Acute Vascular Endothelial Changes Associated with Increased Blood Velocity Gradients

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ABSTRACT

The purpose of this study is to quantify the acute changes in endothelial histology that are associated with an induced increase in blood velocity. A nontraumatic intra-aortic device was designed to produce a rapid convergence of the aortic blood stream into a narrow channel along the ventral aspect of the thoracic aorta in dogs. The endothelial surface overlying this channel was exposed to a broad range of surface shearing stress by the accelerated blood flow. Techniques were developed to quantify the resulting distribution of shearing stress so that the stress to which the endothelial surface was exposed at every point along the channel could be determined. Special histologic techniques were developed using formalin fixation and gelatin embedding of the tissue so that endothelial cytology could be studied and criteria for normal cells established. Using these criteria, cell counts were done to establish the "normal" endothelial cell population density as a function of stress exposure. The stress corresponding to the mode of these cell density distribution curves was defined as the acute yield stress ($\tau_C$). The acute yield stress for endothelial cells was found to be $379 \pm 85$ (SD) dynes/cm$^2$. Exposure to stress in excess of this value for periods as short as one hour resulted in marked deterioration of the endothelial surface consisting of endothelial cytoplasmic swelling, cell deformation, cell disintegration, and finally dissolution and erosion of cell substance. The relationship of these events to cellular rheology and interfacial chemistry is discussed.

ADDITIONAL KEY WORDS endothelial rheology yield stress endothelial injury damage erosion vessel wall shearing stress vascular fluid mechanics turbulence arteriosclerosis blood-endothelial interfacial chemistry thoracic aorta dogs

It is the purpose of this report to describe vascular endothelial changes associated with an acutely induced increase in blood velocity produced by an intravascular device and to propose plausible mechanisms by which these changes come about. The endothelial changes to be described consist of cell deformation, swelling, and ultimate dissolution. We focus attention on hydrodynamic events in the region of the blood-endothelial interface that might produce these changes. Events in the fluid phase of this region can affect the endothelial surface either by the purely mechanical interactions of pressure and shearing stresses on the surface or by the convective properties of the flow which can strongly influence the normal electro-chemical milieu at the surface. Speculation regarding both of these mechanisms is presented after presentation of the results of this study.

Flow Terminology

Depending on location and physiologic circumstances, blood flow may be either laminar or turbulent. In the special circumstances of the present study it is seen that both laminar flow and turbulent flow were produced. Laminar flow is characterized by an orderly progression of the fluid particles along smooth trajectories called "streamlines." The associated pressure and shearing stresses in the fluid
vary smoothly from point to point and from moment to moment. Laminar flow occurs at relatively low flow levels and in regions where the boundary configuration can promote conditions of stability for the flow, e.g., converging boundaries.

If flow levels exceed some critical value or if the configuration of the conduit boundaries are unsuitable for stable flow (e.g., diverge too much), then vortex systems develop near the boundary and, depending on the local geometry, may remain relatively stationary (stationary vortex) or may be convected along by the stream (vortex shedding). The shed vortices are ultimately damped out by the viscous properties of the fluid.

When flow is increased to a somewhat higher level, vortex shedding becomes more intense. Moreover, the shed vortices no longer die away but rapidly spawn new vortices which spread across the entire stream resulting in fully developed turbulence at a point somewhat downstream.

Fully developed turbulent flow is characterized by violent, chaotic, three-dimensional fluctuations in the velocities of the fluid particles. Any attempt to describe the detailed motions of the fluid particles in this situation would be hopelessly complicated and mathematically intractable. Therefore, it is convenient to consider turbulent flow to consist of an average or "time-smoothed" velocity component superimposed upon which is a fluctuating high frequency velocity component. The time period over which this running average is made must be long compared to the period of the lowest turbulent frequency fluctuation, but short compared to the highest physiologic frequency of interest. These conditions prevail in the present study, i.e., there is a large gap in the frequency spectrum between the low physiologic frequencies of cardiac origin and the high frequencies of turbulence. As might be expected, the associated pressure and shearing stress may also be resolved into corresponding "time-smoothed" components and superimposed high frequency fluctuations. This method of looking at turbulent flow has practical as well as theoretical importance.

From a theoretical point of view, this approach converts a mathematically intractable problem into two separate, simpler problems. The first problem is to describe the high frequency fluctuating components with statistical averaging techniques so that one can ignore the tedious and rather pointless, detailed description of the individual particle motions. The second problem is to define the relationships among the time-smoothed quantities and these averaged fluctuating components so that the problem can be handled formally by the mathematics of the simpler laminar flow situation. This approach is discussed further in the Appendix.

From a practical point of view, the time-smoothed quantities are the simplest to measure. Moreover, they are usually an order of magnitude larger than their corresponding high frequency components. Therefore, the time-smoothed hydrodynamic variables represent the major features of interest in most situations. Thus it can be inferred that any mechanical interaction of the flow with the endothelial surface also would be described best by the time-smoothed variables. As shown later, the results of this study tend to bear out this inference.

Evidence also is presented, however, which indicates that the high frequency components may play an auxiliary, if not independent, role in producing endothelial damage. Therefore, in studying the interaction of blood with the endothelial surface, it is of importance to determine the presence and, if possible, to quantify the high frequency components of the blood velocity field, too.

These fluctuating components of velocity, pressure, and shearing stress can be estimated by several indirect methods. The accuracy of any of these is questionable, particularly when applied to measurements in blood. Since all of the high frequency components tend to be correlated with one another, measurement of any one permits an estimate of the others. Pressure is probably the simplest variable to measure. Since turbulent frequencies are in the audible range, a simple heart sound microphone can be used for this measurement.
To recapitulate, the interaction of both laminar and turbulent flow with the endothelial surface is considered. The flow exerts stresses on this surface which may be thought of as consisting of two superimposed components: a time-smoothed component which is measured quite accurately and a high frequency fluctuating component which cannot be estimated accurately. In laminar flow the time-smoothed component is the only component. In this paper, the terms "velocity," "pressure," and "shearing stress" refer to the corresponding time-smoothed quantities.

Methods

Experimental Design
All studies were performed acutely in the descending thoracic aorta of mongrel dogs weighing 20 to 30 kg under chloralose-urethane (48-480 mg/kg) anesthesia. The first group (10 dogs) served as control animals so that standards for normal histology could be established against which the histologic changes found in the second experimental group (22 dogs) would be compared. Each group was subjected to thoracotomy and given the same dosage of Evans blue dye (2 mg/kg).

Evans blue dye was given to form a visual "tag" for albumin. This dye forms a relatively "tight" chemical complex with serum albumin. Therefore, if the normal mechanical or electrochemical barriers at the blood-endothelial surface are altered by hydrodynamic events in this interfacial region, one might expect plasma proteins (including albumin) to pass more easily across the damaged interface. If this occurred, areas of damage should demonstrate an increased staining affinity for Evans blue dye. Aschheim and Zweifach (1), as well as others, have used a similar rationale to detect endothelial injury in the microcirculation.

Each dog in the experimental group also had a specially designed plug carefully maneuvered into the descending thoracic aorta in such a manner that a longitudinal strip of endothelial surface extending from the upper thoracic aorta down to and including the resting site of the plug remained untouched by the plug surface. The purpose of this plug was to increase the blood velocity locally in a controlled manner along the endothelial surface by partially occluding the lumen of the aorta. The plug was cylindrical in shape (Fig. 1) and made of polished lucite. A longitudinal uniform cylindrical channel 0.32 cm in diameter by 3.0 cm long was placed along the outer surface of the plug so that about 40° of its circumference intersected the circumference of the plug (or vessel lumen). Thus, a 0.15-cm × 3.0-cm opening was formed along the
side of the plug by the common intersection of the channel and plug (or vessel lumen) circumference. When the plug was in place, this opening was covered by the adjacent endothelial surface of the aortic wall, as indicated in the upper left of Figure 1. In this manner an attenuated, but continuous, uniform cylindrical passageway was formed for blood flow around the plug. The entrance portions of the channel in the plug were machined to be an elliptical horn having semimajor and semiminor axes of 0.96 and 0.63 cm (see also Fig. 4c). These dimensional parameters calculated from the work of Ling (2) were designed to guide the convergent flow into the channel in a symmetrical and uniform manner.

Fine (0.01 cm deep) longitudinal grooves were milled along the plug surface on the opposite two thirds of the plug circumference (see top of Fig. 1) to act as guiding grooves which would slide along the endothelial surface like fine sled runners to prevent rotation of the plug during its descent in the aorta, thereby leaving unmoled endothelium in the trajectory of the plug channel. Two circumferentially oriented grooves also were placed at either end to help anchor the plug at its resting site (see below).

At the time of thoracotomy the plug was introduced through a purse string opening into the left atrium. The plug was manipulated into the ventricular outflow tract from which the blood stream carried the plug out into the upper descending aorta where it was temporarily arrested and, if necessary, rotated so that the plug channel was on the ventral aspect of the aorta. This was done to "aim" the plug properly so that after its final descent the resting site of the channel would not overlie one of the intercostal ostia. The plug was then released again to travel to a position selected to just occlude the aortic lumen without overstretching the wall. A diagram of this preparation appears in the mid-region of Figure 1. A woven tape 6 cm wide was fastened evenly around the vessel to secure the plug in place, to occlude the shallow longitudinal guiding grooves, and to prevent pulsation of the wall over the flow channel in the plug. An electromagnetic flowmeter was placed around the aorta 4 cm downstream from the plug. The intervening aorta was surrounded by a rigid, tapered plastic splint so that the flow contour recorded by the flowmeter could be assumed to represent that passing through the plug channel.

Two intercostal arteries, one about 2 cm upstream from the plug site and one about 4 cm downstream, were cannulated with pressure catheters so that the lateral aortic pressures and over-all pressure drop across the plug (Δp) could be recorded throughout the study using Sanborn 267B pressure transducers. The intervening intercostal arteries were ligated.

It was not possible to record sound accurately from the experimental animals because of the woven tape and plastic sleeve at and below the plug site. Therefore, 13 separate studies were done without the plastic sleeve and where the tape was replaced by a plastic plate containing small windows through which the sound-sensing probe could be introduced as indicated in the bottom of Figure 1. Sound was recorded from the ventral adventitial surface of the aorta sequentially in 1-cm increments from about 3 cm above to about 6 cm below the plug channel. Sound was also recorded from the vessel surface over the channel itself through the windows in the plastic plate.

The sound recording system consisted of a Sanborn Model 373 crystal microphone attached to a 1 mm i.d. 4 cm long rubber tube at the end of which was a small 1.5-mm metal "bell" which could be placed on the vessel wall like a stethoscope, as shown in the lower right of Figure 1. It was not possible to determine the dynamic accuracy of this recording system. Therefore, the electrical output of this device should be considered only to represent an approximation to the true sound pressure radiated from the vessel wall covered by the stethoscope bell. It is reasonable to assume, however, that the output of the microphone for any given frequency will be some monotonic function of the input. Therefore, it is plausible to interpret the measured sound en-
ergy as an index of "relative sound intensity" at least in the useful frequency range of the microphone (about 5-1500 cps). If we make the added assumption that the radiated sound intensity is a monotonic function of the intensity of the high frequency pressure fluctuations striking the underlying endothelial surface, then we can use the recorded sound energy as a relative index of "turbulence intensity."

The 13 ancillary studies were carried out to examine the relative distribution of turbulence intensity along the aorta, including the flow channel in the plug. All data were recorded on an Ampex FR 600 7-channel FM tape recorder. The tapes were played back through an analog computing device, the output of which represented a smoothed "envelope" of the energy contained in the recorded sound. The use of these data is described later.

