Hemodynamic Influences on Vascular Endothelial Biology* 1

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ABSTRACT

The vascular endothelium resides in a unique biomechanical stress environment resulting from the hemodynamics of the system. In vivo studies indicate that there are regional differences in endothelial biology and that this may be due to the influence of the local hemodynamic environment. To investigate this further, cell culture studies have been conducted using well-defined mechanical stress environments. To study flow effects, we have employed a parallel plate chamber in which endothelial cell monolayers are exposed to laminar flow. In such experiments and concomitant with changes in morphology, there are a variety of other alterations in cell function, including a decrease in the rate of cell proliferation for subconfluent monolayers. Changes in cell behavior due to the direct effect of pressure and in cultured cells which are in a cyclical stress field also have been observed. In the recognition/transduction of such a mechanical signal, the pathway may possibly include a membrane event linked to the control of intracellular calcium. It may be that the same signaling mechanisms are involved both in cytoskeletal/shape changes and in the control of the cell’s growth program and, in exercising such an influence, hemodynamics may have an important role in the response of the arterial wall to injury and the resulting repair and/or disease processes.

Keywords. Endothelial cell; hemodynamics; mechanical stress; shear stress; vascular endothelium

INTRODUCTION

The vascular endothelium is the cellular monolayer which forms the inner lining of an artery. As such, it occupies a strategic location in which it is the interface between the blood flowing through the vessel and the wall itself. Although once viewed as simply a passive barrier between blood and the underlying components of the wall, the endothelial cell is now recognized to play a dynamic role in the vascular system, both in the regulation of normal biology and in the response of the arterial wall to injury and the resulting repair and/or disease process. Thus, in addition to its thrombo-resistant nature and its ability to control blood-arterial wall transport processes, to any list of properties of arterial endothelium we must include such functions as the regulation of vascular tone, the role of the endothelial cell in the recruitment-attachment of inflammatory cells, the synthesis of connective tissue as well as other substances, and its participation in the complex cascade of events initiated when the integrity of the endothelium is violated.

In being the inner lining of an artery, the endothelium is directly exposed to flowing blood. As such, the endothelial cell resides in an environment which is not only biochemical in nature, but which also has a unique biomechanical component to it. This unique mechanical stress environment, resulting from the hemodynamics of the system, will be discussed in the next section. However, if there are to be hemodynamic influences on vascular biology, then, because of its unique placement, one would expect the endothelial cell to be a mediator of any such effects. Thus, if we are to understand any such hemodynamic effects on vascular biology, we must first understand the influence of hemodynamics on the endothelium and on the endothelial cell. It is this topic which will be addressed in this brief review.

THE IN VIVO HEMODYNAMIC ENVIRONMENT

As the heart rhythmically beats, it periodically ejects blood and there is a pressure pulse which
Because of the pulsatile nature of the flow, there is a pulsatile flow wave form and there are hemodynamically-imposed forces acting on the arterial wall. These are illustrated in Fig. 1 and include both arterial pressure and a frictional force which acts tangential to the wall. The frictional force per unit area is called shear stress and the magnitude of this stress is related to the velocity pattern as will be discussed below.

The pressure is a normal stress which acts directly on the endothelium, but which also serves to distend the visco-elastic arterial wall. Because of the propagating nature of the pressure wave, there are both circumferential and longitudinal stresses induced within the vessel wall, resulting in cyclic strains, i.e., extensions per unit length. These extensions are of similar order of magnitude; however, the circumferential strain, associated with what is termed a "hoop stress" in the arterial wall, is somewhat larger and on the order of 10%. This force is borne by the entire arterial wall, and the vascular endothelial cell both "sees" the pressure directly and "rides" a basement membrane which is being cyclically stretched.

