Macromolecular Transport Within Heart Valves

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The present study documents the permeability characteristics of heart valvular endothelium to low-density lipoprotein (LDL), albumin, and horseradish peroxidase (HRP). Using quantitative autoradiography, LDL and albumin concentrations were measured within aortic valves of squirrel monkeys and rabbits after 30 minutes of in vivo circulation. The valvular concentration profiles were analyzed using theoretical mathematical models based on fundamental transport principles. In vivo transvalvular concentration profiles of LDL and albumin displayed the highest tissue concentrations immediately beneath the endothelium and displayed the lowest concentrations near the midline of the valve. Tissue concentrations of LDL and albumin displayed large differences in magnitudes between different regions of individual valve leaflets suggesting marked spatial variation in the permeability properties of the valvular endothelium to LDL and albumin; this was also seen visually with HRP. The results of the theoretical analysis showed that 1) the aortic valvular endothelium limits the uptake of LDL and albumin into the valvular tissue, 2) the permeability of the valvular endothelium differs widely from one region of a valve to another and even from one side of the valve to the other within a single valvular region, and 3) intramural diffusion is the predominant mode of transport for LDL and albumin within the aortic valve, even in valvular regions exposed to large pressure differences across the valve. (Circulation Research 1989;64:1213-1223)

Lipid deposits and tissue calcifications may frequently be identified within heart valves as part of the degenerative processes.1-7 Transport and accumulation of low-density lipoproteins (LDL) within valvular tissue may be an important determinant in the establishment of these deposits. Previous in vivo transport studies have demonstrated heart valvular endothelium to be highly permeable to albumin-bound trypan blue,8 horseradish peroxidase (HRP),9,10 and carbon particles.11 Although these studies suggest that the valvular endothelium may be highly permeable to other macromolecular solutes such as LDL, to date, no studies have examined the permeability of the valvular endothelium to LDL and albumin. In addition, previous studies have been purely qualitative and not quantitative in nature and have not examined the effects of anatomical location within the valve as an important factor in determining the transport properties of macromolecules.

In the present report, transvalvular LDL and albumin concentrations were measured in aortic valves of the squirrel monkey and rabbit after 30 minutes of in vivo circulation. Transvalvular concentration profiles for the 125I-labeled proteins were determined by absolute quantitative autoradiography12 and were compared with theoretical mathematical models based on fundamental transport principles. The best estimate for the relevant transport parameters was achieved using nonlinear optimization techniques. In both rabbits and monkeys, transvalvular concentrations of LDL and albumin suggest that valvular endothelial permeability to these macromolecules is generally high but varies spatially throughout a valve leaflet.

Materials and Methods
Preparation of the Radiolabeled Proteins

Human LDL was isolated from fresh plasma by precipitation techniques as previously described.12-14 Rabbit serum albumin (RSA) was obtained from Miles Laboratory, Elkhart, Indiana. The human LDL and RSA preparations were radioiodinated using iodine monochloride (Na125I) by a modification of the method of MacFarlane.15,16 Physicochemical and immunochemical characterization of the radiolabeled LDL are described elsewhere.17,18 Labeling efficiency of the iodination reactions were
determined by precipitation with 10% trichloroacetic acid; iodination efficiency ranged from 33.5% to 41.1%. The precipitability of the radiolabeled proteins with 10% trichloroacetic acid ranged from 97.2% to 98.3%; the specific activity of the $^{125}$I-LDL ranged from 150 to 410 cpm/ng LDL apoprotein.

**In Vivo Animal Experiments**

**Monkeys.** The in vivo monkey experiments have been previously described.\(^{17,18}\) Briefly, adult male squirrel monkeys approximately 2–5 years old and weighing 0.7–1 kg were obtained from Bolivia by Primate Imports, New York, New York. On the day of the animal experiments, the monkeys were premedicated with intramuscular atropine sulfate (0.1 mg/kg) and intramuscular ketamine hydrochloride (Ketalar®, 20 mg/kg). Inhalation anesthesia was induced with a mixture of $N_2O$, $O_2$, and Halothane. The right external jugular vein and left common carotid artery were isolated and cannulated; the left common carotid artery blood pressure was monitored and recorded with a pressure transducer. The inferior vena cava was isolated at approximately the level of the right renal vein, and a long ligature was placed loosely around it to mark the location. The right external jugular vein and left common carotid artery were isolated and cannulated; the left common carotid artery blood pressure was monitored and recorded with a pressure transducer. The inferior vena cava was isolated at approximately the level of the right renal vein, and a long ligature was placed loosely around it to mark the location. Radiolabeled LDL, 7.8–13 mCi (35–59 mg apoprotein), was injected through a 0.22-μm filter (Millipore, New Bedford, Massachusetts) into the external jugular catheter. After 29 minutes, circulation of $^{125}$I-LDL, HRP (80 mg/kg, Type II, Sigma Chemical, St. Louis, Missouri) dissolved in 10 ml of 150 mM NaCl was injected through a 0.22-μm filter into the venous catheter.\(^{19,20}\) After 30 minutes of circulation of $^{125}$I-LDL (1 minute of HRP circulation), the animal was killed with a 50-ml bolus of 2.5% glutaraldehyde under pressure. The rabbits did not receive HRP. The harvested aortic valves were handled exactly as described in the monkey experiment. Data from a single pair of rabbits is included in which the tissues were fixed after only 10 minutes of circulation of the $^{125}$I-RSA.

