Hydrodynamic Injury of the Endothelium in Acute Aortic Stenosis

ISABELLE JORIS, PhD, THOMAS ZAND, MD, and GUIDO MAJNO, MD

From the Department of Pathology, University of Massachusetts Medical Center, Worcester, Massachusetts

The acute effects of increased shear stress on the endothelium were studied by reducing the lumen of the rat aorta to 20–25% of normal by means of metal clips. Intimal damage in the stenotic area was assessed by light microscopy after perfusion with $AgNo_3$ and study of the endothelium *en face*. Most of the endothelium was lost within 3 minutes; the extent of the damage was not increased after 1 hour. Electron-microscopic examination showed that some endothelial cells became permeable to tracers (thorium dioxide and horseradish peroxidase); platelets adhered to the exposed internal elastic membrane. Focal endothelial changes were represented by myelin figures of various kinds arising from the luminal surface and by "cellular ulcers," superficial erosions of the endothelial cells accompanied by localized cytoplasmic changes. These "ulcers" occurred more frequently over the nucleus and near junctions; they have not been described in other forms of arterial injury. (Am J Pathol 1982, 106:394–408)

THE ARTERIAL INTIMA is constantly submitted to the mechanical friction of the bloodstream; it is obvious that an increase of this physiologic trauma should lead to pathologic changes in the endothelium. The possibility of intimal damage related to flow has long been recognized;[1-7] yet there have been very few experimental studies of the *early* endothelial changes due to increased shear stress or turbulent flow. The earliest work in this field is due to Fry, who devised a model for the study of hemodynamic injury to arterial endothelium *in vivo* and succeeded in correlating the damage—as shown by light microscopy—with calculated values of shear stress.[2] The instrumentation needed for these measurements was applicable only to acute studies; it required the use of a large artery (the aorta of the dog) and complex surgical procedures. To approach the study of hydrodynamic injury at the level of electron microscopy, we chose a simpler animal model that would lend itself more easily to ultrastructural methods and to multiple experimental variations, the abdominal aorta of the rat, constricted by two different types of metal clips. In this paper we will describe the early endothelial lesions produced by these two methods.

Materials and Methods

Animals

We used "lean" male Wistar rats (Charles River Breeding Laboratories, Wilmington, Mass) weighing 200–300 g and fed a maintenance diet of 3 standard pellets of Purina chow per day. With this diet the rats grew normally without becoming obese. When Purina chow was allowed *ad libitum*, the rats developed a pad of retroperitoneal adipose tissue that made surgery on the aorta more difficult and traumatic.

Stenosis was produced with two types of metal clips, which reduced the lumen either by symmetrical transverse compression (*transverse clip*) or by tightly pinching and obliterating the right half of the aortic lumen (*lateral clip*) (Figure 1).

Transverse clips were fashioned from 22 g platinum wire beaten flat to 1 mm width, cut to 7 mm length, and bent into a U around a steel thickness gauge, leaving a gap of 0.45 mm between the arms. The ventral tip of the U was slightly everted. Platinum was used because of its low reactivity with living tissue, and for its rigidity, which allowed it to maintain its shape despite the dilating effect of aortic pulsation. When the clip slipped over the aorta, it reduced the lumen to a transverse slit.

The width of the transverse clip was chosen on the basis of pilot experiments with looser clips (0.80 and

Supported in part by Grants HL-16952 and HL-25973 from the National Institutes of Health and by a subsidy from the Upjohn Company, Kalamazoo, MI.

Accepted for publication October 23, 1981.

Address reprint requests to: Isabelle Joris, PhD, Department of Pathology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01605.

0002-9440/82/0310-0394$01.25

0.68 mm). After 1 hour the resulting endothelial damage was too mild and spotty for reliable sampling.

Lateral clips were fashioned from bezel silver plate 0.008 inch (0.2 mm) in thickness, cut into strips measuring 7 × 1.25 mm, carefully roughened with fine sandpaper, and bent into a wide U over a steel thickness gauge (with a 2–3-mm gap). The free tips of the U were brought over the midline of the aorta, then clamped tight with a hemostat. This reduced the lumen to a rounded channel 0.5–0.7 mm in diameter. (Platinum lateral clips were tested, but their rigidity made the surgical application too clumsy.)

Surgical Procedure

Under ether anesthesia, the rat was laid supine, the abdomen was opened, and the aorta was exposed by displacement of the viscera to the rat's right. Further surgery was performed under a surgical microscope with fiberoptics illumination (Applied Fiberoptics, Southbridge, Mass). With No. 5 jeweler's forceps, in the region of the iliolumbar arteries (below the left renal artery), the pre-aortic peritoneum was carefully opened; while the aorta was supported from beneath with a fine curved forceps, the aortic adventitia was separated laterally from the inferior vena cava (with a 26 G needle), and a passage was prepared for the clip between the two vessels. For both types of clips the aorta was approached from the rat's right side. Transverse clips were held with forceps and slipped on; lateral clips were held with a hemostat and clamped on tightly.

Sham-operated control animals were prepared in two ways: 1) preparation of the passage for the clip around the aorta, but without application of the clip; 2) application of the clip for 2 seconds only.

Perfusion Fixation

A left thoracotomy was performed, and the thoracic aorta was cannulated with an Angiocath 20 G (Deseret Co., Sandy, Utah) in the downstream direction. Solutions were infused at 110 mm Hg. Outflow was through severed jugular veins. In the experimental animals, clips were left in place during perfusion or (in 13 rats) removed just before.

Demonstration of "Silver Lines" for Topographic Study (Light Microscopy)

To ensure the best possible preservation of the endothelium, silver nitrate was applied after glutaraldehyde fixation in the following perfusion sequence: 3% glutaraldehyde for 5 minutes, Ringer's lactate (pH 7.4) for 3 minutes, $AgNO_3$ (0.05–0.125%) for 1 minute, Ringer's lactate for 3 minutes, bromides (NH_4Br 3% and CoBr 1%) for 1 minute, and 3% glutaraldehyde for 20 minutes. The abdominal aorta between the renal and iliac arteries was then excised and immersed in fixative for 4½ hours under a desk lamp with a 60-Watt bulb. After removing the excess connective tissue and loose adventitia, we took off the clip and cut open the aorta (along its left lateral face for the transverse clip, or along the flattened right lateral face for the lateral clip). The cut sides were spread apart, and the vessel was placed between a glass slide and a coverslip, endothelial face up. The specimen was then studied while still wet. We obtained satisfactory permanent preparations by allowing the vessel to dry under the coverslip at room temperature for 24–48 hours, then mounting it in Permount.

Electron Microscopy

After perfusion fixation with 3% glutaraldehyde for 20 minutes the abdominal aorta was excised *in toto*, immersed in 3% glutaraldehyde at room temperature for a total fixation time of 5 hours, washed in cacodylate buffer (0.1 M, pH 7.4), postfixed with 1.3% osmium tetroxide, dehydrated in graded alcohols, then cut transversely into rings or longitudinally into strips selected at the clip site and adjacent areas. Tissue samples were embedded in Polybed 812 (Polysciences, Inc., Warrington, Pa). One-micron-thick sections were stained with toluidine blue; ultrathin sections were cut with a diamond knife on an LKB Ultrotome III, mounted on copper grids, stained with uranyl acetate and lead citrate, coated with carbon, and studied with a Philips 301 or a JEM 100S electron microscope.

Use of Permeability Tracers

Colloidal thorium dioxide (Thorotrast 20–26%, sterilized; Fellows Testagar, Detroit, Mich) was injected intravenously in 0.5-ml aliquots one, three, or four times over 1 hour. Horseradish peroxidase (Type III, Sigma, St. Louis, Mo), 15 mg/100 g body weight, was injected intravenously 10 minutes before perfusion.

Aortas exposed to horseradish peroxidase were incubated and revealed with saturated 3,3′-diaminobenzidine tetrahydrochloride (DAB, Polysciences, Inc., Warrington, Pa) in Tris hydrochloride buffer (0.05 M, pH 7.3) with 0.01–0.03% H_2O_2 for 30–60 minutes, rinsed in Tris hydrochloride buffer for 5–15

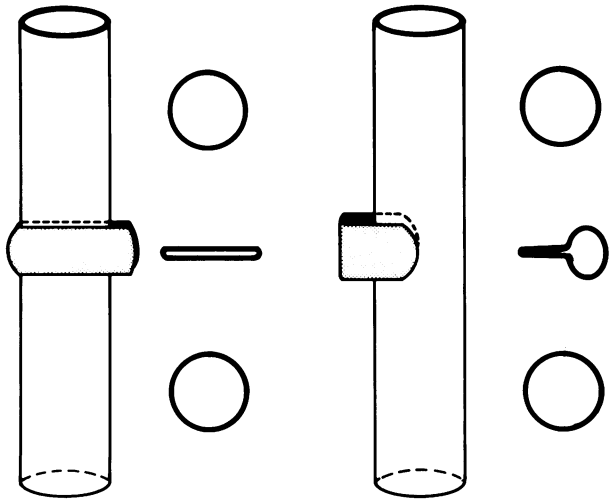


Figure 1—Transverse clip and lateral clip with corresponding contours of the lumen.

minutes, and processed as above. Half of the ultrathin sections were examined unstained.

Blood Pressure Measurements

In 6 rats the mean arterial pressure was measured while the rats were under ether anesthesia, before, during, and after application of a clip, either transverse or lateral. Recordings were taken proximally (through a cannula in the right carotid artery) and distally (through a cannula in the right iliac artery). The cannulas led to a pressure transducer (No. 4-3271, Bell and Howell, Pasadena, Calif) connected to a blood pressure module (Parametron 7150, Roche Medical Electronics, Inc., Cranbury, NJ). Blood pressure recordings were made with a Dual Channel Strip-chart recorder (Model 7274, Roche Medical Electronics Inc., Cranbury, NJ).

