Certain Histological and Chemical Responses of the Vascular Interface to Acutely Induced Mechanical Stress in the Aorta of the Dog

By Donald L. Fry, M.D.

ABSTRACT
The purpose of this study is to quantify certain histologic and chemical responses of the intimal tissues in vivo to acutely induced mechanical stresses. Evans blue dye was given to tag serum albumin and an artificial fat emulsion was infused so that altered fluxes of either serum proteins or the artificial chylomicrons across the vascular interface into the intimal region could be detected. Special histologic and photodensimetric techniques were developed to estimate these fluxes as well as the architectural changes in the endothelial cell population. Architectural changes were quantified by doing endothelial cell counts to quantify the “normal” and “abnormal” endothelial cell population density as a function of stress exposure. The stress corresponding to the greatest rate of change of normal to abnormal cell forms is defined as the acute critical yield stress ($\tau_c$) and was found to average < 420 dynes/cm$^2$. Similarly the stress at which the greatest number of cells are being eroded is defined as the erosion stress ($\tau_e$). The flux of Evans blue dye into the intima increased with pressure or wall strain, with shearing stress, and with increased turbulence. The flux of artificial chylomicrons into the intimal region never occurred in the presence of a normal endothelial cell population and was found to be most heavy in areas of total cellular erosion.

ADDITIONAL KEY WORDS endothelial cell yield stress erosion stress erosion injury damage rheology vascular interface permeability shearing stress chemistry Evans blue dye fat deposition vascular fluid mechanics turbulence pressure thoracic aorta atherosclerosis intravenous fat infusion

It is the purpose of this report to examine the interrelationships among histologic changes, fibrin deposition, and the altered fluxes of artificial chylomicrons and Evans-blue tagged albumin across the intimal surface that are associated with certain acutely induced hemodynamic events.

Methods
EXPERIMENTAL DESIGN
Dogs weighing between 25 and 38 kg were studied acutely under chloralose-urethane (48 to 480 mg/kg) anesthesia. They were subjected to thoracotomy, exposing the thoracic aorta from the heart to the diaphragm. A uniform portion of the descending thoracic aorta was selected as a “test site.” Adventitial suture markers were placed about 12 cm apart at the upstream and the downstream ends of the selected region which could be used as reference markers during subsequent preparation of the tissue for photographic and histologic measurements.

The dogs were then studied in three separate groups. Group 1 (control), which consisted of ten dogs, was studied histologically to determine the distributions of the aforementioned fluxes that could be expected under normal conditions. Following preparation of the test site, Evans blue dye (2 mg/kg bw) was given intravenously followed by an infusion of fat emulsion (Intralipid, 1 g fat/kg bw). In two of these dogs blood samples also were drawn before administering fat emulsion and at selected periods over the ensuing 3-hour period to determine the nature of the blood lipid patterns produced by the fat infusion.

Pressures were recorded at the upstream and downstream ends of the test site throughout...
the experiment using 30-cm PE 20 polyethylene catheters tied into intercostal arteries and attached to P23Db Statham pressure transducers. Flow was monitored at the downstream end of the test site using a 400-cps gated sine wave electromagnetic flowmeter.

At the end of the experimental period, the animals were killed by bleeding. The descending thoracic aorta was carefully removed, dissected free of excess perivascular tissues, opened longitudinally along its dorsal aspect, and mounted on an adjustable tissue-holding rack, upon which it was stretched to its in-vivo dimensions. The stretched endothelial surface of these specimens was immediately photographed on Ektachrome type-B film (developed by E2 process) under uniform illumination (2800°K) for subsequent photodensimetic analysis. Following this, the specimen was placed in a gelatin-formalin fixative for histologic preparation at its in-vivo dimensions as described previously (1).

Group 2, which consisted of ten dogs, was studied to examine the effects of pressure alone on the distribution of the aforementioned fluxes. These dogs were subjected to the same surgical procedure and received the same dosage of Evans blue dye and fat emulsion as the control group. In addition, however, the middle-thoracic aorta was cross-clamped to create a large pressure difference across the clamped region and at the same time to stop blood flow, interfacial shearing stress, and turbulence. After a 2- to 3-hour period, the aortas of these dogs were treated photographically and histologically in the same manner as the control dogs.

Group 3, which consisted of 15 dogs, was studied to determine the effect of increased interfacial shearing stress and turbulence on the distribution of the aforementioned chemical changes. This group was subjected to the same surgical procedures and received the same dosages of Evans blue dye as the previous two groups of animals. However, in five of these no fat emulsion was given. The remaining ten received the same dosage of fat emulsion as the previous two groups. In addition, a specially designed, nontraumatic plug containing a longitudinal groove along its side was installed intravascularly at the test site in the descending thoracic aorta following thoracotomy. The details of this device and its method of installation have been reported elsewhere (1). The purpose of this plug was to accelerate the blood flow around the plug through the small uniform channel along its side so that the overlying aortic endothelial surface was exposed to a measurable broad range of shearing stress by the adjacent blood flow.

The distribution of time-smoothed shearing stress in this hydrodynamic configuration is such that shearing stress on the overlying endothelial surface is most intense at the upstream end of the channel and decays in an approximately exponential manner with distance downstream to relatively low levels at the efflux. This stress distribution was measured immediately after the dog was killed by allowing the blood to flow from the dog through a physical replica of the experimental situation (1).