**Autoradiography**

On the day after the animal experiments, the heart valves were embedded for autoradiography using established methods described elsewhere.\(^{12,17,18}\) Briefly, the samples were warmed to room temperature; regions from each of the valves were selected for plastic embedding. The selected samples were rinsed in 0.1 M cacodylate, fixed in 2 g/dl osmium tetroxide in 0.1 M cacodylate for 90 minutes, rinsed in distilled water, dehydrated through a series of graded ethanol solutions, immersed in propylene oxide, infiltrated in 1:1 (vol/vol) propylene: Epon 812, and embedded in fresh Epon 812. The aortic valves that are thin and membrane-like were embedded in toto in Epon as an entire leaflet or cusp. This was accomplished by dividing the aorta between cusps and embedding the aortic wall and cusp as a unit. In preparation of the Epon blocks for sectioning, the aortic wall was trimmed away and discarded. The aortic valve as viewed from above (aortic aspect) would be a circular structure (Figure 1A). In Figure 1B, the aorta has been divided at a junction between two cusps and opened like a book to display three leaflets or cusps. A cross section through an individual cusp showing the microscopic anatomy is shown in Figure 1C. The aortic valve attaches to the aortic wall at the anulus fibrosus. The aortic valve leaflet is composed of two opposing layers of endothelium and an interposed collagen matrix; the upper aspect (aortic side) of the leaflet is referred to as arterialis and the lower aspect (ventricular side) as ventriculairis.\(^{21}\) The collagen matrix below the linea alba is divided into two regions. The upper layer of collagen matrix (lamina fibrosa) is a densely organized matrix; the lower layer (lamina spongiosa) is a more loosely organized collagen matrix.

As previously described, both uniformly $^{125}$I-labeled gelatin standards of known concentrations and tissue samples were treated in an identical manner for autoradiography.\(^{12,17,18}\) Tissue sections (1 μm thick) that were free of scratches or surface defects were cut with glass knives on an ultramicrotome (Sorvall MT-2, Du Pont, Newton, Connecticut). Ten serial sections were cut and placed in an appropriate location\(^{22}\) on an acid-washed microscope slide. Later, in a completely sealed darkroom, slides containing radioactive tissue sections, nonradioactive rabbit tissue sections, and gelatin standards sections were dipped into melted (40°C)
The measured concentrations of $^{125}$I-LDL were determined by counting grains in the photographic emulsions over the tissue sections at multiple magnification ($\times 440$ to $\times 1,000$). Magnifications were chosen so that the valve cross section was contained within the eyepiece reticle grid; the transvalvular thickness varied from 30 to 160 $\mu$m. For seven to eight sections on a slide, grain densities were determined and recorded as a function of position from one endothelium to the other. Every attempt was made to align the counting grid with the same endothelium (arterialis or ventricularis) for each of the seven to eight sections on a single microscope slide. When present in the autoradiogram, the lamina spongiosa and fibrosa easily orient the endothelium in the autoradiogram histologically with respect to arterialis and ventricularis aspects (Figure 1C). For autoradiograms in which the lamina spongiosa and fibrosa were not present or were not easily identified, the arterialis and ventricularis aspects of the endothelial surfaces were identified by orientation of the leaflet with the Epon block.

Standard calibration curves relating silver grain densities to volumetric isotope concentrations were constructed as previously described. Standard calibration samples were dipped, exposed, and developed at the same time as each group of radioactive tissue slides. Nonradioactive tissue sections of rabbit aortic walls were included with each group on a separate slide as a control for positive or negative chemography. Because the $^{123}$I concentration changed with time (half-life of 60.0 days), the original measured concentration $C_0(0)$ (cpm/ml) of each gelatin calibration sample was corrected to its concentration on the day of the animal experiment by use of the known decay rate for $^{123}$I ($k=0.0115525$/day). A calibration curve was constructed by fitting the data from a series of samples to a straight line, $y=a+bC_0(t_1)$, where $a$ and $b$ are the intercept and slope, respectively, calculated by linear regression analysis. Since the calibration and aortic samples were exposed over precisely the same period, the aortic concentration (cpm/ml) was calculated directly from the measured grain density by use of $C_T(t_1)=(y-a)/b$. The calculated concentrations were normalized with respect to the measured initial plasma isotope concentration on the date of the experiment ($C_P$, cpm/ml) to yield the relative $^{125}$I-LDL concentration, $C_T/C_P$.