Experimental Plan

Stenosis was created in 38 rats, of which 26 were used for topographic assessment by the silver nitrate method (light microscopy, LM); in 12 of this group the transverse clip was applied, in 11 the lateral clip. With each type of clip the rats were killed at 3, 5, 10, 20, 45, or 60 minutes. In 3 *control* rats the transverse clip was applied for 2 seconds only. Twelve rats were used for electron-microscopic (EM) examination, 6 with transverse, 6 with lateral clips. In 13 clipped rats the iliac arteries were clamped shortly after the start of the perfusion. A group of 16 rats were sham-operated (9 LM, 7 EM; those in which the clip was applied for 2 seconds only were studied either immediately or after 1 hour); 17 rats were used as untreated controls (13 LM, 4 EM). Permeability tracers were injected into 11 rats.

Results

Measurements of the stenosis were performed on fixed specimens and on plastic-embedded sections. The standard transverse clip changed the normal lumen of the aorta (diameter 1.5 ± 0.1 mm) into an elongated slit 0.15 mm wide, representing 20% of the normal cross-sectional area. The lateral clip reduced the lumen to a channel about half the normal diameter, corresponding to 25% of the original cross-sectional area. As expected, the degree of stenosis was constant with the transverse clip, slightly variable with the lateral clip, due to the difficulty of evaluating—in the operating field—the precise midline of the aorta as a target for the tips of the clip.

Mean blood pressure before application of a clip (under ether anesthesia) was 80 mm Hg. After a standard clip was applied (transverse or lateral), neither proximal nor distal blood pressures changed. However, if a lateral clip was applied in such a manner as to reduce the lumen to slightly less than 50% of the normal diameter, there was a transient drop in distal pressure of 10–15 mm Hg, with return to normal within 15–20 seconds.

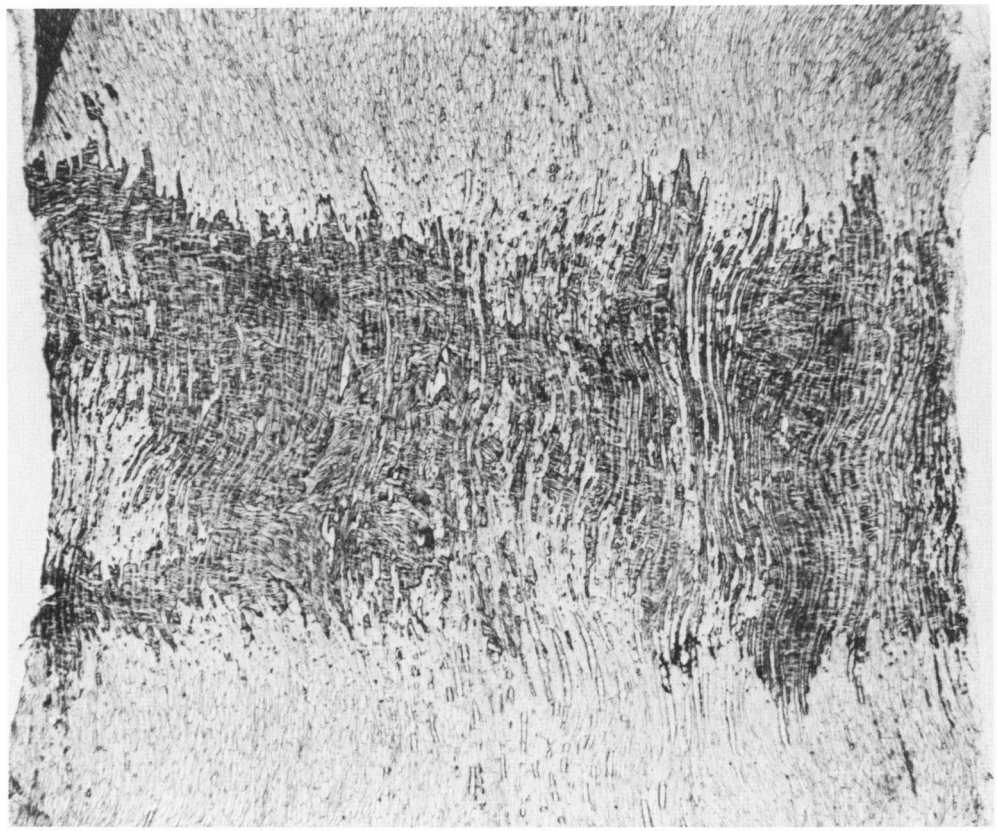
When the "clipped" rats awoke, they showed no obvious sign of distress; the use of the hind limbs was not impaired. All the clips had remained in place.

Light Microscopy

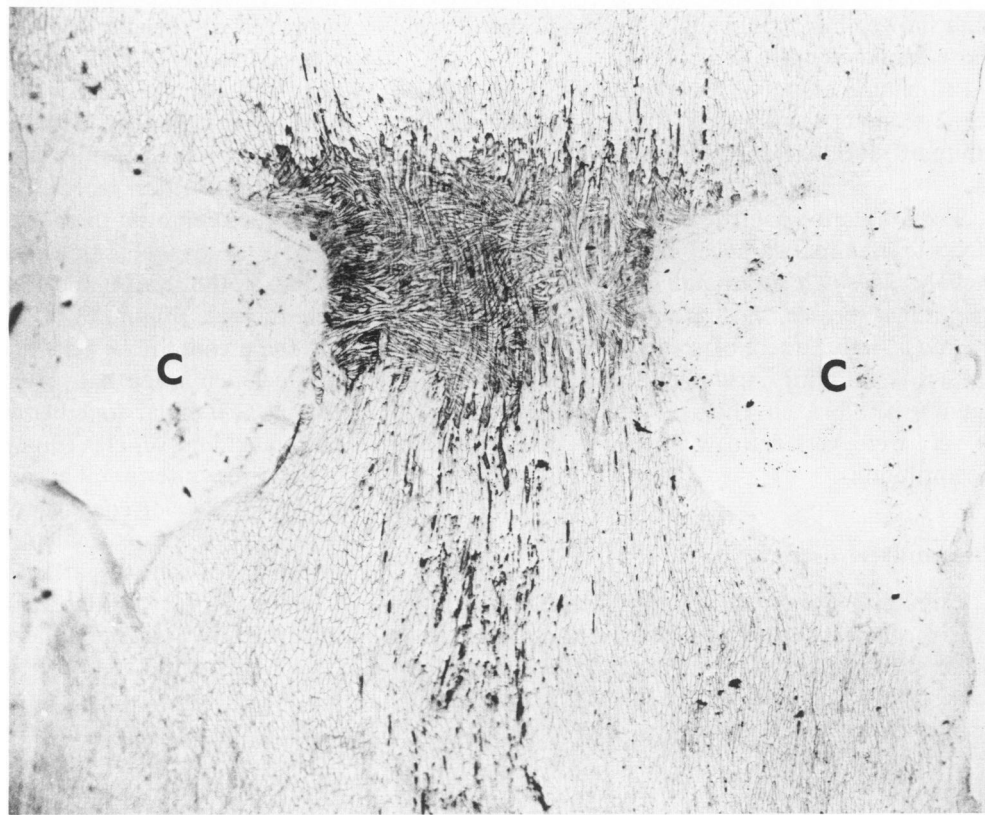
Study of the intima *en face* (silver nitrate method) showed similar changes at all stages between 3 and 60 minutes. At low power (Figures 2 and 3) the shape of the lesion—predictably—differed with the two clips. Stenosis with the transverse clip induced a symmetrical, band-shaped lesion ~1 mm wide (Figure 2); its margins were microscopically frayed, but the transition toward normal intima was relatively sharp; small streaks or patches of injury appeared sometimes distally and more rarely proximally within the range of 1–2 mm. The lateral clip was usually accompanied, on the distal side, by longitudinal streaks of injury (Figure 3), referred to hereafter as *jet lesions*.

Microscopic detail of the acute endothelial damage in the stenotic area, as shown by the silver method, appeared in well-known forms:[8,9] focal widening of the silver lines, loss of cells, and medial staining. At both margins of the lesion, the remains of endothelial "silver lines" were faintly visible over the stained media.

Beyond 1–2 mm above or below the stenosis the in-



2



3

Figures 2 and 3—Inner surface of two rat aortas 1 hour after application of the clip (transverse in **Figure 1**, lateral in **Figure 2**). Silver nitrate method. Direction of flow from top to bottom. Dark areas represent endothelial injury. Note jet lesion in **Figure 2**. *C, C* = areas compressed by clip. (×48)

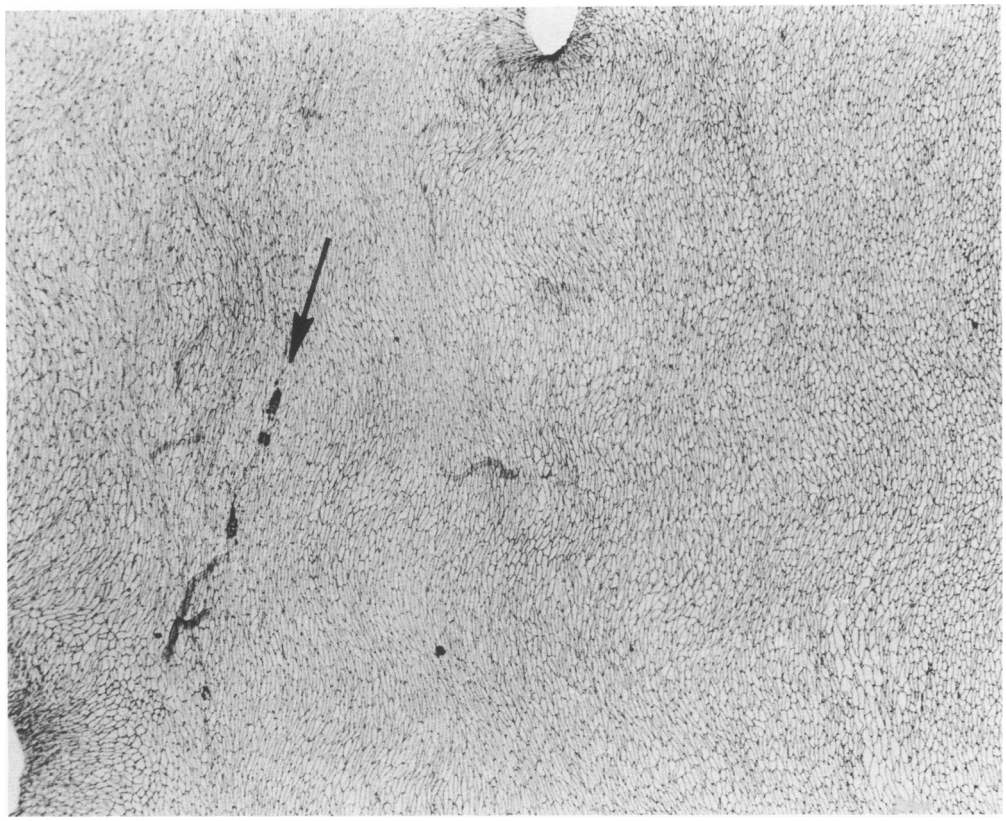


Figure 4—Control. Inner surface of a rat aorta 3 minutes after the application of a transverse clip for 2 seconds. Silver nitrate method. Direction of flow from top to bottom. Endothelial damage is limited to three small areas (*arrow*). The curved shadows in the background represent adventitial blood vessels. (×48)

tima showed minimal changes indistinguishable from those found in untreated controls.

