Transport in rat vessel walls. I. Hydraulic conductivities of the aorta, pulmonary artery, and inferior vena cava with intact and denuded endothelium

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Shou, Yixin, Kung-ming Jan, and David S. Rumschitzki. Transport in rat vessel walls. I. Hydraulic conductivities of the aorta, pulmonary artery, and inferior vena cava with intact and denuded endothelium. Am J Physiol Heart Circ Physiol 291: H2758–H2771, 2006. First published May 26, 2006; doi:10.1152/ajpheart.00610.2005.—In this study, filtration flows through the walls of the rat aorta, pulmonary artery (PA), and inferior vena cava (IVC), vessels with very different susceptibilities to atherosclerosis, were measured as a function of transmural pressure (ΔP), with intact and denuded endothelium on the same vessel. Aortic hydraulic conductivity (Le) is high at 60 mmHg, drops ~40% by 100 mmHg, and is pressure independent to 140 mmHg. The trends are similar in the PA and IVC, dropping 42% from 10 to 40 mmHg and flat to 100 mmHg (PA) and dropping 33% from 10 to 20 mmHg and essentially flat to 60 mmHg (IVC). Removal of the endothelium renders Le(ΔP) flat: it increases Le of the aorta by ~75%, doubles Le of the PA, and quadruples Le of the IVC. Specific resistance (1/Le) of the aortic endothelium is ~47% of total resistance; i.e., the endothelium accounts for ~47% of the ΔP drop at 100 mmHg. The PA value is 55% at >40 mmHg, and the IVC value is 23% at 10 mmHg. Le of the intact aorta, PA, and IVC are order 10^-8 –10^-7, and 5 × 10^-7 cm²/s·mmHg⁻¹, and wall thicknesses are 145.8 μm (SD 9.3), 78.9 μm (SD 3.3), and 66.1 μm (SD 4.1), respectively. These data are consistent with the different wall structures of the three vessels. The rat aortic Le data are quantitatively consistent with rabbit Le(ΔP) (Tedgui A and Lever MJ. Am J Physiol Heart Circ Physiol 247: H784–H791, 1984; Baldwin AL and Wilson LM. Am J Physiol Heart Circ Physiol 264: H26–H32, 1993), suggesting that intimal compression under pressure loading may also play a role in Le(ΔP) in these other vessels. Despite very different driving ΔP, nominal transmural water fluxes of these three vessels are very similar and, therefore, cannot alone account for their differences in disease susceptibility. The different rates of macromolecular tracers convected by these water fluxes into the walls of these vessels may account for this difference. Atherosclerosis is a disease that develops in large arteries and valves (31, 45). It begins with low-density lipoprotein (LDL) cholesterol delivery into the vessel wall and its accumulation there. Blood-borne monocytes enter the arterial intima in regions with high subendothelial lipid concentration, becoming macrophages that attempt to scavenge the extracellular cholesterol (41). When overwhelmed, they form foam cells. Lipid and necrotic cells accumulate to comprise the earliest lesions (33, 34). Such lesions mature and thicken the arterial wall, which, eventually, can compromise the cross section for blood flow and increase the possibility of blockage (41), rupture, or clot formation. Atherosclerosis normally occurs in the large arteries and/or in the aortic valve but not in the pulmonary artery (PA) or in veins. In humans, the PA is vulnerable only in the presence of pulmonary hypertension (14). [Curiously, some data suggest that the rabbit PA appears to be similar to the rabbit aorta in its proclivity toward developing atherosclerosis (32), and a comparison of the PA of the rabbit with that of an animal without this unusual susceptibility is worthy of study.] Veins normally do not develop atherosclerosis. However, when veins such as the saphenous vein, which is frequently used in coronary bypass procedures, are placed in arterial conditions, i.e., high pressure, atherosclerosis often develops (27), along with vessel remodeling. After 6–12 yr, 71% of such vein grafts develop atherosclerosis, and the structures of the deposits resemble arterial fatty streaks (5). If the triggering events for the disease are lipid transport across the vessel endothelium and its subendothelial accumulation, it is natural to wonder whether these processes are radically different in resistant and disease-prone vessels. One of the first issues that one must address to develop an understanding of the mass transport in these vessels is whether such transport is driven by diffusion and/or convection. In previous mass transport studies of the aorta, rats were injected with a macromolecular tracer, such as horseradish peroxidase (HRP), and were killed after different circulation times (11, 35). In an examination of the animals’ aortas en face, HRP was found to traverse the endothelium in rare localized spots, rather than uniformly. Measuring the sizes of the HRP spots as a function of tracer circulation time, these investigators found tracer spot growth that was sufficiently fast as to be consistent only with a convection-dominated transport mechanism; for any reasonable value of the diffusivity, these data were not consistent with a diffusion-dominated process. Huang et al. (17) and Yuan et al. (48) mathematically modeled how macromolecules such as HRP, albumin, and LDL leak through the rare, widened interendothelial junctions, while water passes through all junctions. Huang et al. developed a new convection-diffusion transport model that divided the subendothelial space into the subendothelial intima and media and, with strong ultrastructural evidence, postulated that the intima was much more porous than the media and, thus, offered a much smaller hydrodynamic resistance than the media. With the parameters determined independently, the resulting convection-dominated transport theory predicted the observed growth rate of HRP tracer spots. Thus an understanding of water flow in the artery wall is central.

Again, if lipid transport and accumulation are indeed the keys to triggering early atherosclerosis, a detailed understand-
ing of the relevant transport processes in each vessel might explain the differences in vessel susceptibilities. This study asks the following question: Just how similar are the water filtration processes that drive convective lipid transport in the aorta to those in the PA and the inferior vena cava (IVC)? Our initial hypothesis is that they are different and correlate with the differences in vessel susceptibilities. The PA is normally exposed to a low transmural pressure (ΔP, ∼16 mmHg) and has lower Po2 (40 mmHg) than the aorta (100 mmHg). The wall of the normal PA is thinner than the wall of the aorta: 78.9 μm (SD 3.3) vs. 145.8 μm (SD 9.3) (see RESULTS). The basic structure of the PA is similar to that of the aorta: it consists of a monolayer of endothelial cells with an associated intima and tunica media, which has a repeated structure of elastic and smooth muscle cells (SMCs). These two layers are separated by an internal elastic lamina (IEL) with numerous fenestrae. The IVC has an even lower ΔP (5 mmHg) than the PA and the same Po2 as the PA. Because the vein is exposed to very low pressures, it has a thinner wall than the arteries (25): ∼66.1 μm (SD 4.1) for rat IVC (see RESULTS). The wall structure of veins is different from that of arteries. The vein wall has a larger lumen, fewer SMCs, a lower elastic tissue content, and a much higher distensibility, thereby providing much lower hydraulic resistance than arterial walls (30).

Because of these differences among vessels, it is important to assess whether convection plays as important a role in the mass transport in these lower-pressure vessels as in the large arteries. ΔP is the driving force (per unit area) for convection, and the hydraulic conductivity (Lp) is the critical transport parameter that provides a relation between the water flux and the pressure driving force. If the combination of the measured Lp and ΔP, not Lp alone, for different vessels and conditions leads to transmural flows of magnitudes that correlate with vessel susceptibility to atherosclerosis, this would support the proposition that convective lipid transport is a leading determining factor in the prelesion triggering events. We have noted the lower driving pressures in the PA and IVC and now need to measure their Lp values to assess the roles of convection in these vessels. In particular, if the product of the vein’s very low driving ΔP and its Lp leads to negligible transmural water flow, convection would not be important and diffusion would likely dominate macromolecular mass transport in these vessels.

