Effect of Endothelial Integrity, Transmural Pressure, and Time on the Intimal–Medial Uptake of Serum $^{125}$I-Albumin and $^{125}$I-LDL in an In Vitro Porcine Arterial Organ-Support System

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This report describes a new in vitro, metabolically supported, Sinclair Research Farm minipig aortic preparation in which the intimal-medial uptakes ($M, \text{mg} \cdot \text{cm}^{-2}$ of intimal surface) of porcine $^{125}$I-albumin and normocholesterolemic (nonoxidized) porcine $^{125}$I-low density lipoprotein (LDL) from a stirred, autogenous serum (containing a $^{125}$I-protein concentration of $c_0, \text{mg} \cdot \text{cm}^{-2}$ at 37°C and pH 7.4) were studied as functions of transmural pressure (0 $\leq P \leq$ 150 mm Hg), time (30 $\leq t \leq$ 120 minutes), and endothelial integrity. The following new observations were made: 1) The normalized transendothelial uptakes ($M/c_0, \text{cm}$) of both albumin and LDL across normal intact aortic endothelial surfaces were insensitive to $P$. This indicated that these macromolecular solutes were not readily convected across the normal aortic endothelial surface despite increasing $P$. 2) However, the associated transendothelial $M/c_0$ versus $t$ relations for the normal intact surfaces were shown to increase monotonically with $t$ in a manner consistent with a simple diffusive transport across a large surface barrier into the subjacent media, either with (Cases 2A and 2B) or without (Case 1) an associated transmural water convection. 3) The shapes of these temporal $M/c_0$ curves of albumin and LDL were virtually the same; however, the magnitude of the albumin $M/c_0$ curve was about sevenfold greater than that of LDL. 4) The $M/c_0$ across the injured endothelial surface (Case 2C) not only increased monotonically with $t$ but also increased significantly with $P$, indicating that in the absence of a normal endothelial surface, a very large convective component was added to the transport processes across the exposed aortic endothelial basement membrane and internal elastica. We conclude that: 1) the normal porcine aortic endothelial surface can provide a virtually complete barrier to the transendothelial convective transport of both albumin and LDL, 2) the diffusive barrier of the normal endothelial surface to LDL was sevenfold greater than that to albumin, 3) loss of the endothelial cell layer was associated with a threefold increase in the (P=0) diffusive intimal-medial uptake of serum albumin in contrast to an eightfold increase in the pressurized (P=150 mm Hg) combined diffusive-convective intimal-medial albumin uptake in the same vessel. (Arteriosclerosis and Thrombosis 1992;12:1313–1328)

Key Words • albumin • aorta • arterial temporal uptake • atherosclerosis • arterial mass transport • blood pressure • endothelium • endothelial injury • endothelial permeability • hypertension • intima • low density lipoproteins • media • mathematical models • serum proteins • swine

The arterial transendothelial transport of plasma macromolecules probably plays an important role in the accumulation of atherogenic reactants (such as low density lipoproteins [LDLs]) in the subjacent intimal tissue space as well as in the removal of the atherogenic products from these spaces. The present report describes new work with porcine $^{125}$I-labeled albumin and nonoxidized $^{125}$I-labeled LDL as relatively simple, well-defined, natural serum macromolecules that should be suitable for preliminary efforts to define some of the relevant arterial parameters of transendothelial transport under special in vitro conditions. These conditions provided unique experimental control of pressure, time, nutrient milieu, etc. The present work consisted of experiments in which the porcine aortic intimal–medial uptakes ($M, \text{mg} \cdot \text{cm}^{-2}$) of $^{125}$I-albumin or nonoxidized normocholesterolemic $^{125}$I-LDL across the normal, intact endothelial surfaces and the injured aortic intimal surfaces of Sinclair Research Farm (SRF) minipigs were measured as functions of the duration ($t$, minutes) of intimal surface exposure to the $^{125}$I-protein and as functions of arterial transmural pressure ($P$, mm Hg). These uptake measurements were done in an organ-support system (OSS) at 37°C, pH 7.4.
using stirred, pure, autogenous venous serum (AS) as the nutrient medium. This OSS methodology made it possible to design experimental protocols to examine the relative roles of diffusion, convection, and endothelial integrity on the uptake of 125I-proteins during a "window" of time chosen to be suitable for empirical (but quantitative) linear-regression analyses of the M(t) and M(P) data. More specifically, four sets of such studies were done to examine 1) the effect of endothelial integrity (intact versus injured endothelial surface) and pressure (0 ≤ P ≤ 150 mm Hg) on the uptake of 125I-albumin, 2) the effect of endothelial integrity and time (0 < t ≤ 120 minutes) on the temporal uptake of 125I-albumin, 3) the effect of pressure on the uptakes of 125I-albumin and 125I-LDL across the intact endothelial surface, and 4) the effect of time on the uptakes of 125I-albumin and 125I-LDL across the intact endothelial surface.

Methods

Incubating Reagents

Porcine AS was prepared immediately after jugular phlebotomy and frozen at —80°C until it was used as the nutrient-reagent medium for the intimal surfaces; a 15% solution of AS in a physiologically balanced porcine electrolyte-glucose solution (PES) was used as the vessel-harvest medium; a 25% AS in PES solution was used as the nutrient medium on the adventitial surface of the vessel to simulate the adventitial interstitial fluid. Culture-positive nutrient media and data from culture-positive studies were discarded.

The pH, Pco_2, HCO_3~, and P_O_2 were measured (Radiometer ABL30) before and during each experiment. The initial HCO_3~ concentration of the reagents was adjusted to that of the aortic donor so that the HCO_3~ concentrations of the AS and AS/PES media for a given study were equal to the in vivo value from the vessel donor. The OSS ambient Pco_2 for the reagent-vessel system was controlled in accordance with the HCO_3~ concentration to give pH = 7.4 as detailed subsequently.

Radioactively Labeled Reagents

Purified porcine albumin was obtained from Cappel Organon Teknika, Durham, N.C. Fresh, nonoxidized, normocholesterolemic, SRF minipig LDL was prepared for this project by Dr. W.C. Taddei-Peters at the Biotechnology Research Institute, Organon Teknika, Rockville, Md., as follows. Freshly phlebotomized 1% porcine electrolyte-glucose solution (PES) was used as the vessel-harvest medium; a 25% AS in PES solution was used as the nutrient medium on the adventitial surface of the vessel to simulate the adventitial interstitial fluid. Culture-positive nutrient media and data from culture-positive studies were discarded.