In the animals to which the clip had been applied for 2 seconds the clip site showed minimal injury, limited to the loss of a few endothelial cells (Figure 4).

Sham-operated controls showed, at the sham-operated site, minimal damage, ie, the loss of a few endothelial cells, single or in small groups. Over the rest of the intimal surface, in all groups of rats including *untreated controls*, there often appeared thin longitudinal streaks of injury, usually distal or proximal to the opening of a branch; they varied in number from rat to rat, were very narrow (one or a few cells) and discontinuous.

Electron Microscopy

Intimal changes were identical with the two types of clips. Damage was limited to the stenotic area and 1–2 mm above and below. The intimal changes will be described from the most to the least severe; the latter were found at the margin of the lesion.

Loss of endothelium was prominent in the center of the lesion, where the internal elastic lamina (IEL) was exposed to the lumen, with or without preservation of the endothelial basal lamina. When the IEL was folded, the damage was most severe over the ridges. Islands of damaged or fragmented endothelial cells were usually present (Figures 5 and 6). Platelets were often adherent to the exposed IEL (Figure 6), especially when the clip had been removed before perfusion-fixation. Isolated fragments of endothelial cells were common in the lumen; they evidently belonged to partially detached cells not completely shown in the section and always showed signs of severe damage (Figure 5).

Where endothelial cells had been torn away, their plasma membrane had often remained adherent to the basal lamina, together with the nearest row of plasmalemmal vesicles (Figure 5 inset).

Changes interpretable as *cell death* took two forms: 1) *cellular edema* was accompanied by a diffuse increase of plasmalemmal permeability, which allowed Thorotrast particles to penetrate diffusely into the cytoplasm (Figure 7); sometimes this change affected only a part of the cell (Figure 8); 2) other cells were shrunken, very electron-dense, and their plasma membrane became indistinct; these cells could be diffusely impregnated by horseradish peroxidase (HRP) (Figure 9). In these cells the presence of HRP reaction product was visible as a dark brown stain by light microscopy, in 1-μ sections.

Interendothelial gaps occurred either between cells

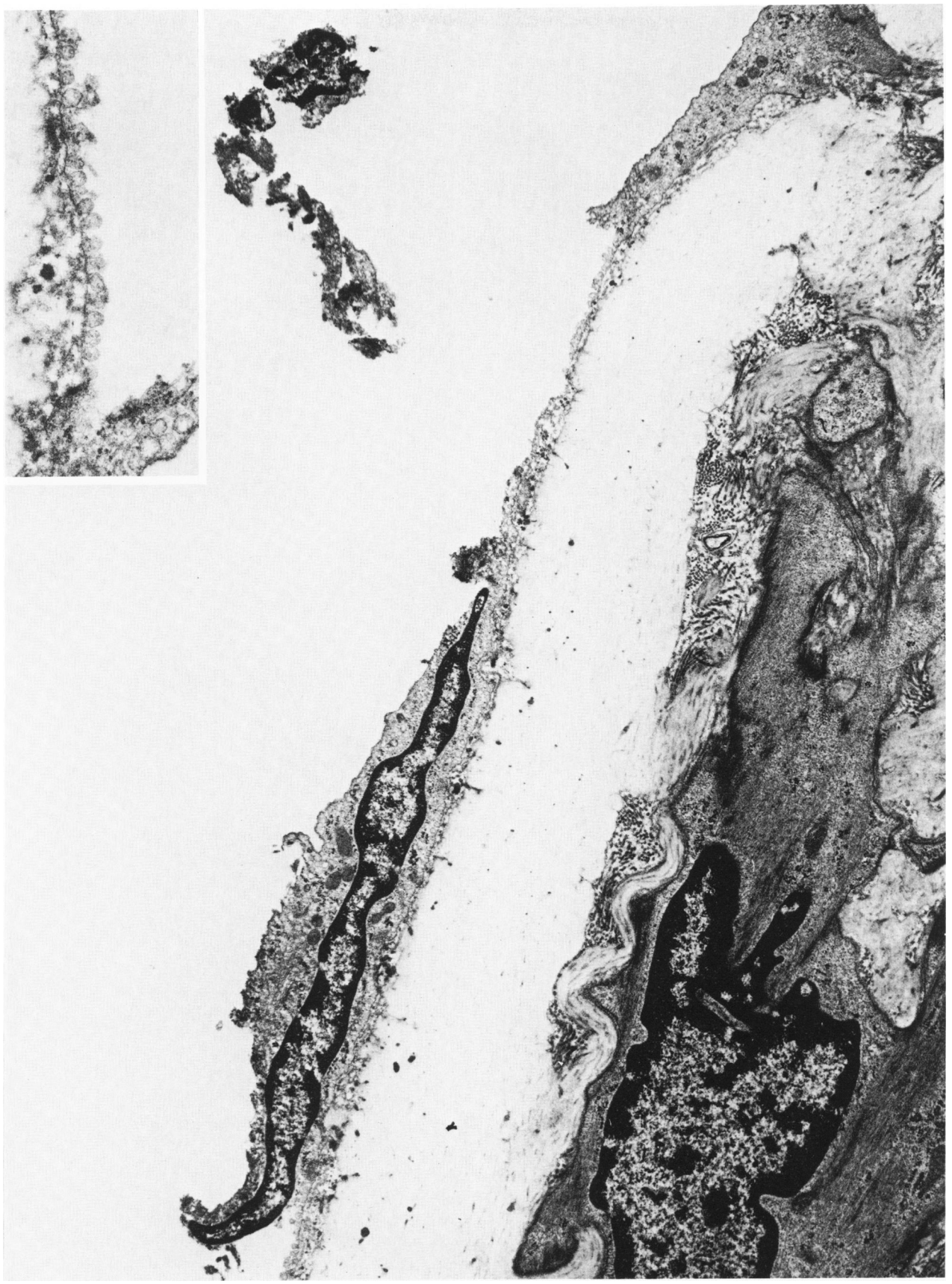


Figure 5 – Lateral clip, 60-minute stage (HRP intravenously). Upper edge of the lesion. *Top*, an endothelial cell was torn off, leaving its plasma membrane and vesicles attached (enlarged in **inset**). A part of an injured endothelial cell appears in the lumen. *Below*, an endothelial cell with extensive "ulcers." (× 12,400; **inset**, × 38,000)