At the time of plug placement 2 mg/kg of Evans blue dye was given intravenously as a simple visible "tag" for albumin as described earlier. The plug was left in place for 1, 2, or 3 hours before the animals were killed, and suture markers were placed on the aortic wall and their separation was measured. The animals were killed by bleeding, and the blood was saved to be used in a calibration stand to establish the in vivo pressure gradient distribution along the channel in the plug.

**METHOD OF ESTIMATING THE IN VIVO "TIME-SMOOTHED" SHEARING STRESS ON THE VESSEL WALL**

If we are to correlate endothelial tissue damage with its associated exposure to stress, then it is necessary to quantify the distribution of shearing stress to which the endothelial surface in the plug channel was exposed. The time-smoothed shearing stress on the wall of a uniform circular channel like that in the plug is not uniform with distance along the tube except at relatively large distances from the entrance. For a given flow, the stress is very high at the entrance and decreases progressively with distance along the tube, asymptotically approaching a uniform value at distances in excess of 50 pipe diameters downstream from the entrance. Therefore, the endothelium overlying the plug channel was exposed to a broad range of shearing stress depending on the location along the channel, i.e., that over the entrance was subjected to many times greater shearing stress than that over the downstream end of the channel. As shown later, the creation of this broad range of shearing stress was an essential part of the experimental design.

Instruments for estimating the shearing stress directly in vivo are not presently available. However, methods do exist for estimating both the instantaneous flow \([Q(t)]\) and the pressure gradient distribution \([\partial p/\partial z]\) along the channel. If we can find a mathematical relationship between these measurable variables and the shearing stress \([\tau]\), then it is possible to estimate the stress distribution indirectly. Atabek derived such a relationship for laminar flow in the entrance region of a circular conduit having a perfectly uniform velocity distribution at its entrance \((3)\). These conditions could not be met in the present study. Turbulent flow as well as laminar flow were encountered, and, moreover, it was not possible to achieve geometrical symmetry of the entrance portions of the plug channel to insure a uniform velocity profile at the entrance. Therefore, it was necessary to rederive the required mathematical relationships to account for turbulent effects from somewhat more fundamental equations of fluid motion than were used by Atabek and to determine experimentally to what degree the asymmetrical entrance geometry influenced the velocity profiles. Detailed hemodynamic studies were carried out on eight different blood specimens to obtain this information. The results of these experiments and necessary mathematical derivations are described briefly in the Appendix.

The shearing stress \((\tau)\) on the wall of the channel formed by the plug in the aorta is shown by

\[
\tau = \frac{R}{2} \frac{\partial p}{\partial z} + \frac{\rho}{2\pi R} \frac{dQ}{dt} + \frac{\rho}{R} \frac{\partial}{\partial z} \int_0^R \omega^2 r dr,
\]

\[1\]

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where $R$ is the channel radius, $z$ is the distance along a coordinate placed on the center line of the tube, $\partial p/\partial z$ is the pressure gradient parallel to the axis of the tube, $p$ is the blood density, $Q$ is the flow, $t$ is time, and $w$ is the blood velocity in the axial direction at a distance $r$ from the center of the tube. The first term on the right of equation 1 may be thought of as the over-all driving force being applied to the blood, whereas, the second and third terms represent the inertial forces due to the local acceleration and the kinetic energy gradient. The difference between the first and the latter two terms represents the drag forces exerted by the stream on the wall. It is this drag expressed in terms of the shearing stress, $\tau$, that is of the most interest in the present study.

The use of equation 1 involves the measurement and computation of the instantaneous velocity field everywhere in the conduit. This presents serious problems in recording and data processing. In view of this, a separate study, which is also presented in the Appendix, was carried out to determine if, under the in vivo experimental conditions of this study, it would be possible to simplify equation 1. The results of this ancillary study indicate that unacceptable errors are not introduced in the restricted circumstances of the present study by ignoring the last two terms in equation 1. Thus, equation 1 can be simplified to

$$\tau \sim \frac{R}{2} \frac{\partial p}{\partial z}. \quad (2)$$

This reduces the problem of estimating $\tau$ simply to that of measuring $\partial p/\partial z$ as a function of time and distance along $z$.

Although greatly simplified, the measurement of $\partial p/\partial z$ in vivo still presents serious technical difficulties, first, since the channel of interest is very small ($R = 0.16$ cm), and, second, since care must be taken not to disturb the tissue overlying the channel. One practical approach to this measurement is to construct a scaled, physical model of the in vivo system in which the problems of access and instrumentation are not hampered by physiologic and histologic technical requirements.

A physical model, which is shown schematically in Figure 2, was designed to be as nearly an exact geometric replica of the in vivo situation as possible. A plug identical to those used in vivo was placed in a lucite tube having an inside diameter equal to the average aortic dimensions encountered in the living animals. The electromagnetic flowmeter probe used in vivo and its associated excised segment of the vessel were placed in the system for calibration as shown. The system was filled with blood from the experimental animal. Flow which was monitored by an orifice meter was generated by a variable speed

![Figure 2](image_url)

**Diagram of test stand for calibration of electromagnetic flowmeter and pressure gradient distribution.** Horizontal lucite tube (aorta) contains, from left to right, orifice plate flowmeter, electromagnetic flowmeter probe on its excised vessel segment, and a replica of the in vivo plug. Evenly spaced pressure taps are placed along the flow channel.
pump. The over-all pressure drop was measured by a Sanborn 267B differential pressure transducer. Similar transducers were used simultaneously to measure the incremental lateral pressure drops between closely spaced (1 cm) points along the channel. The ratio \( \Delta p/\Delta z \) (where \( \Delta p \) was the incremental pressure drop and \( \Delta z \) was the pressure tap separation) was taken to represent the pressure gradient at points halfway between each set of these pressure taps.

In this manner it was possible not only to calibrate the electromagnetic flowmeter using the actual blood and blood vessel from the animal, but also to establish the relationship of \( \frac{d\rho}{dz} \) to both flow \( Q \) and to \( z \) (the distance from the entrance of the plug channel). It was found that this relationship was virtually independent of the diameter of the large lucite (aorta) tube. Thus the fact that the actual aortic diameters (and therefore the plug diameters) varied around the average chosen for the calibration stand was of no importance. The only critical parameters in the simulation were the plug channel dimensions and entrance geometry. The plugs were carefully machined so that they were as nearly identical as possible in these respects.

There are four potential sources of error in the foregoing calibration procedure. The first is the assumption that \( \frac{\Delta p}{\Delta z} = \Delta p/\Delta z \). The second is that temperatures could not be maintained at the in vivo level, thus causing the blood viscosity to deviate somewhat. The third is that the channel dimensions varied slightly within machining tolerances in spite of every effort to the contrary. The fourth source relates to the assumption that the vessel wall over the channel has the same "smoothness" and same rheologic interaction with the adjacent blood as does its lucite analog in the model. Although it is unlikely that any of the foregoing errors are large, the possibility of their occurrence should be taken into account in any detailed interpretation of the final results of this study.

The actual calibration procedure consisted of the following steps. (1) Ranges of flows were generated through the model such that the range of the electromagnetic flowmeter signal bracketed its values in the in vivo situation. (2) Using the orifice meter as a primary standard, actual flow values were assigned to the electromagnetic flowmeter signal (except for animals 307 and 309, discussed later). (3) At each of a number of selected flow levels, the corresponding values of the pressure gradient \( (\Delta p/\Delta z) \) at selected locations \( z \) along the plug channel were recorded. (4) These data were extrapolated between measurement sites along \( z \) by conventional polynomial fitting techniques to give a continuous representation of \( \frac{\Delta p}{\Delta z} \) as a function of \( z \) for each flow level studied. (5) The shearing stress \( \tau \) was then computed from equation 2 as a continuous function of \( z \) using the results of step 4 for \( \frac{\Delta p}{\Delta z} \).

Plots of \( \tau \) as a function of \( z \) for the extreme values of flow, as well as the temporal mean flow encountered in the study, were made for each experiment. These plots, representing the stress distribution along the system, were superimposed on corresponding plots representing the resulting tissue damage.

**Preparation of Tissues for Histologic Examination**

Immediately after the animal was killed, the aorta was removed, opened longitudinally, and in the experimental group of animals the plug was carefully removed so as not to disturb the endothelium. The specimen was then pinned to an adjustable mounting board and stretched to the estimated in vivo length and circumference dimensions using the described markers. In addition, small scalpel nicks were placed neatly across the beginning and end points of the flow channel for orientation, as well as for distance calibration at microscopy.

A number of paraffin embedding and fresh freezing histologic techniques were explored. All of these approaches produced profound dehydration or other distorting artifacts. It was found that formalin-fixed, gelatin-embedded, frozen sections yielded the most consistent and uniform histologic picture.¹

¹This technique was developed by the Baker Histology Laboratories, Great Falls, Virginia.
The stretched specimens were placed into a buffered, 37°C 10% formalin – 2% gelatin fixative for 24 hours immediately after death. The tissues were then placed in formalin-gelatin solutions of successively increasing concentration over the ensuing 48 hours until a 30% gelatin concentration was achieved. The rationale for this incubation period was to achieve fixation and at the same time promote diffusion of the gelatin solution into the endothelial surface to enhance adherence after solidification. Then the temperature was dropped to 20° and the gelatin solidified. The gelatin-embedded tissue was cut out of the stretching racks and placed into formalin for another 72 hours to harden the gelatin further.

The fixed tissue blocks were removed following fixation and placed in a special cutting device. This device was designed with parallel cutting blades and a sighting mechanism such that the portion of the tissue block containing the narrow longitudinal strip of aorta originally covering the flow channel in the plug could be aligned and excised for mounting on the microtome stage. This gelatin-embedded strip was mounted on one edge so that the plane of the cutting blade was common both to the longitudinal axis and the radius of the plug channel. The blade was set for a 20-μ cutting depth. The first 15 sections were discarded to place the cutting plane approximately in the midregion of the flow channel. The next ten sections, located as shown schematically by the heavy longitudinal line in Figure 3, were saved for mounting and staining.

The sections were stained individually with Oil red O (to detect any fat) and counterstained with hematoxlin for study of cytology. Following staining, the sections were carefully mounted longitudinally on slides so that the histologic changes could be quantified for comparison with the associated hydrodynamic parameters along the system using a graduated microscopic stage.

The major problems with this technique are: (1) staining tends to be somewhat uneven, and (2) the gelatin tends to separate from the endothelial surface after sectioning, particularly from normal tissues. Therefore, great care must be exercised in handling the individual sections to achieve technically acceptable slides.

**Results**

A number of the animals developed arrhythmias at the time of intracardiac insertion of the plug. Twenty-two animals survived this procedure. Of these 22 animals only 13 could be included for data analysis. Four of the 22 were excluded because of clot formation in the entrance region of the plug. The presence of the clot violated the assumptions necessary for calculation of shearing stress. Clots occurred mostly in the studies of longer duration (3 hours). Four animals were excluded because the plug did not follow an acceptably straight trajectory to its resting site (vide infra). One experimental animal and two control animals were excluded because staining was too dark to evaluate the histologic sections. All of the remaining 13 animals were analyzed histologically for damage distribution, but only 11 of these could be analyzed for the concomitant stress distribution, since electromagnetic flow meter calibrations were not obtained in two of the initial studies (307 and 309). The importance of immediate post-experimental flow meter calibration was not fully appreciated during the initial phases of this study. Finally it was not possible to obtain an equal sex distribution in either group of animals because of problems in animal procurement.