Yamartino et al. (46) found a low Lp in a slab of rabbit aorta stuck to a rigid porous background. Vargas et al. (42) maintained the rabbit aorta’s cylindrical shape and used a weighing method to measure the transmural flow rate. Tedgui and Lever (38) calculated the transmural flow at 70 and 180 mmHg on the same vessel by measuring the velocity of an air bubble in a horizontal catheter connecting the pressurized water source to the rabbit’s isolated vessel lumen. Baldwin and Wilson (2) extended the pressure range of this experiment on rabbit aorta from 50 to 150 mmHg in 25-mmHg increments. No one appears to have applied this technique to vessels other than the aorta. Both groups found a high Lp at low pressure (the lowest pressure measured by Baldwin and Wilson had a uniquely large error bar), which dropped sharply (44%) by 75 mmHg and then remained pressure independent. This low-pressure high Lp disappeared on nitric oxide (NO) inhibition (2). Mechanical endothelial denudation increased Lp significantly and appeared to make it pressure insensitive. Dhar et al. (13), on the other hand, found that Lp of chemically denuded rabbit aorta decreased with increasing ΔP, although they subjected each vessel to only a single pressure. Finally, Tedgui and Lever used their measurements to infer the Lp of the aortic endothelium and to calculate the percentage of the wall’s total resistance to flow due to the endothelium. Tarbell (36), working in cultured bovine aortic endothelial cells, addressed Lp of the endothelium directly. They found a transient increase in Lp with sudden pressure change that relaxed to background in ∼1 h.

McCandless et al. (29) found a very high Lp (20.1 ± 5.2 × 10−5 cm·s−1·mmHg−1) for cultured sheep PA endothelial cells. Their method may have suffered from large leaks of water around the chamber’s edges, which may explain their reported high value. Moreover, measurement on a cultured monolayer provides Lp of the endothelium and not the other components of the wall. Vargas et al. (43), using dog vena cava, measured Lp by the volume-clamp method. The vessel was hung from a force transducer that was calibrated to measure weight. The volume change of the vessel was constantly monitored. The weight loss was transmitted to a polygraph and a controller; the feedback loop then injected fluid into the vessel lumen to maintain the lumen at constant volume.

By the conservation of mass, the rate of the fluid injected into the vessel equaled the rate of fluid loss through the vessel wall.

To explain the behavior of rabbit aortic Lp with ΔP and with endothelial denudation, Kim and Tarbell (22) developed a theory based on whole wall compaction, whereas Huang et al. (16) proposed a new mathematical model that invoked the ultra-high intimal porosity to restrict pressure compaction to the intima. They predicted that rising pressure compressed the intima, thereby decreasing its void space and slightly increasing its resistance; the compressed endothelium could block IEL fenestrae, altering the water flow and increasing its resistance much more significantly. [Compression of the surface glycocalyx under pressure is likely to be less severe and, therefore, even less significant than compression of the intimal matrix, despite the apparent sharp rise in Lp of frog mesenteric capillaries on enzymatic degradation of its endothelial surface glycocalyx layer (1).] At higher ΔP, the intima is supported by the stiffer collagen and does not compress further; therefore, Lp becomes insensitive to pressure. Huang et al. (18) experimentally confirmed this predicted intimal compaction behavior with increasing ΔP on the rat. This mechanism is also consistent with the disappearance of the high Lp at 50 mmHg (and no change at other pressures) on NO inhibition. With NO, the wall is relaxed, and a low ΔP pushes the endothelium against a media that has some elasticity or give and, thus, does not completely compress the intima; removal of NO stiffens the wall and fosters complete compression at the lower ΔP of 50 mmHg. Vessel-to-vessel variation in the elasticity of the media might then account for the large error bars for the intact vessel at this apparent borderline pressure.

In this study, we examine rat aorta, PA, and IVC. We measure Lp as a function of ΔP for the same vessel with and without an intact endothelium to investigate the extent of similarities or differences among the vessels. Of primary importance is whether transmural convection is very different and, thereby, transports different amounts of macromolecules into the walls of the PA and the IVC than into the aorta. The detailed measurements of Lp for the aorta will complete a
consistent set of data, all taken from the rat, for the model of Huang et al. (16). It will also demonstrate whether the behavior of $L_p$ with pressure and endothelial denudation in rabbit aorta, attributed to intimal compression, also occurs in rat aorta. Although rats do not naturally become atherosclerotic because of their low blood cholesterol, they can develop atherosclerosis when fed an atherogenic diet (44).

**METHODS**

*Experimental Setup and Measurement Technique*

A mercury sphygmomanometer controlled the pressure of a large-volume air reservoir that was connected to a solution reservoir. A three-way connector joined this solution reservoir, a 1-ml syringe, and a horizontal 120-mm Tygon catheter (0.5 mm ID) mounted on a finely graduated ruler. The catheter was inserted into the isolated aorta, which was closed off and immersed in a bathing solution (Fig. 1A). The syringe injected an air bubble into the catheter, and after its velocity reached steady state (∼30 min), its position was recorded five to six times at 5-min intervals. Solution incompressibility implies that the volume (bubble velocity × catheter cross section) that it traces per unit time equals the flow rate through the vessel wall. All chemicals were obtained from Sigma (St. Louis, MO) unless otherwise stated. The solution entering the vessel contained 4% (wt/vol) BSA (fraction V, Fisher Scientific) in PBS, 10−3 M NaNO3 to reduce SMC contraction (2, 4), and 0.03% (wt/vol) trypan blue, which serves two purposes. 1) Blue dye in the adventitia means the vessel has a leak (the dye’s intensity relative to the clear bathing solution made even minute leaks easily visible), which we tried to secure with string. If this was not successful, we discarded the vessel. 2) Trypan blue is a vital stain that penetrates the membrane of nonviable cells (2, 38). The bathing solution was identical, except for the absence of trypan blue, so as to keep the osmotic pressures on both sides of the wall the same. From its molecular size (19), we estimated the hydrodynamic radius of trypan blue to be ∼1 nm and, thus, its reflection coefficient to be ∼0.064 (28), as opposed to albumin’s value of 0.8–1.0 (21, 24). Thus the osmotic pressure contribution of the dye was negligible.

As in all previous similar studies (2, 38), we used mechanical calipers (accurate to ±0.1 mm) to measure diameters and lengths from tie to tie of pressurized excised vessels. This method has the advantage that it does not destroy the vessel and, thus, allows multiple measurements on the same vessel at a series of pressures with and without endothelium. To assess the precision and reliability of this method relative to a method that requires destruction of the vessel, we measured the diameters at three different points along the length of two vessels (an aorta at 100 mmHg and an IVC at 60 mmHg), each three times. We fixed each vessel under pressure, sliced it open along its axis, flattened it on a glass slide, viewed it, took images under the microscope, and measured the edge-to-edge distance along its outer surface at 30 points along its length.