**In Vitro Experiments**

$^{123}$I-LDL and $^{125}$I-RSA were diluted to approximately 10$^6$ cpm/ml using autologous rabbit serum.
These solutions were equilibrated to 37° C in small Petri dishes (10×35 mm, Falcon, Oxnard, California) and with 100% O₂ and 2.7% CO₂ in air at equal flow rates. New Zealand white rabbits (2.5 kg) were killed with 120 mg phenobarbital. The sternum was split, the pericardium was opened, and the heart was removed. The aortic valves were dissected free and divided into leaflets. Each leaflet was immersed in the radiolabeled protein solution for 60 minutes. The valves were removed from the radiolabeled solutions, immersed in 2.5% glutaraldehyde and 0.1 M cacodylate for fixation, and treated exactly as in the in vivo experiments for quantitative autoradiography.

**Modeling**

The transvalvular radiolabeled protein concentration profiles were analyzed using a theoretical mathematical model based on fundamental transport principles of molecular diffusion. This model considers that 1) the valvular endothelium provides a resistance for the macromolecule to enter the tissue (diffusive permeability) and 2) diffusion is the only important transport mechanism to occur within the collagen matrix of the heart valve. A complete description of this model, including definition of variables and parameters, is provided in the "Appendix." The solution to the differential equation is obtained using the standard method of separation of variables. The solution for a time varying boundary condition was obtained using Duhamel's Superposition Integral.

All in vivo concentration profiles were studied with the complete model; in vitro concentration profiles were analyzed with two limiting cases. These two limiting cases assumed 1) complete permeability (no interfacial diffusive resistance) at each endothelial border (Br and B2 are infinity) and 2) complete permeability and a steady state (Br, B2, and τ are infinity). The two remaining parameters in the first limiting case of complete endothelial permeability were ε₁ and τ. The second limiting case assumes both complete permeability and a steady state. This limiting case has only ε₁ as the remaining parameter. Analysis of appropriate concentration profiles in which these conditions may apply allows an estimate for ε₁. For analysis of the in vivo profiles unless otherwise indicated, ε₁ for both 125I-LDL and 123I-RSA was taken to ε₁=0.17. This estimate is consistent with measurements in this study and with previous determinations of ε₁=0.17 for albumin in the thoracic aorta of the rabbit,ε₁=0.15 for the canine thoracic aorta,ε₁=0.09 for the rabbit common carotid artery. The correct value for LDL is probably equal to, or less than, the value for albumin.

Nonlinear least squares optimization techniques were used to adjust Br, B2, and τ for the best fit of the theoretically predicted concentration profile to the actual measured concentration profile. Powell's method was used for searching optimum parameter values in the parameter space. Because of potential errors in the series solution of this undimensional diffusion problem, 25 terms of the series were always calculated for each theoretical value for Ψ(π,τ) and calculations were performed with 16 significant figures. Standard deviations of parameters were calculated using a linearization of the problem about the optimum in parameter space from the Powell subroutine. Model fits were considered successful if residuals (measured data minus theoretical values) were normally distributed about zero and no significant trends were evident from plots of the residuals versus the independent variables. A mass transfer coefficient (k) of 2.6×10⁻³ cm/sec represented the largest number that could be managed by the hardware and was interpreted as infinity (no demonstrable interfacial transport resistance for the solute, complete permeability).

**Statistics**

Comparisons between two groups were made using the t test (two-sided, unpaired); comparisons among groups were made by analysis of variance (ANOVA) and appropriate parametric or nonparametric multiple range testing. The standard deviations for the parameters D and k₁ or k₂ were determined by the method of propagation of error as described elsewhere.

**Results**

In Vivo 123I-LDL Aortic Valve Profiles in Squirrel Monkeys

123I-LDL concentrations were measured within aortic heart valves after 30 minutes of in vivo LDL circulation. In general, transvalvular 123I-LDL concentrations were highest near the endothelial borders and lowest near the midpoint within the valve (Figure 2). There were large differences in the magnitudes of 123I-LDL concentrations within an individual valve or valve leaflet. The valvular concentrations of 123I-LDL were not necessarily symmetric with respect to arterialis and ventricularis aspects of the valves. Five transvalvular concentration profiles from the aortic valve of Monkey 1 are shown in Figure 2. The ordinate is tissue concentration, Cᵣ/Cᵣ; the abscissa is depth into the valve from one endothelial surface, x, divided by the valve thickness, L. An x/L of zero corresponded to endocardium 1 (arterialis) and an x/L of unity corresponded to endocardium 2 (ventricularis). Concentrations were measured at different locations along the length of the valve. Each profile represents mean values for at least 18 profiles taken from the same region. In Curve 1 (Monkey 1), concentrations were greater near the ventricularis endocardium indicating asymmetric permeation into the valve (ventricularis versus arterialis). The other four concentration profiles, on the other hand, were symmetrical across the valve. Transvalvular 123I-LDL concentration profiles from the other monkeys were both symmetrical and...
asymmetrical and displayed different concentration magnitudes (Monkeys 2, 3, and 4). In general, of the more than 500 individual transvalvular profiles examined, no obvious trends could be identified in terms of magnitude of LDL concentration or symmetry of the profile as a function of spatial location within the valve.