**Physiological Observations**

The data appearing in the upper left of Fig.
Figure 4 are representative of the physiologic data obtained in vivo. Reading from top to bottom, the tracings are (1) the upstream pressure ($p_1$), (2) the flow ($Q$), and (3) the pressure drop across the system ($\Delta p$). The average aortic pressure immediately prior to insertion of the plug was 169 g/cm$^2$ for the group as a whole. The same values shortly after placement of the plug averaged 167. Thus, the normal pressure upstream was unaffected by the presence of the plug. The average pressure drop across the system was 61 g/cm$^2$, the mean flow was 15.8 cm$^3$/sec, and the averaged Reynold's number for the plug channel was 1008.

The distribution of shearing stress along the plug (from $z = 0$ to $z = 3$ cm) is shown in the graph on the upper right of Figure 4. The numbers on the three curves refer to the points during the cardiac cycle, shown on the flow curve to the left, for which the stress distribution was calculated. Curve 2 is the temporal mean stress distribution for the entire experimental period, whereas, curves 1 and 3 represent two extreme distributions for the particular heart beat shown. Notice that the stress is most intense upstream, decreasing rapidly with distance along the channel. The mean stress distribution along the uniform portion of the plug channel is replotted in panel c as the solid curve. The dotted extensions of this curve were estimated by assuming equation 2 to hold in the nonuniform portions of the plug. As shown in the Appendix, equation 2 is valid in the uniform portion of the plug and in the uniform portions of the aorta above and below the plug, but it is questionable in the entrance and efflux portions of the plug where the radial component of blood velocity becomes significant. The dotted portions of the curve are included to demonstrate the rapidity with which the stress develops in the entrance region and decays in the efflux portion of the plug.

The distribution of pressure along the system is represented by the upper curve ($p$) in panel c. The pressure can be seen to decay rapidly in the entrance portion of the plug in association with the marked acceleration of the blood from the wide aortic chamber into the attenuated channel of the plug. Although not apparent in this illustration, it can be shown that the pressure distribution just distal to the efflux portion of the plug undulates slightly with distance, suggesting that a rather coarse vortex system sits in this region.

The distribution of sound energy measured in the 13 separate studies described earlier is shown in d of Figure 4. Energy is expressed as the ratio of energy at any point of interest to control values recorded from the upper descending thoracic aorta. If the Reynolds number is used empirically to rank the flows encountered in this portion of the study, certain patterns emerge. For example, the dashed curve at the bottom of Figure 4 represents the mean distribution of sound energy for eight of the animals in which the Reynolds number ($N_R$) exceeded the critical value of 1000 and the lower solid curve, the mean of five animals in which $N_R$ was less than 1000. These curves show that radiated sound energy is maximum in the efflux region for both groups of animals; however, it is approximately six times greater in the animals having Reynolds numbers greater than 1000.

The distribution of energy in the channel portion of the plug also differs between the two groups. In the low $N_R$ group energy remains essentially at control values, suggesting that flow remains laminar. In the high $N_R$ group sound energy in the inlet region is also in the range of control values; however, it increases between $z = 0$ and $z = 1$ cm to a higher level, remaining essentially constant for the remainder of the channel length, i.e., from $z = 1$ to $z = 2.7$ cm. This suggests that flow is laminar in the inlet region but becomes unstable shortly thereafter, developing into classical turbulent flow. This idea is supported by the observation that the correlation between the sound intensity from the portion of the channel between $z = 1$ and $z = 2.7$ cm and the corresponding Reynolds number was 0.87.

It is of interest that the level of radiated sound in this uniform portion of the plug channel correlates with the Reynolds number. This, in itself, is evidence that the intensity of sound...
(a) Representative physiologic data collected during an experiment. From top down: upstream pressure ($P_u$), flow ($Q$) through plug channel, and pressure drop ($\Delta P$) between the upstream and downstream intercostal catheters. Numbered points on the flow curve refer to data in b. (b) Endothelial surface shearing stress ($\tau$) as a function of distance ($z$) along the channel for the three different flow levels indicated by the numbered points on the flow curve in a. (c) A plot showing the temporal mean distribution of pressure (solid curve, $p$, $\mu$/cm$^2$), wall surface shearing stress (solid and heavy dashed curve, $\tau$, dynes/cm$^2$), and assumed turbulent energy (fine dashed curve) along the aortic wall. The ordinate is given as an arbitrary scale, $S$, which may be converted to the desired variable by the relations listed to the right of the scale. A diagram of the aortic segment and plug corresponding to these above plots appears immediately below for reference purposes. The "a" and "b" are the semiminor and semimajor axes of the truncated elliptical horn forming the entrance portion of the plug channel and are numerically equal to 0.63 and 0.96 cm, respectively. (d) This graph shows the distributions of the mean, relative, sound intensity radiated from the aortic surface of the 13 special studies (see text and lower portion of Fig. 1). Solid curve = the average distribution of sound.
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may be used as a measure of turbulence intensity since we know from the laws of hydrodynamics that turbulence intensity should correlate with the Reynolds number in fully developed turbulent flow. This suggests also that the markedly increased sound level in the efflux region indicates an increased turbulence intensity in this region. This possibility is of interest in view of the histological and anatomical data to be presented below. In anticipation of these histologic findings, the sound intensity in this efflux region was also correlated with the channel Reynolds number. The correlation coefficient was 0.64. Thus it appears that the channel Reynolds number does not bear a very strong relationship to the radiated sound in the efflux region.

ANATOMICAL OBSERVATIONS

Figure 5 is a reproduction of a photograph showing a typical aorta from the experimental group of animals, which has been opened, stretched to in vivo dimensions, and prepared for fixation. The specimen was photographed through an orange filter and printed on high contrast paper so that blue-stained areas would appear dark. This shows the typical staining pattern caused by the Evans blue dye in the region of the plug.

The pattern of the plug surface can be seen in the middle of the photograph. The tag is on the upstream end of the specimen and therefore during life flow passed from left to right. The strip of endothelial surface overlying the flow channel in the plug stained most darkly over its upstream portion in the region of high shearing stress (see Fig. 4). Staining becomes progressively less downstream from this point until reaching the highly turbulent efflux region (see Fig. 4) where staining suddenly becomes quite intense again.

The horizontal white stripe on the left of the figure represents the normal, unmolested endothelial surface that lay in the trajectory made by the plug channel during its descent from the upper aorta to its resting site. As can be seen, this tissue remained unstained. On the other hand, the areas of endothelial surface which came into contact with the plug surface during its descent were mechanically abraded (obvious only under the microscope) and stained bright blue as indicated by the dark areas on either side of the white trajectory.

Although microscopic examination of sections under high power failed to demonstrate this staining, examination of the cut surface of the tissue blocks removed from the microtome stage using 50× magnification indicated

FIGURE 5

A photograph of the opened aorta stretched to in vivo dimensions taken immediately post mortem. An orange filter was used so that blue-stained areas appear dark. Flow was from left to right during life. The horizontal white stripe on the left = the normal endothelial surface that lay in the trajectory of the plug groove during its descent. The endothelium on either side of the strip came in contact with the plug and stained blue. The central pattern represents the plug site through the center of which may be seen the strip of endothelium that covered the plug channel. Note: (1) Staining increases abruptly in the upstream end of the channel and then decreases progressively thereafter to the efflux region. (2) Staining again increases in the region of the expanding turbulent jet issuing from the channel. (3) Staining gradually disappears downstream from the efflux region.
that staining was sharply localized to the intimal surface. From study of the data in Figure 4, it is apparent that the topography of staining is coincident with endothelial surface areas that were exposed either to increased hydraulic shear or increased turbulence (or to mechanical contact with the plug surface). This suggests that abnormal amounts of albumin entered the intima in areas of endothelial damage. We shall return to this question later.

Apart from these physico-chemical considerations, the importance of Evans blue dye to the present study was also of a practical nature. The staining pattern was used for two purposes: first, in interpreting the histologic data of this study, it is essential to know that the tissue overlying the plug channel was subjected to no forces except those of the flow, i.e., it must be known that the channel site was not previously damaged by contact with the plug surface due to rotation of the plug. Evidence that this did not occur was obtained by determining that the longitudinal white stripe (on the left of Fig. 5) representing the unstained trajectory of the channel upstream from the plug was aligned with the blue-stained stripe representing the plug channel site.

The second purpose for which the staining was used was to help in aligning the blocks of gelatin-embedded tissue for cutting prior to

**FIGURE 6**

Photomicrographs of formalin-fixed, gelatin-embedded normal endothelial tissue from central descending thoracic aortas of dogs stained with the oil red "O" and hematoxylin. Horizontal bars = 10 μ. Panels a and e-f are longitudinal sections, and b, a circumferential section.
mounting on the microtome stage. Without the staining it is not possible to see the channel site clearly enough in the fixed tissue block to remove the histologic sections properly from the midstream region as described earlier (Fig. 3). Even with Evans blue staining, it was frequently difficult to be sure that the upstream portion of the tissue section corresponded to the light stripe representing the channel trajectory. There was frequent histologic evidence that perfect alignment in this region was not always achieved.

HISTOLOGIC OBSERVATIONS

It is useful first to describe the microscopic appearance of the normal aortic tissues from the animals in group 1 in order to establish the picture of "normal histology" against which the abnormal findings from the experimental group may be compared. Photomicrographs of these tissues taken from the descending thoracic aorta of the normal dogs are shown in Figure 6 (horizontal bars represent 10 μ). Although endothelial architecture and cell distribution tended to vary somewhat depending on location and animal, the majority of cells have the features shown in the top four panels of Figure 6. Panels a, c, and d are longitudinal sections, and panel b is a circumferential section, all from the ventral aspect of the descending thoracic aorta.

The normal endothelial cellular profiles present low-lying, elongated silhouettes which are in tight apposition to the basement membrane. The nuclei are nearly parallel to the basement membrane and have their major axes parallel to the longitudinal axis of the blood vessel, i.e., the nuclei are elongated and oriented with the lines of flow. This elongated shape may be ellipsoidal, sausage-shaped, or spindle-shaped, depending on the particular preparation. The nuclear surface adjacent to the basement membrane usually is somewhat flattened or even slightly concave toward the basement membrane. The nuclear border is usually smooth and sharp, and the contained chromatin material is relatively basophilic, having a finely granular appearing

### TABLE 1

<table>
<thead>
<tr>
<th>Distinguishing characteristics</th>
<th>&quot;Ideal&quot; cells (A)</th>
<th>&quot;Altered&quot; cells (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell profile</td>
<td>Spindle shaped with central nuclear bulge</td>
<td>Irregular shapes</td>
</tr>
<tr>
<td>Nuclear orientation</td>
<td>Major axes parallel to the axis of the blood vessel</td>
<td>Frequently tilted and jumbled</td>
</tr>
<tr>
<td>Cell spacing</td>
<td>Moderately non-uniform</td>
<td>Very non-uniform, occasionally piling up</td>
</tr>
<tr>
<td>Nuclear shape (profile)</td>
<td>Low-lying profile closely adhered to basement membrane. Ellipsoidal to slightly crescentic in shape with flat side toward basement membrane</td>
<td>May be crescentic but usually are bent, irregular, occasionally appearing multinucleated</td>
</tr>
<tr>
<td>Nuclear border</td>
<td>Smooth</td>
<td>Often rough, becoming diffuse and hazy in severe damage</td>
</tr>
<tr>
<td>Nuclear texture</td>
<td>Dense, nearly homogeneous, occasionally with one or more nucleoli</td>
<td>May be very inhomogeneous and granular, occasionally with clumping and fragmentation of chromatin material</td>
</tr>
<tr>
<td>Nuclear staining</td>
<td>Dark blue</td>
<td>Varying from dark to pale blue</td>
</tr>
<tr>
<td>Cytoplasmic border</td>
<td>Sharp, may undulate slightly</td>
<td>Irregular, fuzzy, with occasional adsorbed fat droplets and other granular material</td>
</tr>
<tr>
<td>Cytoplasmic volume</td>
<td>Sparse</td>
<td>Swollen</td>
</tr>
<tr>
<td>Cytoplasmic texture</td>
<td>Homogeneous ground glass appearance</td>
<td>Granular with frequent inclusions such as fat droplets</td>
</tr>
<tr>
<td>Cytoplasmic staining</td>
<td>Uniform pale blue</td>
<td>Variable</td>
</tr>
</tbody>
</table>
texture. One or more nucleoli are frequently visible.