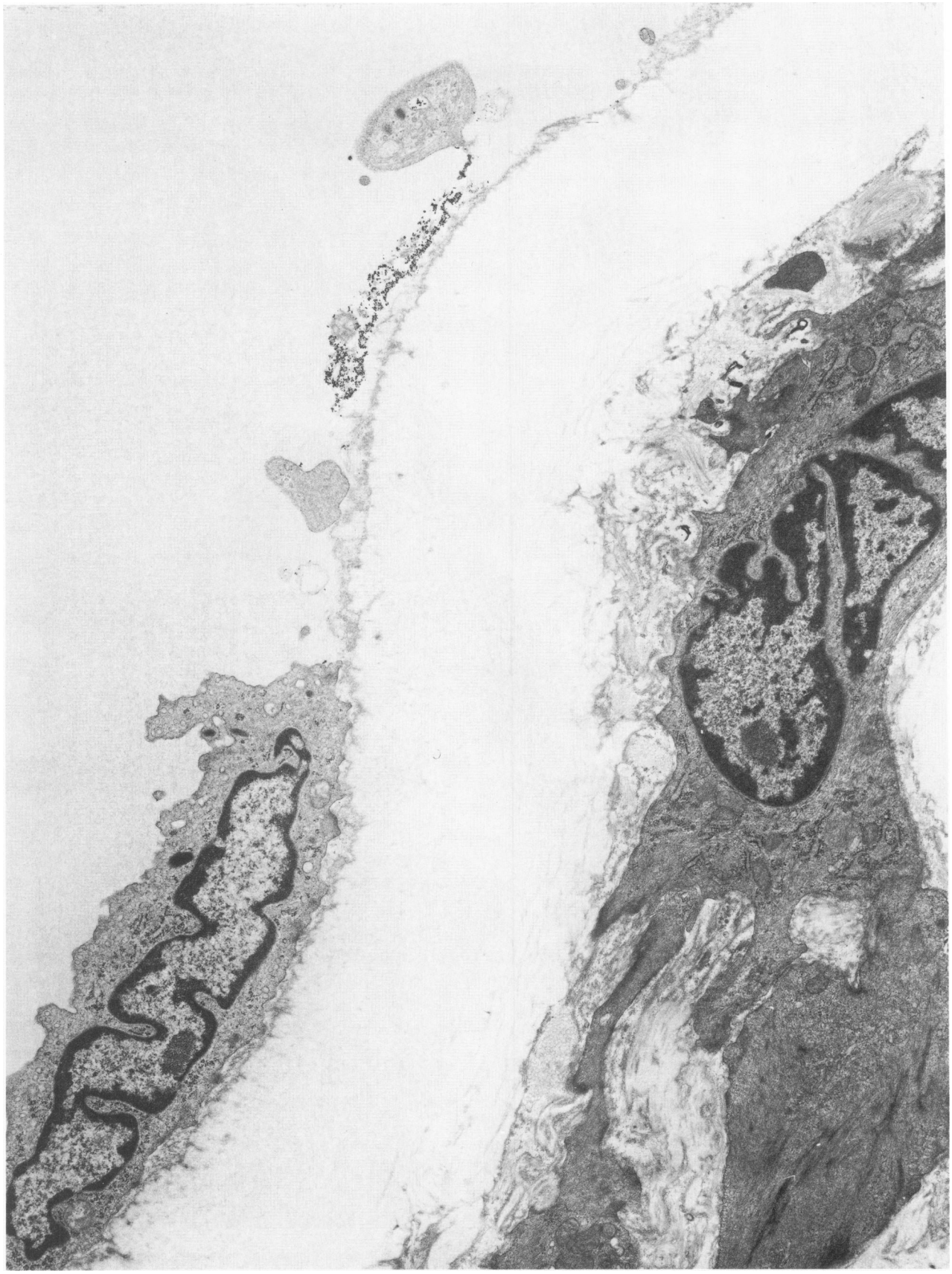
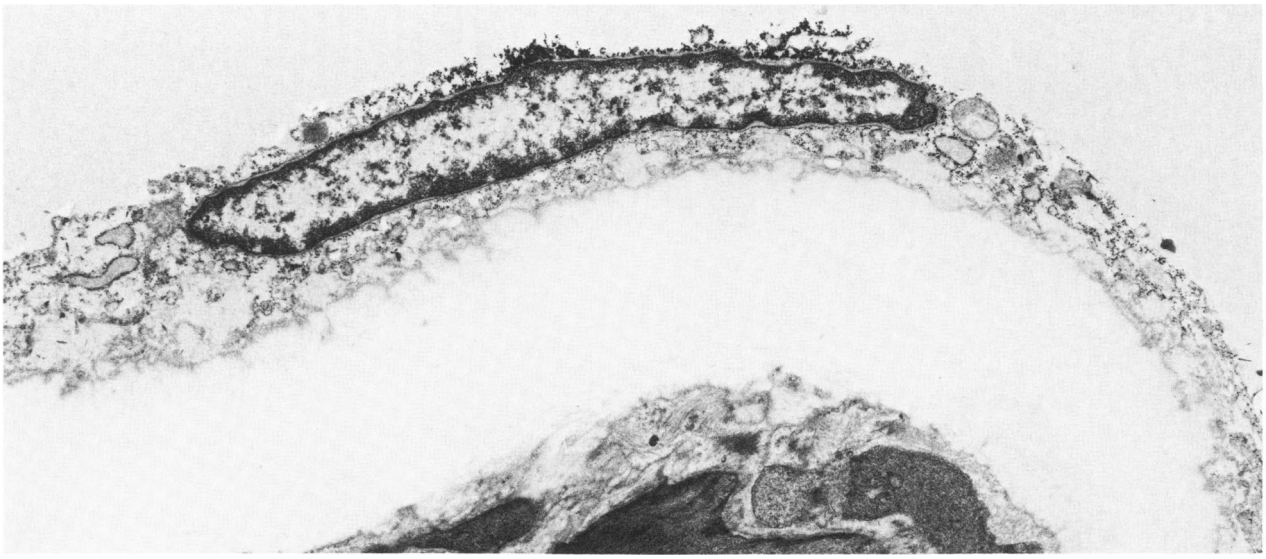
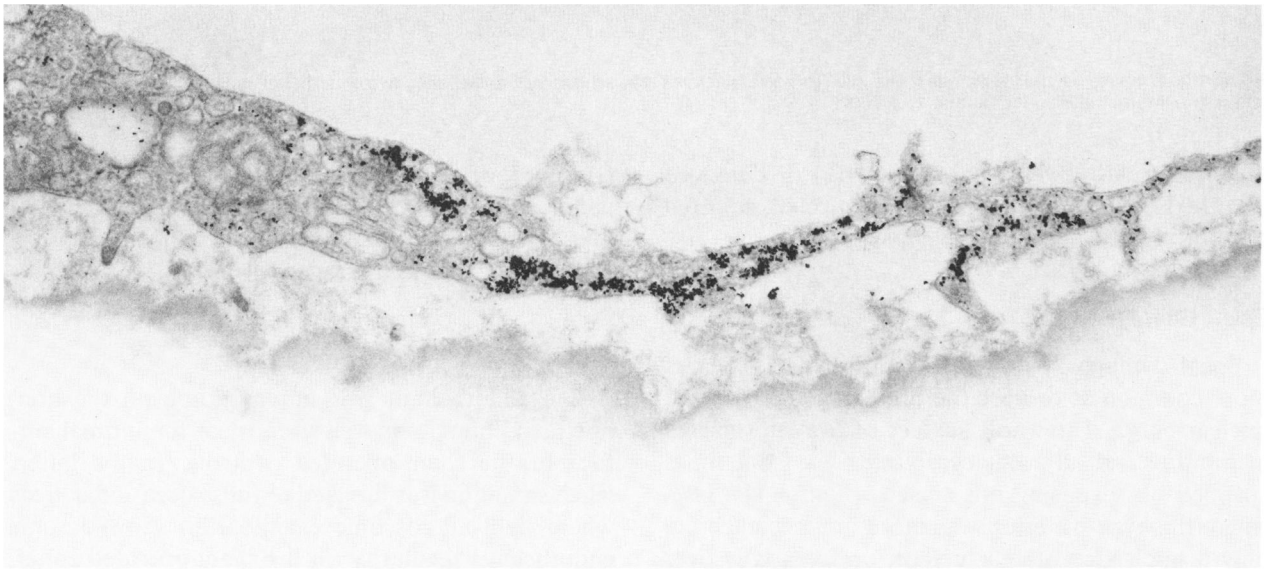


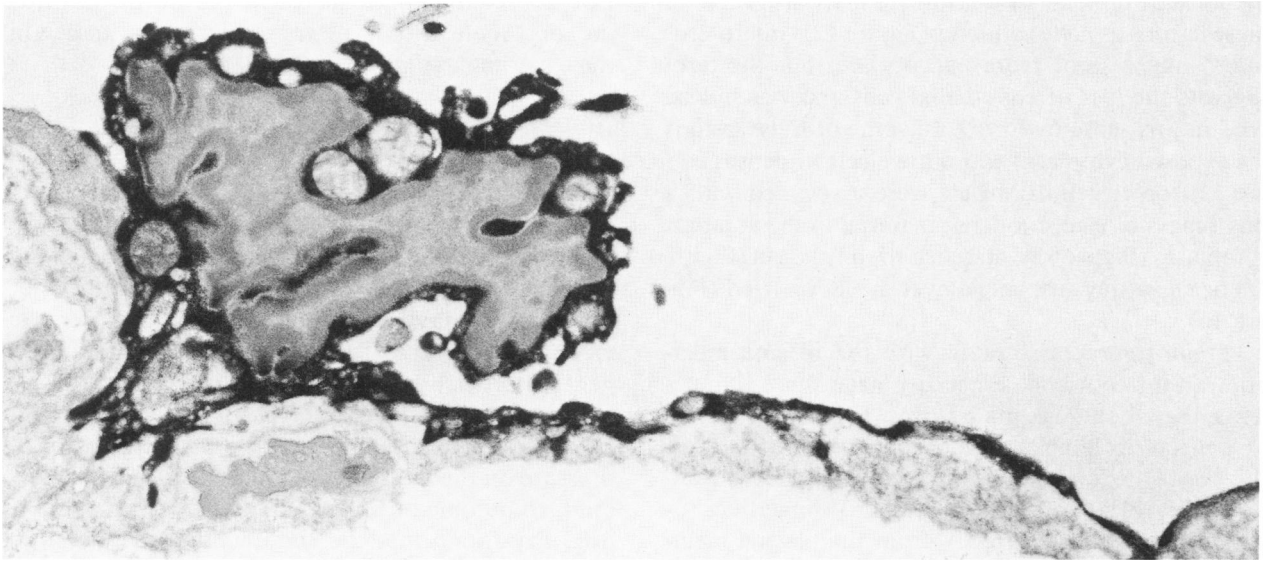
Figure 6—Lateral clip, 60 minutes; clip site (Thorotrast 4 times intravenously). Denudation of the intima over a ridge of the internal elastic membrane. Thorotrast and platelets mark the denuded area, proving that the lesion occurred *in vivo*. (× 10,800)



7



8



9

Figure 7 – Transverse clip, 60 minutes; lower edge of the lesion (Thorotrast 4 times intravenously). Severely injured endothelial cell; note loss of plasma membrane over the nucleus, with penetration of Thorotrast particles. (× 8800) **Figure 8** – Transverse clip, 60 minutes; clip site (Thorotrast 1 time intravenously). Injured endothelial cell; Thorotrast particles are within it. (× 29,700) **Figure 9** – Transverse clip, 60 minutes; clip site (HRP intravenously). Damaged endothelial cell diffusely imbued with HRP. (× 13,500)

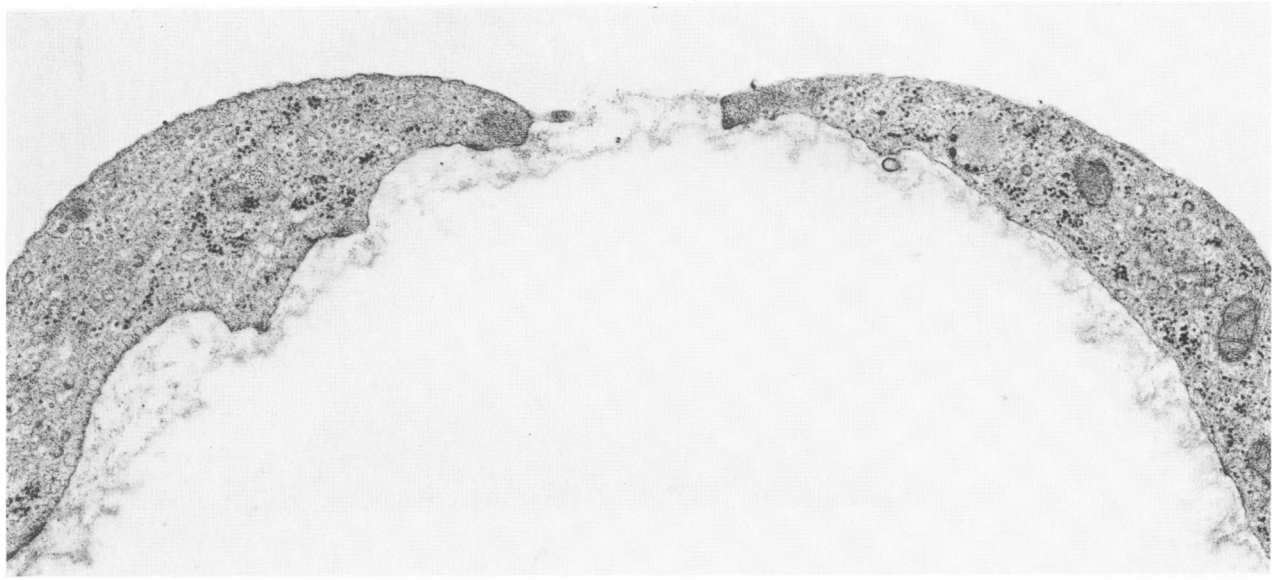


Figure 10 — Transverse clip, 60 minutes; clip site (Thorotrast 4 times intravenously). Gap between two endothelial cells, on a ridge formed by a fold in the internal elastic membrane. (× 22,000)

that were relatively normal (Figure 10) or damaged (Figure 11); when Thorotrast was injected, it often but not always labeled these gaps.