All protocols were approved by the Institutional Animal Care and Use Committee.

*Transmural Flow Rate and Vessel Dimensions*

**Aorta.** Six healthy male Sprague-Dawley rats (250–350 g body wt) on a normal diet were anesthetized with 1% pentobarbital sodium (15 mg/500 g rat ip) and injected with ∼0.5 ml of heparin (1,000 U iv) to prevent blood coagulation. A rodent respirator kept the rat ventilated. After opening the chest and carefully dissecting the fat, we exposed the aorta from the rest of the fat and connective tissue and ligated the first three pairs of intercostal arteries. The aorta was cannulated from the distal end, and the lumen side was rinsed with the PBS solution described above, pressurized at 100 mmHg, the normal aortic pressure, to prevent vessel collapse, which could damage the endothelium. The aorta was ligated at the proximal end and excised. The segment of the aorta was immersed in a solution bath as described above at 37°C. The solution bath was changed every 20 min, and air was continuously bubbled through it to keep the vessel oxygenated. We were able to successfully transfer six of eight rat aortas.

To begin the measurement, an air bubble was introduced into the horizontal catheter at low pressure; then the pressure was increased to the desired value. The experimental pressures ranged from 60 to 140 mmHg in 20-mmHg increments. At each pressure, we determined the flow rate through the vessel wall by monitoring the movement of the bubble’s front meniscus after it became steady. The outer dimensions (length and diameter) of the vessel were measured. The same vessel was deendothelialized by back-and-forth motion of a 2.5-mm-diameter Epon polymer tip on a glass rod that was inserted into the vessel. We recannulated, flushed, and tested the vessel for leaks before restarting the measurements at the same pressures used for the endothelium-intact preparation. At this point, the excised vessel had been outside the rat body for ∼10 h. After the flow rate measurements...
were completed, the vessel was fixed with 2% glutaraldehyde under pressure, stained with Harris’ hematoxylin, and examined under a light microscope to ensure that the endothelium had been totally removed. One Lp experiment was performed without NaNO3, with and without endothelium. The results were within the error range of those with this muscle relaxant.

PA. Twelve healthy male Sprague-Dawley rats (250–350 g body wt) fed a normal diet were used for this protocol: six for the pressure range 20–100 mmHg and six for the pressure range 10–40 mmHg. We began with the former. The procedure was similar to that for the aorta. After anesthesia, the femoral vein was cannulated with Tygon tubing and injected with heparin, and the rat was attached to a rodent respirator as described above. After opening the chest, we carefully dissected out the fat, exposed the PA, and ligated the left pulmonary branch near the pulmonary bifurcation. We cannulated the main pulmonary trunk through the right ventricle with a Tygon catheter and washed the luminal side of the vessel with the reservoir solution containing trypan blue pressurized to 20 mmHg to prevent vessel collapse. The animal was killed with an overdose of pentobarbital, and the right pulmonary artery was tied near the lung. After the vessel was removed, at which point 1–1.5 h had elapsed, the balance of the procedure, except the pressure range, was identical to that for the aorta.

A second set of six rats was used to measure Lp over the pressure range 10–40 mmHg. The procedure was identical, except that both, rather than just one, PA branches were taken at the root of the lung.

IVC. Ten healthy male Sprague-Dawley rats (250–350 g body wt) were used for the procedure for the IVC, which was similar to those described above, except we cannulated the vein through the right atrium with a catheter and again pressurized the vessel lumen at 20 mmHg. Although 20 mmHg is higher than the normal pressure of the vena cava, its dimensions hardly changed (see results) from their values at 10 mmHg. The animal was killed, and the vessel was tied at the diaphragm, removed, and submerged in PBS as described above. The rest of the procedure was as described above, except the pressure range was 10–60 mmHg for the IVC. Because a number of vessels sprang leaks at 50–60 mmHg, we succeeded with and without endothelium in four animals from 10 to 60 mmHg and in two animals with endothelium from 10 to 40 mmHg and in two animals with and two without endothelium from 10 to 40 mmHg. The pressure setup was not stable at lower pressures, such as 5 mmHg.

Structural Study

An overdose of 1% pentobarbital sodium was injected to kill the rat. The blood vessels of interest, i.e., PA, IVC, and aorta, were removed and fixed (at 0.5°C) with 1% and 2% (vol/vol) glutaraldehyde solution for 1 h each and then rinsed with PBS solution to wash out the fixative. The tissue was postfixed with 2% (vol/vol) osmium tetroxide (Ted Pella) for 90 min. After fixation, the tissue was rinsed three times (~1 min each) with distilled water, dehydrated with ethanol in series [30, 50, 75, 80, 90, and 100% (vol/vol)] and propylene oxide (Ted Pella), and, finally, infiltrated and embedded in Epon 12 (Ted Pella).

Using a glass knife with an ultramicrotome (model MT-1, Dupont), we sectioned the Epon blocks to a thickness of ~100–500 nm [purple reflection (6, 18)] and glued the sections to a glass slide with 2% (wt/vol) gelatin (Ted Pella). We then applied orcein stain to specifically highlight the elastic fibers in the vessel walls (18). The slides were dried and observed under light microscopy. The vessel wall thicknesses were measured using NIH ImageJ. For the aorta and PA, which contain large amounts of elastic tissue, we used a 0.4% (wt/vol) orcein solution (0.4 g of orcein dissolved in 100 ml of 70% ethanol to which 0.6 ml of concentrated HCl was added); for the IVC, which contains much less elastic tissue, we used a 1% orcein solution.

Calculations

For the aorta, the IVC, and the first population of PAs, we assume that the vessel is cylindrical; thus its outer surface area (AS) = π(OD)L, where OD is the outer diameter of the vessel and L is its length. The second set of extracted PAs included both pulmonary branches, forming a V shape, with the pulmonary trunk as the base. We calculated AS of the vessel by separating it into four parts. First, we calculated the area of each branch, as if the two branches had met in a V shape. Each of these areas is the product of the perimeter of the branch cross section π(OD)i (i = 1,2) and the average of the smaller and larger lengths [l + (l + Δl)/2, (i = 1,2), where the two branches would meet in a V at the far end of the vessel. To this we add AS of the trunk [π(OD)TR], from its far end to the point where it joins the branches. Finally, because the two branches meet the trunk before actually completing the V shape, we subtract the area near the tip of the V. We approximate this tip area as that of a cone of base diameter OD equal to that of the trunk (ODT) and of cone angle θ, where θ is the half angle of the branch bifurcation. Thus AS = π(OD)T[(l + l + Δl)/2 + π(OD)TR] − π(OD)T2/4sinθ (Fig. 1B). The bubble velocity V was determined as follows: V = 4πl/3t, where l is the distance the bubble travels in time t. Let ID be the inner diameter of the catheter. Because the solution is incompressible, the flow rate Q at steady state through the vessel wall equals that traced out by the bubble in the tube, i.e., Q = π(ID)2V/4. Q = Lp × AS × ΔP allowed us to determine Lp as function of ΔP. For the intact vessel, it represents the overall Lp or Lp(i), for the deendothelialized vessels, it represents Lp for the media + IEL or Lp(i+1). If, as is standard in the field (38, 43), one assumes that the specific resistance 1/Lp adds similar to linear resistors in series, one calculates Lp for the endothelium + intima or Lp(i+1) from 1/Lp(i+1) = 1/Lp(1+1) + 1/Lp(i+1). To calculate the percent resistance of the endothelium + intima, we take the average intact vessel Lp for a particular rat at a particular pressure and call it Lp(i), do the same for Lp(1+1), and then use this formula to calculate Lp(i+1) for that rat at that pressure. The fractional resistance of the endothelium (which, from the steady-state relation, equals the fraction of the pressure drop taken up by the endothelium) is [1/Lp(i+1) − 1/Lp(i)]/1/Lp(i), where 1/Lp is a resistance and the quantity enclosed in <> signifies an average over all the subject rats. As such, the average Lp values do not satisfy the defining relation for Lp(1+1).