Panels a and b are from the same animal and demonstrate typical relative nuclear dimensions. The nuclear dimensions are variable ranging from 8 to 20 μ long, 3 to 5 μ in the direction of vessel circumference, and 1 to 3 μ in the direction of vessel radius. The center-to-center spacing of the nuclei is even more variable, being around 15 to 50 μ in the longitudinal direction and 10 to 30 μ in the circumferential direction.

The cytoplasm presents a sharp border which trails off from the edge of the nuclei in all directions and is bounded below by the refractile basement membrane. The border occasionally appears to undulate somewhat as in Figure 6a, but more frequently it is smoothly curved as shown in Figure 6c and d. The cytoplasm stains pale blue and has a homogeneous, ground-glass appearing texture. This can be seen most clearly in the higher magnification shown in Figure 6d. The salient features of this normal endothelial histology are summarized in the left-hand column of Table 1 under the heading of “Ideal” cells for subsequent reference.

The intimal region immediately below the endothelial cells consists of fine refractile fibers which are relatively homogeneously distributed and are usually strongly oriented in the longitudinal direction. These fibers appear to be embedded in a pale-blue, homogeneous matrix of ground substance. The fibers are also associated with elongated nuclear forms which are assumed to represent fibrocytes, smooth muscle cells, and primitive undifferentiated cells. No distinction is made among these “connective tissue cell” types which, for brevity, are referred to hereafter simply as “CTC.” The nuclei of these CTC are about the same size but much more irregularly shaped than endothelial nuclei, as if conforming to the mechanical constraints of the adjacent bundles of connective tissue. This region of intima extends approximately 5 to 50 μ into the wall, following which the fiber orientation assumes a more circumferential orientation.

The circumferentially oriented fibers extend through the rest of the wall width. These fibers tend to be clustered in bundles and follow a more undulating course than in the immediately sub-endothelial region. Aside from this, the nuclear characteristics and ground substance appear to be identical. Minor exceptions to the foregoing picture are seen occasionally in the ventral aspect of the aorta and more commonly on the dorsal aspect, particularly in proximity to the intercostal artery ostia. On the dorsal aspect of the aorta, the thickness of the layer of longitudinally oriented fibers immediately under the endothelial cells is considerably greater, particularly immediately downstream from the intercostal ostia. Moreover, the number of cell nuclei both on the endothelial surface as well as in the subjacent connective tissue is considerably more sparse.

The adventitial region appears to be richly supplied with small arteries. However, the associated capillary beds never penetrate below the outer third of the wall. One infers that the inner two-thirds of the aortic wall meets its metabolic needs primarily by diffusion of substances from the vessel lumen on the one side and the capillary bed in the outer wall on the other side. One concludes from this that the intimal regions of the tissues overlying the plug channel in the experimental group of animals were properly nourished.

The foregoing descriptions represent the most common histologic pattern seen in the normal tissues. Areas interpreted as “pathologic” in light of subsequent findings appeared to occur spontaneously in certain areas of some of the normal dogs. Two types of spontaneous lesions were seen. The first consisted of “abnormal” endothelial forms such as shown in Figure 6e. These abnormal forms were usually characterized by the appearance of moderate degrees of cytoplasmic swelling and distorted nuclear architecture. The second was the appearance of areas in which the endothelial cells appeared to be completely eroded from the basement membrane. An example of this is shown in Figure 6f. Here one finds the absence of endothelial cells, and,
if one studies carefully the border between the lumen and the vessel wall, one sees that the ground substance stains more lightly, as if the blood flow had washed the fibers in the interstitial region partly free of their surrounding matrix. Nuclei of CTC appear to be concentrated in this region and no longer assume their usual either longitudinal or circumferential orientation, but they appear to be tilted as if migrating toward the denuded area. Changes such as these were frequent in the normal sections and logically might be considered as fixation artifacts if it were not for the observations to be presented later. It follows that the spontaneous occurrence of "pathologic" changes in normal tissues must be born in mind when evaluating histologic changes related to any particular experimental intervention.

Photomicrographs of tissues from the experimental group of animals are shown in Figure 7. For purposes of orientation, it is convenient to refer again to Figures 3 and 4. In Figure 4,c it can be seen that endothelial tissues located upstream from the plug, say at values of \(z\) less than \(-0.5\), were exposed to normal pressures, no detectable turbulence, and a lower than normal shearing stress. Figure 7,a and b, are photomicrographs of tissues in this region upstream from the entrance portion of the plug. These tissues were in the unmolested trajectory of the groove of the plug corresponding to the white stripe shown on the left of Figure 5. Comparing Figure 7,a and b, with Figure 6,a, c, and d, and with the criteria listed in the left-hand column of Table 1, it can be seen that the tissues from this upstream region cannot be distinguished from the normal tissues.

The exposure to shearing stress corresponding to the remaining photomicrographs in Figure 7 increases with ascending alphabetical order of the panels (from above downward). The tissues in Figure 7,c and d, were exposed to the lowest levels of shearing stress and represent some of the earliest changes seen. Although the cytoplasmic border remains sharp, there appears to be early swelling and deformation of the cytoplasm. These changes, though more numerous in the high shear region, are identical to those occurring spontaneously in certain regions of normal tissues as were shown in Figure 6,e.

With a slightly increased exposure to stress, the sharp cytoplasmic border disappears, and the apparent texture of the cytoplasm becomes granular, frequently containing fat droplets and other inclusions as shown in Figure 7,e, f, and g. The CTC on the right of Figure 7,f, appears to be migrating from the immediately subadjacent connective tissue into the endothelial region near the damaged endothelial cell on the right half of the panel. Migrating cellular forms such as these were commonly seen with all levels of damage in the "high" shear regions but were almost never seen in the normal tissues.

With a somewhat greater exposure to stress, the cytoplasm becomes progressively disintegrated (Fig. 7,g, h, and i). At this stage there is usually altered nuclear staining and marked deformation of the nuclear architecture (h and i). The nuclear forms (h and i) appear to be flowing slowly along the surface of the basement membrane. Piles of tangled endothelial nuclear forms such as seen in h may occasionally extend 50 to 100 \(\mu\) into the lumen and 100 to 400 \(\mu\) along the basement membrane. With still greater exposure to shearing stress, the nuclear staining properties become very severely altered as shown in i, finally becoming invisible, as shown in j, where only the remnants of cellular debris are adherent to the basement membrane. With greater exposure, the debris is usually washed away, leaving a completely exposed basement membrane as shown in k.

With continued exposure to shearing stress, this denuded surface may demonstrate further changes along one or both of two avenues. The less common avenue appears to be progressive erosion of the surface resulting in fragmentation of its fibrillar structure (early evidence of which may be seen on the right of Fig. 7,k) and loss of the light, blue-staining ground substance. The sharply defined interfacial border (basement membrane) is re-
NORMAL STRESS EXPOSURE

IN ORDER OF INCREASED STRESS EXPOSURE

10 μ
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placed by a fragmented, fibrillar, bushy interface.

The more common avenue appears to be the deposition of blood elements on the eroded surface such as shown in Figure 7. The deposited material consists of fibrin, platelets, blood cells, and other unidentified debris. The original sharp interfacial border is frequently still discernible under the deposited material.

Progressing downstream from \( z = 0 \), i.e., along the uniform portion of the plug channel, the reverse sequence of histologic changes previously described occurs. Thus in the upstream portion (near \( z = 0 \)) deposition of fibrinous matter, total erosion of endothelial cells, and fragmentation of the basement membrane can be seen. Somewhat downstream from this point, markedly distorted endothelial cells begin to reappear. Still farther downstream, a progressive increase in endothelial cell density occurs associated with the gradual reappearance of relatively normal endothelial cell forms.

The distribution of mechanical events associated with these histologic changes is best seen by referring again to Figure 4.c. It is apparent that the associated shearing stress drops in an exponential fashion along this portion of the system (i.e., along the uniform portion of the plug channel). The corresponding pressure also decreases, but much less so. If the Reynolds number is low, no turbulent energy appears until the efflux region. If the Reynolds number is sufficiently high, turbulent energy can appear in the upper portion of the channel. This energy remains at a detectable but low level until the lowermost region of the channel is reached (i.e., between \( z = 2.5 \) and \( 3.0 \)). In this region turbulence increases precipitously in all cases, reaching a peak value about 1 cm below the efflux of the plug channel. In this efflux region (at about \( z = 3.5 \) to 4.5), the pressure continues to decline or rise transiently very slightly. The shearing stress drops to negligible values.

It was of interest to find that in all animals cellular changes of varying degrees occurred in the efflux region of low shearing stress but high turbulence. These histologic changes were indistinguishable from those occurring upstream in the low-turbulence, high-shearing stress region. (As discussed later, the high frequency velocity components of turbulence can produce shearing stresses independent of the "time-smoothed" component, as well as increased chemical convection). Proceeding downstream from the region of the plug channel efflux, evidence of cellular damage as well as turbulent energy progressively decreases.

Therefore, it appears that the aforementioned endothelial histologic changes are related to stress exposure, to the time-smoothed component in the uniform channel, and to turbulence in the efflux region. These changes may be summarized according to increasing exposure to shearing stress approximately in the following order: (1) endothelial cytoplasmic swelling, (2) ordered cellular deformation (bent nuclear forms and apparent cellular flow), (3) disordered cellular deformation (cytoplasmic and nuclear granulation and distintegration), (4) early reparative attempts by migrating cellular elements from store of subjacent CTC, (5) reduction of endothelial cell to amorphous cellular debris, (6) erosion of all cellular vestiges from the basement membrane, which occasionally may go on to (7) further erosion and fragmentation of basement membrane and subjacent vessel material, but more often goes directly

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FIGURE 7

Photomicrographs of formalin-fixed, gelatin-embedded endothelial tissue removed from ventral aspect of aorta as indicated in Figure 3. Tissues stained with oil red "O" and hematoxylin. Horizontal bar at bottom = 10 μ for all panels; panels a and b = tissue removed from plug trajectory (white stripe on left of Fig. 5); panels c-1 = tissues removed from plug channel region. Increasing exposure to surface shearing stress corresponds to increasing alphabetical order of panels (from above downward).

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to (8) deposition of blood elements on the eroded surface.

If any meaningful correlation between these histologic changes and the magnitude of stress exposure is to be made, then one must devise some method of quantifying this tissue damage. In principle, the foregoing sequence could be used in ascending order as a measure of "damage," provided these events were truly serial events. Actually many of the events appeared to be progressing in parallel. This was particularly true of the changes in endothelial cellular architecture (represented by events 1 through 5) suggesting that all cells are not identical but have variable susceptibilities to damage. Any method of quantifying damage, therefore, must take into account this distribution of properties among the cell population. With this in mind, the following scheme was adopted.