Flow was measured continuously in vivo using the same electromagnetic flowmeter system as used in the former two groups. Pressure was measured continuously from a point 4 cm upstream and a point 4 cm downstream from the test site using Statham P23Db pressure transducers. The reader is referred to the previous publication for a more detailed description of this experimental approach (1).

**OPTICAL MEASUREMENTS**

The normal endothelial surface does not stain readily with Evans blue. Since this dye forms a relatively stable chemical complex with serum albumin (2), an increased blue staining of the endothelial surface is usually assumed to represent an increased flux of serum albumin across this surface. Methods do not exist for measuring the Evans blue concentration in the intimal tissue directly. Until they do, methods of estimating intimal staining will remain indirect and unfortunately inaccurate. For lack of a better approach, an indirect photodensimetric technique was used to estimate the Evans blue dye concentration in the intima using a photographic color transparency instead of performing the measurement in the specimen itself. To do densitometry on the fresh tissue would have required prolonged exposure of the tissue to intense light and possible surface drying. Moreover, it was necessary to get the tissue specimens into the fixative as soon after death as possible to avoid post-mortem histologic changes. Before describing the details of this method, it will be useful first to anticipate the results to be presented later and review the characteristic surface staining patterns that occur in the above animal preparations.

The endothelial surfaces of the control dogs usually developed a uniform, generalized, faint-blue tinge and on occasion showed localized darker staining regions particularly around branch points. Group 2 usually showed a diffusely increased blue staining in the higher pressure region. The staining pattern of group 3 is somewhat more complicated. A black and white photograph of a freshly mounted tissue specimen taken from group 3 is shown in the upper portion of Figure 1 and demonstrates...
The vascular interface at the test site. Upper portion of figure is photograph of tissue specimen on stretching rack taken through a red filter so that blue-stained areas appear as dark gray. Drawing of photograph appears in lower portion of figure showing the reference z axis extending along the center of the specimen from left-to-right in the direction of flow. Uniform portion of plug channel corresponds to segment between 0 and 3 cm as indicated.

The “butterfly”-shaped pattern in the middle of the picture represents the resting site of the intravascular plug. The region to the left is the upstream portion of the specimen and can be seen to stain bright blue except for the horizontal longitudinal white stripe along the axis of the specimen. This unstained strip represents the normal unmolested endothelial surface that originally lay in the trajectory of the rectangular slit over the opening of the plug channel during its descent from the upper aorta to the test site. The blue-stained regions on either side of this trajectory can be shown microscopically to have been abraded by the plug surface during its descent.

Toward the right of the white trajectory in the entrance portion of the plug channel, the endothelial surface develops an increased staining affinity which is associated with the increased shearing stress of the blood flow as it accelerates into the uniform portion of the channel. The staining is most intense in the entrance region (at \( z = 0 \), see lower diagram in Fig. 1) and then decreases downstream concomitant with the exponentially decreasing shearing stress along the channel. Just downstream from the channel efflux region and endothelial surface again manifests an increased staining affinity associated with the turbulent discharge from the channel.

It can be concluded from these group 3 studies that the endothelial surface develops an increased staining affinity for Evans blue dye 1) if it is mechanically abraded, 2) if it is exposed to increased shearing stresses from the
adjacent blood flow, and 3) if it is exposed to chaotic forces of turbulence. It is the increased staining affinity associated with the latter two events that is most interesting from a physiologic viewpoint. Since these mechanical events can be quantified along the axis of the plug flow channel (1), the optical density distribution of interest will be that occurring along the corresponding axis of the transparency.

Referring to the drawing in the lower portion of Figure 1, the optical density of the transparency at a wavelength of 630 nm was measured as a function of distance along an imaginary dashed line drawn from left to right through the flow channel as indicated. This line corresponds to the locus of the histologic sections to be studied as well as the surface over which the in-vivo distribution of pressure and shearing stress were calculated using the techniques described earlier (1).

This line will be referred to as the "z axis" of the system, and all data will be referred to this coordinate. The origin of this z coordinate is placed at the beginning of the uniform portion of the plug channel as also indicated in the figure (z = 0). Therefore, data upstream from the plug channel will correspond to negative values of z and events downstream to positive z.

The transparency was mounted on a flat 4 x 5 x 1/2-inch plate of lucite, one surface of which was scoured to a ground-glass texture with an abrasive to diffuse light for viewing purposes. The entire assembly was mounted clear-side down (transparency and viewing surface up) on the stage of a photobinocular microscope. A photovolt model 520M photometer was placed in one of the eyepieces of the microscope to measure the light coming through the 10X objective of the microscope. A Zeiss graduated interference light filter was placed in the substage light path and adjusted to 630 nm (red). This wavelength was chosen to minimize the variability in optical density due to the red dyes in the photographic emulsion representing hemoglobin in the tissue and yet retain good optical density for those representing the Evans blue-stained regions.

The instantaneous coordinates of the stage were sensed electrically and recorded continuously on magnetic tape. Under visual guidance, it was possible to maneuver the stage such that the z coordinate on the transparency was made to pass under the objective lens over its entire

![Graph](image-url)
MECHANICAL STRESS AND INTIMAL CHANGE

97

length. The electrical signal from the photometer was plotted against that from the stage position sensor on a Sanborn Model 670A x-y plotter to obtain a light transmission (at 630 nm) vs. z curve. All measurements were repeated at least once to establish the reproducibility of the curves, examples of which are shown as the superimposed light traces in Figure 2. A moderate amount of high-frequency "noise" was always present, and therefore, final curves were constructed for analysis by visually fitting a smoothed curve to these data as shown by the heavier line in Figure 2.