The parameters ($B_1$, $B_2$, and $\tau$) that yielded the optimum fit of the theoretical model to the data for each of the in vivo $^{125}$I-LDL aortic valve monkey transvalvular concentration profiles are listed in Table 1. The table includes estimates of the three parameters, standard deviations of the parameters, and estimates of $D$ (cm$^2$/sec), $k_1$ (cm/sec), and $k_2$ (cm/sec). The average diffusion coefficient, $D$, was $5.2\pm3.4\times10^{-10}$ cm$^2$/sec; the average transendothelial permeability coefficient ($k$) was $2.3\pm1.8\times10^{-8}$ cm/sec if the lowest and the highest values are excluded.

TABLE 1. Monkey $^{125}$I-Low-Density Lipoprotein In Vivo Aortic Valve After 30 Minutes of Circulation

<table>
<thead>
<tr>
<th>Curve</th>
<th>$B_1$</th>
<th>$B_2$</th>
<th>$\tau$</th>
<th>$D\times10^{10}$</th>
<th>$k_1\times10^9$</th>
<th>$k_2\times10^8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.26±0.58</td>
<td>30.000±5×10$^7$</td>
<td>0.16±0.08</td>
<td>3.5±1.6</td>
<td>25±57</td>
<td>3×10$^9$±5×10$^9$</td>
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<tr>
<td>2</td>
<td>0.25±0.04</td>
<td>0.11±0.03</td>
<td>0.15±0.03</td>
<td>6.3±1.1</td>
<td>30±7</td>
<td>14±4</td>
</tr>
<tr>
<td>3</td>
<td>0.076±0.017</td>
<td>0.089±0.019</td>
<td>0.096±0.021</td>
<td>2.9±0.8</td>
<td>5.1±1.7</td>
<td>6.0±1.9</td>
</tr>
<tr>
<td>4</td>
<td>0.12±0.01</td>
<td>0.14±0.01</td>
<td>0.18±0.14</td>
<td>3.1±2.4</td>
<td>11±9</td>
<td>13±10</td>
</tr>
<tr>
<td>5</td>
<td>0.16±0.03</td>
<td>0.12±0.03</td>
<td>0.14±0.03</td>
<td>9.7±2.6</td>
<td>24±7</td>
<td>18±6</td>
</tr>
<tr>
<td>6</td>
<td>0.31±0.02</td>
<td>0.33±0.03</td>
<td>0.14±0.01</td>
<td>4.4±0.3</td>
<td>30±3</td>
<td>32±3</td>
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<td>43±9</td>
<td>6.6±2.4</td>
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<tr>
<td>8</td>
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<td>0.27±0.08</td>
<td>0.35±0.10</td>
<td>3.9±1.1</td>
<td>40±16</td>
<td>40±16</td>
</tr>
<tr>
<td>9</td>
<td>0.095±0.016</td>
<td>0.101±0.016</td>
<td>0.100±0.016</td>
<td>5.2±0.8</td>
<td>8.7±2.0</td>
<td>9.3±2.1</td>
</tr>
<tr>
<td>10</td>
<td>0.02±0.028</td>
<td>0.012±0.013</td>
<td>0.15±0.03</td>
<td>13±2</td>
<td>39±8</td>
<td>2.1±2.4</td>
</tr>
<tr>
<td>11</td>
<td>1.5±0.2</td>
<td>0.92±0.5</td>
<td>0.12±0.02</td>
<td>1.3±0.2</td>
<td>73±16</td>
<td>0.0004±2</td>
</tr>
</tbody>
</table>

Monkey 1 (Curves 1–5); Monkey 2 (Curves 6–7); Monkey 3 (Curves 8–9); Monkey 4 (Curves 10–11). $B_1$, $B_2$, and $\tau$ are dimensionless; $D$, effective diffusivity coefficient in tissue (cm$^2$/sec); $k_1$ and $k_2$, apparent mass transfer coefficient for diffusive transport across endothelium 1 and 2, respectively (cm/sec). Mean±SD.
In Vivo 125I-LDL Aortic Valve Profiles in Rabbits

The transvalvular concentration profiles after 10 minutes of in vivo circulation of 125I-LDL in the rabbit were similar to profiles in the squirrel monkey in shape and the wide variations in concentration magnitudes (Figure 3). Three concentration profiles taken from valves in two rabbits are shown. As shown in Table 2, an average D was 4.1±0.7×10⁻⁹ cm²/sec (mean±SD, n=3) and was slightly lower than an average D for the 30-minute rabbit profiles, 1.7±1.1×10⁻⁹ cm²/sec (Table 3). The average permeability coefficient (k) was slightly lower than an average k for 30-minute rabbit profiles, 1.5±1.6×10⁻⁷ cm/sec (Table 3).