Statistics

Paired Student’s t-tests were used to compare Lp with and without endothelium. The same method was used to compare the values at different AP of each Lp, OD, and AS of the vessels with and without endothelium. P < 0.05 was chosen as the criterion for statistical significance. Values are means (SD).

RESULTS

Accuracy of Caliper Measurements and Other Sources of Error

The en face measurements of the sliced-open aorta [2.61 mm (SD 0.01)] and IVC [2.88 mm (SD 0.03)] were in excellent agreement with the caliper measurements: 2.56 mm (SD 0.02) and 2.87 mm (SD 0.04), respectively. This level of accuracy is critical to the Lp measurements, because the diameter and length are smaller for the rat than for the rabbit aorta, for which our Lp technique was developed and previously used. Similarly, the bubble in the catheter moves more slowly in experiments on the smaller vessels. Fortunately, according to Bretherton’s classic theory (7), the frictional losses due to the
bubble motion in the catheter scale as the capillary number (the product of the bubble velocity and the fluid viscosity divided by the interfacial tension) to the two-thirds power. Our estimates show that this resistance is negligible ($-10^{-4}$ mmHg) compared with the other resistances in the system, even for the lowest pressures. Also, small holes in the vessel’s endothelium can cause a larger fractional change in the measured $L_p$ in vessels with smaller AS at the same $\Delta P$. This requires special vigilance in searching for leaks in rat vessels. These issues are the most critical for the rat PA, which is the smallest of the vessels. Finally, temperature control is important for reproducibility of vessel condition, and the measured temperature variation never exceeded 2–3°C.

**Structure**

The structure of the vessel wall plays an important role in determining its $L_p$. Figure 2A shows a cross section of the aorta stained by orcein, a specific stain that highlights/colors the elastic sheets in the aortic media red (black in Fig. 2). The gaps between elastic sheets that contain SMCs and proteoglycans are ~11.4 $\mu$m (SD 2.7), roughly the diameter of the SMCs. The aorta has a complete, 4.5-$\mu$m (SD 1.6)-thick IEL and 5.71-$\mu$m (SD 2.14)-thick continuous elastic layers. Total wall thickness is 145.8 $\mu$m (SD 9.3).

Transverse sections of the PA also show extensive orcein staining (Fig. 2B). The 3.4-$\mu$m (SD 1.9)-thick elastic sheets in

![Fig. 2](Image)

**Fig. 2.** Vessel wall structure: light-microscopic views of 100-nm-thick sections stained with orcein, which specifically highlights elastic tissue in red (shown here in black). A: aorta, with wall thickness of 145 $\mu$m at zero pressure. Note complete internal elastic lamina [IEL, thickness 4.6 $\mu$m (SD 1.6)] and continuous layers of elastic tissue (0.4% orcein stain). B: PA, with wall thickness of 78.9 $\mu$m (SD 3.3) at zero pressure. Note complete IEL [3.2 $\mu$m thick (SD 0.7)] and continuous wavy layers of elastic tissue (0.4% orcein stain). C: inferior vena cava (IVC), with wall thickness of 66.1 $\mu$m (SD 4.1) at zero pressure. Note incomplete IEL (broken darker line labeled IEL below lumen) and only sparse bits of elastic tissue throughout (1.0% orcein stain).
the PA media account for much of the elasticity of the artery. The largest space between two adjacent elastic layers is 14.9 μm (SD 2.9), which is again roughly the diameter of the SMCs. The elastic tissue and SMCs are the two major components of the media, in addition to proteoglycans and collagen. The wall thickness is ~78.9 μm (SD 3.3) at 0 mmHg. The thickness of the IEL is 3.2 μm (SD 0.7).

The IVC lives under a low physiological pressure of ~5 mmHg. The structure of the veins (Fig. 2C) is quite different from that of the PA and aorta. The vein has a thinner wall [66.1 μm (SD 4.1) at 0 mmHg], a discontinuous IEL, sparse elastic tissue (note very sparse orcein staining, despite higher orcein concentration), and fewer SMCs. Because the thickness of the IVC wall is only 66.1 μm (SD 4.1) and because it contains far less elastic tissue than the PA, we would expect it to present much less resistance to transmural water flow than even the PA.

**OD and AS**

The aorta expands dynamically with increasing pressure. The outer AS, normalized by its value at 60 mmHg, expands fairly linearly by ~42% from 60 to 140 mmHg ΔP (P < 0.05; Fig. 3A). Because the OD of the aorta expands only ~20% from 60 to 140 mmHg (P < 0.05), the balance of the area expansion is due to a change in the length of the vessel.
Removal of the aortic endothelium does not change these values significantly ($P > 0.05$).

The OD of the PA increases fairly linearly by 32.4% from 20 to 100 mmHg ($P < 0.05$). [It is not plotted for the Y-shaped vessels, although the OD varies little between adjacent pressures, e.g., from 80 to 100 mmHg ($P > 0.05$).] Again, because the length of the vessel also varies with changing ΔP, Fig. 3B plots the vessel’s absolute diameter and the ratio of its outer AS as a function of ΔP compared with its value at 20 mmHg [AS(20)]. The outer AS of the PA decreases monotonically from 20 mmHg to a maximum at 100 mmHg of ~1.65 times AS(20). AS increases 71% from 10 to 40 mmHg ($P < 0.05$). Before and after deendothelialization, the OD of the vessel displays a similar increasing trend, but the significance is lower ($P > 0.05$) with deendothelialization. The deendothelialized diameter increases ~26.7% from 20 to 100 mmHg ($P < 0.05$). The difference between the outer AS of the vessel with and without its endothelium is within the error bars of the curves ($P > 0.05$) at each ΔP over the entire range 10–100 mmHg, and the difference between the two groups of rats is statistically insignificant ($P > 0.05$). Because the endothelium comprises a very small fraction of the vessel wall, it is no surprise that removal of the endothelium has no effect on AS, despite some wall hydration.