The reproducibility of the photographic technique was examined by placing monochromatic test strips on the tissue racks and photographing them under lighting and exposure conditions identical to those used for the tissue. The variability of the resulting light transmission scans was always within ± 5% of the mean light transmission over the total length of z. It can be concluded that the photographic and light measuring techniques are acceptably reproducible and "noise-free" for present purposes. The interpretation and further analysis of these smoothed light transmission vs. z curves will be discussed in the Results section.

HISTOLOGIC MEASUREMENTS

The histologic picture associated with the above staining pattern was studied from formalin-fixed, gelatin-embedded, 20-μ thick, hematoxylin- and oil red "O"-stained, longitudinal tissue sections that originally lay along the z axis of the tissue specimen as described previously (1). The histologic slides were placed on a specially designed servomotor-driven, calibrated, electrically recording microscope stage. The motion of the stage could be controlled by small motions of a spring-restrained toggle switch operated with the palm, leaving fingers free to focus the microscope continuously up and down to scan the entire depth of the tissue section. The two electrical signals representing the coordinates of the point in the center of the visual field of the microscope were recorded continuously on two channels of an Ampex FR 600 FM magnetic tape system.

The right hand was free to operate a keyboard of microswitches which were used to send various electrical signals to the remaining channels on the tape system to represent counts of certain cell species or the presence of deposited fat or fibrin. For example, as the slide is maneuvered through the visual field, each cell is identified and its presence signaled on the appropriate channel as an electrical pulse from the keyboard. Similarly the presence of intimal fat or of surface-adhered fibrin passing under the center point of the ocular reticule was signaled by coincident depression of the appropriate key and the appearance of a steady voltage on the associated tape channel corresponding to the "duration" of the lesion.

Using cytologic criteria described previously (1), the following cell species could be uniquely identified and were counted: normal endothelial cells, abnormal endothelial cells, and adhered white blood cells. A computer program was written to convert these counts to their corresponding population densities (cells/mm² of interfacial surface). The deposited fat and fibrin almost always involved the full depth (20μ) of the section when it occurred. Therefore, the "density" of fat or fibrin involvement was expressed as the average percent interfacial involvement by calculating a running average with respect to z of the digitized voltage on the particular channel of interest. Computer programs were written to accomplish this also.

Using the foregoing procedure, it was possible to quantify the histologic changes as cell population density distributions and as percent area fat or fibrin involvement distributions. These distributions can be compared directly either to the optical density distribution of the Evans blue staining patterns or can be related to the corresponding distribution of pressure and shearing stress along z.

PHYSIOLOGIC DATA

As discussed above, the physiologic data recorded continuously during the in-vivo period of these studies were the flow and the pressures at the two extremes of the test site. As would be expected, typically normal pressure and flow patterns were observed in the control group of animals. Moreover, the mean pressure difference between the extremes of the test site was insignificant. One concludes that the endothelial surface was exposed to essentially identical mechanical events along its entire length, i.e., the mean pressure was at a physiologic level (165 ± 12 [SD] g/cm²) and did not vary along the test site; the mean shearing stress was uniform, remaining in the normal range (< 100 dynes/cm²) (3), and there was no acoustical evidence of turbulence (1). Therefore, any cytologic or chemical changes found in this group may be attributed to variability in the preparation of the histologic material or in the properties of the tissue itself.
**DISTRIBUTION OF FLUID FORCES ALONG SYSTEM (Z)**

- **a**
  - Upstream
  - Entrance portion of plug
  - Uniform portion of plug channel
  - Efflux portion of plug
  - Downstream

- **b**
  - Injury concentration of Evans Blue along Z

---

**Equations and Units**

- $p = 5 \times 10^{-2} \text{ dyne/cm}^2$
- $\tau = 5 \times 10^{-1} \text{ dynes/cm}^2$

---

Circulation Research, Vol. XXIV, January 1969
MECHANICAL STRESS AND INTIMAL CHANGE

In group 2, the obvious major physiologic observation was the large mean pressure difference (131 ± 15.5 [sd] g/cm²) between the two aortic chambers created by the cross clamp. The mean shearing stress as well as turbulence may be assumed to be negligible in these animals. Therefore, any cytologic or chemical changes observed above and below the clamp in this group may be attributed to the effects of the associated pressure difference alone.

In summary, the physiologic observations in groups 1 and 2 are entirely consistent with intuition and need not be examined at this point in any greater depth. The physiologic data from group 3, on the other hand, are more interesting.

Of the 15 animals in group 3, three developed thrombus in the entrance portion of the plug and could not be analyzed for the distribution of mechanical variables. The distribution of pressure and shearing stress along the aortic test site for a representative experiment from the remaining 12 is shown in Figure 3, a. Pressure (p) and shearing stress (τ) are on the ordinate and the z axis on the abscissa. A schematic diagram of the vessel surface, with the imprint of the plug site and z axis as shown in Figure 1, is redrawn as the inset at the top of this illustration for reference purposes. Flow is from left to right, and the origin of the z coordinate (z = 0) is at the beginning of the uniform part of the plug channel.