The endothelial cell population was simply divided into two groups. Cells having all of the histologic criteria outlined in the left-hand column of Table 1 were considered to be ideal cells and were placed in group A. Endothelial cells having any histologic criteria suggesting the architectural changes represented by events 1 through 5 were placed in group B. These histologic criteria are summarized in the right-hand column of Table 1 for comparison with the "normal criteria." The population density of each of these two groups of cells was then determined as follows:

A ratchet device was placed on the microscope stage so that the slide could be moved smoothly in 1-mm increments. By focusing up and down, the entire tissue depth could be scanned. Using a blood cell counter, the number of "A" cells and "B" cells per linear millimeter of tissue section were counted and tabulated as a function of distance along the slide (or as a function of z by aligning the slide with the nicks made at the time of tissue mounting). Since the tissue sections were 20μ in thickness, these 1-mm counts were multiplied by 50 to convert to cells per square millimeter. The thickness of any deposited material from the blood phase that was present was estimated to the nearest 5μ and also recorded.

To check the reproducibility of the cell-counting technique, random sections of each slide were reread several weeks later without reference to the previous tabulation sheets. After recounting, the cell densities at each site along z were correlated with the same readings done previously. The correlation coefficients between the two readings for each cell type and for each animal appear in Table 2. The correlation coefficients for A cells ranged between 0.76 and 0.99 and for B cells between 0.60 and 0.98. The correlation coefficients for pooled readings for all animals were 0.93 and 0.83 for A cells and B cells. Thus the counting technique using the criteria in Table 1 affords an acceptably objective measure of the different cell populations along z in all of the studies except perhaps in the case of dog 329.

Cell counts were done also on tissue sections from five of the normal animals to establish the normal population density of A and B

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<thead>
<tr>
<th>Dog. no.</th>
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<th>B cells</th>
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<tbody>
<tr>
<td>307</td>
<td>.987</td>
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</tr>
<tr>
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<td>.989</td>
<td>.951</td>
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<td>.603</td>
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<tr>
<td>All animals pooled</td>
<td>.933</td>
<td>.831</td>
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</table>

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Upstream</th>
<th>Downstream</th>
<th>t value</th>
<th>p value</th>
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<tbody>
<tr>
<td>A</td>
<td>1435</td>
<td>1306</td>
<td>0.69</td>
<td>0.50</td>
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<tr>
<td>B</td>
<td>375</td>
<td>236</td>
<td>2.40</td>
<td>0.02</td>
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<tr>
<td>A + B</td>
<td>1808</td>
<td>1603</td>
<td>0.98</td>
<td>0.40</td>
</tr>
</tbody>
</table>

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cells and to explore whether these densities vary systematically with distance along \( z \). This was determined by comparing the mean cell densities in the upstream region (\( z < -1.0 \)) with those in the downstream region (\( z > 4 \)). These data are summarized in Table 3. The cell densities appeared to decrease slightly with distance downstream. However, the decreases in total density \((A + B)\) and density of \( A \) cells were not statistically significant. The decreased density of \( B \) cells appeared to be somewhat more significant. The \( A \) cells make up 80% and the \( B \) cells 20% of the normal endothelial cell population. The \( B \) cells in these normal tissues were all of the types shown in Figure 6,e and 7,c and d, i.e., were cells showing the "earliest" types of histologic change. Evidence of more severely changed forms such as shown in Figure 7,e-\( j \) was not seen in normal sections.

Unlike the foregoing, the densities of \( A \) and \( B \) cells in the experimental group of animals were distributed in patterns which depended very much on the location along \( z \) and the associated hydrodynamic parameters. Moreover, the population of \( B \) cells was much more heterogeneous containing more severely altered cell forms (Fig. 7,e-\( j \)).

Typical cell density distributions are shown in Figure 8 plotted in the same format as the mechanical parameters plotted in Figure 4,c. A five-point running average was used to convert the cell density histograms to the "continuous" curves shown in the figure. The total cell density may or (as in this case) may not decrease in the region of highest shearing stress. The population of \( A \) cells always drops precipitously in the plug entrance region \((-1 < z < 0)\) reaching a minimum in the region of highest shearing stress (around \( z = 0 \)). As the shearing stress decreases, the population of \( A \) cells increases, frequently returning to normal values, as it does in this case, just upstream from the channel efflux. In the efflux region the density of \( A \) cells usually dips again to another minimum corresponding to the appearance of significant turbulent energy, increasing again to normal values somewhat further downstream. Notice that the pressure variations along the system do not bear any relationship to the observed cell damage pattern. Moreover, along the splinted portion of the system the \( A \) cell population density swings through two complete abnormal to normal cycles. The foregoing suggests that neither the rigid configuration itself nor the pressure was related to the observed damage. The effect of evenly applied pressure in a rigid vessel is considered further in the Discussion portion of this report.

As would be expected from the discussion of Figure 7, the density of \( B \) cells (dotted curve in Fig. 8) tends to be related to the decrease in \( A \) cells, at least for moderate stress exposure. Thus the density of \( B \) cells rises in the entrance region and, if erosion is not severe, reaches a maximum around \( z = 0 \), decreasing gradually thereafter, approaching normal values just upstream from the efflux. The density distribution of \( B \) cells increases again in the region of the turbulent efflux (around \( z = 3.5 \)).

With more severe exposure to stress, one finds decreases in the densities of both \( A \) and

\[\text{FIGURE 8}\]

Plots representing endothelial cell population density distribution \((A, B \text{ and } A + B)\) and thickness of deposited material \((\lambda)\) superimposed on the corresponding pressure \((p)\) and surface shearing stress \((\tau)\) distributions along a vessel-plug system such as shown in Figures 1 and 3. The arbitrary scale, \( S \), on the ordinate may be converted to the desired variable by the relations listed at the top of the figure.
$B$ cells indicating erosion of the endothelial surface. As would be expected, this is seen most commonly around $z = 0$ and reflects the decrease in total cell density ($A + B$) mentioned earlier. If the total cell density becomes too low, layers of deposited material frequently appear. It follows that deposited material also will be most common in the region around $z = 0$ or in the turbulent efflux.

These results are summarized in the schematic diagram of Figure 9,a. The distribution of $A$ cells and the associated distributions of shearing stress and turbulent intensity suggest several important conclusions. First, notice the two distinct minima in the $A$ cell density distribution curve which occur under clearly different hydrodynamic situations. The first occurs in a region of high shearing stress and low turbulent intensity which corresponds approximately to the bracketed region $-0.5 < z < 2.5$ cm in Figure 9,a. The second occurs in a region of low shearing stress but high turbulent intensity which corresponds to the bracketed region $3 < z < 6$ cm in Figure 9,a.

We conclude, on the one hand, that shearing stress can produce endothelial damage independently of turbulence (there was no turbulence in animals having $N_p < 1000$), and, on the other hand, that turbulence can produce damage independently of the time-smoothed shearing stress.

Endothelial cells appear to have varying susceptibility to damage. In plotting the population density of $A$ cells vs. shearing stress as shown in Figure 9,b, the steepest part of this curve (arrow) would occur at a point corresponding to the greatest rate of change of $A$ cells with respect to shearing stress, i.e., the stress at which there is the greatest rate of conversion of $A$ cells to $B$ cells. The stress at which this occurs, therefore, could be used to describe the most common mode of cell behavior with respect to stress. We call this the acute critical stress ($T_p$).

This point may be determined by differentiating the curve in Figure 9,b and measuring its maximum as indicated in Figure 9,c. Since the cell counts represented discrete measurements, it was necessary to replace these data with a continuous smooth function prior to forming the derivatives. The data were fitted to a 5-term polynomial using "least-squared" deviation criteria and then differentiated analytically to yield smooth curves similar to that shown in Figure 9,c. The acute critical stress ($T_p$) estimated in this way for each animal appears in Table 4 along with other pertinent parameters.

If animal no. 329 is excluded, the values of $T_p$ have a mean value of $379 \pm 85$ (sd) dynes/cm². Animal 329 is 10 sd away from this mean and, therefore, was excluded from subsequent statistical considerations. Simple inspection of the data in Table 4 shows no obvious trends.
To check this impression various statistical techniques were used to search for relationships among the acute critical stress and the other parameters in the table. Significant relationships could not be found. This was true whether data were separated into groups according to duration ($T = 3$ was not tested because it contained only two entries), or whether it was pooled (to include $T = 3$). In particular, the mean $\tau_c$ for $T = 1$ was $437 \pm 102$ (SD) and for $T = 2$ was $346 \pm 43$ (SD). The difference between these means was not significant ($P > 0.9$). This suggests that the "time constant" describing the process by which shearing stress produces cell damage is probably less than 1 hour. Moreover, no correlation was found between $\tau_c$ and the channel Reynolds number ($N_R$). The correlation coefficient was $-0.06$ for $T = 1$, $-0.04$ for $T = 2$, and $0.16$ for the pooled data. This suggests that turbulence in the channel does not play a significant role in enhancing the damaging effect of the time-smoothed shearing stress. Although it is possible that a larger number of experiments might show some trend, for example, a decrease of $\tau_c$ with duration of experiment, there is no evidence of any trend in the present data. One concludes from this (at least for the present) that the acute critical stress ($\tau_c$) may be considered a constant representing some sort of parameter that defines the resistance of endothelial cells to damage by the time-smoothed shearing stress and that appears to be independent of both the high frequency components of turbulence as well as the duration of the applied stress in excess of 1 hour, i.e., the process appears to have a relatively short "time constant."

Endothelial changes also occur just below the channel efflux that are histologically identical to those occurring in the upstream portion of the plug channel. We do not have a direct measure of turbulent intensity in the efflux region. If we make the assumption, supported by the rather weak correlation discussed earlier, that turbulent intensity in the efflux region bears some monotonically increasing relationship to the channel Reynolds number, then one might expect this Reynolds number to correlate with the degree of damage in the efflux region. The "degree of damage" was defined simply as the maximum percent of damaged cell density in the efflux region. This was calculated as percent damage = $(A_N - A_{min}) / A_N \times 100$, where $A_N$ is the normal mean A cell density in this same region (from Table 3, row 1, column 2) and $A_{min}$ is the minimum cell density occurring for $z > 3$.

The correlation coefficient between $N_R$ and percent damage for the pooled data was $-0.05$. It appears that no correlation exists between cell damage and an assumed turbulent intensity estimated as described above. If turbulence does cause the observed damage, we

<table>
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<th>Age*</th>
<th>$T$ (hr)</th>
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<th>Critical stress ($\tau_c$)</th>
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<td>340</td>
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*Age was divided into young (Y) and middle-aged (M) animals. Old animals were not used in this study.

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conclude that the responsible components are not related directly to the channel Reynolds number. Since the radiated sound intensity over the efflux did not correlate well (0.64) with the channel Reynolds number, it is possible that in the experiments in which the tissues were studied histologically, the percent damage might still be related to the intensity of the radiated sound if it were possible to make this measurement in these experiments.

Two basic conclusions may be drawn from the results of this study. The first is that there appears to be a critical value of time-smoothed shearing stress to which endothelial cells "yield" and develop altered structural and chemical characteristics as manifested by changes in architecture, population density, staining properties, and the passage of Evans blue dye. The yielding process appears to have a relatively short "time constant" (probably less than 1 hour). This acute critical stress may be considered a rheologic property of endothelial cells representing their resistance to the shearing forces of the adjacent flow. The acute critical stress is independent of the presence of turbulence, i.e., the critical stress did not decrease with increasing channel Reynolds number (which in turn was shown to correlate with the intensity of turbulence).

