms. These geometries were either planes or cylinders (see Fig. 2). More specifically, these boundary conditions were as follows: 1) the aforementioned sets of inside dimensions of the well walls and endothelial surfaces (the dimensions for each of the lateral well walls were 12 $\times$ 12 $\times$ 29 mm); 2) the dimensions of the corresponding well partitions were 12 $\times$ 12 $\times$ 25 mm high; 3) the dimensions of the endothelial surface in the bottom of each well were 12 $\times$ 12 mm; 4) the outer surfaces of the cylindrical in-flow efflux pipes in the fluid were 26 $\times$ 3 mm diameter; and 5) the vertical flow manifold channels on the left and right surfaces of the FA flow configuring device were 15 $\times$ 1 mm on the right and 9 $\times$ 1 mm on the left (refer also to Fig. 2); 2) each varying flow patterns are of most relevance to the flat under surfaces of the flow applicator mounts, which extended 2 mm into each well. Thus the upper boundary of the flow in all wells was 29 mm from the endothelial surface.

Of these boundaries, the separation of the FA device from the endothelial surface (\delta; in mm) is the most critical measurement. This gap was set on the FA applicator mount to be 0.65 mm above the TWA well bottom. However, using a dental casting material, the endothelial intimal surface layer was found to extend ~0.1 mm into the well chambers. Accordingly, \delta was chosen to be 0.55 mm for the present computations. These flow boundaries were assembled using the special Gambit software tools, e.g., boxes, cylinders, spheres, etc., to create the more complex FA-R and F flow geometries.

Use of the steady entrance flow condition was based on the following considerations: the pulsatile flow velocity and shear-stress fields are most intense in the FA system. Accordingly, the deviation of flow from constant flow was estimated by the Womersley unsteadiness number (\alpha) in the FA flow; \alpha was found to be a modest 1.3. The deviation from laminar flow conditions was estimated by the Reynolds number (\textit{N}_R). The mean flow \textit{N}_R was 51.9, and the peak flow \textit{N}_R was 86.3. At these peak values of 0 < \textit{N}_R < 86.6, and \alpha = 1.3 for the FA flow field, flow would be laminar (45) and for many computational purposes could be approximated by the mean values of the experimental pulsatile flow (51).

Moreover, the use of the constant inlet flow assumption in the present analysis was also consistent with the ultimate objectives of the present research, i.e., relationships of different chronic shear-stress fields to atherogenesis. For example, raised atherosclerotic lesions (atheroma) develop gradually over relatively long periods of time, e.g., months. These lesions occur in a branching vascular system that is continuously exposed to widely varying, unknown or, at best, poorly defined diurnal patterns of pulsatile flows along its entire extent. Because we do not have specific detailed knowledge of such chronic diurnal flow patterns, present purposes are adequately served by assuming that the mean long-term values of such varying flow patterns are of most relevance to atherogenesis. In any case, the “chronic” spatial distributions of endothelial shear stresses in the present work were computed using the mean flow (52 ml/min) as the entrance condition for computation of the simulated chronic endothelial shear-stress exposure patterns in the FA-R and F wells. These patterns and their significance are considered in more detail in the Discussion.

The author gratefully acknowledges the people that helped with the many different tasks of this labor-intensive research. Were it not for the dedicated, 24 h/day “espirit de corps” of the author’s technical team, it is unlikely that this research could have been done. The author expresses heartfelt gratitude to Carolyn Groff, who has been deeply involved in every phase of the planning, methodological development, and execution of each of these complicated experiments, Steve Spencer and Chris Thompson for laborious collection of blood, useful suggestions, and careful preparation of the large volumes of sterile pooled porcine serum-MCDB nutrient media, Art Sayre for fabrication and maintenance of the specially designed instruments and equipment, and Paul Clingan for assistance with FIDAP computation of the velocity and shear-stress fields. Steve Spencer also worked out the immunostaining of the GMA embedded tissues. The author also gratefully acknowledges helpful discussions with Dr. Kenneth Jones regarding apoptotic processes, assistance of Brian Kemmenoe with the TEM, John Mitchell with the SEM, and the generous access to porcine blood at Falters Packing Plant (Columbus, OH).

This research was supported National Heart, Lung, and Blood Institute Grant HL-29095 and the Ohio State University Development Fund.

REFERENCES