Limitations in the accuracy of our pressure setup dictated a lowest ΔP of only 10 mmHg for the IVC, rather than its physiological value of ±5 mmHg. For the four rats measured over the entire pressure range with and without endothelium, Fig. 3C shows that the OD of the vein monotonically increases 11.3% over its pressure range ($P < 0.05$). The outer AS, relative to its value at 10 mmHg, increases monotonically by only 25% over the pressure range 10–60 mmHg, but this increase is not significant ($P > 0.05$). Removal of the endothelium changes the measured OD values insignificantly ($P > 0.05$), even though the curve appears much flatter, having increased only 2.1% over the pressure range ($P < 0.05$). The normalized AS of the vessel surface also increases similarly, albeit apparently more slowly, than that for the intact vessels (Fig. 3C), falling within the error bars of the curve for endothelium-intact vessels ($P > 0.05$). Again, the slower increase in OD than in AS represents lengthening of the vessel with pressure.

$L_p$

For the aorta, PA, and IVC, we measured $L_p$ for approximately half of the vessels by starting from the lowest pressure and proceeding to higher values and for the other half of the vessels by starting at the highest pressure and proceeding to lower values. The time to steady state at each pressure did not significantly depend on the order of the pressures, nor did $L_p$ measured at all except at the lowest pressure tested for a given vessel, where vessels measured from low to high pressures tended, on average, to have very slightly higher $L_p$ than those measured from low to high pressure. This is likely a small preconditioning effect. Because the trends of $L_p$ with pressure discussed below were independent of pressure order, we did not separate the data from these two groups.

For the aorta at 100 mmHg (time-averaged physiological pressure), the average ($n = 6$) $L_p$ with intact endothelium is $2.79 \times 10^{-8}$ cm·s$^{-1}$·mmHg$^{-1}$ (SD 0.72). Average $L_p$ with intact endothelium at 60 mmHg is higher [4.69 $\times 10^{-8}$ cm·s$^{-1}$·mmHg$^{-1}$ (SD 1.20)] than at the other levels of ΔP. The average $L_p$ decreases with increasing pressure, dropping ~40% from 60 to 100 mmHg (Fig. 4A; $P < 0.05$), but remains flat thereafter ($P > 0.05$). Deendothelialization increases $L_p$ by roughly three-fourths ($P < 0.05$). The average $L_p$ without endothelium at 100 mmHg is $4.89 \times 10^{-8}$ cm·s$^{-1}$·mmHg$^{-1}$ (SD 1.01). In contrast to intact vessels, $L_p$ variation over the pressure range is insignificant (Fig. 4A; $P > 0.05$).

The aorta, measurements of $L_p$ with and without endothelium allow a decomposition of the resistance roughly into an endothelial and a medial component. $L_{p(e + i)}$ at 100 mmHg is $5.9 \times 10^{-8}$ cm·s$^{-1}$·mmHg$^{-1}$ (SD 3.5), which translates to ~47% of the total wall resistance deriving from the endothelium at that pressure; i.e., the endothelium accounts for ~47% of the total pressure drop.

We measured $L_p$ of the PA in six rats at five pressures from 20 to 100 mmHg with intact endothelium, deendothelialized the PA, and then repeated the measurements at the same five pressures. Unfortunately, the time required to extend these measurements to include 10 mmHg, including waiting for steady state at this low pressure, was long enough so that the vessel was no longer viable for both sets of measurements. We repeated the procedure on a second population of the same rats at 10, 20, and 40 mmHg. As shown in Figs. 3B and 4B, values from the two populations agree very well (no significant difference, $P > 0.05$) in the overlap region. Thus we have pooled both sets of data.

$L_p$ with intact endothelium is $1.90 \times 10^{-8}$ cm·s$^{-1}$·mmHg$^{-1}$ (SD 0.36) at 20 mmHg. It drops ~31% from 20 to 40 mmHg ($P < 0.05$). Beyond 40 mmHg, $L_p$ varies little with pressure ($P > 0.05$; Fig. 4B). $L_p$ of the intact vessel is $2.34 \times 10^{-7}$ cm·s$^{-1}$·mmHg$^{-1}$ (SD 0.27) at 10 mmHg and drops 42% from 10 to 40 mmHg ($P < 0.05$).

The average magnitude of $L_p$ for the deendothelialized PA over the entire pressure range is more than double that for the endothelium-intact PA ($P < 0.05$). At 20 mmHg, the average $L_p$ is $2.99 \times 10^{-7}$ cm·s$^{-1}$·mmHg$^{-1}$ (SD 0.49), but it does not vary significantly with increasing pressure ($P > 0.05$; Fig. 4B). The same is true for the second group of rats; there was no significant difference ($P > 0.05$) between the rats in the two pressure ranges. From $L_p$ of the intact and deendothelialized walls, one calculates the endothelial $L_{p(e + i)} = 6.02 \times 10^{-7}$ cm·s$^{-1}$·mmHg$^{-1}$, or ~32% of the total wall specific resistance at 20 mmHg, in contrast to its average value of 55% from 40 to 100 mmHg.

The average $L_p$ of the vein with intact wall is $5.79 \times 10^{-7}$ cm·s$^{-1}$·mmHg$^{-1}$ (SD 1.53) at 10 mmHg in eight rats, including two rats that were not subsequently subjected to deendothelialization; if data from these two rats are excluded, this value and the subsequent values in this section change insignificantly. As shown in Fig. 4C, $L_p$ drops 33.2% at 20 mmHg ($P = 0.012 < 0.05, n = 8$) and varies little with increasing ΔP ($P > 0.05$) until 50 mmHg, where $n = 4$ and the error bars are larger; thus the (increasing) value is less meaningful. If we consider this rise, it may be appropriate to consider the average curve to be flat. Because some of the vessels sprang leaks at 50–60 mmHg, only four vessels were available over the entire range. If the higher $L_p$ at low pressure is real, the shape for the $L_p$ vs. ΔP curve is similar in all vessels, at least for the lower four IVC pressures (Fig. 5A).

$L_p$ of deendothelialized IVCs at 10 mmHg is $24.94 \times 10^{-7}$ cm·s$^{-1}$·mmHg$^{-1}$ (SD 5.91) ($n = 8$), roughly four times its intact value ($P < 0.05$). The average $L_p$ in the denuded vessels
is relatively flat ($P > 0.05$) and insensitive to pressure change (Fig. 4C), although the individual traces show some (inconsistent) variation. The calculated average ($n = 8$) $L_p$ of the IVC endothelium $[L_p(e + i)]$ is $7.80 \times 10^{-7}$ cm$^2$ s$^{-1}$ mmHg$^{-1}$ (SD 2.53), representing $\sim 74\%$ ($n = 8$) of the total wall resistance at 10 mmHg. $L_p$ vs. $\Delta P$ trends for all deendothelialized vessels are similar (Fig. 5B).

**DISCUSSION**

Lipid transport from the lumen to the arterial wall and its binding to the extracellular matrix appear to be the earliest triggering events for atherogenesis. It is natural to suspect that the very disparate susceptibilities of different vessels to atherosclerosis derive from differences in these processes. Experimentation...
imental and theoretical evidence implies that LDL transport in large susceptible arteries is convection dominated, with \( L_p \) the governing parameter for transmural water flow. The primary goals of this study are to measure and compare \( L_p \) for the rat aorta, PA, and IVC and, with the known \( \Delta P \) typically experienced by these vessels, estimate and compare transmural convection in these vessels. To address these questions, we have measured the transmural water flow and the outer AS of isolated rat aorta, PA, and IVC as a function of pressure. We subjected each vessel to a full set of measurements at six different pressures, with intact and denuded endothelium, and calculated \( L_p \) at each pressure for both vessel conditions. Figure 3 shows the geometric variation.