Pressure (p) is seen to decrease rapidly in the entrance portion of the plug associated with the acceleration of the blood as it enters the plug channel. Pressure drops less rapidly along the uniform portion of the channel and is seen to increase slightly in the efflux region as the blood decelerates into the expanding flow channel provided by the aorta below the plug. The shearing stress (τ) upstream from the plug is seen to be quite low but rises precipitously to a peak value as the blood enters the uniform portion of the flow channel. Thereafter shearing stress can be seen to decrease progressively along the plug channel, finally dropping to low values again in the efflux region. The solid portion of this curve is an accurate representation of the shearing stress, whereas the dashed portion contains a certain amount of error since it also was calculated from the pressure gradient. As discussed elsewhere (1) the necessary conditions for calculating τ from the pressure gradient are violated in these "entrance" and "efflux" regions.

As reported earlier (1) these time-smoothed quantities, i.e., the time-smoothed pressure and time-smoothed shearing stress, are associated with corresponding superimposed high-frequency components when the flow becomes unstable and turbulence develops. A typical distribution of "turbulent intensity" taken from the previous study (1) is shown by the stippled envelope in the diagram. In experiments in which the channel Reynolds number remains below the critical value of 1,000, the flow remains stable and turbulence only appears in the chaotic efflux from the channel. In animals with channel Reynolds numbers in excess of the critical value, turbulence develops in the upstream portion of the channel and remains essentially constant along the rest of the channel until reaching the efflux region wherein it becomes greatly increased. Since the particular experiment chosen for illustration in Figure 3 had a channel Reynolds number in excess of 1,000, the latter

FIGURE 3
Relationship of mechanical and optical variables to location along channel. (a) Diagram of tissue specimen shown in Figure 1 appears at the top with reference z axis. Graph immediately below the diagram shows corresponding distributions of pressure (p) shearing stress (τ) and approximate distribution of turbulent intensity as estimated from previous study. (b) Lower graph shows distribution of injury concentration (C) as function of distance along the test site. The corresponding light transmission, from which the injury concentration was calculated, is shown as the fine curve (I) redrawn from Figure 2. The shearing stress (τ) and turbulent intensity are also redrawn from the graph above for reference purposes. Actual values of variables may be assigned to the ordinate (S) of each graph using the corresponding relationships shown at the right of each graph.
situation presumably obtained as is indicated in the diagram.

**EVANS BLUE DYE DATA**

The photographic transparencies from the three experimental groups of animals were scanned for optical transmission at 630 nm along the z axis of the specimen as described earlier. In the first two groups of animals the log of the ratio of the average transmitted light in the upper one-third of the tissue specimen to the average transmitted light in the lower one-third of the specimen (i.e., difference in optical density) was calculated for each animal and compared to zero, i.e., no optical difference. In group 1 the average value of the difference in optical density was $-0.023 \pm 0.038$ (SEM). Comparing each difference in optical density to zero, no significant difference was found ($0.6 < P < 0.5$). One concludes from this that there is normally no difference in the staining properties of the tissue in the upper and the lower end of the aortic test segment, or, if there is, it is too subtle to detect by these techniques and therefore may be ignored.

The photographic data from group 2 were processed in an identical manner. The average difference in optical density between the upper (high pressure) third and the lower (low pressure) third of the aortic test segment was $-0.366 \pm 0.14$ (SEM). Comparing each difference in optical density to zero, a significant difference was found ($0.05 < P < 0.02$). Thus, the light transmission is significantly decreased in the region above the clamp where the pressure was high. One concludes that there is a positive correlation between lateral pressure or associated wall strain and the intimal concentration of Evans blue dye, i.e., pressure (or its associated wall strain) in the absence of other mechanical events is associated with an increased flux of Evans blue dye into the intimal tissue layer.

Photometric analysis of the data from group 3 revealed a consistent pattern of light transmission. A typical light transmission vs. z plot has been shown in Figure 2. The smoothed light transmission curve has been redrawn as the light solid curve (I in Fig. 3, b). Referring to the left-hand portion of this curve, it can be seen that the light transmission is relatively constant over the tissue surface corresponding to the "white" stripe representing the trajectory of the plug-channel shown in Figure 1. Light transmission then drops rather precipitously in the region corresponding to the entrance of the plug channel, reflecting the appearance of the increased blue staining in this region. Note that this corresponds closely to the peak of the superimposed shearing stress ($\tau$) curve which has been redrawn from Figure 3, a. Light transmission then increases along the uniform ($0 < z < 3$) portion of the plug channel until reaching the efflux region wherein it again dips to a minimum associated with the turbulent efflux from the channel as indicated by the stippled envelope. Light transmission then increases progressively downstream approaching a value which is higher than that measured over the "white" trajectory above the plug.

Referring to the pressure distribution which is shown in Figure 3, a and referring also to the dye-staining data obtained from the clamp group of animals described above, it is reasonable to attribute a large part of the difference in light transmission between the extreme left-hand side and the extreme right-hand side of Figure 3, b to the associated differences in lateral pressure (or circumferential wall strains) that occurred across the plug in the animals of group 3. However, the intervening staining pattern, i.e., between $z = -1$ and $z = 6$, has a form which obviously cannot be correlated with the pressure alone. The pattern in this region appears to have some relationship also to the pattern of shearing stress in the region $-1 < z < 3$ and to turbulence in the region $3 < z < 6$. In summary, it appears that the intimal concentration of Evans blue dye in the animals of group 3 must be related to all three variables, i.e., to the pressure, or circumferential wall strain distribution, or both, to the shearing stress distribution, and to the distribution of turbulence.