The pH, Pco_2, HCO_3~, and P_O_2 were measured (Radiometer ABL30) before and during each experiment. The initial HCO_3~ concentration of the reagents was adjusted to that of the aortic donor so that the HCO_3~ concentrations of the AS and AS/PES media for a given study were equal to the in vivo value from the vessel donor. The OSS ambient Pco_2 for the reagent-vessel system was controlled in accordance with the HCO_3~ concentration to give pH = 7.4 as detailed subsequently.

Transport Methodology

All procedures used in this study were carried out in accordance with institutional guidelines regarding the use of experimental animals and were done under aseptic conditions. All animals were fully anesthetized with ketamine (~40 mg/kg body wt) plus acepromazine (~0.04 ml/kg body wt) followed by maintenance doses of sodium pentobarbital as necessary before any surgical procedures. Femoral arterial blood gases were monitored throughout surgery for appropriate ventilatory control (Harvard respirator with O_2-enriched air) to maintain P_O_2 >200 mm Hg and 7.37<pH<7.43. The descending thoracic aorta was carefully excised through a left thoracotomy from 22 (74±11 kg, mean±SD)
FIGURE 1. Diagram of tissue preparation and tissue holding device (THD) for the organ-support system. Panel A: Excised vessel is opened longitudinally along dorsal line. Panel B: Vessel is opened flat with the endothelial side up and slowly stretched to its in vivo length. Panel C: Vessel is clamped along the longitudinally cut edges in the THD, after which it is slowly stretched in the circumferential direction to restore its in vivo dimension. Panel D: Tissue is captured between upper (intimal wells) and lower (adventitial chambers) parts of the well assembly (WA), thereby isolating 18 contiguous wells of 1.2x1.2-cm portions of intimal and corresponding adventitial surfaces along the vessel for study. Transmural pressure is applied, using appropriate negative gauge pressure to pressure lines (P) leading to each adventitial chamber. Reprinted with permission from Reference 1.

young adult (2.3±0.2 years old, mean±SD) SRF minipigs (University of Missouri, Columbia, Mo.). The excised vessel was slowly relaxed (to minimize potentially damaging viscoelastic and myogenic stresses in the tissue), immersed in a 37°C AS/PES bath, and dissected free of excess adventitial tissue.

Referring to Figure 1, the vessel was then opened longitudinally along its dorsal aspect through the intercostal orifices (panel A), stretched slowly to its in vivo length (panel B), and clamped as a flat sheet along each longitudinally cut edge, endothelial side up, in a specially designed adjustable tissue-holding device (THD) (panel C). The THD was then adjusted slowly to restore the former in vivo circumferential dimension. (During this and the following procedures, the intimal surface was maintained under oxygenated AS/PES.) As shown in Figure 1D, the sheet of tissue was then captured in a well assembly (WA) consisting of two devices with matching intimal and adventitial well chambers that partitioned the arterial wall into 18 1.20-cm square independent regions for study. In those studies in which uptakes across the injured intimal surface were to be examined in specified wells, the endothelial cells were removed mechanically with a squeegee device.

The adventitial chambers were filled with the simulated interstitial fluid (25% AS in PES). Each of the matching intimal chambers (wells) was filled with 100% AS as noted above for incubation periods in accordance with the experimental protocols described below. The desired transmural pressure was obtained by attaching an appropriate negative (gauge) pressure source (P, Figure 1D) to the corresponding adventitial chamber. In the present study, wells were pressurized at P=0, 50, 100, and 150 mm Hg to simulate various blood pressures. An oscillatory-flow assembly was attached to the top of each well in the WA for continuous stirring of the nutrient media in the wells at ~70 cycles/min to approximate in vivo blood flow and to abolish chemical gradients in the nutrient media.1,2

After these preparative procedures, the THD containing the tissue in the WA was enclosed in the chamber of the OSS to begin a 1-hour equilibration period with unlabeled AS. The OSS consisted of a transparent chamber in which the temperature and composition of the gas environment surrounding the THD were rigorously controlled at 37°C, 100% humidity, P02 >200 mm Hg, and a PCO2 to produce a pH of 7.4 for the HCO3− concentration of that particular tissue donor.1 After the 1 hour of incubation at the specified pressure (P=0, 50, 100, or 150 mm Hg), the nutrient reagent in each well was sampled for culture, chemistry (Roche Biomedical Laboratories, Inc., Columbus, Ohio*), pH, and gas partial pressures. Then in accordance with the protocols described subsequently, the nutrient media were aspirated completely and replaced with the corresponding fresh incubation media containing the porcine 125I-albumin or 125I-LDL. The end of the 125I-albumin or 125I-LDL exposure period, reagent samples were taken from each well to recheck the specific activity of the AS and relevant chemical parameters (i.e., the electrophoretic mobilities, radioactive protein purity, tyrosine, and iodide concentrations) using SDS-PAGE, PAGE, and thin-layer chromatography.6 In all cases, it was found that the TCA-soluble radioactivity concentration was composed entirely of iodide, and in no cases was 125I-tyrosine measurable.

The electrophoretic mobilities of 125I-albumin, EBD-albumin, and the native albumin of the serum were identical and remained unchanged after 2 hours of incubation on the vessel.

After we obtained the necessary reagent samples, the balance of the reagent was completely aspirated, and the well and intimal surfaces were quickly and vigorously rinsed with a jet of buffered saline solution to remove intimal surface contamination by residual radioactive reagent. Immediately after this brief rinse (~3 seconds), the saline was replaced with 10% TCA to arrest further movement of the labeled protein. (In a few cases, 3% glutaraldehyde in phosphate buffer was used to allow for subsequent electron microscopy.) Immediately after this, the pressure-induced centerpoint deflection (w0, cm) of the intimal surface was determined by using a special displacement measuring device.1,7 The system was then depressurized and the WA removed. After 3 hours of fixation in TCA, the THD with tissue was transferred to a phosphate-buffered 3% glutaraldehyde bath for completion of an 18-hour period of tissue fixation and further elution of free iodide.