As noted earlier, the shear stress is due to friction between the flowing blood and the vessel's inner surface, i.e., the endothelium. It is associated with the velocity pattern; to be specific, if one approximates blood as a Newtonian fluid, then the shear stress is directly related to the velocity gradient in the blood in the immediate vicinity of the wall. Because the blood velocity is not only time-dependent, but also spatially varying, there is a wide range in the level of shear stress to which the endothelium is exposed. In a straight section of artery, the time-averaged mean stress is approximately 15–20 dynes/cm², and there may even be a shear stress regulation of lumen diameter which is endothelial-dependent. Because of the pulsatile nature of the flow, the peak shear stresses may be considerably higher. The highest shear stresses are believed to be encountered by the endothelium on the flow divider in a region of bifurcation, where the peak shear stress may easily be in excess of 100 dynes/cm². On the other hand, on the outer wall of a bifurcation there are regions where the shear stress may be quite low, in some cases with a mean value approximately equal to zero. However, even in such a localized, low shear region, the flow may be highly oscillatory, with large excursions in shear stress as the flow "sloshes" back and forth.

If one compares the magnitude of the shear stress to that of pressure, the former is much less, being on the order of no more than one-thousandth that of mean arterial pressure. Still, in acting directly on the vascular endothelium, shear stress is believed to be an important component of the hemodynamic environment, and there has been considerable focus on its influence on vascular endothelial cell biology as will be discussed in the next 2 sections. However, one must not lose sight of the fact that pressure, both in acting directly on the endothelium and in distending the underlying wall, may equally well have an important influence; there are reports in the literature focusing on such investigations. These effects will also be discussed.

In Vivo Studies of Vascular Endothelium

There have been a series of studies over the past 20 years suggesting an influence of hemodynamics on the endothelium. These possible hemodynamic effects are summarized in Table I. One area of interest has been the possible relationship of endothelial cell morphology to the characteristics of blood flow. The first demonstration of this was the study of Flaherty et al (16) who reported that endothelial cells aligned with the direction of flow. Silkworth and Stiehens (52) also investigated this qualitatively as did our laboratory. In our own work, through a variety of animal studies and using the vascular casting technique, no endothelial denudation was ever found, except in cases where we had caused it through some type of traumatic insult. Furthermore, in a careful examination of regions of rabbit inter-costal ostia, the pattern of orientation and the shape of endothelial cells were found to be suggestive of the complicated flow patterns expected to be present at such branch points based on fluid dynamic considerations. These observations indicated that in vivo endothelial cells 1) aligned with the direction of flow which one would expect in the immediate vicinity of the wall and 2) had greater elongation in regions of high shear than in those of low shear.

To test this further, an experiment was conducted in which an aortic stenosis was produced in the dog by banding the thoracic aorta, and endothelial cell geometry and orientation were determined as a function of position relative to the stenosis. In order to quantitatively determine differences in cell shape,
we used shape index (S.I.) as a parameter, where S.I. = 4πA/P^2. A is cell area and P its perimeter, and the smaller the shape index, the more elongated the cell. What was found was highly elongated endothelial cells in the throat of the stenosis, i.e., the point of minimum area; here, where the shear stress is the highest, the shape index was determined to be 0.11, representing a very elongated cell. On the other hand, immediately downstream of the throat of the stenosis where the flow is separated and the mean shear low, the shape index was 0.75, a characteristic value for more polygonal, round cells. Finally, further downstream the shape index returned to a value of approximately 0.30, similar to that found in unstenosed control specimens. This experiment thus demonstrated that, by changing the geometry of the aorta to produce a specific alteration in the hemodynamics, the resulting endothelial cell pattern was consistent with what one would expect based on the fluid dynamics of a stenosed flow and the hypothesis that cells are more elongated in the presence of a high shear stress.

Using the rabbit as an animal model, Kim et al (28) found that the F-actin microfilament distribution was, in a very similar way, altered by the change in flow pattern associated with the introduction of an aortic stenosis. In high shear regions, where we found highly elongated cells, they found cells of similar shape and with F-actin stress fibers significantly thicker and longer than those in cells from control aortas. On the other hand, in the low, fluctuating shear region immediately distal to the throat of the stenosis, they found endothelial cells to be polygonal in shape and with both a peripheral microfilament band and central stress fibers.