It would be of considerable interest to...
analyze these relationships with statistical techniques to gain greater insight into the individual staining mechanisms related to these three variables. Unfortunately this sort of analysis would require prior, rather detailed, knowledge of the very mechanisms which are being sought. Although this dilemma frustrates efforts to analyze the present data with any rigor, a crude analysis will be done to separate the Evans blue staining in the photographic image into two components, one which as was shown in group 2 can be explained on the basis of the pressure distribution alone and a residual component which cannot. This latter component will be called the "injury" concentration \( (C_r) \) of Evans blue-dye in the intima. This will be accomplished by assuming that the difference in optical density between the upstream end of the white stripe on the left of Figures 1 and 2 and a point downstream away from the turbulent efflux region can be attributed solely to the corresponding pressure difference in a manner analogous to the data from group 2. Since the exact functional relationship between pressure and staining is not known, a linear approximation to this relationship will be made using the above difference in pressure and optical density to calculate the necessary proportionality constant. Multiplying the pressure at any point along \( z \) by this value gives the value of the optical density that could have been expected to be due solely to the pressure distribution. The difference between this predicted pressure-related optical density and the actual optical density at that point is an estimate of the added optical density (injury concentration, \( C_T \)) which could be related to the corresponding shearing stress and turbulence.

A representative injury concentration \( (C_r) \) vs. \( z \) curve calculated in the above manner is shown in Figure 3, b as the heavy solid curve along with the corresponding shearing stress \( (\tau) \) distribution for that animal. The light transmission curve \( (I) \) from which \( C_r \) was calculated is shown as the light solid curve. The injury concentration is seen to increase to a peak value in the entrance region of the flow channel \( (z = 0) \). In most of the animals the injury concentration decreases progressively from this point on to the efflux. In all of the animals the injury concentration shows an increase in the efflux region similar to that shown in this figure. Inspection of these \( C_r \) vs. \( z \) profiles suggests that \( C_r \) is probably closely related to the shearing stress exposure in the plug channel and to turbulence in the efflux region.

Although it was not possible to measure the intensity of the turbulence directly, it was possible to measure the shearing stress exposure along the uniform portion of the plug channel by the methods described earlier. The visual impression that \( C_r \) is related to the shearing stress \( (\tau) \) along the part of the system upstream from the efflux region \( (z < 3) \) was tested by calculating the correlation between these variables for each animal. The correlation coefficients and the \( P \) values for these coefficients from these calculations appear in Table 1. A significantly positive correlation is seen in each case.

It can be concluded that when the trend in optical density that might be expected just on the basis of the pressure distribution is removed, the residual optical density \( (C_T) \) can be correlated with the intensity of the shearing stress. Moreover, from simple inspection there also appears to be a correlation between

### Table 1

Correlating \( C_r \) with \( \tau \) for Upper and Channel Sections

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>No. of points</th>
<th>Correlation coefficient</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>401</td>
<td>42</td>
<td>0.98</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>409</td>
<td>35</td>
<td>0.95</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>410</td>
<td>34</td>
<td>0.95</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>419</td>
<td>38</td>
<td>0.94</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>421</td>
<td>39</td>
<td>0.96</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>426</td>
<td>33</td>
<td>0.83</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>427</td>
<td>39</td>
<td>0.91</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>428</td>
<td>35</td>
<td>0.85</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>429</td>
<td>33</td>
<td>0.42</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>439</td>
<td>43</td>
<td>0.79</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>440</td>
<td>30</td>
<td>0.47</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>446</td>
<td>29</td>
<td>0.77</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Circulation Research, Vol. XXIV, January 1969
Composite illustration showing the relationship of the measured distribution of variables along the reference z axis in the graph at the top to the corresponding histologic picture shown as the series of photomicrographs in the columns and rows below. Referring to the graph, S is a nondimensional ordinate which may be converted to the desired variable by the relationships shown at the far right of the graph. The distribution of the variables, pressure (p), shearing stress (r), A-cell density (A), B-cell density (B), injury concentration (C), and fat (Fat), are shown as the corresponding coded curves in this figure. The light vertical lines divide the graph into regions along the test site, labeled with the lower case letters a through j, which refer to the regions from which the tissue specimens for the photomicrographs appearing below were taken. The photomicrographs are arranged in four major rows. Rows numbered 1, 2, and 3 each contain three sub rows which are numbered on the left to indicate the animal from which the specimens were taken (426, 428, and 440). Each major row is divided into columns indicated by the lower case letters at the upper left of the top photomicrograph in each column. These letters correspond to the regions indicated at the top of the graph shown above. Row 4 shows photomicrographs taken from animals not included in the quantitative part of this study because of thrombus formation. These sections show the coexistence of thrombus and fat deposition.
Cₚ in the efflux region and exposure to turbulence; however, since turbulence was not estimated in these particular studies, the nature of this correlation cannot be expressed explicitly.

**HISTOLOGIC DATA**

The histologic data from groups 1 and 2 did not vary from that described previously for normal canine aortas (1). In group 2 the endothelial architecture remained normal both above and below the clamp. There was no evidence of intimal fat deposition in either group.