Calculation of 125I-Albumin Uptake (M, mg · cm−2)

After the fixation period in the stretched, unpressurized state, the tissue of each well bottom was carefully Reprinted with permission from Reference 1.

*The reproducibility and accuracy of the chemistry values from Roche Biomedical Laboratories were checked frequently by periodic submission of blind duplicate samples as well as known calibration solutions.
excised along the well margins and weighed to obtain the weight \((w_0)\) of the square piece of tissue comprising the entire well bottom. Then the aforementioned dark blue-stained areas of overt intimal injury adjacent to the well walls were generously trimmed, leaving an unstained central rectangular specimen, as described in greater detail elsewhere.\(^1\) In two preparations from each set of experiments, extra portions of the remaining trimmed specimens were removed for examination by light microscopy and study of ultrastructure by scanning electron microscopy and transmission electron microscopy.

The weight \((w_t)\) of the remaining trimmed tissue was used to calculate the \((\text{unfixed, stretched, } P=0)\) intimal area \((A_t)\) of the tissue specimen from the following relation:

\[
A_t = 1.44(w_t/w_0) \text{ cm}^2
\]  

(1)

in which 1.44 cm\(^2\) was the \((\text{known})\) intimal area of the unfixed, stretched, tissue that originally comprised the bottom of the well, \(w_t\) was the weight of that specimen, and \(w_0\) was the weight of the final trimmed specimen.

The radioactivity \((\mu Ci)\) of the trimmed specimen was measured (LKB CompuGamma gamma counter, Pharmacia/LKB Instruments, Gaithersburg, Md.) to obtain the intimal-medial uptake of radioactively labeled albumin or LDL that had accumulated during the specified duration \((t)\) at pressure \((P)\) at that well site. The corresponding measured uptake \(M_m, \text{ mg } \cdot \text{cm}^{-2}\) of the labeled protein was calculated from the measured radioactivity \((\mu Ci)\) and the intimal area \((A_t)\) of the specimen (in the unfixed, \(P=0\) state) by

\[
M_m = \mu Ci / (K_0 \times A_t) \text{ mg } \cdot \text{cm}^{-2}
\]  

(2)

where \(K_0\) is the specific activity of the reagent \((\mu Ci/\text{mg protein})\), which was calculated from

\[
K_0 = \mu Ci / c_0 \cdot V
\]  

(3)

in which \(\mu Ci\) is the radioactivity of an aliquot of the reagent, \(V\) \((\text{ml})\) is the volume of this aliquot, and \(c_0\) \((\text{mg/ml})\) is the concentration of the protein of interest in the liquid phase of the reagent.

The values of the measured uptake \(M_m\) from Equation 2 were corrected for the added pressure-induced surface \((\text{area})\) strain that existed during the pressurized state of the study by

\[
M = F(w_0) \times M_m
\]  

(4)

in which \(M\) is the \((\text{pressure-strain-corrected})\) uptake, \(w_0\) \((\text{cm})\) is the centerpoint deflexion of the pressurized intimal surface from its unpressurized position, and \(F(w_0)\) is a function that was evaluated numerically for each measured value of \(w_0\).\(^1\) The average value for the \(P=100\) mm Hg correction factors, \(F(w_0)\), that were calculated for the present studies was 0.966.\(^1\) Uptake data will be presented below in normalized form, i.e., \(M/c_0 \text{ cm, in which } M (\text{mg } \cdot \text{cm}^{-2})\) is the intimal-medial uptake from Equation 4, and \(c_0\) \((\text{mg } \cdot \text{cm}^{-2})\) is the serum albumin or LDL concentration in the liquid phase of the reagent.

**Light Microscopy**

After the above procedures, the fixed and trimmed tissue specimens from all wells of each study were prepared for structural studies. Each specimen was trimmed, one portion for light microscopy and two portions for electron microscopy. The tissues for light microscopy from each experiment were embedded as an ensemble of 18 tissues (wells) in one block of glycol-methacrylate from which 4-\(\mu\)m-thick sections were cut, mounted on glass slides, and examined with three types of plastic section stains: 1) modified Lees, 2) methylene blue–azure II, and 3) Culpepper’s elastin stain.

**Electron Microscopy**

Comparative scanning electron microscopy and transmission electron microscopy were done in two of the experiments from each set of experiments to assess ultrastructural changes associated with the imposed experimental conditions noted above. Standard electron microscopic methodologies were used as detailed previously.\(^1\)

**Protocols**

As summarized in the last paragraph of the “Introduction,” the four sets of protocols for this research were designed to measure the intimal–medial uptake \([M(t,P)]\) of \(^{125}\text{I}-\text{albumin}\) or \(^{125}\text{I}-\text{LDL}\) as functions of pressure \([M(P)_{120 \text{ minutes}}]\), time \([M(t)_{100 \text{ mm Hg}}]\), and endothelial integrity. Since it has been shown that uptake tends to decrease with distance \((z)\) along the aorta (Reference 8 and D.L. Fry, unpublished observations), the desired pressures \((P=0, 50, 100, \text{ and } 150 \text{ mm Hg})\) for the \(M(P)_{120 \text{ minutes}}\) studies and the desired times \((t=30, 60, 90, \text{ and } 120 \text{ minutes})\) for the \(M(t)_{100 \text{ mm Hg}}\) studies were assigned to the wells along the vessel in a replicated, rotational format. For example, measurement of \(M\) for a given \(P\) typically would be replicated at three equidistant points along the vessel; this spacing pattern would then be shifted by one or two wells with each succeeding tissue preparation. Thus, the final group-averaged data for a set of studies were averaged with respect to \(z\) (but not \(t\) or \(P\)), i.e., the spatial variations of uptake among the preparations in each set of experiments would tend to average out in the final group mean \(M(P)_{120 \text{ minutes}}\) and \(M(t)_{100 \text{ mm Hg}}\) curves. The same assignment strategy was used for the sets of experiments to examine the role of endothelial integrity on the \(M(P)_{120 \text{ minutes}}\) and \(M(t)_{100 \text{ mm Hg}}\) data for \(^{125}\text{I}-\text{albumin}\), except that alternate wells were injured and the same values of \(P\) or \(t\) were assigned to adjacent injured and normal wells so that paired “2-averaged,” normal and injured endothelium data from the same tissue preparation would be reflected in the final group mean \(M(t)_P\) and \(M(P)_t\), normal-versus-injured curves.