Focal Cellular Injury

Focal damage of individual endothelial cells was more common at or near the junctions and over the nuclear bulge. The whole surface of the cell (plasma membrane and adjacent cytoplasm) was sometimes stripped off, exposing the nucleus (Figure 11); elsewhere this type of lesion was more limited, affecting only a small area of cell surface, preferentially over the nucleus or close to the junction; we will refer to these lesions as *cellular ulcers* (Figure 12) "micro-erosions" might seem more appropriate, but the term suggests the loss of endothelial cells and was indeed used in this sense by Fry[10]). In some of these lesions the exposed cytoplasm was more electron-dense (Figure 12); other cellular ulcers were associated with a small mass of electron-dense material in the adjacent cytoplasm (Figure 13), attached to the margin (Figure 14) or appearing suspended over the lesion itself (Figure 15).

Myelin figures associated with the plasma membrane were common, especially near the cell junctions; they took three main forms: 1) rounded masses of concentric lamellas attached to the cell surface (Figure 16); 2) blebs filled with smaller vesicles ("multivesicular blebs") (Figure 17); and 3) fingerlike, tubular shapes arising directly from the plasma membrane (Figure 18).

Medial changes were mild and uncommon; they consisted of occasional vacuoles lined by two cell membranes, of the type known as cell-to-cell hernias;[11] they were found only at the clip site and with transverse clips, in the outer media.

Controls

The endothelium was unremarkable in the nonoperated controls as well as most of the intimal surface of the sham-operated controls. In the latter, when sampling was directed to those occasional areas where light-microscopic examination showed some endothelial irregularity (in the sham-operated zone), EM examination showed recent cell death, cell lifting, and some cellular ulcers similar to those seen in the experimental group.

Discussion

It would be a highly complex undertaking to define, in terms of hydrodynamics, the precise changes in the quality and quantity of flow through a living stenotic artery; the instrumentation required is not applicable to small laboratory animals. However, the effects of stenosis on local blood flow can be stated in general terms.[12-14] As the blood enters the throat of the stenosis, its velocity increases, with a concomitant increase in shear stress and a *decrease* in lateral pressure on the intima; distal to the stenosis, the flow tends to become turbulent. In his experiments on the dog, Fry found that the endothelium was damaged and eventually abraded within 1 hour if exposed to a shear stress exceeding 379 ± 85 dynes/sq cm (acute

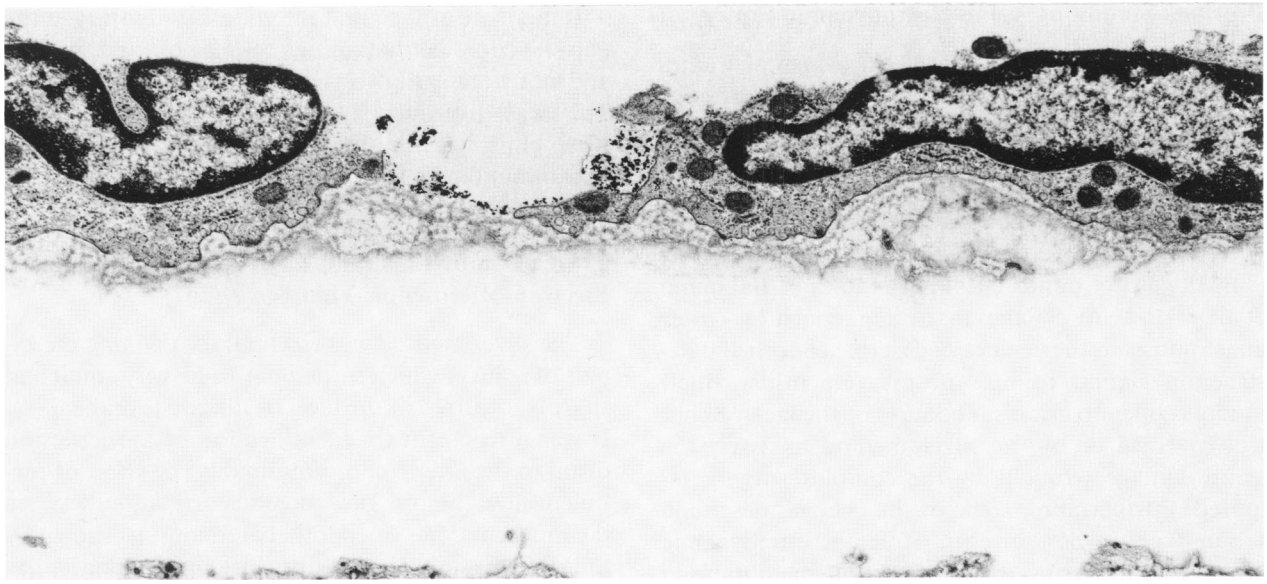


Figure 11 – Transverse clip, 60 minutes; just above the clip site (Thorotrast 1 time intravenously). A zone of injury between two cells, marked by Thorotrast; note loss of cytoplasm over the nucleus of both cells. (× 13,100)

yield stress).[2,10] In our model, we found empirically that if the cross-sectional area of the aorta was reduced by 75–80%, the endothelium was abraded within 3 minutes; we can therefore safely assume that we have exceeded the value of acute yield stress for the endothelium of the rat aorta (which may or may not be the same as that for the canine aorta).

The findings of normal distal pressure despite a 75–80% reduction in lumen area is in line with the known pathophysiology of arterial stenosis: the so-called *critical stenosis* (percentage reduction of lumen area below which there is a significant reduction of distal pressure and flow) has been reported to lie between 69% and 75%.[14]

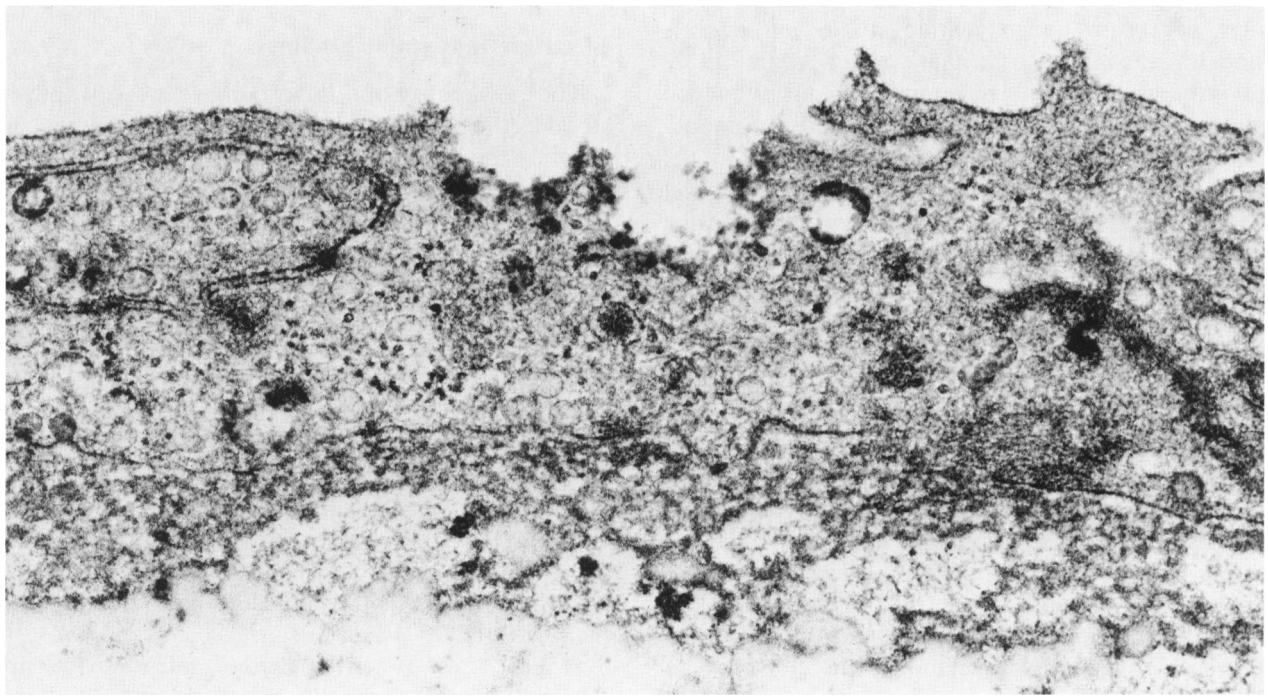


Figure 12 – Lateral clip, 60 minutes; below the clip site (HRP intravenously). Ulceration of the cell surface near a junction; note the electron density of exposed cytoplasm. (× 51,300)

Previous Studies of Acute Hemodynamic Injury

After the work of Fry[2] most experimental studies concentrated on chronic effects, using either arteriovenous fistulas[15-17] or stenosis by partial ligature[18-20] or an *in vitro* model.[21] Acute 30-minute changes were studied by scanning electron microscopy (SEM) in dog coronary arteries;[22] the results are compatible with ours, but transmission electron microscopy (TEM) was not done. The TEM studies of Baumann et al[23,24] on acute changes in the dog renal artery after anastomosis to the vena cava describe endothelial destruction similar to our most severe injury; their model is open to criticism because of the lack of control by SEM or by the silver method to assess the acute damage wrought by the complex surgery required. Furthermore, some of the changes described are unlikely to be the result of acute hemodynamic injury (multilayering of the basement membrane in 15 minutes, degeneration of the internal elastic lamina in 30 minutes). Spontaneous lesions were found in the aorto-iliac junction in young pigs;[25] by EM the changes were similar to the most advanced lesions seen in our study. Endothelial ulcers (at the cellular level) were not described, possibly because the lesions were of a more chronic nature.