Schwartz and his co-workers, more than fifteen years ago, in a series of studies using the pig as the animal model and reviewed in reference 47, showed a correlation between endothelial cell shape, cell turnover rate, and the permeability of the endothelium to the transport of macromolecules. In essence, thoracic aortic regions of high permeability, identified by the uptake of the protein-binding Evans blue dye, also exhibited increased cell turnover, enhanced uptake of 3H-albumin, 131I-fibrinogen, and 3H-unesterified cholesterol, and greater intimal cholesterol accumulation. This was as compared to aortic white regions, i.e., those which did not stain with Evans blue. Furthermore, whereas in the white regions the endothelial cells were characterized as elongated, in the blue regions, cells were more polygonal. Although the evidence in terms of any hemodynamic effect is clearly circumstantial, it is not unreasonable, based on the other studies noted above, to characterize the thoracic blue regions with polygonal shaped cells as corresponding to low shear and the white regions with elongated cells as corresponding to high shear. Certainly, whatever the nature of the mechanisms involved, there are striking focal differences which could be due, at least in part, to differences in the biomechanical environment imposed by the hemodynamics of the system.

There have been other studies of endothelial cell replication in vivo. Schwartz et al found the thymidine index for a 24-hr period to range from 0.3-1.5%, with the location of dividing cells to be nonrandomly distributed (49, 50). They suggested that local hemodynamic conditions might be one possible cause for this clustering of dividing cells. Wright (62, 63) also found spatial variations in her measurements of the pattern of mitosis in aortic endothelium, with the mitotic frequency being higher in regions of branching (62).

In a more recent study by Gerrity et al (19), preferential intimal penetration by blood monocytes has been observed in Evans blue areas as compared to white aortic areas. This indicates a focal nature to the recruitment of monocytes, and suggests another role for the endothelium which may be hemodynamically modulated.

Finally, Thubrikar et al (57) investigated the inhibition of atherosclerotic lesion formation in rabbits associated with the reduction of intramural arterial wall stress. They concluded that there not only was a significant effect, but one quite possibly mediated by alterations in endothelial biology, associated with the changes in cell morphology which they had observed (4). This suggests that the influence of flow is not just through shear stress, but also due to stresses in the underlying wall.

Although such in vivo studies are informative, they do indicate that, before any firm conclusions can be made with regard to the existence of an influence of a mechanical stimulus in vascular biology, in injury repair, and in the etiology of vascular disease, an evaluation of endothelial cellular responses to well-defined mechanical stress conditions is necessary. Vascular endothelium is not only relatively inaccessible in vivo to experimental manipulation, but also resides in a biomechanical environment which can only be defined qualitatively and not quantitatively. Thus, recent in vitro studies of endothelial cell dynamics have been initiated, using cultured populations of vascular cells studied under well-defined mechanical stress conditions. These are discussed below.

**In Vitro Studies of Vascular Endothelial Cells**

As noted above, in order to investigate the influence of hemodynamics under conditions where the biomechanical environment can be quantitatively
Table I.—Selected properties for arterial endothelium for which in vivo data suggest a hemodynamic influence (related references in parentheses).

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<td>1. Cell Shape and Orientation (16, 34, 40, 52)</td>
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<td>2. Actin Microfilament Localization (28, 60)</td>
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<td>3. Trans-endothelial Macromolecule Transport (47)</td>
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<td>4. Cell Turnover (47, 50, 62)</td>
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<td>5. Monocyte Recruitment (19)</td>
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Table II.—Endothelial cell responses to flow-imposed shear stress as determined through in vitro cell culture studies (related references in parentheses).