As implied above, the temporal uptake \([M(t)_P]\) studies were done at a pressure of \(P=100 \text{ mm Hg}\), and the uptake-versus-pressure \([M(P)_t]\) studies were done with an exposure period of \(t=120 \text{ minutes}\). All wells were stirred (to ensure solute mixing and oxygenation at the intimal surface) and maintained under physiological conditions as detailed elsewhere.\(^1\) Initially, the wells were pressurized at their assigned pressures with the stirred, nonlabeled AS for an equilibrium period of at least 1 hour, after which the intimal surfaces were exposed to the corresponding AS media with the radioactively labeled protein of interest for the assigned durations of exposure. The sets of average, normalized \(M/c_0 \text{ cm (x10)}^3\) versus \(t\) or \(P\) data are presented below in...
The subjacent basement membrane, ground substance, connective tissue, and smooth muscle cells also appeared to be normal. No differences were found among pressurized, nonpressurized, 125I-LDL, or 125I-albumin tissues. Transmission electron microscopy of the injured tissues showed that the collagen and elastin in the interfacial region of the injured surface appeared intact; however, there appeared to be some expansion of the spaces below the exposed endothelial basement membrane and subjacent intimal inter fibrillar spaces with some loss of ground substance, extending approximately 20 μm below the basement membranes. The medial ultrastructure beyond this region (i.e., beyond about 20 μm) appeared unaffected by the deendothelialization.

Referring to the scanning electron microscopy photomicrographs of the mechanically injured intimal surfaces in Figure 2, note the absence of endothelial cells and the presence of a mostly intact and slightly fenestrated basement membrane system (see also Figures 3 and 4, pp 482–483 of Reference 5). Comparing Figure 2A (well 1) with Figure 2B (well 16), the surface structure of the basement membrane appeared to become somewhat less fibrillar and fenestrated, i.e., more “membrane-like,” in the downstream (Figure 2B) portion of the vessel.

### Results

#### Mass-Transport Data: Overview of Presentation

The results from the four sets of uptake studies mentioned earlier will be presented below in graphic form (Figures 3–6) and in corresponding tabular form (Tables 2–5). In each of the graphs, the y-axis values of the points represent the group mean values of the M/c0 (±SEM) data for the corresponding values of either t minutes (at P=100 mm Hg) or P mm Hg (at t=120 minutes) as indicated on the corresponding x axes. The SEM bars represent not only the interanimal variability in M/c0 but also the residual z dependence that survived the aforementioned z-averaging experimental design. The four tables contain the linear-regression coefficients that summarize the magnitudes and trends of the M/c0 data for the individual experiments in each of the corresponding four sets of experiments. The group mean coefficients (intercept, slope, $R^2$) appear at the bottom of each column. The relatively weak $R^2$ values for the component studies reflect mostly the strong $z$ dependence of M/c0 in the individual. Note that in Tables 3 and 5, representing the temporal uptakes across normal surfaces, the regressions were computed without (t seconds)$^{1/2}$ in addition to (t seconds) as the independent variable.

#### Effect of transmural pressure (P) and endothelial integrity on the intimal–medial uptake of 125I-albumin

Figure 3 represents the M/c0 versus P relations for the normal (lower curve) and paired injured (upper curve) intimal surfaces. For the injured surfaces, uptake is shown to rise monotonically with pressure, whereas uptake remains virtually constant with pressure for the normal, intact endothelial surface. The ratio $M(P)/c_0$ for the injured surfaces was about three times greater than that for the normal surface at P=0 and about 7.5 times greater at P=150 mm Hg. This demonstrates that injury of the endothelial surface is associated not only with an increased diffusive component but also with the appearance of a large, pressure-driven, convective component to transmural transport.

### Table 1. Chemical Changes in the Nutrient Medium

<table>
<thead>
<tr>
<th>Component</th>
<th>$t_0$</th>
<th>$t_{int}$</th>
<th>Difference after 1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.45±0.03</td>
<td>7.38±0.02</td>
<td>-0.07 meq/l</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>30.9±1.10</td>
<td>30.3±1.10</td>
<td>0.06 meq/l</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>31.0±2.61</td>
<td>28.86±3.03</td>
<td>1.24 mg/dl</td>
</tr>
<tr>
<td>Glucose</td>
<td>89.0±9.00</td>
<td>87.0±9.00</td>
<td>0.00 mg/dl</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.13±0.06</td>
<td>4.21±0.06</td>
<td>0.08 meq/l</td>
</tr>
</tbody>
</table>

The corresponding (paired) serum Na, K, Ca, Mg, total cholesterol, albumin, total protein, and lactate dehydrogenase concentrations did not change significantly (n=7, p>0.05) during the same incubation periods. Mean serum albumin and mean total cholesterol values for all autogenous serum reagents used in this research (n=22) were 4.00±0.35 (±SD) g·dl$^{-1}$ and 57.7±12.3 (±SD) mg·dl$^{-1}$, respectively.

#### Structural Characteristics

Detailed light microscopic examination of 4-μm glycolmethacrylate-embedded tissue sections (stained with modified Lees, methylene blue-azure II, and Culpepper's elastin stains) was done in all studies. No differences were found among the tissues subjected to the same incubation periods. Mean serum albumin and mean total cholesterol concentrations did not change significantly (n=7, p>0.05) during the same incubation periods. Mean serum albumin and mean total cholesterol values for all autogenous serum reagents used in this research (n=22) were 4.00±0.35 (±SD) g·dl$^{-1}$ and 57.7±12.3 (±SD) mg·dl$^{-1}$, respectively.

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With reference to Table 2, the linear-regression analysis of the M(P)/c₀ data from each individual showed that 1) the mean intercepts of the normal and injured M/c₀ versus P data were significantly different from each other and 2) although the slopes of the injured M(P)/c₀ data differed significantly from zero and were all positive, the slopes of the normal M(P)/c₀ data were virtually zero or slightly negative. These data quantify the observations from Figure 3 that pressure-driven convective macromolecular transport across the normal intact porcine aortic endothelial surface appears to be relatively negligible from P=0 to P=150 mm Hg but becomes very significant with endothelial loss.