**OD and AS**

Because the agreement of the two methods for measuring vessel geometry is excellent, we employed the vessel-preserving caliper-based method. The vessel diameter adjusts to a change in \( \Delta P \), which enters into the definition of \( L_p \) from the measured data of total flow vs. \( \Delta P \). AS of the aorta increases ~42% (\( P < 0.05 \)) from 60 to 140 mmHg, more than its OD, indicating that the vessel lengthens as well (Fig. 3A). The same is true of the PA, but both percent increases are a bit larger than those for the aorta. Endothelial denudation did not change this behavior in either vessel (\( P > 0.05 \); Fig. 3B) (38). These expansions of OD and AS are due to the elastic properties of the complete and continuous elastic sheets of the artery wall. The stiffness of the collagen, having been stretched to its maximum, causes the high-pressure plateaus (40). The endothelium makes up \(<1\%\) of the total wall thickness and accounts for a negligible amount of the vessel’s hoop stress; consequently, denudation does not significantly change the pressure dependence of the OD and AS.

In contrast, AS of the IVC increases much less (only ~25%) from 10 to 60 mmHg, and this increase is relatively linear (Fig. 3C). This means that the vein expands uniformly, with an increase in pressure, with and without endothelium. This is consistent with the pressure-volume relation developed by Brown and Heistad (9), who found that the volume of the vena cava did not increase significantly with pressure beyond 5 mmHg. The limited area increase of the IVC may be due to its lack of continuous elastic tissue, which can account for vessel elasticity, and to its abundance of collagen fibers, which accounts for its stiffness, both in contrast to arteries (40).

**\( L_p(\text{int}) \) in Intact Vessels**

Recently, DeMaio et al. (12) studied the sealing effect that transiently reduces the \( L_p \) of bovine aortic endothelial cells cultured at zero pressure after a sudden rise in \( \Delta P \). In the present study, we measured \( L_p \) of vessels accustomed to and extracted under physiological pressure. In the study of DeMaio et al., the transients returned to background in ~60 min. We assessed the flow rate for 30 min at a given pressure until steady state appeared to have been reached and then measured transmural flow five to six times at 5-min intervals to infer \( L_p \). We observed transients in this first ~30 min, but these appeared negligible in subsequent measurements.

Figure 5 shows \( L_p \) for all three types of vessels. The average \( L_p \) is \( 3.16 \times 10^{-8} \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1} \) (SD 0.87) for rat aorta \((n = 6)\) over all pressures, \( 1.85 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1} \) (SD 0.49) from 10 to 40 mmHg and \( 1.48 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1} \) (SD 0.13) from 40 to 100 mmHg for rat PA \((n = 6)\), and \( 4.85 \times 10^{-7} \text{ cm} \cdot \text{s}^{-1} \cdot \text{mmHg}^{-1} \) (SD 1.10) from 10 to 40 mmHg for rat IVC \((n = 8)\).

To explain the large increase in \( L_p \) from aorta to PA to IVC, we examined the wall structures of these vessels. The structure of the media of the PA appeared to be similar to that of the aorta, with parallel sheets of elastic tissue. The IEL [3.2 \( \mu \text{m} \) (SD 0.7)] and other elastic tissues [3.4 \( \mu \text{m} \) (SD 1.9)] of the PA appeared thinner than those of the aorta [4.5 \( \mu \text{m} \) (SD 1.6) and...
5.7 μm (SD 2.1); Fig. 2, A and B). Because the wall of the PA [78.9 μm (SD 3.3)] is thinner than that of the aorta [145.8 μm (SD 9.3)] at zero pressure but the distance between adjacent sheets is similar (~10 μm, the diameter of the SMCs in the media), the PA has fewer elastic sheets than the aorta. The structure of the vein is very different from that of the arteries (Fig. 2C). The media of the vein contains no discernable IEL and only sparse bits of elastic fibers, as well as collagen fibers and SMCs. It is not nearly as densely packed as the media of the aorta or PA, as indicated by the albumin porosities reported by Lever and Jay (25) and by the medial 125I-labeled (125I-LDL) concentrations reported by Tompkins et al. (39), and the IVC has the thinnest wall [66.1 μm (SD 4.1)]. These factors contribute to and are consistent with the relative magnitudes of $L_p$ in the three vessels.

Previously, we reported a zero-pressure thickness of the aortic wall of 107 μm (18), in contrast to the present value of 145.8 μm (SD 9.3). The reason for the difference is that, in our earlier study, the vessel was perfusion fixed at near zero pressure while still in the rat so as to be comparable with aortas fixed in the animal under pressure. In contrast, the present values were determined by histological study of fixed excised vessels. In the rat, the vessel is stretched; when it is excised, this stretching is released, allowing the vessel to retract and, thus, become thicker. Each set of measurements was reproducible.

Shape of the $L_p$–ΔP Curve

Trends. We measured $L_p$ on the aorta from the same animal, with and without endothelium, as a function of its ΔP. As noted above, $L_p$ of the intact rat aorta drops ~40% from 60 to 100 mmHg and remains pressure independent thereafter. For the PA, we examined pressure from 10 to 40 mmHg and from 20 to 100 mmHg, because the mean physiological pressure of the PA is 16 mmHg but can exceed 100 mmHg for pulmonary hypertension (8), when the PA becomes susceptible to atherosclerosis. In the PA, $L_p$ drops ~42% from 10 and 40 mmHg (P < 0.05) and is flat thereafter (P > 0.05). In the IVC, $L_p$ drops ~33.2% (n = 8) from 10 to 20 mmHg and remains flat until it rises at the large error-bar values of 50 and 60 mmHg; these error bars may indicate that the curve should be considered flat. Endothelial denudation sharply increases $L_p$ by ~175% in the aorta, >200% in the PA, and >400% in the IVC and renders $L_p$ independent of pressure, so that the water flux varies linearly with ΔP in all the vessels (Fig. 4). These trends are clearly parallel in the aorta and the PA and similar in the IVC, despite the differences in vessel wall structure and absolute magnitude of $L_p$ (Fig. 5). The lack of a complete IEL in the IVC (see below) argues against a drop in $L_p$ from 10 to 20 mmHg; such a drop would suggest an even higher $L_p$ at the vessel’s normal physiological pressure of 5 mmHg.