The transvalvular concentrations in rabbits after 30 minutes of in vivo circulation of 125I-LDL were higher in magnitude than after circulation for 10 minutes (Figure 4). The maximum concentration achieved was a Cᵣ/Cᵣm of 0.150, which was much greater than any concentrations of LDL measured after circulation for 10 minutes. All the transvalvular profiles in rabbits were taken from regions above the linea alba (Figure 1). As with the monkey data, the concentration magnitudes between profiles differed widely between regions within a valve and between animals. Transvalvular concentration profiles (Curves 1 and 2) were symmetric with respect to endothelial boundaries and had intermediate concentration magnitudes. As in the case of the monkeys, the parameter k₁ and k₂ varied widely and indicated large differences in transendothelial permeability (Table 3). The lowest k (least permeability) was 2×10⁻¹³ cm/sec, the largest k (high permeability) was 4.4×10⁻⁷ cm/sec, and the average k was 1.5±1.6×10⁻⁷ cm/sec. The estimated diffusivity coefficient did not vary as widely. The maximum D was 3.7×10⁻⁹ cm/sec, the lowest D was 0.23×10⁻⁹ cm²/sec, and the average D was 1.7±1.1×10⁻⁹ cm²/sec (Table 3).

In Vivo 123I-RSA Profiles in Rabbits

123I-RSA transvalvular concentration profiles generally showed higher endothelial permeability in vivo and resulted in consistently higher transvalvular tissue concentrations of 123I-RSA than were found with 125I-LDL (Figure 5). 123I-RSA concentrations taken from the aortic valve of Rabbit 1 were higher than 125I-LDL concentrations at 30 minutes in rabbits. The best fit parameters, D and k, for the 123I-RSA transvalvular concentration profiles were generally higher than for the 125I-LDL parameters found in either the rabbit or the monkey (Table 4). The average diffusivity coefficient, D, was 11±7×10⁻⁹ cm²/sec; the average permeability coefficient, k, was extremely high (1±3×10⁻³ cm/sec). The large standard deviation of k reflects the large variation of values seen for k.

In Vitro Transport in Heart Valves

To determine the eᵣ (accessible volume/total volume) for each solute (125I-LDL and 123I-RSA), 60-minute in vitro incubations were performed. In these studies, rabbit aortic valves were harvested from animals and immediately exposed to high concentrations of radiolabeled LDL and albumin for 1 hour in vitro. Concentration profiles across these valves was determined as in the in vivo experiments using quantitative autoradiography. The in vitro 125I-LDL 60-minute transvalvular concentration profiles generally showed the same large variability in concentration magnitudes but were flat (Figure 6). These flat transvalvular profiles were fitted with Bᵣ, Bᵣᵣ, and τ as infinity and eᵣ the single optimizing parameter; estimates of eᵣ were from 0.03 to 0.155 for LDL and were 0.102, 0.174, and

<table>
<thead>
<tr>
<th>Curve</th>
<th>Bᵣ</th>
<th>Bᵣᵣ</th>
<th>τ</th>
<th>D×10⁹</th>
<th>k₁×10⁹</th>
<th>k₂×10⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.68±0.17</td>
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<td>0.047±0.011</td>
<td>3.5±0.8</td>
<td>61±21</td>
<td>77±26</td>
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<tr>
<td>2</td>
<td>0.44±0.08</td>
<td>0.43±0.08</td>
<td>0.092±0.016</td>
<td>4.8±0.8</td>
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<tr>
<td>3</td>
<td>0.45±0.11</td>
<td>0.54±0.12</td>
<td>0.078±0.017</td>
<td>4.1±0.9</td>
<td>56±18</td>
<td>67±21</td>
</tr>
</tbody>
</table>

Rabbit 1 (Curve 1); Rabbit 2 (Curves 2,3). D, effective diffusivity coefficient in tissue (cm²/sec); k₁ and k₂, apparent mass transfer coefficients for diffusive transport across endothelium 1 and 2, respectively (cm/sec). Mean±SD.
0.176 for RSA. Five in vitro profiles displayed curvature (data not shown) and were fitted by $B_1$, and $B_2$ as infinity and optimizing both $\tau$ and $e_0$.

**In Vivo HRP Accumulation**

Brown staining of horseradish peroxidase reaction product was seen in each aortic valve examined in either the squirrel monkey or in the rabbit; an example is shown in Figure 7A. The brown staining of the valves was not absolutely uniform but appeared in patches; in some cases, these patches were confluent appearing as uniform staining (Figure 7B).

**Discussion**

This study represents the first quantitative analysis of uptake and distribution of LDL or serum albumin into aortic valves in vivo or in vitro. Very high concentrations of both these radiolabeled proteins were found within the valves after a 30-minute concentration period. Analysis of the measured concentration profiles using a theoretical transport model resulted in quantitative estimates for endothelial permeability (permeability coefficient, $k$) for the endocardial aspect of the valve ($k_1$) and for the ventricular aspect of the valve ($k_2$), and the effective diffusion coefficients ($D$) for transport both in vivo (28 profiles) and in vitro (14 profiles). As discussed below, these results suggest that the macromolecules (LDL and albumin) entered the aortic valves by crossing the valvular endothelium and were transported within the valves by molecular diffusion. The $k$ was found to have large local variations for different endothelial regions, indicating large differences in permeability to LDL and albumin; in contrast, the effective diffusion coefficient ($D$) for both LDL and albumin did not vary and was consistent among different valvarum regions.