Effect of time (t) and endothelial integrity on the intimal-medial uptake of 125I-albumin. As shown above, uptake by the normal surface was insensitive to pressure. Thus, the temporal uptake of 125I-albumin by the normal surface was studied only at a physiological pressure of P=100 mm Hg. For comparison, the (paired) injured wells were also studied at P=100 mm Hg. The group mean uptake [M(t)/c₀±SEM] versus t relations for intact and paired injured intimal surface preparations are summarized in Figure 4. The upper curve represents the M/c₀ versus t relation for the injured intimal surface data and the lower curve the corresponding relation for the normal, intact endothelial surface data.

As mentioned earlier, each set of these M(t)/c₀ data was analyzed by linear regression of M/c₀ against t (seconds) and against t² (seconds²). These analyses are summarized in Table 3. Comparing the R² values in Table 3A with those in Table 3B suggests that the normal-surface data were marginally but not convincingly fitted better with t² (seconds²) than with t (seconds). Comparing these R² values with the R² values in Tables 3C and 3D suggests that the injured-surface data could be fitted equally well with t or t². Referring now to the slopes in Table 3, it can be seen that the slopes were all significantly positive, i.e., M/c₀ rose monotonically with time for both normal- and injured-surface data. The corresponding intercepts for both normal- and injured-surface data were significantly positive when fitted with t (Tables 3A and 3C) but not with t² (Tables 3B and 3D). The intercepts in Table 3B (normal surface) were all negative, whereas those in Table 3D (injured surface) did not differ significantly from zero. These regression analyses will be discussed further subsequently.
tion ($M/c_0$ versus $P=0$-150 mm Hg), and the lower curve represents the corresponding mean±SEM $^{125}$I-
LDL uptake-versus-P data. Note particularly that the uptakes, $M(P)/c_0$, for both proteins appeared to be
insensitive to pressure. The uptake of LDL was only about 15% of the albumin at each pressure.

The corresponding individual regression analyses ($M/c_0$ versus $P$) are summarized in Tables 4A and 4B for LDL. The mean intercepts (at P=0) were significantly different from each other and from zero. In contrast, neither of the slopes varied significantly from zero.* Thus, the normal transendothelial uptake data of both albumin and LDL do not vary significantly with pressure, i.e., convective macromolecular transport across the normal aortic endothelial surface appears to be negligible from P=0-150 mm Hg, particularly for LDL.

**Effect of time (t) on the intimal-medial uptakes of $^{125}$I-albumin and $^{125}$I-LDL across the normal intact intimal surface.** The effects of $^{125}$I-protein exposure duration (t) on the normal transendothelial uptakes of $^{125}$I-albumin and $^{125}$I-LDL are summarized by the mean±SEM $M/c_0$ versus t (minutes) data shown in Figure 6. The $M(t)$ relations for the two proteins appear to be geometrically

![Figure 4](https://example.com/fig4.png)

**FIGURE 4. Line plot showing the relation of the normalized intimal-medial uptake [M(t)/c_0×10^3 cm] of $^{125}$I-albumin to duration (t, minutes) of exposure to the $^{125}$I-albumin for the injured (upper curve) and the paired normal intact (lower curve) intimal surface studies at P=100 mm Hg. Vertical bars are SEM.**

*Inspection of Figure 5 indicates that simple linear-regression statistics were entirely appropriate for analysis of the LDL $M/c_0$ versus P data. However, the use of this analysis for the corresponding albumin data could be questioned. The albumin $M/c_0$ at P=0 deviates sufficiently from the obvious linear trend suggested by the data at P=50, 100, and 150 mm Hg that it places in doubt the appropriateness of a linear-regression analysis that includes the P=0 $M/c_0$ data as was done for Table 4A. Therefore, to examine the albumin ($M/c_0$) P dependence further, linear-regression analyses were repeated without the P=0 data. The mean intercepts of these new analyses changed from $0.444×10^{-3}$ to $0.408×10^{-3}$ cm, the slopes from $0.089×10^{-4}$ to $0.220×10^{-3}$ cm·sec^{-1}, and the $R^2$ values from 0.472 to 0.653. Thus, the fit improved by about 18%. These new slopes also did not differ significantly from zero (p>0.30). The difference between the mean of the new intercepts (at P=0) was compared with the mean of the corresponding $M/c_0$ data at P=0. A significant difference could not be demonstrated between these two means (p>0.30). Thus, the population of the P=0 $M/c_0$ data could not be distinguished from the population of the extrapolated P=0 $M/c_0$ values represented by the new intercepts.

The same analysis was done with the P=50, 100, and 150 mm Hg LDL $M/c_0$ data, with results virtually identical to the values in Table 4B, which included the P=0 $M/c_0$ data. Moreover, comparison of the LDL $M/c_0$ at P=0 was shown to be identical to the mean of the new intercepts (p=0.994). We conclude that the validity of the conclusions as stated in the text is substantiated.
TABLE 3. Effect of Time (t, Seconds and t\(^2\), Seconds\(^2\)) on Albumin M/c\(_0\) Across Paired Intact and Injured Intimal Surfaces

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Intercept (\times 10^3) (cm)</th>
<th>Slope (\times 10^6) (cm/sec)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal surface</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>91042</td>
<td>0.123</td>
<td>0.067</td>
<td>0.902</td>
</tr>
<tr>
<td>91043</td>
<td>0.041</td>
<td>0.070</td>
<td>0.943</td>
</tr>
<tr>
<td>91044</td>
<td>0.142</td>
<td>0.056</td>
<td>0.815</td>
</tr>
<tr>
<td>91045</td>
<td>0.231</td>
<td>0.060</td>
<td>0.510</td>
</tr>
<tr>
<td>91046</td>
<td>0.220</td>
<td>0.062</td>
<td>0.995</td>
</tr>
<tr>
<td>Mean</td>
<td>0.151</td>
<td>0.063</td>
<td>0.833</td>
</tr>
<tr>
<td>SEM</td>
<td>0.035</td>
<td>0.002</td>
<td>0.086</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injured surface</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>91042</td>
<td>0.664</td>
<td>0.208</td>
<td>0.922</td>
</tr>
<tr>
<td>91043</td>
<td>0.595</td>
<td>0.145</td>
<td>0.828</td>
</tr>
<tr>
<td>91044</td>
<td>0.521</td>
<td>0.118</td>
<td>0.810</td>
</tr>
<tr>
<td>91045</td>
<td>0.382</td>
<td>0.273</td>
<td>0.818</td>
</tr>
<tr>
<td>91046</td>
<td>0.725</td>
<td>0.154</td>
<td>0.775</td>
</tr>
<tr>
<td>Mean</td>
<td>0.577</td>
<td>0.180</td>
<td>0.831</td>
</tr>
<tr>
<td>SEM</td>
<td>0.060</td>
<td>0.028</td>
<td>0.025</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 5. Line plot showing the comparative relation of the normalized intimal–medial uptakes [M(P)/c\(_0\)\(\times 10^3\) cm] of \(^{125}\)I-albumin (upper curve) and \(^{125}\)I-low density lipoprotein (LDL) (lower curve) across the normal intact endothelial surface to transmural pressure (P, mm Hg) at t=120 minutes. Vertical bars are SEM.