Critique of Our Model

The pattern of blood flow through a stenosis depends heavily on the geometry of that stenosis. In view of future hydrodynamic studies on a scale model of the rat aorta, it might have appeared more advisable to adopt an axisymmetric stenosis,[14] which can be produced very simply by applying a nonoccluding ligature.[18-20,22,26,27] We discarded this model because a ligature would have caused longitudinal folding of the intima, resulting in compression of the endothelium between the folds and other cellular changes,[28] which would greatly complicate the interpretation of the endothelial damage induced by shear stress. The transverse clip has the drawback of causing, in the long run, atrophy of the media and therefore, possibly, endothelial changes secondary to this atrophy; the present study, limited to 1 hour, could scarcely be affected by this factor. Furthermore, the lesions obtained were the same as with the lateral clip, to which the criticism does not apply.

In a study of arterial stenosis, it is conceivable that artifacts may be caused by perfusion-fixation: the fixative could continue to induce shearing injury *post mortem*. This is why we removed the clip prior to perfusion in one group of rats and clamped the iliac arteries in another group to reduce the flow while maintaining a pressure of 110 mm Hg. These precautions, however, proved unnecessary, because the results were identical.

It might be argued that the endothelial injury at the clip site could be due to the presence of the clip itself and not necessarily to the narrowing of the lumen. This was disproven by our pilot experiments with wider clips, whereby endothelial damage was reduced to a minimum.

Correlation of Light and Electron-Microscopic Findings

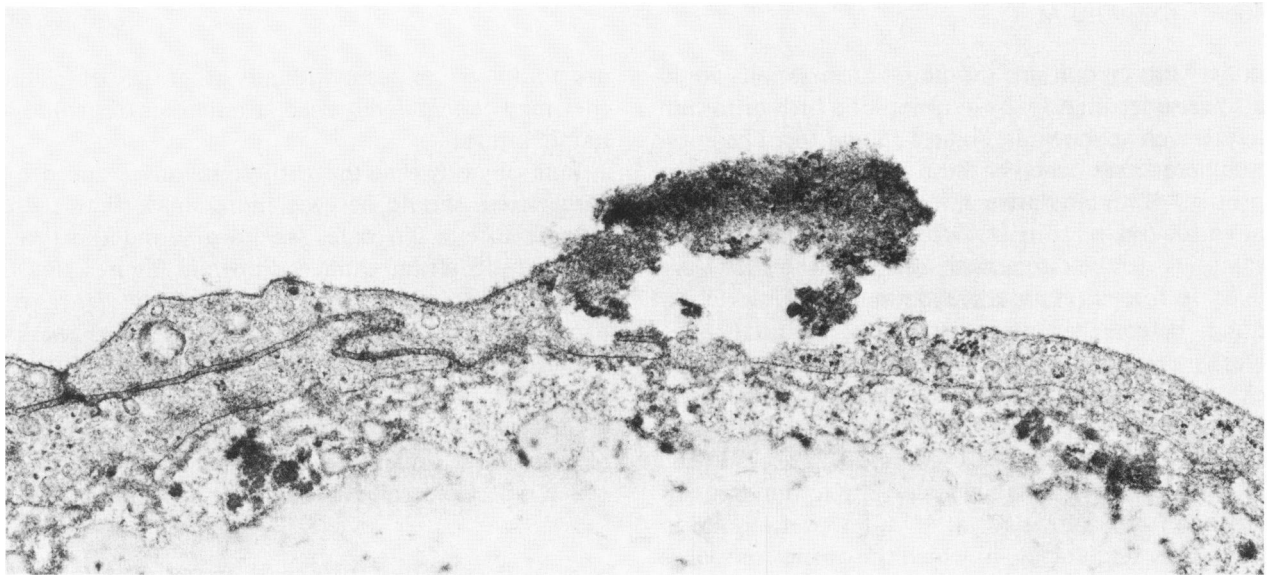
The silver method was essential for defining the extent of injury, which turned out to be very small and sharply localized at the level of the clip (Figures 2–4). It was also useful for identification of the jet lesion distal to the lateral clip, presumably the effect of turbulent flow (Figure 3). A precise correlation between the different types of endothelial lesions, as shown by the silver technique, and ultrastructural changes, has never been attempted; we are presently undertaking it. The silver method does not appear to demonstrate the intracellular lesions that we identified ultrastructurally as cellular ulcers.

Some of the cellular changes seen by EM were too discrete to be identified on histologic sections, even on 1-μ Epon sections (eg, the intercellular gaps and the cellular ulcers). The two changes easily visible by LM were endothelial lifting and denudation. All the changes observed by us were compatible with those described by Fry in dog aortas.[2,12]

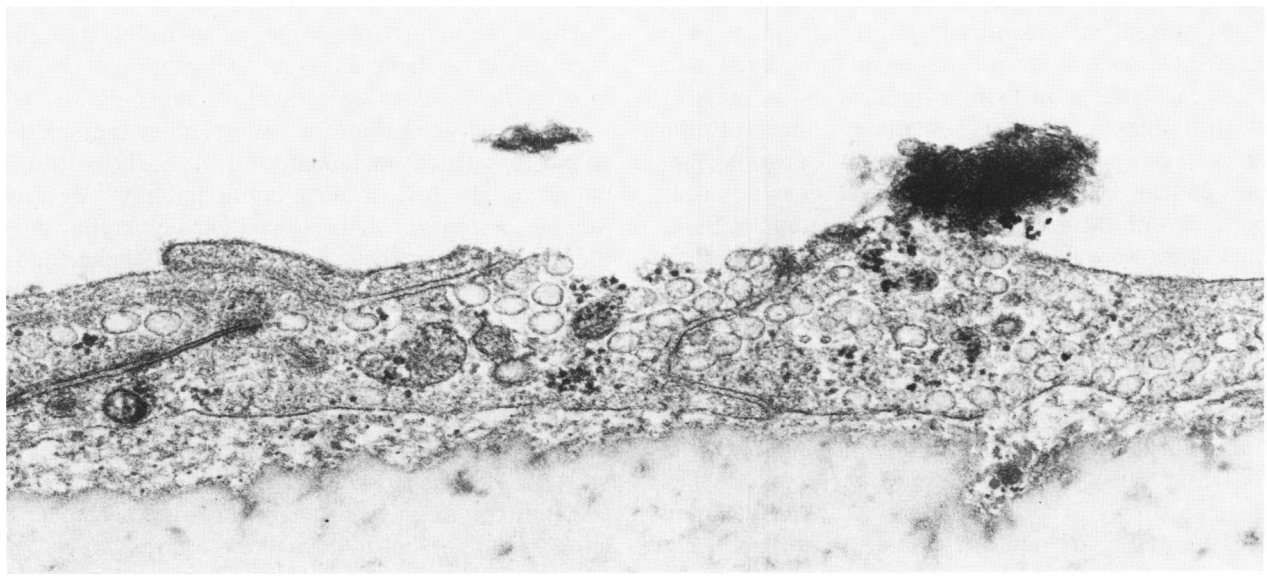
Electron-Microscopic Findings

The entire range of cellular damage is easily understood as the result of an increased shearing force. Ultimately the cells are torn off (Figure 5). In this process they show how tightly they are attached, because often, as they peel off, their plasma membrane remains attached to the basal lamina (Figure 5, inset). We have never seen a normal cell being torn off; the sequence appeared to be cell damage leading to cell desquamation. The latter was surely not an artifact, as proven by the adhesion of platelets and/or of Thorotrast particles to the denuded surface (Figure 6). Denudation of the intima was also observed by Gertz et al,[22] who submitted dog coronary arteries to 40–50% constriction with a silk ligature; 30 minutes later, by SEM, clusters of platelets had become attached. Adhesion of scattered platelets was found also in spontaneously denuded patches of intima in the pig aorta.[25]

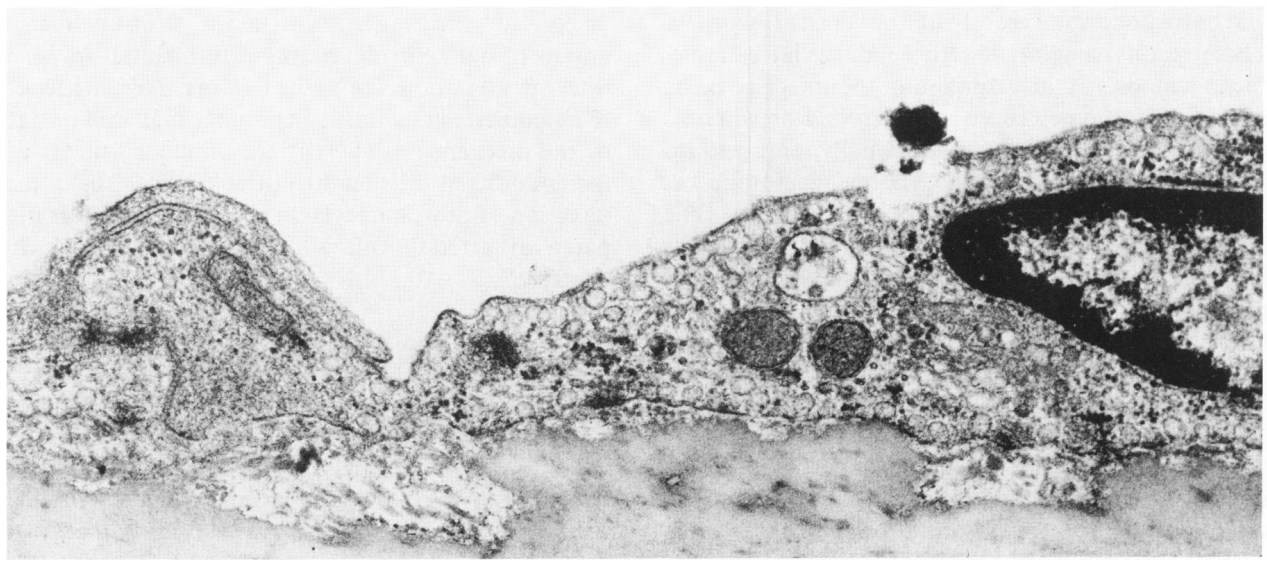
If shear stress of a given degree is adequate to strip off whole endothelial cells, a lesser degree of shear should erode the surface of the cells without removing them altogether. This was precisely what was found; the *cellular ulcers* (Figures 12–15) consisted of a focal loss of cell membrane and of a thin layer of