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<td>1. Cell Shape and Orientation (10, 14, 32, 33, 35)</td>
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<td>2. Cytoskeletal Localization (11, 45)</td>
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<td>3. Cell Mechanical Stiffness (45)</td>
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<td>4. Cell Migration (2, 10, 15)</td>
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<td>5. Cell Proliferation (2, 3, 9, 10, 35, 36, 53)</td>
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<td>6. Endocytosis (8, 53)</td>
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<td>7. Fibronectin (21, 24, 59)</td>
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<td>8. Intracellular Signaling (1, 20, 42)</td>
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<td>9. Ion channels (41)</td>
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<td>10. Secretion (12, 13, 18, 23)</td>
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defined, a number of research laboratories have initiated experimental efforts in which vascular endothelial cells are studied in cell culture systems. Cell culture is not a physiologic model; however, it is a model where biologic responses can be observed under carefully designed and well-defined laboratory conditions. Clearly, a goal of investigators who do such work should be to engineer cell culture experimental systems so as to make these biologic models more physiologic. In such experimentation, the inclusion of mechanical stress in the cell culture environment may be essential since, in vivo, the endothelial cell resides in the presence of flow which provides the unique biomechanical environment previously described. In fact, a flow environment is the natural environment of the endothelial cell and, in that sense, the environment of static cell culture is an artificial one.

The first effort in studying cultured vascular endothelial cells in the presence of flow was that of Dewey et al (10, 11) who used a cone-plate viscometer to study shear stress effects. Others have joined in the use of cell culture as a way to study the effects of a cell's biomechanical environment, including those due to shear stress, to the direct effect of pressure, and to cyclic stretch.

The majority of the studies reported for vascular endothelial cells have represented investigations of the effect of a fluid-imposed shear stress. Both bovine aortic endothelial cells (BAECs), after multiple passage, and human umbilical vein endothelial cells (HUVECs), either primary or early passage, have been used in these reported studies, and the responses of such cells to flow have been dynamic and of an active nature. A noninclusive list of the changes observed in endothelial cells as an adaptation to their flow environment is given in Table II.

The most visible effect of shear stress on cultured endothelial cells is the rather dramatic change in shape and orientation. BAECs, in response to an initiation of flow, elongate and align their major axis with the direction of flow. This has been demonstrated by a number of groups (10, 14, 32, 33) and is illustrated in Fig. 2. For the studies illustrated by the image in Fig. 2, the media included 20% fetal bovine serum and the substrate was Thermanox, a polyester material. If one uses such images to determine the shape index characterizing such cell populations, one calculates a value of approximately 0.80 for the polygonal shape cells of static culture, a shape index of 0.55 for cells in the presence of a steady laminar shear stress of 30 dynes/cm² for 24 hr, and a value of 0.50 for a steady shear stress 60 dynes/cm², again with a time duration of 24 hr (33). Extending the duration of exposure to 48 hr resulted in cells only slightly more elongated and beyond 48 hr, there was no additional effect. Furthermore, the degree of elongation and, thus, the shape index is influenced not only by the magnitude of the shear stress and the duration of exposure, but also by the composition of the media and the substrate to which the cells are anchored (33).

Comparing the shape index values noted above with the in vivo values provided in the previous section, it is clear that cells in static culture, at best, are like those from a region of very low flow. Furthermore, endothelial cells exposed to a steady laminar shear stress, with a value comparable to the mean shear stress associated with a straight section of artery, are not as elongated as cells in vivo, i.e. the shape indices for cultured BAECs exposed to flow are on the high side of the range of values measured in vivo. This could be due to many things including the choice of culture media, the substrate employed, and/or the nature of the flow which in vivo is pulsatile and not steady.

In order to investigate the influence of pulsatile flow on the shape of endothelial cells in culture, confluent BAEC monolayers were exposed for 24 hr to a simple nonreversing pulsatile flow, one with a sinusoidal waveform and a frequency of pulsation of 1 Hz. For a pulsatile flow condition with a peak shear stress of 45 dynes/cm², a mean shear stress of 30 dynes/cm², and a minimum value of 15 dynes/cm², the shape index value for the BAEC monolayer was approximately 0.45 (35). Although these cells, in general, were still not as elongated as those found in vivo, they were more elongated than those exposed...
to a steady shear stress, even those exposed to a steady shear stress equal to the peak value of the pulsatile flow.