Modeling

The foregoing sets of results contain the main messages of this report. Since experimental measurements of arterial M/c\(_0\) as functions of time (t) and pressure (P) have not been published previously, it is difficult to relate these new data to other vascular mass-transport data in the literature. In an effort to provide the reader with some basis for such comparisons, a greatly simplified mathematical model of arterial intimal–medial uptake of radioactively labeled serum proteins is presented in the “Appendix” (Equation 6) along with essential mass-transport parameter definitions. Certain limiting forms of Equation 6, subject to various simplifying assumptions, are derived to approximate the present M/c\(_0\)-versus-t data. Each of these forms results in a simple two-parameter, linear expression relating M/c\(_0\) to t (or t\(^2\)) and to the main parameter groups that control the behavior of Equation 6. The linear nature of these expressions allows one to relate the intercepts and slopes from the simple linear-regression analyses of the M(t) data (Tables 3 and 5) to the particular groupings of the physical parameters that appear in these expressions, provided one makes the rather severe assumption that the “window” of time (30<t<120 minutes) for the present data corresponds to “large” values of t in the model (see “Appendix”). These parameter groupings in the model are D\(_A\)eF, v\(_A\)eF, and \(\phi\), in which D\(_A\) is the medial diffusion coefficient, eF is the medial distribution coefficient, v\(_A\) is the pressure-driven medial convective velocity, and \(\phi\) is the endothelial–intimal diffusive permeability coefficient.

Four hypothetical cases, which are consistent with the present protocols and uptake data, are described in the first column of Table 6. In all cases, it is assumed that medial chemical reactions may be ignored (\(k_0=0, \beta_0=0, \gamma_0=1\); see “Appendix”). Cases 1, 2A, and 2B represent situations in which pressure-driven transintimal convection of solute is prevented (e.g., see Figure 5) because...
the normal endothelium either has prevented transintimal convection of both water and solute (Case 1) or has acted like a perfect macromolecular sieve to exclude solute but to allow water convection (Cases 2A and 2B). Case 2B is similar to Case 2A, except that a significant portion of the uptake at early times is assumed to be due to rapid reversible intimal uptake and binding of the labeled solute. Case 2C represents the case of combined diffusion and pressure-driven convection of solute across the (normal or injured) endothelial surface, e.g., see Figures 3 and 4.

The second column in Table 6 indicates the 125I-protein being considered. The mean values of the regression coefficients (A and B) and their sources appear in columns 3 and 4. The last three columns contain the numerical values of the three-model parameter groupings, with their particular source or equation (“Appendix”) with which they were calculated. The limitations of these efforts are detailed in the subsequent “Discussion.”

Discussion

The relevance of the present work to the pathobiology of arterial disease is reasonably well documented. First, the porcine arterial system has been shown to be a useful model in which humanoid atherogenesis occurs spontaneously and can be accelerated by relatively mild diet-induced hypercholesterolemia. Second, the validity and limitations of the OSS methodology as used in the present work have been well documented. Third, the present and previous 125I-albumin M/c0 data obtained by the OSS methodology agree closely with the corresponding in vivo porcine values of Bell et al.

Finally, the characteristic topographic pattern of atherosclerotic lesion incidence along the arterial trees of hypercholesterolemic SRF minipigs is strikingly similar to the in vivo patterns of increased endothelial permeability in normcholesterolemic cohorts, as judged from patterns of transmural concentration distributions. Furthermore, the characteristic topographic pattern of atherosclerotic lesion incidence along the arterial trees of hypercholesterolemic SRF minipigs is strikingly similar to the in vivo patterns of increased endothelial permeability in normcholesterolemic cohorts, as judged from patterns of transmural concentration distributions. Finally, the characteristic topographic pattern of atherosclerotic lesion incidence along the arterial trees of hypercholesterolemic SRF minipigs is strikingly similar to the in vivo patterns of increased endothelial permeability in normcholesterolemic cohorts, as judged from patterns of transmural concentration distributions.

Study of postulated mechanisms to explain these observations requires, among other things, mathematical modeling of the $M(t,P)/c_0$ uptake relations. Although many imaginative models and techniques to describe the associated transmural concentration distributions $c(x)/c_0$ have been described for this purpose, only one has been suggested for $M(t,P)/c_0$ data. For present purposes, this model was rewritten as Equation 6 in the “Appendix.” In view of its mathematical complexity, it was hoped that certain simpler linear equations, representing various limits of Equation 6 (for large t), might be fitted to the present linear-regression data to yield
approximate model-parameter values for comparison with other transport parameter values in the literature. The parameter values calculated from these efforts are summarized in the last three columns of Table 6. With reference to Table 7, some of the parameter values reported in the literature (subscripted with “L”) may be related to the values in Table 6 by the following equations: $D_A e_L^2 = D_L e_L$ cm$^2$ · sec$^{-1}$ (in which $D_L$ is an apparent diffusivity and $e_L$ is an assumed solute distribution coefficient) and $P = P_L$ cm · sec$^{-1}$, where $P_L$ represents the various published endothelial diffusive permeability or diffusive mass-transfer coefficients (e.g., $k_i$, $K_E$, $k_p$, $P_E$, etc.). If one uses the value for $e_L$ that was assumed in each reference for this comparison, the values in Table 6 do not agree well with the corresponding published in vivo values of $D_L$, $P$, or $P_L$ for albumin in rabbits$^{25,27,28}$ or LDL in rabbits$^{36}$ or monkeys.$^{35}$ The values of $D_A e_L^2$ for the normal surface are about two orders of magnitude smaller than published values for rabbits and monkeys.$^{25,28,33,36}$ Except for Case 2B, the associated values for endothelial permeability ($P$) in the last column of Table 6 are about one order of magnitude higher than the corresponding published in vivo values for the normal endothelial surface.