The second major conclusion is that endothelial cells demonstrate an impressive but ill-defined susceptibility to damage in the region

(a) Typical stress-strain curve for an elastic substance showing "elastic limit" (S1) and "yield stress" (S2). (b) Schematic representation of endothelial surface under static conditions or under simple hydrostatic pressure in rigid, splinted vessel. (c) Shearing strain of endothelial surface produced by surface shearing stress (τ) of adjacent blood flow. (d) Compression-elongation strain of endothelial surface produced by simple increase of hydrostatic pressure in distensible unsplinted vessel. (e) Two models of endothelial cell shearing strain produced by the high frequency components of turbulence. Lower left inset shows shear by bending due to uneven pressure. Lower right inset shows shear by surface drag of adjacent high-frequency flow component.

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of the highly turbulent efflux from the plug channel. The time-smoothed shearing stress was negligible in this region. Although the level of damage in this area did not correlate with the Reynolds number for the channel immediately upstream, it is probable that the damage was related to the turbulent energy of the efflux, perhaps to some band of frequencies or amplitudes. This and other aspects of the problem will be discussed in greater detail later.

Discussion

It was shown that endothelial cells appear to yield to the abnormal forces of the adjacent fluid flow. In considering the yielding of any substance to an applied force, two questions arise, the magnitude of the imposed stress and the strength of the material. Both of these questions are considered in relation to the results of this study. For tutorial purposes, the discussion begins by considering endothelial cells to be simple elastic bodies to focus attention on the modes by which stresses can act on a deformable system. Following this, the rheologic properties of a more realistic cell system are reviewed. Finally, speculation regarding the possible relationships of certain physico-chemical mechanisms to cell rheology will be presented.

EFFECT OF STRESS ON SIMPLE ELASTIC CELLS

In engineering practice it is customary to study the strength of a material by placing a sample of the substance in question into a machine which can apply an increasing but controlled stress to the sample. The resulting stress, as well as strain, are continuously monitored and plotted on a stress-strain diagram which might look like the one shown in Figure 10,a. Below a certain level of stress ($S_1$), the stress-strain relationship is reproducible and linear. However, above this stress, $S_1$, the substance exhibits permanent deformation and no longer returns to its original shape when the stress is removed. If stress is progressively increased beyond $S_1$ to some stress $S_2$, the substance can be seen to flow and no longer exhibits elastic behavior. This stress is sometimes called the yield stress of the material.

If the applied stress is a tensile stress, then the observed strain is elongation. If the stress is a shearing stress, then the resulting strain is a shearing strain. The effect of shearing stress on a greatly simplified endothelial surface is to be considered. For illustrative purposes, endothelial cells are considered to be simple parallelepiped structures like bricks in a wall, as indicated in Figure 10,b. If a shearing stress is applied to the surface of these cells from the overlying flow, the cells develop a shearing strain and appear rhomboidal (Fig. 10,c). If the stress remains constant, one would expect the cells to maintain a fixed rhomboidal attitude as indicated in the figure.

If, on the other hand, the stress is increased to some new constant value in excess of the critical or yield value, one would expect the cells to exhibit continuous deformation such that the cell partitions now would rotate and elongate continually to the right, ultimately becoming unstable and washing away. The foregoing is fairly obvious and is analogous to events in the uniform portion of the plug channel.

Consider now the effect of pressure stress on the endothelial surface. If the cells in Figure 10,b, are subjected to an evenly applied increase in hydrostatic pressure ($p$), one would expect two different patterns of strain, depending on whether the vessel wall was rigid or compliant. If the vessel wall were rigidly splinted, as it was over the plug channel and immediately downstream from the plug, the cells would be exposed to a simple increase in hydrostatic pressure and would respond by a simple change in volume. Since all tissues that have been studied have been shown to be highly incompressible, one would expect the endothelial cells to show negligible strain under these circumstances. Thus the cells would remain as they are in Figure 10,b.

On the other hand, if the vessel wall is not splinted and thus can dilate when exposed to an evenly applied increased intraluminal pressure, such as would be the case in normal vessels or in the vessel segment immediately up-
stream from the plug site, quite a different situation can occur. Under these circumstances, the endothelial cells would be expected to suffer an associated compressional strain in the radial direction and elongating strains in the longitudinal and circumferential directions. This situation is indicated in Figure 10.d. The amount of strain for a given increase in pressure depends on a number of factors such as the vessel dimensions and the elastic properties of the wall which supports the endothelial surface.

In the present study the greatest population of normal cells occurred in the upstream portion of the system where the pressure-related strains were greatest. Moreover, the greatest damage occurred in the plug channel region where the cells were afforded the greatest protection from the pressure related strains. In conclusion, evenly applied pressure stress bears no direct relationship to the cellular changes observed in this study.

When the pressure is applied unevenly as it is, for example, in turbulence, quite a different situation can arise. If the instantaneous pressure distribution along the wall of the channel in turbulent flow were "photographed," the pressure would be seen to vary acutely from point to point. The endothelial surface would tend to conform to this uneven pressure distribution, appearing somewhat like the surface of a puddle in a downpour. This situation is shown schematically in Figure 10.e. The curved arrows indicate an assumed turbulent velocity distribution. The graph indicates an assumed instantaneous pressure distribution around the time-smoothed value (the horizontal dashed line). The endothelial cells are shown conforming to this pressure distribution (increasing pressure is in the downward direction). Notice that the resulting undulating configuration of the surface is associated with rhomboidal deformation of the cells similar to that shown in Figure 10.c. This deformation may be thought of as being produced by a shearing stress acting across the cell partitions as indicated in the left-hand insertion at the bottom of Figure 10. Thus, uneven pressure distribution can produce shearing stresses on the endothelial cell.

Moreover, notice that the turbulent velocity components associated with the uneven pressure distribution also can produce shearing stresses on the endothelial surface identical to those shown in Figure 10.c, for the time-smoothed shearing stress. The turbulent pressure distribution and associated velocity distribution tend to be correlated such that their effect is additive in producing shearing stress on the endothelial cell. We conclude that the high frequency pressure and velocity components of turbulence could act independently of their time-smoothed components to produce high frequency shearing stresses on the endothelial cells which might have magnitudes sufficient to exceed the critical stress. It follows that if the time constant of the yield process is sufficiently short, the endothelial cells might suffer changes identical to those produced by the time-smoothed shearing stress. The foregoing situation is analogous to the hydrodynamic events in the eflux region.

This discussion could be extended to consider a number of other added factors such as the ballistic effects of the suspended blood cells, a more detailed and perhaps realistic analysis of possible stress systems acting on and in the endothelial cells, and a more sophisticated discussion of turbulence, particularly in the expanding jet located in the eflux region. The resolution of the present measurements probably does not justify this; therefore such discussion is deferred until more detailed studies are done.

CELL RHEOLOGY

In the previous discussion endothelial cells were assumed to be simple elastic bodies to avoid unnecessary detail in discussing the modes by which hydraulic stresses can produce deformation of the endothelial surface. The actual properties of endothelial cells are probably much more complex.

The rheology of endothelial cells and their adjacent structures in the intimal region have not been studied previously. Although the physical properties of tissue cells vary quantitatively among different cell types, it is
reasonable to assume that there are no qualitative differences. If one accepts this belief, then one can apply the knowledge of rheology developed from studies of certain special cell types to the cells of the intimal region. Since certain cell forms are quite large, it was possible to study the physical properties of living cytoplasm by a number of ingenious micro manipulative techniques (4).

A cell is bounded exteriorly by a thin membrane called the unit membrane. This membrane is so thin (it can only be seen with an electron microscope) that it apparently does not contribute significantly to the structural rigidity of the cell. On the other hand, it probably plays a central role in maintaining the chemical integrity of the cell. It is possible that the mechanical forces of the adjacent flow might produce stresses in this delicate structure in excess of its critical stress resulting in tears and exposure of the underlying cytoplasm to the mechanical and chemical forces of the adjacent blood flow. This event might be expected to be followed by profound structural and chemical changes as described.

It is generally felt that immediately under the unit membrane is a relatively thick “rubbery” layer of protoplasm called the cell cortex or ectoplasm which not only buttresses the unit membrane but also provides the structural rigidity of the cell. The cell cortex has the properties of a “gel.” An important physical property of such substances is that they have both a gel phase (essentially an elastic solid) and a sol phase (essentially a viscous fluid), depending on the temperature, other ionic constituents, and the state of mechanical stress (5). For example, if a gel is subjected to a small enough shearing stress, it deforms “visco-elastically” much like rubber, returning after a period of time to its original shape when the shearing stress is removed. On the other hand, if a shearing stress in excess of some critical value \( \tau_c \) is applied, the gel “collapses” into a viscous fluid (usually non-Newtonian) which flows under the influence of the stress, thus becoming permanently deformed. It follows that the amount of deformation depends now not only on the magnitude but also on the duration of the stress. Following removal of the stress, the sol returns to its gel state, again becoming a “visco-elastic” body, however, with a shape different from its initial state.

The gelatinous cortex encases the cell endoplasm which contains a number of semi-solid inclusions such as the nucleus, mitochondria, etc. The entire structure is traversed by a fine canal system called the reticulum. The endoplasm appears to be somewhat inhomogeneous. At different times and at different locations in the cell, the endoplasm may be in a gel phase, although it usually appears to be a “sol.” In the sol phase “protoplasmic streaming” can be detected particularly in certain plant cells and in mobile or migrating animal cells. Protoplasmic streaming, as well as other normal physiologic processes within the cell, appear to depend on the maintenance of a normal ectoplasmic-endoplasmic interface. For example, removal of endoplasm from within the cell cortex causes streaming in the endoplasm to cease (4). Collapse (“solation”) of the cortical gel into a sol by application of chemical or mechanical irritants or by altering the external chemical milieu (such as the calcium ion concentration) is associated with sudden, profound, chemical changes within the cell (5).

The foregoing facts are of possible significance in the interpretation of the histologic data presented. Endothelial cells subjected to the minimal shearing stress of the normal blood flow are expected to show a slight viscoelastic deformation in vivo. However, after death this deformation disappears with the elastic return to equilibrium conditions associated with the cessation of blood flow and its attendant shearing stress. Thus, the geometry of the cells seen in normal histologic slides should represent the normal mechanical resting state of the endothelial cells (plus certain unknown artifacts resulting from tissue shrinkage, etc., associated with the fixation process).

If blood velocity were increased carefully such that the stress slightly exceeded the critical shearing stress \( \tau_c \), the cell sub-
stance would begin to transform to its sol phase either with or without associated damage to the unit membrane. Thus the cell would be expected gradually to "flow." Tissue sections removed under these conditions would be expected to show early signs of permanent cell deformation since in the sol state the elastic properties to return the cell to its original shape disappeared. As discussed above, transition of the cell from a gel to sol phase is associated with profound physico-chemical changes throughout the cell. Therefore, deformed cells should also manifest altered chemical or staining properties. Most notable of these would be cell swelling secondary to the increased osmotic pressure associated with the generation of new chemically "unsatisfied" moieties in the gel to sol transformation.

Finally, if stress in excess of the critical value is maintained for a sufficient period of time, deformation proceeds to some value at which point the cell becomes mechanically unstable and separates from its mooring. In this manner endothelial cellular substance gradually is washed away, exposing the underlying fibers embedded in the "mucinous" ground substance of the intima to similar eroding processes. The gelatinous or mucinous ground substance should be eroded first, leaving the naked fibrillar structures exposed directly to the mechanical and chemical forces of the blood stream.

Thus, there are many parallelisms in the rheologic behavior of real cells and the simple elastic-body type of cells discussed earlier; however, there are also significant differences in that real cells have apparent physico-chemical changes associated with their deformations.

**CHEMICAL CONSIDERATIONS**

Up to this point the implication was that the purely mechanical effect of the shearing stress itself was the sole agent in producing the observed histologic (and chemical) changes. Another possibility is that the endothelial cell becomes "weakened" by some event associated with the increased shearing stress (either the time-smoothed or high frequency components), thus potentiating the damaging effect of stress.