Interpretation. Earlier investigators (2, 38) found similar trends for $L_p$ (ΔP) for the rabbit aorta, and we have confirmed that the $L_p$ of the rat aorta is qualitatively and quantitatively similar to that of the rabbit. The average values for endothelium-intact and -denuded rat aorta are almost identical to the rabbit data of Tedgui and Lever (38) (Fig. 6) used by Huang et al. (17) and, thereby, provide Huang et al. with a complete set of rat-derived data. The error bar for the high value at low pressure reported by Baldwin et al. (2) was uniquely large (Fig. 6), reflecting that the trend was not consistent in all individual traces. Their $L_p$ values were roughly double those of Tedgui and Lever. A quantitative factor of 2 is also roughly the variation in magnitude that one can observe between the aortas of different animals of the same species, even when their trends with varying ΔP and with deendothelialization are qualitatively similar. Kim and Tarbell (22) attempted to explain the trend with a wall compaction hypothesis that invoked a variation of void fraction with distance into the wall. Huang et al. (16) constructed a theory based on a large observed disparity in matrix structure and void volume between the intima (~90% void for albumin) and media (a few percent void for albumin) (37). Their theory suggested that the intima easily compresses under pressure until its stiff collagen matrix carries the load and allows no further compression, but the dense media is nearly incompressible at these pressures. The compressed intima would make it denser and more resistant (a small effect), and the juxtaposition of the endothelium and the IEL would block the fenestrae of the IEL and, thereby, severely curtail transmural flow. Clearly, removal of the endothelium eliminates this effect and makes $L_p$ pressure independent. This theory is consistent with the large error bar at 50 mmHg of Baldwin et al. (2) and their use of an NO inhibitor (2), which eliminated the high $L_p$ at 50 mmHg but left $L_p$ at other pressures unchanged, as explained in the introduction. Thus this theory successfully explained the rabbit aorta data, and the similarity in structure and $L_p$ trends between rat and rabbit aortas suggests that the theory is equally valid in rat aorta. Huang et al. (18) confirmed intimal compaction under pressure load in rat experiments, finding that its intima indeed compresses 80–85% from 0 to 100 mmHg.

The similarity in wall structures of the PA and aorta, both with complete, fenestrated IELs and discernable intimae, suggests a similar qualitative explanation for the $L_p$ (ΔP) curves for the PA. The IVC, in contrast, does not have a fenestrated IEL;
therefore, fenestral blockage by an impinging endothelium is not a potential explanation for the drop in \( L_p \) with \( \Delta P \). Intimal compaction could still increase the contribution of the intima to flow resistance, because there is evidence (unpublished observations) from the valve leaflet that the absence of an IEL does not preclude the existence of a thin, sparse, subendothelial intima. Moreover, such an increase in intimal resistance would be more significant in the vena cava, because \( L_{p(e+i)} \) accounts for a much larger fraction of the total resistance in the vein. This theory is further supported by Tompkins et al. (39), who injected a radioactive \(^{125}\text{I}\)-LDL tracer into a squirrel monkey 30 min before it was killed and used quantitative autoradiography to measure the average LDL concentration vs. distance from the endothelium for different vessels, including the aorta, PA, and IVC. Each of these profiles had a high immediately subendothelial (intimal) value, a sharp drop near the luminal side, and then a flat, low value in the media. The profile of the aorta differed from the PA and IVC, only in being lower in magnitude. This sharp concentration drop is consistent with a high-void subendothelial intima juxtaposed against the dense matrix in all three vessels. LDL may also bind with the intimal extracellular matrix (15, 47), thereby magnifying the difference between intimal and medial concentrations.

**Observations of Dhar et al.**

In contrast to the literature results on the rabbit, Dhar et al. (13) observed that \( L_p \) of denuded rabbit aorta decreased with increasing \( \Delta P \). They attributed this drop in \( L_p \) to media compressibility and constructed a corresponding theory. In contrast to our experiment, Dhar et al. used solutions of different albumin and saline concentrations on opposite sides of the vessel wall. Then they made a major assumption that the media of the aorta was very porous, so that they could neglect the reflection coefficients and, thus, the osmotic pressure difference across the rabbit aortic wall. The media is roughly 42% porous to sucrose but only a few percent porous to albumin (37). This indicates that most of the albumin cannot traverse the aortic wall. Knox et al. (24) and Karmakar and Lever (21) estimated a reflection coefficient of 0.8–1.0 for a 4% BSA solution in PBS, which directly contradicts the assumption of Dhar et al. Thus the albumin will result in an osmotic pressure drop across the aortic wall, which is consistent with the finding of Lever and Sharifi (26) that aortic wall \( L_p \) is lower with a higher albumin gradient across the wall. At higher pressures, the initial transmural flow would be higher and could thus lead to a larger steady unsiirred layer than at lower pressures. Neglecting the resulting transmural osmotic pressure difference would lead to an underestimate of \( L_p \), and the magnitude of this underestimate would increase with \( \Delta P \); this could account for their observed drop in \( L_p \) with \( \Delta P \).

Table 1. **Average \( L_p \) of rat aorta, PA, and IVC**

<table>
<thead>
<tr>
<th></th>
<th>( n )</th>
<th>Pressure, mmHg</th>
<th>( L_{p(e+i)} \times 10^6 \text{ cm}^2 \text{ cm}^{-1} \text{ mmHg}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>6</td>
<td>60–140</td>
<td>3.16 (0.87)</td>
</tr>
<tr>
<td>PA</td>
<td>8</td>
<td>10–100</td>
<td>16.9 (3.6)</td>
</tr>
<tr>
<td>IVC</td>
<td>8</td>
<td>10–60</td>
<td>48.5 (11.0)</td>
</tr>
</tbody>
</table>

Values are means (SD); \( n \), number of rats. \( L_p \), hydraulic conductivity; \( L_{p(e+i)} \), \( L_p \) of intact vessel; \( L_{p(m+i)} \), \( L_p \) of denuded vessel [media (m) + interval elastic lamina (I)]; \( L_{p(e+i)} \), \( L_p \) calculated from \( L_{p(e+i)} \) and \( L_{p(m+i)} \) [endothelium (e) + intima (i)]; PA, pulmonary artery; IVC, inferior vena cava.
resistance of the PA and IVC. These factors are much larger than the ratios (1.2 for PA and 2.9 for IVC) of $L_{p(i)}$ values, the inverse of the resistance of the media, or the ratio of their media thicknesses (1.8 for the PA and 2.2 for the IVC). These large differences in media resistance between the arteries and the IVC must be the result of the different media structures. They would be consistent with higher medial porosities in the PA and IVC, which appear to be indicated by the significantly higher medial concentrations of the tracer $^{125}$I-LDL observed by Tompkins et al. (39) in the PA and (especially) in the IVC than in the aorta. The data of Lever and Jay (25) showing significantly higher albumin concentrations in the PA and IVC media are also consistent with the much higher media porosities of the PA and IVC than of the large arteries.