How does one explain these discrepancies with other workers’ published in vivo values? 1) Are these differences related to the present $Mc_0$ data, e.g., is there an acute increase in endothelial permeability associated with the in vitro state? Perhaps so, but if true, how does one explain a) the close agreement between the in vivo porcine $Mc_0$ data of Bell et al$^8$ with the present (as well as previously published$^4$) normal endothelial surface $^{125}$I-albumin $Mc_0$ measurements, b) the present decreased value of $D_A e_L^2$, rather than an increased value that would be associated with injury,$^{23,24}$ and c) the demonstrated maintenance of a virtually perfect endothelial convective barrier to transport? The foregoing observations and arguments suggest that in vitro changes are not the major source of these discrepancies. More likely explanations appear to be related to differences in 2) the animal species, 3) the methods of mensuration, 4) the mathematical models, and/or 5) the various “simplifying” approximations used throughout the literature and/or those made in the present report (particularly the assumption that $t$ is large). Resolution of these discrepancies will require careful reexamination of assumptions with continued experimental challenge.

In the meantime, the main focus of the present report should be directed to the data per se, as summarized in Figures 3–6. Several important new observations have been made. 1) With reference to the lower curve in Figure 3 and to Figure 5, it was shown that the transendothelial uptake ($Mc_0$) of albumin and particu-
Table 6. Use of Linear-Regression Coefficients From $M(t)$ Data to Estimate Physical Parameters

<table>
<thead>
<tr>
<th>Protein</th>
<th>$A$ (cm)</th>
<th>$B$ (cm $\cdot$ (sec$^{1/2}$)$^{-1}$) or (cm $\cdot$ sec$^{-1}$)</th>
<th>$D_{AEF}$ (cm$^2$ $\cdot$ sec$^{-1}$)</th>
<th>$v_{AEF}$ (cm $\cdot$ sec$^{-1}$)</th>
<th>$\Theta$ (cm $\cdot$ sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>$M_{c0}=A_0+B_0t^{1/2}$</td>
<td>Alb $-0.106 \times 10^{-3}$ (Table 5B)</td>
<td>$0.731 \times 10^{-5}$ (Table 5B)</td>
<td>$0.420 \times 10^{-10}$ (Equation 24)</td>
<td>0 (Assumed)</td>
</tr>
<tr>
<td>Normal endothelial surface</td>
<td>$v_0=s_0=0$</td>
<td>Pure diffusion</td>
<td>LDL $-0.120 \times 10^{-4}$ (Table 5D)</td>
<td>$0.941 \times 10^{-6}$ (Table 5D)</td>
<td>$0.694 \times 10^{-12}$ (Equation 24)</td>
</tr>
<tr>
<td>Case 2A</td>
<td>$M_{c0}=A_0+B_0t$</td>
<td>Alb $0.114 \times 10^{-3}$ (Table 5A)</td>
<td>$0.570 \times 10^{-7}$ (Table 5A)</td>
<td>$0.420 \times 10^{-10}$ (Equation 24)</td>
<td>0 (Assumed)</td>
</tr>
<tr>
<td>Normal endothelial surface</td>
<td>$v_0=0$</td>
<td>LDL $0.160 \times 10^{-4}$ (Table 5C)</td>
<td>$0.740 \times 10^{-8}$ (Table 5C)</td>
<td>$0.694 \times 10^{-12}$ (Equation 24)</td>
<td>0 (Assumed)</td>
</tr>
<tr>
<td>Case 2B</td>
<td>Same as Case 2A except for rapid intimal binding, i.e., $A=M_{c0}$ (see Equation 5)</td>
<td>Alb Assumed to be small; see Equations 35–37</td>
<td>$0.570 \times 10^{-7}$ (Table 5A)</td>
<td>Independent</td>
<td>$v_{AEF} \gg B_2 &gt; A_0$</td>
</tr>
<tr>
<td>Normal endothelial surface</td>
<td>$v_0=0$</td>
<td>LDL Assumed to be small; see Equations 35–37</td>
<td>$0.740 \times 10^{-8}$ (Table 5C)</td>
<td>Independent</td>
<td>$v_{AEF} \gg B_2 &gt; A_0$</td>
</tr>
<tr>
<td>Case 2C</td>
<td>$M_{c0}=A_0+B_0t$</td>
<td>Alb $0.577 \times 10^{-3}$ (Table 3C)</td>
<td>$0.180 \times 10^{-6}$ (Table 3C)</td>
<td>$0.107 \times 10^{-9}$ (Equation 39)</td>
<td>$0.180 \times 10^{-9}$ (Equation 38)</td>
</tr>
<tr>
<td>Injured surface</td>
<td>$v_0=0$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R=1$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$A$, Intercept; $B$, slope; $D_{AEF}$ (in literature); $v_{AEF}$, superficial solute convective velocity ($V_s$ in literature); $\Theta$, endothelial diffusive permeability coefficient corresponding to $k_i$, $K_E$, $k_p$, etc., in literature; $v_s$, medial convection coefficient; $v_i$, intimal convection coefficient; $s_p$ and $s_i$, medial and intimal distribution coefficients, respectively; $R$, $v_s/v_i$ (the intimal/medial convection ratio); Alb, albumin; LDL, low density lipoprotein.

This was particularly true for LDL data and virtually true for the albumin data (see Figure 5, Table 4, and associated footnote in "Results"). Although it was not possible to establish statistical validity that was contrary to the aforementioned conclusion, it should be noted that inspection of the albumin $M_{c0}$ versus $P$ relation in Figure 5 might suggest that $M_{c0}$ gradually increased with pressure for pressures in excess of that (e.g., $P=50$ mm Hg) required to maintain normal arterial matrix "compaction." This gradual increase could be explained either by an increased $\Theta$ due to increased surface stretch with $P$ and/or to a small amount of transendothelial convection of albumin. The present data do not allow resolution of this statistically unsubstantiated scenario but do suggest areas for further study.