As discussed above, the rigidity of cells (at least certain cells) depends very much on the concentration of certain ions in the vicinity of their surface. If this ionic milieu were changed by hydrodynamic events, the structural properties of the cell might also be altered. It is possible that the normal metabolism and "structural strength" of endothelial cells depend on a local chemical milieu different from that of the bulk phase of the blood.

Simple physical chemical considerations lead to the conclusion that an electro-chemical gradient must exist in the neighborhood of the cell surface. First of all, the cell membrane has an ordered protein structure which should contain some sort of fixed charge system associated with the generation of new chemically "unsatisfied" moieties in the gel to sol transformation.

Secondly, blood contains a mixture of complicated molecules which appear to have differing degrees of affinity for water. For example, if a neutral salt is added to plasma, it competes for the available water. Molecules with a lesser affinity for water precipitate, i.e., they are forced out of the liquid phase. For example, in the case of the plasma proteins, fibrinogen appears to have the least affinity for water since it precipitates first during such a "salting out" process.2

Even in the normal situation a molecule such as fibrinogen is competing for water. Since its affinity for water is lower than that of the other moieties, it tends to be forced into regions where there are fewer free water molecules by the more "aggressive" hydrophilic substances, e.g., albumin. The fewest free water molecules occur at a free surface and probably also at an interface where water molecules are more tightly bonded to themselves forming a rather "ice"-like structure. Consequently one might suspect that an in-

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2There is increasing evidence that normally about 1% of the fibrinogen may be in an altered form called "profibrin," which has an even lower affinity for water (6, 7).
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creased concentration of substances such as fibrinogen might occur in an interfacial region. Thus, there are at least two reasons to expect small chemical concentration gradients to exist normally at the interface between the blood and the endothelial cell surface. It is possible that the maintenance of these gradients within certain limits is vital to the cell's survival.

The increased mechanical convection associated with (1) large blood velocity gradients, (2) "vortex shedding," (3) vortex systems that are convected along the wall in the lee of small protuberances, and (4) the large transverse mass transfer associated with fully developed turbulence can greatly increase the apparent "diffusivity" of molecular and ionic species contained in the blood stream. This increased apparent diffusivity (increased effective chemical potential) may be sufficient to erode the natural electro-chemical gradients at the cell surface, permitting an unbalance of the normal local chemical milieu with resultant changes in cell properties.

The Evans blue staining patterns described earlier were considered to be secondary to endothelial cell damage. The above notions are consistent with still a different interpretation in which the staining reflects a primary mechanism in producing cell damage. This interpretation is based on the assumption that Evans blue dye binds only with the more hydrophilic moieties in the blood, e.g., albumin. Normally the endothelial surface would be shielded from these dye-carrying moieties by the adjacent electrochemical gradients. However, in the face of increased velocity gradients, the associated increased convection would tend to erode and penetrate this protective "hydrophobic" chemical blanket, allowing the dye-carrying constituents to reach and penetrate the endothelial surface, thus, unfavorably altering the local chemical milieu.

In summary, factors have been discussed which are involved in the yield of a structure to an applied force with particular reference to the results of the present study. The time-smoothed shearing stress obviously can produce a simple shearing strain in the endothelial cells. It was pointed out that the high frequency pressure and velocity components of turbulence can act also to produce shearing stresses on the endothelial cells independently of the time-smoothed components. Shearing stress would appear to be the dominant mode by which forces are applied to the endothelial cells. It is plausible to assume that the cells react mechanically to the applied stress deforming like visco-elastic bodies (gelatinous bodies in their gel phase), provided the stress does not exceed some critical value, \( \tau_c \). Above this value the cells lose their elastic properties and are transformed into viscous bodies which continually deform under the influence of the applied stress finally "washing away." This transformation is associated with physico-chemical changes which may be related to the gel-sol transformation or to an altered local chemical milieu brought about by the increased chemical convection of the adjacent blood flow. Experiments with other cell types suggest that if the local chemical milieu is altered, the structural rigidity of the cell also is decreased.

Conclusion

The foregoing was purely speculative and, apart from offering plausible explanations for the observed events in the present study, serves mostly to suggest avenues of inquiry for future, more detailed studies. The present study does, however, establish two points of departure: First, endothelial cell degeneration can be produced acutely either by turbulence or by increased shearing stress on the cell surface from the adjacent blood flow. Second, as long as endothelial cells are exposed to a time-smoothed shearing stress below some critical value \( (379 \pm 85 \text{ (SD) dynes/cm}^2) \), most of the cells appear to remain normal (at least in the acute situation and in the absence of extremely high turbulent energy). A time-smoothed shearing stress in excess of this value causes rapid deterioration of the endothelial surface.

Whether shearing stresses of this magnitude can occur in the normally functioning vascular bed is unknown. It is possible that at certain local sites such as at branches and bends
or in vessels supplying regions in which the demand for flow can become very great, such as the renal and coronary arteries, unstable flow and high wall shearing stresses can occur. Methods to explore these problems are currently under investigation in this laboratory.

Appendix

DERIVATION OF THE WALL STRESS FORMULA

It is the purpose of this presentation first to derive a mathematical expression to relate the shearing stress on the endothelial surface in the plug channel to other measurable variables and, second, to summarize experimental data supporting this derivation.

For this derivation, as well as for subsequent data analysis, it is necessary to establish a frame of reference. It is useful to imagine that a cylindrical coordinate system, such as shown in Figure A1, exists in the uniform, circular part of the flow channel so that the longitudinal z axis coincides with the centerline of the channel. The origin of the system is at the channel entrance, as shown in the lower part of Figure A1. The radial r coordinate is perpendicular to the z axis and will traverse any radius of the channel. The θ coordinates are circles concentric with the center of the channel in planes perpendicular to the z axis.

In addition to a coordinates system, certain assumptions are required in the derivation. Let it be assumed:

1. Blood is an incompressible fluid.
2. The conduit is circular and uniform.
3. The pressure varies only along z, but not along r or θ.
4. The velocity distribution is symmetrical about the z axis. This implies that derivatives with respect to θ, derivatives of the θ velocity component, and the θ component of velocity may be ignored.
5. The radial velocity component (w) is always small compared to the longitudinal velocity component (u).

Although assumption 1 is well established, assumptions 2-5 should be verified experimentally.

With the foregoing assumptions, the equations of motion in the z and r directions may be written in cylindrical coordinates as:

\[
\frac{\partial p}{\partial z} + \rho \left[ \frac{\partial \omega}{\partial t} + \omega \frac{\partial \omega}{\partial z} + u \frac{\partial \omega}{\partial r} \right] = 0 \quad A1(a)
\]

\[
\frac{\partial p}{\partial r} = 0, \quad A1(b)
\]

where t is time, \(p\) is fluid density, \(p\) is the time-smoothed pressure, and \(\omega\) and \(u\) are the time-smoothed velocities in the z and r directions.

The first bracketed quantity on the left of equation A1(a) represents the total acceleration of the fluid at a point. The \(\tau_r\) appearing in the second bracketed quantity of equation A1(a) are stresses acting on the surfaces of the infinitesimal fluid element. These surfaces correspond to infinitesimally separated coordinate planes surrounding the point of interest. Therefore, lines normal to opposite sets of surfaces correspond to the three coordinates. The subscript "r" refers to the particular normal coordinate (set of surfaces), and the subscript "θ" refers to the coordinate direction in which the stress is acting. Thus, \(\tau_r\) is a stress on the surface normal to the r coordinate and acting in the θ direction.

The \(\tau_u\) may be resolved into the sum of two components:

\[
\tau_u = \tau_u(L) + \tau_u(T), \quad A2
\]

Diagram showing relationship of cylindrical coordinate system to plug channel.
where \( \tau_0(L) \) are the surface stresses on the fluid element related to the time-smoothed velocities, and the \( \tau_0(T) \) are the "Reynolds stresses" (8). The \( \tau_0(L) \) are literally time-smoothed stresses, whereas, the \( \tau_0(T) \) are "virtual" stresses representing the flux of momentum between adjacent fluid elements. These virtual stresses arise from the inertial terms of the original equations of motion as a result of the averaging procedure outlined earlier. The important point for present purposes is that when considering the time-smoothed properties of the flow, the Reynolds stresses have a significance identical to the true shearing stresses in that their presence exerts the same tractile force across surfaces as do "true" shearing stresses. It follows that the shearing stress on the endothelial surface (\( \tau_{re} \) at \( r = R \)) is determined by the effects of both the true and the virtual stresses.

Since we shall be estimating \( \tau_{re} \), its components need not be considered further. However, it is necessary to consider the components of \( \tau_{re} \) more explicitly in terms of measurable variables. These are given by

\[
\tau_{re} = \tau_{re}(L) + \tau_{re}(T), \tag{A3(a)}
\]

where

\[
\tau_{re}(L) = 2\mu \frac{\partial \omega}{\partial z}, \tag{A3(b)}
\]

\[
-\int_0^R \frac{1}{r} \frac{\partial}{\partial r} (ru) + \frac{\partial \omega}{\partial z} = 0 \tag{A4}
\]

and an identity relating the total flow \( Q \) to the velocity distribution which is given by

\[
Q = 2\pi \int_0^R w r dr, \tag{A5}
\]

where \( R \) is the tube radius.

Finally, equation A1(a) can be rewritten in an equivalent form which will simplify subsequent integration:

\[
\left[ \frac{1}{r} \frac{\partial}{\partial r} (\tau_{re}) + \frac{\partial}{\partial z} (\tau_{re}) \right] = 0. \tag{A6}
\]

The reader can verify that equation A6 is equivalent to A1(a) by carrying out the indicated differentiation operations in the first bracketed quantity and noticing that the new terms generated in excess of those in equation A1(a) are identically equal to equation A4 which is zero.

We are prepared now to turn more directly to our central task, i.e., calculation of the shearing stress on the endothelial surface. Referring back to the coordinate system described earlier and referring to the significance of the subscripts in \( \tau_{re} \), it can be seen that the pertinent shearing stress is the \( \tau_{re} \) in equation A6 at \( r = R \), the location of the endothelial surface. Equation A6 can be solved for \( \tau_{re} \) by integration with respect to \( r \) as follows:

Rearranging and multiplying through by \( r dr \) gives

\[
-\int_0^R \frac{\partial \tau_{re}}{\partial z} + (r dr) \frac{\partial \tau_{re}}{\partial z} + \rho (r dr) \frac{\partial \omega}{\partial t} + \rho (r dr) \frac{\partial \omega}{\partial t} = 0 \tag{A7}
\]

where the fourth term arises from the simple identity

\[
\frac{\partial}{\partial z} \left( \frac{\omega^2}{2} \right) = \frac{\partial \omega}{\partial z} \tag{A8}
\]

Since \( r, z, \) and \( t \) are the independent variables, \( r dr \) behaves like a constant in any differentiation with respect to \( z \) or \( t \). Therefore, it is permissible to multiply \( r dr \) into the dependent variables under the differentiation signs.
in the third, fourth, and sixth terms such that we have

\[-d(\tau_{rr}) + \frac{\partial p}{\partial z} (r \, dr) + \rho \frac{\partial}{\partial t} (w \, rdr) + \frac{\partial}{\partial z} (pw^2 rdr) + \rho d(rwu)\]

\[-\frac{\partial}{\partial z} (\tau_{zr} r dr) = 0.\]

Similarly, we are permitted to integrate with respect to \(r\) under the differentiation signs.

\[\tau_w = \frac{R}{2} \frac{\partial p}{\partial z} + \frac{\rho}{2\pi R} \frac{dQ}{dt} + \frac{1}{R} \frac{\partial}{\partial z} \int_0^R \rho w^2 r dr - \frac{1}{R} \frac{\partial}{\partial z} \int_0^R \tau_{zr} r dr.\]

Thus

\[-\int_0^r d(\tau_{rr}) + \frac{\partial p}{\partial z} \int_0^r r dr + \frac{\rho}{2\pi} \frac{\partial}{\partial t} \int_0^r 2\pi w r dr + \frac{\partial}{\partial z} \int_0^r \rho w^2 r dr + \int_0^r \rho d(rwu)\]

\[\tau_{zr} r dr + g(z,t) = 0,\]

where \(g(z,t)\) is an arbitrary function of integration.