Transport of LDL and albumin into the aortic valve in monkeys and in rabbits may be explained solely on the basis of transendothelial permeability and intravalvular molecular diffusion. For each profile, the highest concentrations were measured immediately beneath the valvular endothelium at either the ventricular aspect and/or the arterial aspect of the valves and the lowest concentrations were found within the valve. Because pressure gradients exist across certain regions of the valves (below the linea alba), pressure-driven convection, which was originally considered in the theoretical transport model, was found to have a negligible contribution to the transport of LDL or albumin. The $D$ for $^{125}$I-LDL in the aortic valve ($5.2 \pm 3.4 \times 10^{-9}$ cm$^2$/sec in the monkey, 30-minute circulation; $4.2 \pm 0.7 \times 10^{-9}$ cm$^2$/sec, in the rabbit, 10-minute circulation; and $1.7 \pm 1.1 \times 10^{-9}$ cm$^2$/sec in the rabbit, 30-minute circulation) were much higher (factor of 2 to 8 times higher) than the average $D$ measured for LDL in the aorta, peripheral arteries, and veins in the same squirrel monkeys ($6.2 \pm 3.7 \times 10^{-10}$ cm$^2$/sec). The larger $D$ for LDL in heart valves suggests differences in tissue structure between the heart valves and the arteries and veins, with diffusion being

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**Figure 4.** In vivo $^{125}$I-low density lipoprotein transvalvular concentration profiles after 30-minute circulation time in rabbits; Rabbit 1 (Curves 1, 4, 6, and 7); Rabbit 2 (Curve 2); Rabbit 3 (Curves 3 and 5). Parameter values (Table 3).
hindered by a smaller effective pore size and/or by adverse fixed charge elements in the arteries and veins. In the aortic valves, the in vivo Ds for LDL were in good agreement with the average of two estimates of D (4.8x10^{-9} cm²/sec) from in vitro experiments (data not shown) in which steady state was not achieved and therefore an estimate of D could be made. The agreement of the in vivo and in vitro estimates for D suggests that arterial pressure gradients have little effect on the intravalvular transport of LDL and albumin within heart valves.

In albumin transport experiments, the data also suggests that the primary intravalvular transport mode for albumin was diffusion. The effective D for albumin in the valves was 1.1±0.7x10^{-8} cm²/sec; this D was larger than the D for LDL in the valves (1.7±1.1x10^{-9} cm²/sec). This value agrees well with an estimate of D for albumin in rabbit thoracic aorta by Truskey (1.1±0.4x10^{-8} cm²/sec).26 The larger D for albumin probably reflects the smaller size (diameter, 18 nm; molecular weight, 50,000) of albumin compared with that of LDL (diameter, 22 nm; molecular weight, ~2 million).

The magnitudes of the concentration profiles for LDL and albumin in both the monkeys and rabbits showed large variations within a single valve leaflet. These large differences in concentration magnitudes may be the result of either 1) large differences in endothelial permeability or 2) large differences in εf.

If the endothelial boundaries were completely permeable to LDL and if the effective diffusivity coefficient for LDL was large enough, then flat profiles would represent steady-state tissue concentrations and under these conditions, C/CPo would be εf. In vitro transvalvular concentration profiles at 60 minutes were measured to determine εf. Six LDL concentration profiles were flat and had a C/CPo that varied from 0.03 to 0.16. If the assumptions of complete permeability and steady state apply, then εf was 0.03 to 0.16. Similarly, in vitro transport of albumin was studied to determine εf. Three flat transvalvular 125I-RSA profiles were observed with values of C/CPo of 0.17, 0.18, and 0.10. Some albumin profiles, which were not completely flat, were also analyzed assuming complete permeability for each endothelium but assuming that a steady state had not been reached. This analysis yielded estimates of εf of 0.18, 0.21, and 0.23, suggesting that εf for albumin varies between 0.10 and 0.23. Thus, it is not clear whether the spatial variation in magnitude is due to changes in endothelial permeability or changes in εf.

The k of the endothelium to LDL was much higher than the k of the adjacent endothelium of the aorta to LDL.9,23 The average k from multiple determinations in the aorta and major arteries of these same monkeys was 1.7±0.4x10^{-9} cm/sec, a value much smaller than the average k of the

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**Table 4.** 125I-Rabbit Serum Albumin In Vivo Aortic Valve After 30 Minutes of Circulation