The histologic data from group 3 may be presented most compactly by the composite illustration shown in Figure 4. The histologic changes in the five animals in group 3 not receiving fat emulsion were identical to the other ten that received fat emulsion except for the occurrence of intimal fat deposition in this latter group. To avoid redundancy, histologic sections are shown only for three of the fat-infused animals.

For reference purposes the distribution of the various mechanical and histologic parameters described earlier are shown at the top of Figure 4 for animal 426. The light vertical lines on this graph bound regions (identified by the lower case letters at the top) from which the tissues shown in photomicrographs appearing in the series of panels below were taken. Photomicrographs are shown for dogs 426, 428, and 440 as indicated by the numbers at the left of each row of panels. These are arranged in three major rows indicated by the three large numerals at the extreme left of each row. Row 4 at the bottom contains selected photomicrographs from the three animals in which fibrin deposits formed in the plug channel.

Each major row contains subcolumns indicated by the lower case letters, a through f, appearing in the upper left-hand corner of each subcolumn. These letters refer to the lettered regions of the graph shown above and indicate the approximate locations along the flow system at which the corresponding photomicrographs were taken. The relative location of the origin of the z axis is indicated at the extreme right of row 1 and extreme left of row 2 by the open vertical bars. The location of the z = 3 cm point indicating the end of the uniform portion of the flow channel in the plug is indicated by the vertical bar in the middle of row 3. Therefore, the photomicrographs in row 1 represent tissue upstream from the plug. Those in row 2 represent tissue in the upper portion of the uniform channel in the plug. The two subcolumns on the left of row 3 represent tissue in the lower portion of the uniform channel, and finally the last two columns in row 3 represent tissues downstream from the plug as indicated. Thus, by referring to the letters at the head of each subcolumn and their positions along the flow channel (indicated by the lettered regions of the graph) one is able to study the histologic picture and its association with the corresponding mechanical variables, distributions of endothelial cells, fat deposition, and injury concentration of Evans blue dye. Although the graph shown was taken from animal 426, the corresponding data from animals 428 and 440 were qualitatively the same.

At location "a", the mechanical parameters are essentially normal and the corresponding histologic picture is similarly normal. At locations b and c, the histologic picture shows evidence of endothelial-cell damage associated with the increased exposure to shearing stress produced by the blood as it accelerates to enter the entrance portion of the plug. Thus from subcolumn a to subcolumn c one sees the progression of histologic changes related to shearing stress exposure which have been described in detail in the previous publication (1).

Subcolumns d, e, and f are taken from the upstream portion of the flow channel where total erosion of endothelial cells is seen frequently. Moreover, in animals which have received intravenous fat emulsion, such as these three animals, impressive deposits of fat may be seen between the fibrillar bundles of connective tissue immediately subjacent to the basement membrane region. The endothelial cells that are remaining appear to be in the
process of yielding, melting, or dissolving. Referring to the graph above, it can be seen that this region of the channel was exposed to the highest shearing stress. Since the photomicrographs in subcolumn f show endothelial cellular erosion and yet do not show any subjacent fat deposition, one concludes that fat deposition depends not only on endothelial cellular erosion but is also related to the duration and intensity of exposure to shearing stress. These relationships will be discussed in greater detail below.

At location g, endothelial cells reappear showing varying degrees of damage. Finally, at location h, where shearing stress has decreased to relatively low levels, endothelial cellular architecture returns to an essentially normal picture. Notice from the graph above, however, that the injury concentration, although decreased from the more severely damaged area, remains high. This observation was seen consistently in all studies and may indicate that the permeability of the interface for certain proteins increases at stress exposures below those associated with detectable histologic change.

Location i corresponds to the turbulent efflux from the plug channel and is usually associated with endothelial cellular damage and erosion typical of that shown in these panels (1). Still further downstream at location j, where shearing stress and turbulence have abated, one sees the return to a normal histologic picture.

The photomicrographs in row 4 were taken from animals which had to be excluded from detailed analysis of mechanical events because of thrombus formation. The sections are presented to show the coexistence of fat deposition and surface adherence of fibrin and blood cellular elements. It is of some interest to note that, although both types of lesions (lipid and thrombotic) can occur independently, when they do occur together as in these photomicrographs, the lipid deposits occur under the thrombus formations suggesting that they preceded the formation of the thrombus. Fibrin deposition never occurred in the presence of normal endothelial cells. The heaviest deposits always occurred in regions of total cellular erosion, i.e., in areas of high stress exposure.

In the two animals selected for serum lipid determinations, no significant alterations in the \( \alpha \) and \( \beta \) lipoprotein patterns or free fatty acid concentrations occurred over a 3-hour period. We conclude, therefore, that the fat deposited in the intima of the animals of group 3 came directly from the artificial chylomicron pool resulting from the emulsion infusion.