13



14



15

Figure 13 – Lateral clip, 60 minutes; below the clip site (HRP intravenously). Cellular ulcer near junction; the lifted portion of cytoplasm is electron-opaque. (× 29,700) **Figure 14** – Lateral clip, 60 minutes; below the clip site (Thorotrast 4 times intravenously). The dense material is probably injured cytoplasm which was torn from the surface of a cell creating an ulcer. (× 51,300) **Figure 15** – Lateral clip, 60 minutes; above the clip site (Thorotrast 4 times intravenously). Cellular ulcer with overlying dense material. (× 40,500)

underlying cytoplasm. (Some of these lesions could also arise secondarily by avulsion of a bleb or myelin figure such as shown in Figures 15 and 16.) The question here arises, whether these abrasions could be the product of manipulative damage. The answer is negative for five reasons: 1) Mechanical trauma during sampling can be discounted, because the aortas were fixed *in toto* in glutaraldehyde and postfixed in osmium before they were sampled; occasional tissue cracks at the edge of the blocks produced during the chopping of fixed tissue were easily recognized as artifacts and lacked the characteristics listed below. 2) Cellular ulcers are very common in the stenotic segments, whereas we never saw any in hundreds of blocks of normal rat aorta;[11,29] a few were found in sham-operated aortas, in which the aortic contour is briefly distorted during the operative procedure. 3) The exposed cytoplasm usually shows an increase in electron density (Figure 13), suggesting a reactive or secondary change in living cytoplasm. 4) A mass of electron-dense material is sometimes attached to the margins or appears to float free over the erosion (Figures 13–15). We interpret it as denatured cytoplasm, perhaps with the addition of plasma protein. 5) Similar lesions were found in a study of chronic stenosis to be published shortly.[30]

Assuming that the cellular ulcers were "open doors" into the cell, we probed their permeability to Thorotrast and to HRP. The relatively large particles of colloidal thorium dioxide did not penetrate; with horseradish peroxidase the result was equivocal: the marginal density that sometimes occurred was not distinguishable from denatured cytoplasm (Figure 12). However, the enzyme did penetrate massively into cells that, from the appearance of their nuclei and mitochondria, were "dying" or "dead" (Figure 9). These results suggest that the endothelial cell succeeds temporarily in maintaining the integrity of its internal environment even when eroded or when its margin is severely damaged; eventually, and perhaps suddenly, the barrier of the plasma membrane becomes diffusely permeable to large molecules. The penetration of HRP into "dead" or "injured" cells has been previously noticed in the intestinal lining,[31,32] in mouse peritoneal cells,[33] and in ischemic myocardial fibers.[34]

As far as we know, the cellular ulcers have not been described before. However, Fry did postulate "a progressive erosion of cell substance"[12] and actually described, by light microscopy, an early "decrease in sharpness of the cytoplasmic border."[2] One cellular ulcer, although not mentioned, appears in an electron micrograph from a study of spontaneous aortic damage in the rabbit (Figure 19).[35] This is evidence that cellular ulcers can occur spontaneously in areas of disturbed flow, and not only in the rat. Since they are not known to occur with any other type of insult, they may be tentatively used as a marker of hemodynamic injury.

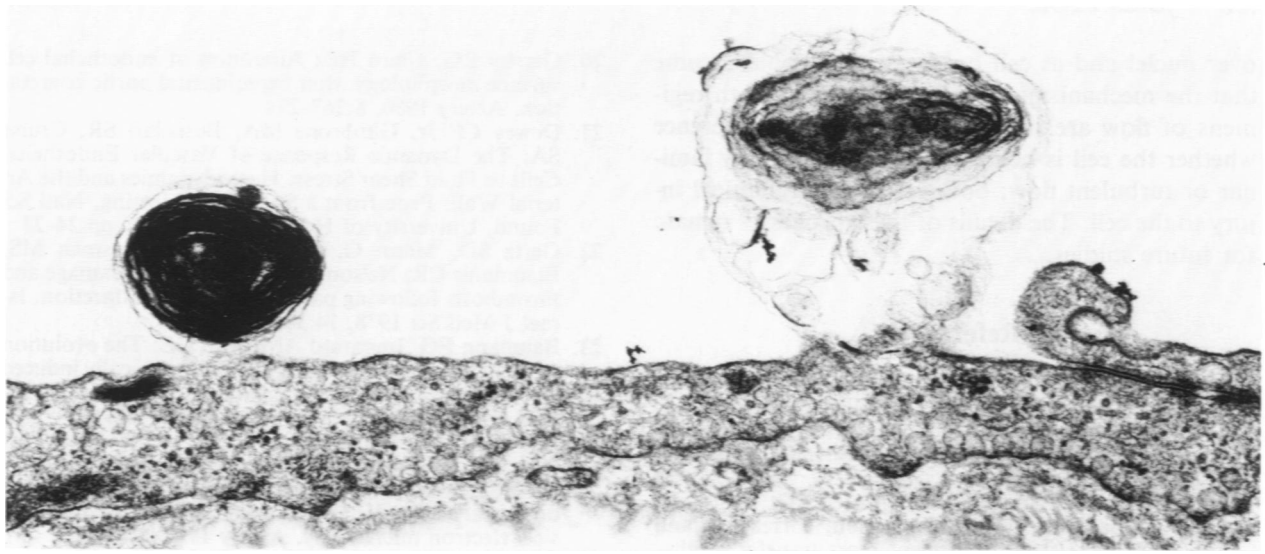
It is obvious that the earliest lesions induced by shear stress should be even more subtle than these cellular ulcers. To date, we have found the three types of membrane changes shown in Figures 16–18: they amount to different forms of *myelin figures* of decreasing complexity. These membrane changes occur also in controls, but certainly not as frequently as in the stenotic aorta. It was not surprising to see these milder lesions also in controls, since they presumably represent the normal attrition of the endothelial cells, which is accelerated in areas of increased shear stress.

Sites of Predilection for Shear Stress Injury

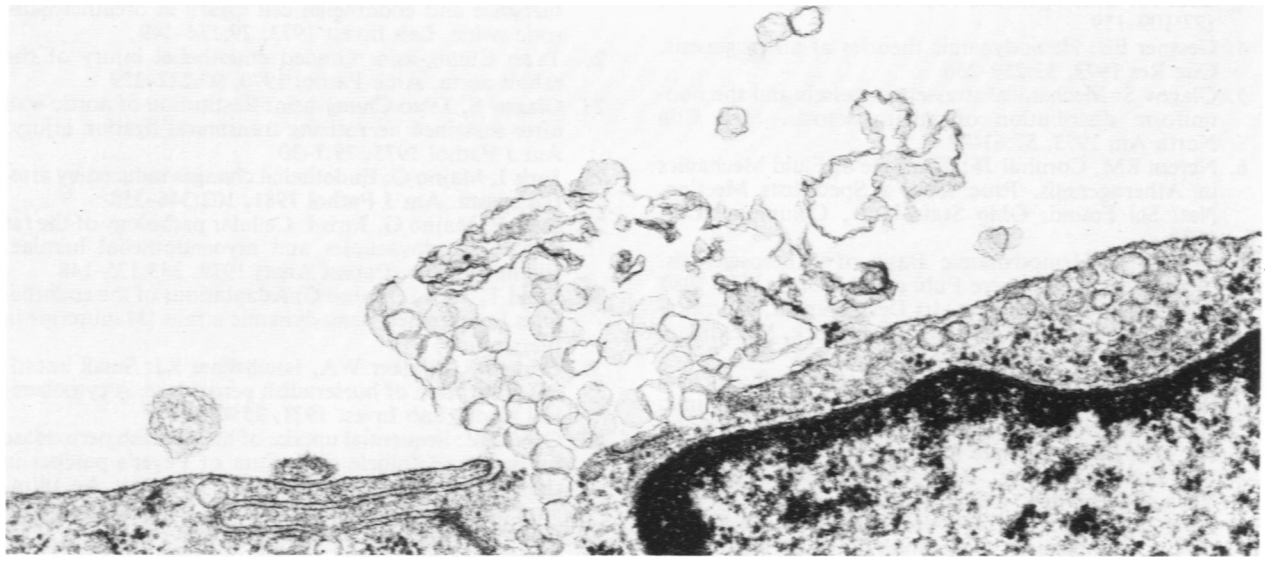
The three main target zones of hydrodynamic injury appear to have clear-cut explanations. 1) The *tops of the intimal ridges* are presumably exposed to higher shear stress than the valleys, since they correspond to a narrower lumen, and thus—in hydrodynamic terms—to a thinner boundary layer. 2) The *junctional areas* probably owe their vulnerability to the fact that the margin of one or both of the endothelial cells is often extended by a flap, a common sight by electron microscopy. The hemodynamic shearing forces (and conceivably also the increased drag of turbulent flow) will increase the tendency of the cell border to be lifted, with subsequent lifting or disruption of the cell itself. 3) The nuclear bulge. It is odd that the nucleus should become a liability to the cell, but the frequency of erosions over the nucleus left no doubt. Like rounded stones on the bottom of a stream, *nuclear bulges* also represent preferential targets for shearing forces. In the electron micrographs of our controls, endothelial thickness was 3–4 times greater at the site of the nucleus. This impact of the bloodstream on the nuclear bulge may well be one of the mechanisms whereby the elongated nuclei of the endothelial cells tend to orient themselves in the direction of flow, and even to reorient themselves if a patch of arterial wall is artificially rotated by 90 degrees.[36]