<table>
<thead>
<tr>
<th>Panel*</th>
<th>B1</th>
<th>B2</th>
<th>t</th>
<th>Dx10^-10</th>
<th>k1x10^9</th>
<th>k2x10^9</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.9±0.570</td>
<td>2.6±1.7</td>
<td>0.137±0.048</td>
<td>4.3±1.8</td>
<td>9.000±60.000</td>
<td>250±190</td>
</tr>
<tr>
<td>B</td>
<td>60,000±0.07</td>
<td>2.6±2.71</td>
<td>0.19±0</td>
<td>2.5±0.5</td>
<td>5x10^{6}±5x10^{6}</td>
<td>230±240</td>
</tr>
<tr>
<td>C</td>
<td>0.004±0.6</td>
<td>4.1±2.9</td>
<td>0.42±0.35</td>
<td>21±17</td>
<td>2±200</td>
<td>1,500±1,700</td>
</tr>
<tr>
<td>†</td>
<td>2.01±0.5</td>
<td>2.24±0.55</td>
<td>0.101±0.018</td>
<td>15±3</td>
<td>330±100</td>
<td>350±110</td>
</tr>
<tr>
<td>†</td>
<td>5.0±2.6</td>
<td>6.7±3.9</td>
<td>0.15±0.04</td>
<td>13±4</td>
<td>880±570</td>
<td>1,200±740</td>
</tr>
<tr>
<td>†</td>
<td>6.8x10^{4}</td>
<td>2.04</td>
<td>0.20</td>
<td>6.7±3.3</td>
<td>1x10^{2}±2x10^{6}</td>
<td>300±350</td>
</tr>
<tr>
<td>†</td>
<td>7.0</td>
<td>0.002</td>
<td>0.87</td>
<td>12</td>
<td>3,000</td>
<td>1</td>
</tr>
</tbody>
</table>

Rabbit 6 (Figure 5A); Rabbit 7 (Figures 5B and 5C). D, effective diffusivity coefficient in tissue (cm²/sec); k1 and k2, apparent mass transfer coefficient for diffusive transport across endothelium 1 and 2, respectively (cm/sec).

*Mean±SD.

tCurve not shown.

---

**Figure 5.** In vivo 125I-rabbit serum albumin transvalvular concentration profiles after 30-minute circulation time in Rabbit 6 (A) and Rabbit 7 (B and C). Valve thickness (μm) and number of individual profiles averaged are indicated. Smooth curves represent results of theoretical model; parameter values (Table 4).
valvular endothelium \((2.3\pm1.8\times10^{-8}\text{ cm/sec})\). This permeability was also much less than the LDL permeability of the pulmonary artery and inferior vena cava of these same monkeys (average \(k_1\), \(2.9\pm0.3\times10^{-9}\text{ cm/sec}\)). In addition for the aortic valvular endothelium, \(k_1\) and \(k_2\) were not significantly or consistently different even though the ventricularis endothelium is exposed to fluid flow (high shear rates) during ventricular ejection.

Focal regions of enhanced permeability to LDL have been identified throughout the cardiovascular system in these monkeys.\(^{17,18}\) The average permeability for these focal regions of vessels was \(6\pm12\times10^{-8}\text{ cm/sec}\), which is similar to the average LDL permeability of the aortic valvular endothelium \((2.3\pm1.8\times10^{-8}\text{ cm/sec})\). Unlike the usual aortic endothelium, which is impermeable to LDL, the average aortic valvular endothelium has an enhanced permeability as compared with the aortic endothe-lium to LDL. These results suggest that LDL concentrations in some regions within the aortic valves may be at tissue saturation under normal physiological conditions at all times. Why such high tissue concentrations of LDL do not initiate atherosclerosis in the heart valves is not entirely clear.

One possible explanation is that, although lipids and lipoproteins may be present within the valve tissue, important cellular components normally present in other vascular tissues and in older valve tissue are not present in the valves of younger animals. It has been postulated that LDL becomes fully atherogenic only in a form which has been oxidatively modified by cellular components within the tissue. Oxidized LDL may be rapidly cleared by the acetyl-LDL or "scavenger" receptors of tissue macrophages,\(^{31}\) and this macrophage-modified LDL may be transported to smooth muscle cells, resulting in fatty deposits and atherogenic lesions.\(^{32}\)

The high permeability of the aortic valvular endothelium to LDL and albumin is not surprising given the previous reports of valvular permeability to trypan blue (molecular weight, 900), HRP (molecular weight 40,000; Stokes-Einstein diameter, 4 nm), and colloid carbon (Stokes-Einstein diameter, >30 nm).\(^8-11\) This study combined with those previous studies demonstrates large quantitative differences in macromolecular permeability of the aortic valves as compared with the thoracic aorta. That LDL normally resides in high concentrations within vascular tissues that only rarely develop atherosclerosis strongly suggests that local factors such as extracellular matrix or the presence of particular cell types (such as smooth muscle cells) may play major roles in the development of atherosclerosis.