In summary, it appears that the normal endothelial cell can withstand shearing stresses up to some critical value below which they behave essentially as elastic bodies remaining tightly adhered to the basement membrane in an orderly and oriented pattern. The permeability of this surface to certain proteins in the blood phase may increase with increasing pressure (or stretch) and with increasing exposure to shearing stress while appearing to remain histologically normal. In the presence of a normal A-cell population, the surface functions as an effective hydrodynamically competent fluid boundary in that the surface rejects cellular elements, emulsified fat, and thrombus formation. When the endothelial surface is exposed to shearing stresses in excess of some critical value, the cells begin to suffer continuous structural changes which might be described variously as “yielding,” “melting,” “dissolving,” or “changing chemically.” This yielding process continues until the cell appears to become mechanically unstable and is washed away from its moorings to the basement membrane either in toto as suggested in Figure 4, no. 440 column b, or by progressive erosion of cell substance as suggested in many of the other panels, e.g. no. 428 column c. In any case, if exposure to these levels of stress continues, the basement membrane becomes denuded and exposed directly to the forces of the flow. Continued exposure appears to produce erosion of the subjacent intrafibrillar matrix material which is associated with changes in the apparent electrofibrillar properties of this surface as evidenced by
the appearance of a strong affinity for fat emulsion, blood cellular elements, and deposition of thrombotic material. In these areas of heaviest fat deposition, the blue ground substance between the collagen fibers also appeared to be depleted in most instances; however, better histochemical and microphotodensimetric techniques must be developed to prove this.

Thus, a consistent pattern of histologic events occurs along the channel. The foregoing data can be summarized compactly if they are analyzed with respect to two derived parameters, the acute critical yield stress \( (\tau_c) \) and the erosion stress \( (\tau_e) \). The first of these, the acute critical yield stress \( (\tau_c) \) for the endothelial-cell population, has been described previously (1). The acute critical yield stress was defined as that stress \( (\tau_c) \) at which there is the greatest conversion of normal \( (A) \) cell forms to abnormal \( (B) \) cells. In an analogous manner, it is possible to define the erosion stress \( (\tau_e) \) of the endothelial cell population from the \( B \)-cell population density curve as that stress at which the greatest number of endothelial cells are disappearing. Thus, stresses in excess of \( \tau_e \) will correspond with areas of severe damage and erosion. The erosion stress will be a function of time since time is required for the cellular destructive process to occur. Consequently, \( \tau_e \) will be high for short exposures and small (approaching \( \tau_c \)) for long exposures. This is in contrast to the critical yield stress \( (\tau_c) \) which, except for a probable initial period of transition, appears to be virtually independent of duration of exposure (1).

The acute critical yield stress and erosion stress for each animal were calculated in the manner described above and are shown in Table 2. The "greater than" or "less than" signs in the table indicate that the stress range occurring in that particular experiment did not extend far enough up to reach \( \tau_e \) or low enough to reach \( \tau_c \). The mean value of \( \tau_e \) for these studies was \(<420 \text{ dynes/cm}^2\) as compared to \(379 \pm 85 \text{ (SD)}\) for the previously reported studies (1). The significance of the difference in these means could not be tested because of the "less than" entries in the table. The values of \( \tau_e \) vary over a wide range. This variability cannot be explained only by the differences in the durations of the experiments and must also reflect a normally occurring wide variation in the time constants of the yielding processes of the endothelial cells among individual animals.

Since these parameters are defined in

### Table 2

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Wt. (kg)</th>
<th>Sex</th>
<th>Age*</th>
<th>Time (hrs)</th>
<th>Reynolds number †</th>
<th>( \tau_c ) (dynes/cm²)</th>
<th>( \tau_e ) (dynes/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>401</td>
<td>37.6</td>
<td>M</td>
<td>M</td>
<td>1</td>
<td>1212</td>
<td>470</td>
<td>830</td>
</tr>
<tr>
<td>409</td>
<td>31.4</td>
<td>M</td>
<td>M</td>
<td>0.5</td>
<td>963</td>
<td>730</td>
<td>&gt; 1020</td>
</tr>
<tr>
<td>410</td>
<td>28.5</td>
<td>M</td>
<td>M</td>
<td>0.5</td>
<td>1146</td>
<td>375</td>
<td>810</td>
</tr>
<tr>
<td>419</td>
<td>28.6</td>
<td>M</td>
<td>M</td>
<td>1</td>
<td>1199</td>
<td>&lt; 340</td>
<td>560</td>
</tr>
<tr>
<td>421</td>
<td>33.0</td>
<td>M</td>
<td>M</td>
<td>1</td>
<td>1205</td>
<td>460</td>
<td>1320</td>
</tr>
<tr>
<td>426</td>
<td>28.6</td>
<td>M</td>
<td>O</td>
<td>1</td>
<td>1113</td>
<td>560</td>
<td>830</td>
</tr>
<tr>
<td>427</td>
<td>32.9</td>
<td>M</td>
<td>M</td>
<td>3</td>
<td>773</td>
<td>&lt; 200</td>
<td>560</td>
</tr>
<tr>
<td>428</td>
<td>28.5</td>
<td>M</td>
<td>M</td>
<td>3</td>
<td>1205</td>
<td>510</td>
<td>1240</td>
</tr>
<tr>
<td>429</td>
<td>32.8</td>
<td>M</td>
<td>M</td>
<td>3</td>
<td>1297</td>
<td>&lt; 300</td>
<td>1105</td>
</tr>
<tr>
<td>439</td>
<td>29.1</td>
<td>F</td>
<td>Y</td>
<td>1</td>
<td>1664</td>
<td>580</td>
<td>1610</td>
</tr>
<tr>
<td>440</td>
<td>26.3</td>
<td>F</td>
<td>Y</td>
<td>1</td>
<td>635</td>
<td>300</td>
<td>430</td>
</tr>
<tr>
<td>448</td>
<td>25.3</td>
<td>M</td>
<td>Y</td>
<td>1</td>
<td>1113</td>
<td>&lt; 220</td>
<td>1120</td>
</tr>
</tbody>
</table>

*Y = young, M = mature, O = old. †Reynolds number calculated on basis of plug channel radius.