With the change in shape which endothelial cells undergo in response to flow, there also is an alteration in cytoskeletal structure (11, 45). The dense peripheral actin bands characteristic of cells in static culture disappear, and actin stress fibers form which are aligned with the direction of flow. In addition, using the micropipette technique to measure the mechanical stiffness of cells detached from substrate, endothelial cells from monolayers exposed to flow retain an elongated shape upon detachment and are an order of magnitude stiffer than cells from static culture, which when detached take on a spherical shape (45). Furthermore, cells exposed to a simple
pulsatile flow, such as described earlier, are even stiffer. Since we believe that the mechanical stiffness of these cells is a reflection of the degree of organization and the extent of the F-actin within the cells, these micropipette measurements provide a quantitative indication of the changes in cytoskeletal structure.

These changes in cell shape and in the localization in F-actin due to shear stress are consistent with the results of the in vivo investigations discussed in the previous section. Furthermore, in experiments with BAECs treated with cytochalasin B, a cytoskeletal inhibitor which disrupts actin assembly, we have shown that there is little elongation in response to shear stress (33). Thus, there is a coupling between cell shape and its internal cytoskeletal structure. Finally, there also are reported studies on the effects of shear stress on the organization of and the amount of cell-associated fibronectin (21, 24, 59). These results suggest an important influence on both synthesis, release and degradation of extracellular matrix components.

In addition to such changes in morphology, cytoskeletal structure, and extracellular matrix in cultured endothelial cells exposed to shear stress, other more functionally-related changes have been observed. Davies et al (8) reported increased pinocytosis of horseradish peroxidase due to changes in flow. Sprague et al (53) observed an influence of laminar shear stress on the endocytosis of low density lipoproteins (LDL), specifically the receptor-mediated binding, internalization, and degradation of LDL.

Several groups have investigated the secretion of prostacyclin by cultured endothelial cells exposed to flow and demonstrated its enhancement (18, 23). In addition, a recent report presents data providing evidence of increased expression of tissue plasminogen activator, both product and messenger RNA, due to the influence of shear stress, with no effect on plasminogen activator inhibitor, type 1 (12, 13).

In the context of any role of flow in a response to injury, the question of an influence of shear stress on endothelial cell proliferation also is of interest. As noted earlier, differences in cell turnover rates have been measured in vivo for regions of differing endothelial morphology. In order to determine whether or not exposure of a confluent BAEC monolayer to a laminar shear stress induces cell proliferation, ³H-thymidine uptake into confluent BAEC cultures was measured after exposure to shear stress (53). The results indicated that ³H-thymidine incorporation into endothelial cell DNA was not significantly different for BAECs exposed to a steady state laminar wall shear stress of 30 dynes/cm² for 24 hr as compared to either low shear or no shear cell populations. The cell replication rate as measured by ³H-thymidine autoradiography confirmed these results; this was also shown for laminar flow by Davies et al (9). Thus, a laminar shear stress acting on a confluent monolayer appears to not enhance cell proliferation or turnover.

This is in contrast to the turbulent shear results of Davies et al (9), who, in the only study to investigate the effect of turbulence, found enhanced cell proliferation for a confluent endothelial cell monolayer exposed to a turbulent shear stress. A turbulent flow is characterized by a broadband, random unsteadiness which includes both velocity and pressure fluctuations. Thus, in this experiment of Davies et al (9), it is unclear whether the effect of the turbulent flow is due to shear stress, pressure, or both effects. In the only reported study of pressure effects on cultured endothelial cells with oscillatory pressure (100 ± 20 mm Hg), there was a loss in cell-cell contact and a commensurate increase in cell division (31). Certainly, there needs to be further investigations of the direct effect of pressure, in particular oscillatory pressure, and which include the range of frequencies important to the composition of the in vivo waveform.