Appendix

The model considers transport across the endothelium at the boundary of the valvular tissue as a convective boundary condition (a thin region at the boundary of the valve which determines concentration at the edge of the valve tissue). The conservation differential equation describes transport within the valvular tissue. Only a diffusion term is included; preliminary investigations showed that a convec-tion term was unnecessary to explain or model measured concentration profiles. Irreversible or reversible binding to cellular or extracellular tissue elements is also not included; preliminary investigations showed that inclusion of such terms was not justified by the measured concentration profiles. The variables are

- \(C_r\), concentration of solute. Note that concentration is averaged over all phases present in the media (mol/ml of tissue)
- \(\varepsilon_r\), volume fraction available to solute in extracellular phase (volume of aqueous phase available to solute/unit volume of tissue)
- \(L\), valve thickness (\(\mu\)m)
- \(D\), effective diffusivity coefficient in tissue (cm²/sec)
- \(x\), spatial coordinate (cm)
- \(t\), time (sec)
- \(C_p\), concentration of solute in plasma (mol/ml)
- \(C_{p0}\), initial concentration of solute in plasma (mol/ml)
- \(k_1\), apparent mass transfer coefficient for diffusive transport across endothelium 1 (cm/sec)
- \(k_2\), apparent mass transfer coefficient for diffusive transport across endothelium 2 (cm/sec)

Dimensionless Groups used in Mathematical Model

- Distance: \(\eta = x/L\); time: \(\tau = D t/L^2\); concentration: plasma, \(\phi_p = C_p/C_{p0}\); tissue, \(\phi = C_r/C_{p0}\varepsilon_r\).
FIGURE 7. Open views of horseradish peroxidase (HRP)-stained heart valves of squirrel monkeys. Upper panels: HRP reaction product stained aortic valve of Monkey 4. The aortic valve is displayed in an "open" view as shown in Figure 1B. The three cusps show patches of brown staining of HRP reaction product and in other regions of the cusps, the valves are left unstained and appear normally transparent. The brown muscular structure below the leaflet is residual myocardial muscle. Lower panels: HRP reaction product stained pulmonary valve of Monkey 1. Two cusps of this valve are shown in an "open" view. Background is blue velvet backing; adjacent aortic wall is unstained white. The valve leaflets are stained brown with HRP reaction product with some areas staining more deeply than others.

Biot number: Endothelium 1, \( \text{Bi}_1 = k_1 L/D \); endothelium 2, \( \text{Bi}_2 = k_2 L/D \).

The dimensionless conservation equation that describes transport within the valve tissue is

\[
\frac{\partial^2 \phi}{\partial \eta^2} - \frac{\partial \phi}{\partial \eta} = 0
\]

Given a step change in plasma concentration (\( \phi_p = 1 \)) and boundary conditions:

\[
\begin{align*}
\eta &= 0; & \frac{\partial \phi}{\partial \eta} &= \text{Bi}_1 (1-\phi) \\
\eta &= 1; & \frac{\partial \phi}{\partial \eta} &= \text{Bi}_2 (1-\phi)
\end{align*}
\]

Initial condition:

\[
\tau = 0 \quad \phi_p = 1; \quad \phi = 0
\]

The solution for this problem was obtained using standard techniques of separation of variables.\(^{24,25}\)

For a plasma step change:

\[
\phi(\eta, \tau) = 1 - \sum_{n=1}^{\infty} C_n \left[ \sin(\lambda_n \eta) + \frac{\lambda_n}{\text{Bi}_1} \cos(\lambda_n \eta) \right] \exp(-\lambda_n^2 \tau)
\]

where

\[
C_n = -2 \frac{\text{Bi}_1^2 (\lambda_n^2 + \text{Bi}_2^2) ((1-\cos \lambda_n) + (\sin \lambda_n)/\text{Bi}_1)}{\lambda_n^4 + \text{Bi}_1^2 (\lambda_n^2 + \text{Bi}_1^2 + \text{Bi}_2) + \text{Bi}_1^2 (\lambda_n^2 + \text{Bi}_2^2)}
\]

And where the eigenvalues, \( \lambda_n \), are evaluated by

\[
\lambda_n \cos \lambda_n - (\lambda_n^2 \sin \lambda_n)/\text{Bi}_1 = -\text{Bi}_1 (\sin \lambda_n + (\lambda_n \cos \lambda_n)/\text{Bi}_1)
\]

Though the plasma concentration was constant during the experiment, for completeness, the above solution of \( \phi(\eta, \tau) \) was generalized for a time-varying boundary condition, \( \Psi(\eta, \tau) \), with Duhamel's Superposition Integral.\(^{24,25}\)

\[
\Psi(\eta, \tau) = \sum_{k=1}^{2} A_k(\tau) - \sum_{n=0}^{\infty} C_n \left[ \sin(\lambda_n \eta) + \frac{\lambda_n}{\text{Bi}_1} \cos(\lambda_n \eta) \right] \exp(-\lambda_n^2 \tau) + \sum_{j=1}^{2} B_j(\tau)
\]
Using plasma decay parameters, $a_1$ and $b_1$, then,

$$A_1 = a_k \exp (-b_k \tau)$$

$$B_1 = \frac{ab}{b_1 - \lambda^2} \left[ \exp (-b_1 \tau) - \exp (-\lambda^2 \tau) \right]$$

The above solution may be found in Carslaw and Jaeger's (Section 3.11 Equations 20-24) for a heat transfer problem without a partition coefficient.

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