_Circulation Research, Vol. XXIV, January 1969_
terms of changes in endothelial cell architecture, they may be used as a convenient method of dividing the data from all animals into groups having common histologic properties. In particular, the data may be ordered into sets according to whether they fall into the four stress intervals defined by 

\[ \{ \sigma < \sigma_{\text{min}} \} , \quad \{ \sigma_{\text{min}} < \sigma < \sigma_{c} \} , \quad \{ \sigma_{c} < \sigma < \sigma_{e} \} , \quad \{ \sigma_{e} < \sigma < \sigma_{\text{max}} \} \]

and \( \sigma_{\text{min}} \) and \( \sigma_{\text{max}} \) are the stress at \( z = 3 \) and \( z = 0 \), respectively. Data for the first set were obtained from the tissue lying in the "white" trajectory above the plug for \( z < -1 \). Thus, the data from all of the experiments may be ordered into four separate groups according to ascending histologic criteria of cellular damage by using the parameters, \( \sigma_{\text{min}} \), \( \sigma_{c} \), \( \sigma_{e} \), and \( \sigma_{\text{max}} \). This places the data from all experiments on a common base which permits averaging of the data across the animals. The mean values for A-cell density, B-cell density, injury concentration, and fat deposition for each of the four sets of data are plotted as the histogram in Figure 5. Although stress could not be calculated for the animals having fibrin deposition, the trend of the fibrin deposition curve would have been parallel to and to the right of the fat deposition curve. The light vertical bars represent \( \pm 1 \) SD. The mean points are connected by the coded lines as indicated to act as a visual aid to display the pattern of each variable with respect to stress. These mean patterns are typical of those seen in each individual. The most important features of the data summarized in this histogram are 1) the absence of fat (or fibrin) deposition in the presence of normal endothelial cell density, 2) the rapid appearance of fat (or fibrin) deposition in the presence of damaged endothelial cells beginning at stresses just below \( \sigma_{e} \), and 3) the progres-
sive increase of the injury concentration of Evans blue dye beginning at levels of stress below that associated with discernible structural changes in the endothelial cell.

Discussion

One must proceed cautiously in applying the inferences drawn from data obtained in an experimental preparation to the normal situation. Accordingly the data from the present study should be viewed as the product of a test preparation which was designed to preserve the normal metabolic milieu of the tissue as nearly as possible while simultaneously controlling and measuring the mechanical variables of interest. To a certain extent these objectives were compromised by a number of variables which unavoidably accompany many in-vivo studies. The necessity of using anesthesia and performing widespread surgical procedures may produce metabolic effects which conceivably might influence the events observed in the present study. Moreover, it is difficult to control the blood pH, oxygen tension, and temperature with great precision. It is possible that the relationships among cell rheology, chemical behavior, and stress exposure may depend on one or more of these variables. Consequently, the variability of the rheologic parameters as measured in the present study may not necessarily represent an actual inherent variability in the properties of the tissues from dog to dog but may reflect the influence of one of the uncontrolled factors. One must be cautious in interpreting the absolute values of the parameters presented earlier until these have been confirmed under more physiologic circumstances.

On the other hand, it was shown clearly that within a given individual consistent relationships exist among the density distributions of the endothelial cell population, the injury concentration of Evans blue dye, the intimal deposition of fat and fibrin, and the stress exposure. In spite of possible distortions in the general chemical milieu of the entire system, one sees a consistent pattern emerge among the variables as summarized in Figure 5. It is most probable that the same patterns would hold for the physiologically perfect system, though with somewhat different absolute numerical values. One cannot escape the conclusion that an increased shearing stress at the blood-wall interface is associated with architectural changes in the interface, an increased flux of Evans blue dye and fat particles across the interface, and an increased affinity of this surface for blood cellular elements and fibrin. Of these, one of the most interesting but perhaps least accurate observation is that the flux of Evans blue dye increases to significant levels at exposures to stress which are less than the acute critical yield stress. If this is true, then it is possible that the flux of certain other molecular species in the blood such as lipoproteins are also coupled to the stress on the endothelial surface under normal physiologic circumstances.

Acknowledgments

The author gratefully acknowledges the many long hours spent on this project by his associates. In particular he wishes to thank Mr. Joseph M. Pearce and his staff, Messrs. Leander Brown and George Johnson, for their untiring technical assistance in the execution of the experiments. Mr. Joseph Janicki and his assistant, Mrs. Cecile Floyd, for their computer programming and data processing. Mr. Fred Plowman for his help in design and meticulous care in the fabrication of the various instruments and devices that were necessary, and Drs. Robert I. Levy, Donald S. Frederickson, and their associates for making available the serum lipid analyses.

References

Circ Res. 1969;24:93-108
doi: 10.1161/01.RES.24.1.93

Certain Histological and Chemical Responses of the Vascular Interface to
Acutely Induced Mechanical Stress in the Aorta of the Dog
DONALD L. FRY

The online version of this article, along with updated information and services, is
located on the World Wide Web at:
http://circres.ahajournals.org/content/24/1/93

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles
originally published in Circulation Research can be obtained via RightsLink, a service of the
Copyright Clearance Center, not the Editorial Office. Once the online version of the published
article for which permission is being requested is located, click Request Permissions in the
middle column of the Web page under Services. Further information about this process is
available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/