In another attempt to investigate the influence of flow on cell proliferation, we have studied the effect of laminar shear stress on sub-confluent BAEC monolayers (36). Here, we have shown that there is no influence of shear stresses of 15 dynes/cm² or less for BAEC on Thermanox. This is in agreement with the earlier low shear stress results of Dewey et al (10). For shear stresses of 30 dynes/cm² and higher, we found a marked decrease in the rate of proliferation as is illustrated in Fig. 3. Thus, endothelial cell proliferation may be not only contact-inhibited, i.e., due to cell density, but also inhibited by the shear stress environment. This is not totally surprising since shear stress influences cell shape and a possible role of cell shape in growth control has been previously recognized (17). Furthermore, since survival of an anchorage-dependent cell requires maintaining its attachment to substrate, then its first priority may be the reorganization of its cytoskeleton. From this perspective, cell proliferation may be of a lesser priority, even though the monolayer is sub-confluent and cell mitosis, thus, is not contact inhibited.

It also should be noted that, for BAEC in the simple pulsatile flow described earlier, there is an even further decrease in the rate of cell proliferation (36). This is consistent with our other results which indicate that for a non-reversing pulsatile flow, the effects of a laminar steady shear stress are accentuated when pulsatility is added to the flow.

Also important to the function of an endothelial...
cell is its ability to migrate. As shown by Young and Herman (65) in their postinjury motility experiments, cell migration is directly affected by the composition of the extracellular matrix. Thus, if flow influences the extracellular matrix of endothelial cells, then it should influence cell motility.

Dewey et al (10) were the first to investigate cell migration in the presence of flow and demonstrated that there is an effect of flow on this event. Ando et al (2) also have studied monolayers in which there is cell migration and also cell proliferation. More recently, Eskin et al (15), in their postinjury studies, found endothelial cells to migrate twice as fast when moving with the direction of flow than when moving against flow.

In addition to cell culture studies of the influence of flow on vascular endothelial cells, there also have been investigations of cyclic stretch effects. In such experiments, cells are cultured on a compliant membrane which is cyclically stretched, either in a uniaxial mode or in a biaxial mode. The intent of such experiments is to simulate the cyclic stretching of the basement membrane to which the endothelial cell is anchored. This cyclic stretching is associated with the propagation of the pressure pulse in an artery which on every heart beat distends the arterial wall, thus producing a cyclic stress.

The results of these investigations are summarized in Table III. As can be seen, there are a variety of effects on endothelial cells due to cyclic stretch. These include morphological alterations, e.g., cell shape and orientation, F-actin reorganization, changes in the level of cell-associated fibronectin, and prostacyclin synthesis (7, 22, 26, 51, 55, 56). In regard to cell orientation, in the presence of uniaxial stress, endothelial cells align perpendicular to the direction of stretch, whereas with biaxial stretch, due to the nature of the stress field, there is no similar orientation effect. There also are reported effects of cyclic stretch on cell proliferation, with the rate being enhanced compared to a similar static control population (54).

From these data, it appears that endothelial cells can discriminate between different types of mechanical stress environments and, having recognized the difference, transduce this into different changes in behavior. A basic question then is: How does an endothelial cell recognize a fluid mechanic signal and, having done so, transduce this signal into a change in structure and function? The recognition is perhaps a membrane event, possibly linked to the control of Ca\(^{2+}\) metabolism. In fact, there are reports of a transient elevation in intracellular calcium due to the influence of mechanical stress (1). An important biochemical pathway controlling Ca\(^{2+}\) mobilization is the phospholipase C-mediated hydrolysis of polyphosphoinositides. In this pathway, activation of a cell surface receptor stimulates a phosphoinositide-specific phospholipase C to hydrolyze phosphatidylinositol 4,5 bisphosphate (PIP\(_2\)) to form inositol 1,4,5, trisphosphate (IP\(_3\)) and diacylglycerol, a stimulator of the Ca\(^{2+}\)/phospholipid-dependent enzyme, protein kinase C. Recently, it has been demonstrated that there is shear stress stimulation of this pathway, with a peak in IP\(_3\) being measured shortly after the onset of flow (42).