Shear Stress Versus Turbulent Flow

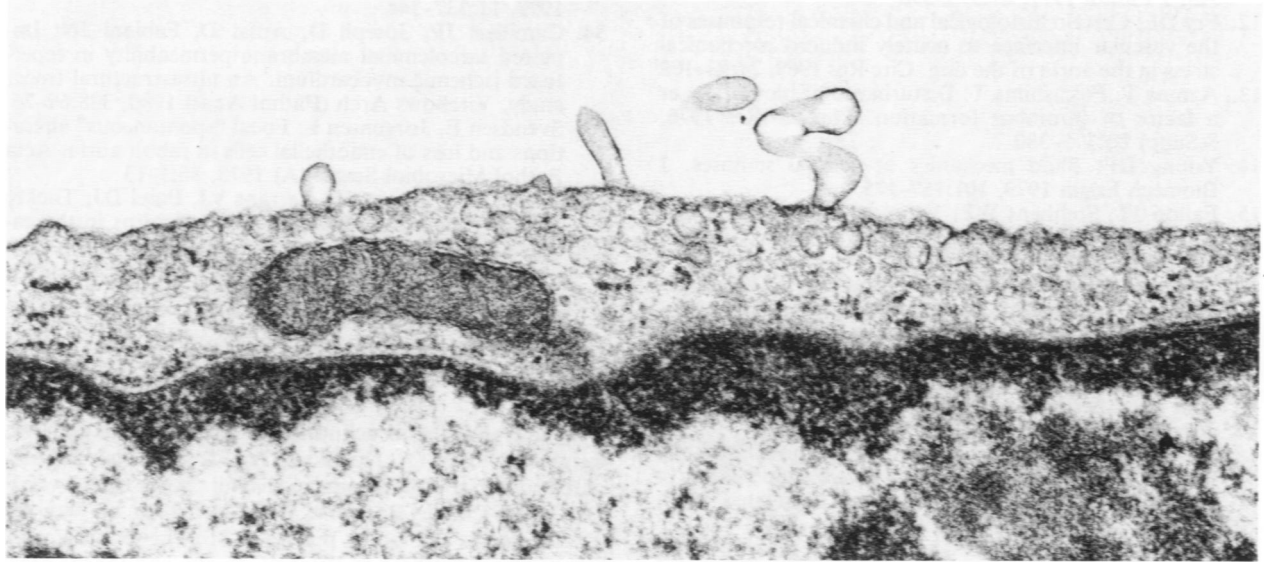
In his studies on the dog, Fry found indistinguishable histologic changes in the region of high shear stress and in the efflux region of low mean shear stress but high turbulence.[2] In our lateral clip model, lesions were more severe in the area of presumed high shear (the throat of the stenosis) and milder in the area presumably exposed to turbulent flow (jet lesion). However, the quality of the lesions was the same (cellular erosions, gaps, prevalence of lesions



16



17



18

Figure 16 – Transverse clip, 60 minutes; clip site (Thorotrast 4 times intravenously). Complex myelin figures arising from the endothelial surface. (× 41,800) **Figure 17** – Lateral clip, 60 minutes; below the clip site (Thorotrast 4 times intravenously). Bleb filled with microvesicles. (× 41,800) **Figure 18** – Transverse clip, 60 minutes; just below the clip site (Thorotrast 4 times intravenously). Simple tubular myelin figures arising from the endothelial surface. (× 51,300)

over nuclei and at cell borders); one would assume that the mechanisms of cellular injury in both regimens of flow are similar.[2] It makes little difference whether the cell is being excessively tugged by laminar or turbulent flow; both result in mechanical injury to the cell. The details of these processes remain for future studies.

References

1. Schwartz CJ, Mitchell JRA: Observations on localization of arterial plaques. Circ Res 1962, 11:63-72
2. Fry DL: Acute vascular endothelial changes associated with increased blood velocity gradients. Circ Res 1968, 22:165-197
3. Caro CG, Fitz-Gerald JM, Schroter RC: Atheroma and arterial wall shear. Observation, correlation and proposal of a shear dependent mass transfer mechanism for atherogenesis. Proc Roy Soc Lond B 1971, 177:109-159
4. Gessner FB: Hemodynamic theories of atherogenesis. Circ Res 1973, 33:259-266
5. Glagov S: Mechanical stresses on vessels and the nonuniform distribution of atherosclerosis. Med Clin North Am 1973, 57:63-77
6. Nerem RM, Cornhill JF: The Role of Fluid Mechanics in Atherogenesis. Proc from a Specialists Meeting, Natl Sci Found, Ohio State Univ, Columbus, OH, 1978
7. Texon M: Hemodynamic Basis of Atherosclerosis. New York, Hemisphere Publ and McGraw-Hill, 1980
8. Altschul R: Endothelium. Its Development, Morphology, Function and Pathology. New York, MacMillan, 1954
9. Gottlob R, Zinner G: Ueber die regeneration geschaedigter endothelien nach hartem und weichem trauma. Virchows Arch (Path Anat) 1962, 336:16-32
10. Fry DL: Hemodynamic forces in atherogenesis, Cerebrovascular Diseases. Edited by P Scheinberg. New York, Raven Press, 1976, pp 77-95
11. Joris I, Majno G: Cell-to-cell herniae in the arterial wall: I. Pathogenesis of vacuoles in the normal media. Am J Pathol 1977, 87:375-398
12. Fry DL: Certain histological and chemical responses of the vascular interface to acutely induced mechanical stress in the aorta of the dog. Circ Res 1969, 24:93-108
13. Azuma T, Fukushima T: Disturbance of blood flow as a factor of thrombus formation. Thromb Res 1976, 8(Suppl II):375-380
14. Young DF: Fluid mechanics of arterial stenoses. J Biomech Engin 1979, 101:157-175
15. Fallon JT, Stehbens WE: Venous endothelium of experimental arteriovenous fistulas in rabbits. Circ Res 1972, 31:546-556
16. Stehbens WE: Haemodynamic production of lipid deposition, intimal tears, mural dissection and thrombosis in the blood vessel wall. Proc R Soc Lond B 1974, 185:357-373
17. Stehbens WE: The ultrastructure of the anastomosed vein of experimental arteriovenous fistulae in sheep. Am J Pathol 1974, 76:377-400
18. Reidy MA, Bowyer DE: Scanning electron microscopy of arteries. The morphology of aortic endothelium in haemodynamically stressed areas associated with branches. Atherosclerosis 1977, 26:181-194
19. Reidy MA, Langille BL: The effect of local blood flow patterns on endothelial cell morphology. Exp Mol Pathol 1980, 32:276-289
20. Gerrity RG, Naito HK: Alteration of endothelial cell surface morphology after experimental aortic coarctation. Artery 1980, 8:267-274
21. Dewey CF Jr, Gimbrone MA, Bussolari SR, Cruise SA: The Dynamic Response of Vascular Endothelial Cells to Fluid Shear Stress. Hemodynamics and the Arterial Wall. Proc from a Specialists Meeting, Natl Sci Found, University of Houston, TX, 1980, pp 24-27
22. Gertz SD, Merin G, Pasternak RC, Gotsman MS, Blaumanis CR, Nelson E: Endothelial cell damage and thrombosis following partial myocardial infarction. Israel J Med Sci 1978, 14:384-388
23. Baumann FG, Imparato AM, Kim GE: The evolution of early fibromuscular lesions hemodynamically induced in the dog renal artery: I. Light and transmission electron microscopy. Circ Res 1976, 39:809-827
24. Baumann FG, Imparato AM, Kim GE, Yoder M, Grover-Johnson N: A study of the evolution of early fibromuscular lesions hemodynamically induced in the dog renal artery: II. Scanning and correlative transmission electron microscopy. Artery 1978, 4:67-99
25. Gutstein WH, Farrell GA, Armellini C: Blood flow disturbance and endothelial cell injury in preatherosclerotic swine. Lab Invest 1973, 29:134-149
26. Ts'ao Chung-hsin: Graded endothelial injury of the rabbit aorta. Arch Pathol 1970, 90:222-229
27. Glagov S, Ts'ao Chung-hsin: Restitution of aortic wall after sustained necrotizing transmural ligation injury. Am J Pathol 1975, 79:7-30
28. Joris I, Majno G: Endothelial changes induced by arterial spasm. Am J Pathol 1981, 102:346-358
29. Stetz E, Majno G, Joris I: Cellular pathology of the rat aorta: Pseudovacuoles and myoendothelial herniae. Virchows Arch (Pathol Anat) 1979, 383:135-148
30. Zand T, Joris I, Majno G: Adaptations of the endothelium to increased hemodynamic strain (Manuscript in preparation)
31. Cornell R, Walker WA, Isselbacher KJ: Small intestinal absorption of horseradish peroxidase. A cytochemical study. Lab Invest 1971, 25:42-48
32. Owen RL: Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: An ultrastructural study. Gastroenterology 1977, 72:440-451
33. Geyer G, Schmidt H-P, Biedermann M: Horseradish peroxidase as a label of injured cells. Histochem J 1979, 11:337-344
34. Camilleri JP, Joseph D, Amat D, Fabiani JN: Impaired sarcolemmal membrane permeability in reperfused ischemic myocardium. An ultrastructural tracer study. Virchows Arch (Pathol Anat) 1980, 388:69-76
35. Svendsen E, Jørgensen L: Focal "spontaneous" alterations and loss of endothelial cells in rabbit aorta. Acta Pathol Microbiol Scand (A) 1978, 86:1-13
36. Flaherty JT, Pierce JE, Ferrans VJ, Patel DJ, Tucker WK, Fry DL: Endothelial nuclear patterns in the canine arterial tree with particular reference to hemodynamic events. Circ Res 1972, 30:23-33

Acknowledgments

It is a pleasure to acknowledge the generous and very helpful advice of Dr. Donald L. Fry. John J. Nunnari provided superb technical assistance in all phases of this work. Dr. Marie-Claude Badonnel expertly helped us with the measurements of blood pressure. For the photographic prints we are indebted to Peter W. Healy, and for the preparation of the manuscript to Jane M. Manzi.