$L_p$ of the Endothelium

Removal of the endothelial cells removes one layer of flow resistance. In terms of Huang et al. (18), it also removes the possibility of fenestral blockage at high $\Delta P$. As a result of these two effects, the latter, $L_p$ without endothelium exceeds that with endothelium. For the rat aorta, ~47% of the resistance comes from the endothelium + intima at 100 mmHg; the rabbit has about the same percent endothelial resistance (2). In contrast to the large disparity of $L_{p(i)}$ and $L_{p(m+1)}$ between vessels, Table 1 shows that average calculated $L_{p(e+i)}$ for the PA and IVC are very similar, although average $L_{p(e+i)}$ for the aorta is somewhat smaller. It accounts for 34% (PA average over 10–40 mmHg), 55% (PA average over 40–100 mmHg), 78.4% (n = 8, IVC average 10–60 mmHg), and 37% (aorta average over 60–140 mmHg) of total wall resistance. In principle, the inherent $L_p$ of the endothelium may be pressure dependent (e.g., the PA), but the higher $L_{p(e+i)}$ at the lowest $\Delta P$ are more likely the result of intimal compaction (see below). Because the medial resistance falls sharply from aorta to PA to IVC, the fractional contribution of the endothelium + intima to the total resistance of the vessel increases accordingly, dominating in the case of the IVC. McCandless et al. (29) measured $L_p = 20.1 \pm 5.2 \times 10^{-7} \text{ cm}^2 \text{s}^{-1} \text{mmHg}^{-1}$ for confluent monolayers of cultured sheep pulmonary artery endothelial cells. As noted in the introduction, these high values may be the result of leaks. Our calculated aortic $L_{p(e+i)}$ [16.0 $\pm$ 7.3 $\times$ $10^{-8}$ cm$^2$s$^{-1}$mmHg$^{-1}$ (SD 7.3) at 60 mmHg] is consistent with values reported by Tedgui and Lever (38) [15.76 $\times$ $10^{-8}$ cm$^2$s$^{-1}$mmHg$^{-1}$ at 70 mmHg] and Vargas et al. (42) (11.9 $\times$ $10^{-8}$ cm$^2$s$^{-1}$mmHg$^{-1}$ at 100 cmH2O) and with measurements on cultured human umbilical venous endothelial cells [6.57 $\pm$ 0.72 $\times$ $10^{-7}$ cm$s^{-1}$mmHg$^{-1}$ at 10 cmH2O (10)] and dog vena cava [1.27 $\pm$ 0.06 $\times$ $10^{-7}$ cm$s^{-1}$mmHg$^{-1}$ at $\leq$120 cmH2O (43)].

The slightly lower $L_{p(e+i)}$ for the aorta than for the PA and IVC may suggest that $L_{p(e+i)}$ depends on the long-term pressure in the vessel. More likely, the smaller value of $L_{p(e+i)}$ for the aorta may reflect the fact that its measurement by difference means it includes not only the conductance/resistance of the endothelium but also contributions from the intima. Whereas the sparse intima normally contributes negligible resistance to water flow, Huang et al. (16) suggest that intimal compaction by $\Delta P$ loading can force the endothelium to block IEL fenestrae, thereby drastically increasing wall resistance to water flow and causing the measured $L_{p(e+i)}$ to be significantly smaller than $L_p$ of the endothelium alone [$L_{p(e)}$]. Because the aorta has a complete and thick IEL, this effect would be strongest in the aorta. Huang et al. (see Fig. 7 in Ref. 18) show that, with the intimal compactions and other parameters typical of the aorta, $L_{p(e+i)}$ will be on the order of one-third to one-fifth $L_{p(e)}$ at 100 mmHg, thereby bringing our aortic $L_{p(e)}$ in line with that of the other vessels. In addition, this line of reasoning would explain why $L_{p(i)}$ at the lowest pressure, where the intima is not fully compressed and its IEL fenestrae are not blocked, for the aorta and PA change very little on denudation: without fenestral blockage, the endothelium contributes far less resistance than the artery’s media; thus denudation hardly changes $L_p$. In addition, John Lever (personal communication) noted that removal of the endothelium likely leads to medial hydration and, consequently, expansion. Because the dense media comprises almost the entire aortic vessel wall, even a small percentage of hydration can lead to a measured $L_{p(m+1)}$ higher than its intact vessel value. This effect intensifies as the media becomes thicker and denser. Thus the true endothelial $L_p$ in intact aorta may indeed be consistent with our PA and IVC measurements, and it is wise to treat $L_{p(e+i)}$ (resistance) calculated in this way as lower (upper) bounds, rather than as precise values.

Transmural Water Flux in Aorta, PA and IVC

Typical $\Delta P$ in aorta, PA, and IVC are 100, 16, and 5 mmHg, respectively. The products of the $L_p$ values above and the corresponding pressures yield estimates of 31.6, 27.2, and 24.3 $\times$ $10^{-7}$ cm/s, respectively, for transmural water fluxes, a small quantitative, but not qualitative, difference. Interestingly, despite the very different pressure driving forces (per unit area) in these vessels, the water fluxes seem to be surprisingly similar. It is difficult to imagine that these small differences by themselves determine susceptibility; rather, if convective tracer transport is significant in the aorta, it ought to have the potential to be significant in these other vessels as well. However, the very different medial structures and, consequently, $L_{p(m+1)}$ of these vessels suggest that tracer (in particular, LDL) convected across the vessel endothelium may distribute more readily between the intima and media and even more efficiently drain out of the PA and IVC than the aorta. As a result, the local concentration of LDL in the intima, which presumably controls the rate of the disease triggering event of binding to extracellular intimal matrix, may still be much lower in the PA and IVC than in the aorta.

In summary, for an understanding of LDL transport into and accumulation in vessel walls, the putative triggering events in atherogenesis, it is necessary to first investigate transmural water transport to assess the potential importance of convective LDL transport. Here we measure and compare $L_p$ of the aorta, where convective LDL transport is known to be dominant, with $L_p$ of the PA and IVC, where the role of convective transport is not fully understood. The wall structure of the PA is similar to that of the aorta but is thinner and has fewer and thinner elastic layers. The structure of the vena cava is much different from that of either of these arteries, with only sparse bits of elastic tissue and a thinner wall. The aorta and PA show a high $L_p$ at low $\Delta P$ with a sharp drop followed by a plateau as $\Delta P$ increases. Removal of the endothelium increases $L_p$ significantly in all vessels and renders it independent of $\Delta P$. The
medial resistance is largest for the aorta, significantly lower for the PA, and even lower for the IVC. In contrast, endothelial \( L_p \) values of all the three vessels are similar in magnitude at their lowest pressures (which is consistent with studies of cultured endothelial cells), with that of the aorta appearing to be slightly smaller than the others. We argue that this lower aortic endothelial \( L_p \) may be an artifact of the measurement technique. Otherwise, it may suggest that the endothelial \( L_p \) is \( \Delta p \) dependent. No studies have been done to measure \( L_p \) of the cell monolayers as a function of the \( \Delta p \) under which the cells were cultured.

The PA experiences a smaller driving \( \Delta p \) (16 vs. 100 mmHg) than the aorta, and the vein is under an even lower physiological pressure (5 mmHg). The data suggest that the balance between a lower driving \( \Delta p \) and a higher \( L_p \) leads to surprisingly similar transmural water fluxes in these vessels. This similarity seems consistent with the similar 30-min LDL profiles across the walls of the three vessels reported by Tompkins et al. (39). It implies that transmural water fluxes alone cannot account for the different susceptibilities of these vessels and begs the question: What can? The relative rates of spread of a macromolecular tracer after it crosses the endothelia of these three vessels would provide extremely useful data as to how vessel structure and the transmural water flows determined here combine to direct macromolecular motion and concentration in the subendothelial regions of these different vessels. One possibility may be that the higher porosity (25) and larger \( L_p \) of the PA and IVC media than the aortic media may permit easy drainage of the macromolecules and prevent their accumulation in the intimae of the former vessels. Measurements of the rates of tracer spot growth in the PA and IVC and a detailed, quantitative convection-diffusion model incorporating the present measurements of transport parameters to explain those future measurements should allow us to quantitatively assess the difference in tissue delivery and concentration of LDL among these vessels. Therein may lie the potential resolution.

GRANTS

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