Notice in the second term that \(\partial p/\partial z\) can be left outside the integration sign in view of assumption 3. Thus, carrying out the indicated integration from 0 to \(r\), we obtain

\[-\tau_{rr} + \frac{\partial p r^2}{\partial z} + \frac{\rho}{2\pi} \frac{\partial}{\partial t} \int_0^r 2\pi w r dr + \frac{\partial}{\partial z} \int_0^r \rho w^2 r dr + \rho w u - \frac{\partial}{\partial z} \int_0^r \tau_{zr} r dr + g(z,t) = 0.\]

Since this equation must be valid at every point across the stream, it must hold also at \(r = 0\). At \(r = 0\) the first, second, and fifth terms are obviously zero, and, since the remaining integral terms are all with respect to \(r\), they too must be zero. We conclude that the arbitrary function of integration, therefore, must also be zero.

Returning to equation A10(a) and now integrating all the way from \(r = 0\) to the boundary at \(r = R\), we obtain

\[-R \tau_{w} + \frac{R^2}{2} \frac{\partial p}{\partial z} + \frac{\rho}{2\pi} \frac{dQ}{dt} + \frac{\partial}{\partial z} \int_0^R \rho w^2 r dr + 0 - \frac{\partial}{\partial z} \int_0^R \tau_{zr} r dr = 0,\]

where \(\tau_w\) is the value of \(\tau_{rr}\) on the wall, \(Q\) is the total flow (compare the third integral in equation A10(a) with equation A5), and the fifth integral is zero at \(r = R\) because \(u = 0\) on the boundary.

Rearranging and dividing by \(R\) give the final integrated form of the equation

Equation A12 relates the shearing stress \(\tau_w\) to the pressure gradient (first term on right), the time rate of change of flow (second term), and the mean sectional gradients of kinetic energy (third term) and \(\tau_{zr}\) (fourth term) along the tube. With the exception of \(\tau_{zr}\), all quantities are directly measurable by the techniques used in the present study.

Two tasks remain. The first is to examine the assumptions listed earlier that were required in the derivation of equation A12. The second is to estimate the relative order of magnitude of the four terms in equation A12 to determine if any terms can be deleted, particularly the last term which cannot be measured directly. These objectives can be achieved by making direct measurements of the velocity fields in a physical model of the in vivo system using fresh blood and studying flow ranges that bracketed those encountered in the animal studies.
Methods

The test stand described earlier in connection with Figure 2 was used to study the detailed hydrodynamic properties of the plug-conduit system. A plug having entrance portions and channel dimensions identical to those used in vivo was placed in a lucite tube having the average dimensions of the aortas that were studied (1.2 cm in diameter).

Blood from each of eight experimental animals was made to flow through the system in eight separate studies using a variable speed pump to bracket the flow levels achieved in vivo. Flow was monitored with an orifice meter at the efflux of the artificial aorta (lucite tube).

The pressure gradient distribution along the system was measured using the access ports shown in Figure 2 as described in the body of the paper. The corresponding velocity distributions across the tube (along r) were measured at each access port (along z) using a specially designed Pitot tube made from fine steel tubing (0.030 cm o.d.). Using standard data smoothing techniques, these data were fitted to simple polynomial functions so that the blood velocity field in the z direction \([u(z, r)]\) for each flow level studied could be expressed as a continuous function of both \(r\) and \(z\).

Since by integration of the continuity equation the velocity \(u(r, z)\) in the radial direction can be related to \(w(r, z)\) by:

\[
\int_0^r w(r, z) \, dr = -\frac{1}{\tau} \frac{\partial p}{\partial z},
\]

it was possible to express \(u\) also as a continuous function of \(r\) and \(z\) using the foregoing polynomial representations of the data.

Results

1. The velocity fields were examined for symmetry. The mean absolute deviation from symmetry along the channel for all flow levels was less than 1% of the mean velocity (range 0.5 to 3.0%). These data strongly support assumption 4 (symmetrical velocity distribution) and by inference assumption 2 (conduit is circular). Therefore, geometric asymmetries that had to be introduced in the in vivo system do not appear to have seriously affected the symmetry of the velocity profiles.

2. The magnitude of the radial velocity field \([u(r, z)]\) was examined. The values of \(u\) ranged between 0 and 0.3% of the corresponding average velocity \([w]\) in the z direction. These results indicate that the radial component of velocity was very small for all flows and at all locations in the conduit. (It follows that the spatial derivatives were also small.) This supports assumption 5. Moreover, since the pressure gradient in the radial direction (described by the radial equation of fluid motion) depends on the radial velocity and its derivatives, one can conclude that the radial pressure gradient also is small. This supports assumption 3.

Thus the first major conclusion that can be drawn from this study is that none of the five necessary assumptions listed earlier are seriously violated by the in vivo experimental situation. Therefore, equation A12 may be considered to represent a valid relationship between the wall shearing stress and the other variables indicated on the right.

The second objective of this experimental portion of the Appendix was to determine the relative magnitudes of the terms in equation A12. With the exception of the last term (containing \(\tau_{oz}\)), these terms can be evaluated directly from the pressure-flow-velocity data already described.

Turning first to the term containing \(\tau_{oz}\), it is seen from equation A3(a) that \(\tau_{oz}\) can be considered to consist of the sum of two terms, a laminar stress term \(\tau_{oz}(L)\) and a Reynolds stress term \(\tau_{oz}(\tau)\). The laminar stress term is given by equation A3(b). Since blood has a viscosity (\(\mu\)) of about four times that of water, it is possible to evaluate the magnitude of \(\tau_{oz}(L)\) from equation A3(b) by carrying out the indicated operation of differentiation on the aforementioned polynomial representations of the velocity field. This was done for the entire field in the conduit for all flow levels. These values for \(\tau_{oz}(L)\) were compared to the corresponding measured values of first term, containing \(\partial p/\partial z\) using the techniques outlined earlier.

The grand mean absolute value of the laminar part of the last term in equation A12 divided by the first term was of the order \(10^{-4}\). The range was 0 to \(10^{-4}\). We conclude that the laminar component of \(\tau_{oz}\) can be neglected.

The magnitude of the second component of
the last term in equation A12 cannot be estimated quite so easily nor with any great confidence since it was not possible to measure the value of $\frac{\partial \bar{w}}{\partial z}$ directly. Therefore, it is necessary to resort to approximations taken from the literature.

Measurements of $w'$ done in other fields indicate that its value is characteristically from 5 to 15% of the centerline velocity. A plot of one such measurement is given by Schlichting in Figure 18.3 on page 466 (8) and another by Hinze in Figure 7-33 on page 522 (9). If the data in these plots are scaled to the present flow system, then it should be possible to calculate a crude order of magnitude for the Reynolds stress part of the last term in equation A12 for each of these sets of data as follows.

It will be recalled from Figure 4,d, that in the animals having a Reynolds number greater than critical the turbulent intensity in the plug channel becomes constant with $z$ about 1 cm from the entrance. It is reasonable to assume that turbulence has become fully developed along this uniform part of the system. The mean channel velocity for this group of animals was 235 cm/sec. Scaling this to the data in the above mentioned references, plots of $\frac{\partial \bar{w}}{\partial z}$ were constructed from which the integration indicated in the last term of equation A12 was done graphically. Assuming that $\tau_{\infty}(\tau)$ increased linearly between $z = 0$ and $z = 1$, the estimated value of the last term from either of these references was less than 30 dynes/cm$^2$.

Since the turbulent intensity remained constant from $z = 1$ to about 2.7 cm, it can be assumed that the value of this term was zero for most of the rest of the channel, i.e., for $1 < z < 2.7$. Therefore the turbulent component of the last term is most important in the upstream portion of the channel where, however, it only amounts to about 3% of the first term in equation A12. We conclude that since both the laminar and turbulent components of the last term are very small, both parts of the last term in equation A12 may be neglected.

Evaluation of the two inertial terms (terms 2 and 3) in equation A12 was much more straightforward. Term 3, the kinetic energy gradient, was programmed for automatic machine computation using the aforementioned fitted polynomial field equations for $w(\tau, z)$. Steady flow was assumed, so that the second term on the right of the equation could be ignored for the moment. The value of the third term on the right never exceeded 10% of the first term for any of the three flow levels studied, and this occurred only in the uppermost portion of the channel, becoming negligible from the mid-portion to efflux. It should be noted, however, that if it were possible to have estimated the velocity field at more closely spaced points, particularly near $z = 0$, a somewhat greater variation of $w$ with $z$ might have been found. (See, for example, Figure 13, page 27 of reference 10.) Consequently, the third term may have been somewhat larger than estimated in the present study. Therefore in a more critical analysis than is presently necessary this term probably should be retained, particularly for the study of events near $z = 0$. In the present study, interest centered about regions downstream from this point, i.e., $\tau_e$ always occurred in the region $0.5 < z < 2.5$ cm. Thus in the interest of economy, this term could be neglected in the present study.

The value of the second term on the right of equation A12 is purely a function of time and does not vary along $z$. Since flow through the in vivo channel was a periodic function of time, the value of the second term can be represented by a truncated Fourier series calculated from the corresponding Fourier series representation of the flow contours. Fourier analysis was done on flow contours from each of the experimental animals. The moduli were found to decrease rapidly with increasing harmonic number, usually becoming negligible after the eighth harmonic. Moduli for like harmonics were then averaged to give a representative value for the modulus of each harmonic for the experimental group as a whole. Substituting the appropriate values into the second term of equation A12 and carrying out the indicated differentiation operation, numerical values for the moduli of the
corresponding shearing stress series were obtained. The values for harmonics 1-8 were 8, 8, 6, 6, 5, 5, 3, and 2 dynes/cm², respectively. These values are less than 5% of the lowest values obtained for the first or pressure gradient term in equation A12. It is concluded that the time-varying stress term also can be ignored without introducing serious error.

Summary of Appendix

An equation was derived which relates the shearing stress on the wall of the plug channel to other measureable variables. The longitudinal and radial velocity fields were measured in a physical model of the in vivo experimental situation using eight different blood specimens over flow ranges that encompass those encountered in the in vivo studies. Although the in vivo conduit geometry was unavoidably asymmetrical, the velocity profiles remain essentially symmetrical (range: 0.5 to 3% deviation from symmetry). The radial component of velocity remained very small (<0.3% of the mean axial velocity component) for all flows and at all locations. This indicates also that the pressure distribution across the conduit was uniform. Thus the pressure gradient can be considered to be constant with respect to the radial coordinate. From the foregoing it appears that the necessary conditions (the five assumptions listed earlier) for the validity of equation A12 are adequately met by the in vivo experimental situation.

Furthermore, numerical evaluation of the various terms in equation A12 indicates that the fourth term representing the average longitudinal normal stress gradient can always be ignored, and, moreover, in the restricted circumstances of the present studies it is also permissible to ignore the two inertial components (terms 2 and 3) of equation A12, thus greatly simplifying the use of this equation for computational purposes. If accuracy greater than 10% is needed, however, these latter two terms must be retained.

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