Furthermore, recent studies in our laboratory have implicated protein kinase C (PKC) as part of the signaling pathway linking a shear stress-related mechanical signal to the intracellular events underlying alterations in cell morphology (20). The phosphorylation of cytoskeletal proteins by various protein kinases, including PKC, is thought to play a role in cytoskeletal assembly and cytoskeletal protein interactions essential to cellular morphological changes (25, 46). We have shown that exposure to shear stress causes a translocation of PKC from the cytosol to a particulate component (20). Since phospholipids and, presumably, membrane association are required for PKC activation, it is likely that the primary targets for phosphorylation by activated PKC would be concentrated in proximity to the mem-
brane-associated PKC. We have not yet determined the nature of the components of the particulate fraction with which PKC associates in response to shear stress. However, flow-induced morphological changes in BAECs have been shown in our laboratory to be inhibited when the cells were subjected to a shear stress of 30 dynes/cm² for 24 hr in the presence of the PKC inhibitors, sphingosine or staurosporine. The cells were rounder in shape, than those exposed to flow in the absence of inhibitors, and the shear stress-related actin microfilament reorganization from dense peripheral bands into stress fibers also was inhibited. These studies suggest that PKC-dependent phosphorylation may be responsible, at least in part, for the dynamic changes in cytoskeletal structure and cell morphology which occur during exposure to a flow environment.

The intracellular events that regulate the endothelial cell's growth program in response to shear stress are not known. However, in many cell types, hydrolysis of phosphoinositides and PKC activation are thought to be involved in growth control (61). It may be that the same set of signaling mechanisms described above is involved in an influence of shear stress on endothelial cell growth.

**DISCUSSION**

It is clear from what has been presented up to this point that the behavior of vascular endothelial cells is influenced by the hemodynamic environment in which the cells reside. Not only is cell shape and F-actin distribution different under differing conditions of flow, but there are a wide variety of other responses. From *in vivo* studies, we know that local hemodynamic phenomena appear to modulate both endothelial permeability and cell turnover. *In vivo* results even provide evidence that these 2 phenomena are related, and it has been suggested that local regions of the endothelium in which cell turnover is in the act of taking place may be "leakier" in regard to the transendothelial transport of macromolecules (5, 38). This is due to the enlarged junctions associated with the process of cell turnover.

*In vitro* cell culture studies have been complementary to *in vivo* studies and have allowed the study of vascular endothelial responses to flow under well-defined mechanical stress conditions. A number of different laboratories have investigated seemingly a myriad of things related to the ability of the endothelial cell to respond to the influence of shear stress. These results suggest that the endothelial cell, in adapting to a change in flow environment, would appropriately alter its morphology, extracellular matrix, and metabolism. These changes would be so pervasive as to extend down to the gene expression level (3, 13) and would include an influence of hemodynamic factors on cell cycle and on the growth program of endothelial cells (3, 36).

It should again be emphasized that the influence of hemodynamics on the vascular endothelium is not solely due to the effect of shear stress. Even though it is the influence of shear stress which has been studied most extensively, as noted earlier, there are at least 2 other aspects of the endothelial cell's biomechanical environment worthy of consideration. These are the direct effect of pressure and the distension of the underlying wall, which also is an effect of pressure. Much more needs to be learned about the effects on cell behavior of these components of the endothelial cell's biomechanical environment; however, the limited data reported up to the present suggest that these effects are both important.

Recognizing the important influence of a cell's biomechanical environment on its structure and function, it is then important in *in vitro* studies to engineer cell culture systems so as to make them more physiologic. This engineering challenge is not limited to just including a simulation of the biomechanical environment in which the cell finds itself *in vivo*, for there are many other aspects of the *in vivo* condition which are inadequately simulated in cell culture. However, the biomechanical environment of a cell is one very important aspect, and this is not just true for the endothelial cell. There is every reason to believe that it also would be an important consideration for all cells. In regard to vascular biology, this would include the smooth muscle cell, the monocyte-macrophage which is recruited into the arterial wall, and the other formed elements of blood.

One final area of discussion relates to the role of hemodynamics in processes of injury and repair. This includes the response to injury hypothesis (43, 44) believed to be a key to our understanding the genesis of atherosclerosis, the role of the endothelial cell in this response, as well as in responding to injury in general (48), and the influence of hemodynamics in this event (64).

To start with, one can ask whether or not there can be hemodynamic injury. Are the forces such that endothelial denudation can take place? In its earlier form, the response to injury hypothesis included the concept of traumatic injury to the endothelium, with accompanying denudation. Although there were many possible injury mechanisms, one was certainly hemodynamics. However, it is now believed that, although endothelial denudation could occur under extreme conditions, this is more likely to take place in more advanced stages of disease and that initial events involve injury of much more subtle form. Certainly, in all the *in vivo* studies
our laboratory has performed in which vascular casts showing endothelial morphology were made, there has been absolutely no evidence of denudation. The endothelium has always shown itself to be a continuous, intact monolayer. Furthermore, investigations designed to measure the magnitude of shear stress, required to cause endothelial denudation, indicate levels far in excess of that encountered physiologically (58).

In a recent publication, Libby et al (37) presented a cartoon illustrating 2 endothelial cells, one a “happy,” inactivated non-thrombogenic cell, the other a “frowning,” activated, pro-coagulant cell. In looking at that caricature, it seems that the “happy” endothelial cell must be the one which is in the presence of flow and that the “frowning” activated one must be in a low flow region, perhaps even involving flow stasis. In this sense, it may be that hemodynamic injury is more likely to be associated with extremely low flows. The use of the term “injury” may be in fact misleading. It at best may be only injury of a very subtle form, and, thus, the condition may be better described as “abnormal” or “unhealthy.” To put it a different way, there may be a range of flow conditions within which an endothelial cell has the ability to appropriately adapt and thus be “happy.” Outside of that range, its “mood” changes. Although excessively high flow conditions are nonphysiologic, their occurrence is still possible during stages of advanced disease. However, for “normal” conditions it is primarily low flows which cause “injury” and the endothelial cell to “frown,” and with that goes a decrease in cell life time and thus an increase in cell turnover rate.

Of course, there are influences which could alter this picture. There could be mechanical injury associated with interventional devices. Ando et al (2), employing an injury model in cell culture, have shown that the influence of flow is to enhance EC proliferation. This is in contrast to our own results (36), but this difference may be due to the injury stimulating growth factor secretion which in turn may be flow regulated. There also could be bacterial and viral infection or chemical injury from toxic substances. In the latter case, the importance of hemodynamics is that an endothelial cell’s response to injury may be different, depending on the hemodynamic environment in which it resides.

Even without endothelial injury, there still can be injury to the vessel wall and the endothelial cell could still be a participant. This may be the case in the genesis of atherosclerosis where the focal nature of the disease is believed to be due, at least in part, to the influence of hemodynamics. Here, although the endothelial cell may not be truly “injured” and thus still be functioning normally, i.e., as a “happy” cell, differences in endothelial cell behavior in regions of differing hemodynamic environment could result in it playing an altered role as a mediator of arterial wall processes. Thus, in the early stages of atherosclerosis, an intact endothelium could participate by exhibiting an enhanced permeability to macromolecule transport, by a role in the recruitment of the monocyte-macrophage, and/or by the altered secretion of growth factors and extracellular matrix components.

If for whatever the reason there is endothelial denudation, then repair will take place through the processes of cell migration and proliferation. Suffice it to say that these would be expected to be influenced by the local hemodynamic environment. Although there is much to be learned about this, as reviewed earlier, there already is evidence from both in vivo and in vitro experiments which indicates an influence of hemodynamics on cell proliferation. There also are cell culture studies which suggest an effect on cell migration. Thus, although there is much to be learned, there is evidence of an important influence of hemodynamics on those processes important to any repair of an injured endothelium.

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