(LCD2 across the "normal" porcine aortic endothelial surface was insensitive to transmural pressure, indicating that these macromolecular solutes and perhaps water are not readily convected across the normal aortic intimal surface.* Therefore, transendothelial transport appears to remain predominantly diffusive despite increasing pressure and possible water convection. 2) The associated intimal–medial temporal uptakes across the normal endothelial surface were shown to increase monotonically with time, consistent with a scenario of dominant diffusive transport across a large endothelial surface barrier into the subjacent media, either with (Cases 2A and 2B) or without (Case 1) an associated transmural convective flux of water. The temporal rate of albumin uptake across the normal endothelial surface was about sevenfold greater than that of LDL. 3) In contrast, the $M_{c0}$ across the injured endothelial surface (Case 2C) increased monotonically not only with time but also with pressure, indicating that in the absence of a normal endothelial surface, a significant convective component is added to the transmural transport processes across the exposed aortic endothelial basement membrane and internal elastic layers. For example, loss of the endothelial surface was associated with an approximately threefold increase of the purely diffusive ($P=0$ mm Hg) uptake of $^{125}$I-albumin in contrast with an approximately eightfold increase of the ($P=150$ mm Hg) combined diffusive and convective uptake in the same artery.

These direct observations raise several cogent questions: 1) Are the subjacent arterial tissues normally protected from the convective tides of plasma substances associated with the day-to-day blood pressure fluctuations associated with exercise, anxiety, etc.? 2) Is the normal convective barrier achieved by endothelial macromolecular sieving and/or exclusion of transendothelial water flux? 3) Are the varying metabolic needs of the wall normally met by the modulation of transendothelial diffusive transport by various biological mediators or other "self-correcting" physiological feedback mechanisms? 4) Does the endothelium allow convection only under dire metabolic requirements or injury? 5) Is the appearance of significant transendothelial convection the hallmark of early endothelial response to altered hemodynamics or to pathobiological mediators, such as those associated with hypercholes-
terolemia, smoking, inflammation, etc.? 6) Is transendothelial convection (with associated integral accumulation of solutes) a necessary initiating condition for atherogenesis? Answers to such questions will require, among other things, development of various experimental and mathematical techniques for quantitative assessment of the associated transport mechanisms. Although the foregoing observations suggest many avenues of speculation regarding the roles of tissue constitutive properties, structure, pressure, etc., in mechanisms of arterial disease, further speculation will be deferred until the relevant data become available. It is hoped that the present data provide an experimental base from which more elegant and detailed inquiry can be launched. This report with its simplified interpretations and conclusions are offered to help focus and stimulate such inquiry.

**Appendix**

The derivation of a greatly simplified model of arterial one-dimensional (x direction normal to surface) transmural transport of a radioactively labeled protein of interest having a plasma concentration of $c_0$, mg cm$^{-3}$, has been described previously. The model prescribes the arterial medial transmural concentration distribution, $c(x,t,P)$, mg cm$^{-3}$, and the corresponding medial uptake, $M_A(t,P)$, mg cm$^{-3}$, of the labeled protein as functions of time (t) and pressure-driven (P) convection. Cardinal assumptions in the model are that 1) concentration and its gradient remain negligible at the adventitial-medial interface, 2) tissue layers of interest are homogeneous, and 3) times for intimal steady-state and virtual accumulation or binding of the radioactively labeled solute. will be of most interest and is rewritten in a normalized and more straightforward form ($M(x|dA_0,\alpha)$):

$$M(t,v_A(P)) = \frac{c_0}{h_0 \gamma_0} \left( \frac{\sqrt{D_A}}{h} + \frac{\sqrt{D_A + b}}{h} \right)$$

$$\left( -2 + \text{erfc} \left( \frac{\sqrt{D_A}}{h} \right) + \left( \frac{v_A}{2D_A} \right) - \sqrt{\frac{h}{D_A}} \right)$$

$$\left( \frac{v_A}{2D_A} \right) + \sqrt{\frac{h}{D_A}}$$

$$\left( \frac{v_A}{2D_A} \right) - \sqrt{\frac{h}{D_A}}$$

$$\left( \frac{v_A}{2D_A} \right) + \sqrt{\frac{h}{D_A}}$$

In which all constitutive parameters refer to locally volume-averaged (or measured) tissue concentrations and area-averaged fluxes of mobile solute, as explained in greater detail elsewhere.

**Table 7. Various Published In Vivo Transport Parameter Values for the Descending Thoracic Aorta**

<table>
<thead>
<tr>
<th>Species</th>
<th>Radioactively labeled protein</th>
<th>$D_A$ (cm$^2$ sec$^{-1}$)</th>
<th>$e_L$</th>
<th>$D_{A,t} = \frac{D_A e_L}{e_L}$ (cm$^2$ sec$^{-1}$)</th>
<th>$P_L = \frac{e_L}{e_L}$ (cm$^2$ sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Rabbit albumin</td>
<td>0.70 x 10$^{-8}$</td>
<td>0.17*</td>
<td>0.12 x 10$^{-8}$</td>
<td>0.40 x 10$^{-7}$</td>
</tr>
<tr>
<td>LDL</td>
<td>Rabbit Human LDL</td>
<td>0.30 x 10$^{-8}$</td>
<td>0.17*</td>
<td>0.12 x 10$^{-8}$</td>
<td>0.51 x 10$^{-8}$</td>
</tr>
<tr>
<td>Colton</td>
<td>Rabbit Human LDL</td>
<td>0.12 x 10$^{-8}$</td>
<td>0.42†</td>
<td>0.50 x 10$^{-8}$</td>
<td>0.16 x 10$^{-6}$</td>
</tr>
<tr>
<td>Tomkins</td>
<td>Monkey Human LDL</td>
<td>0.50 x 10$^{-9}$</td>
<td>0.17</td>
<td>0.85 x 10$^{-10}$</td>
<td>0.11 x 10$^{-8}$</td>
</tr>
</tbody>
</table>

* $e_L$ was not reported in these papers. A value was assumed so that $D_A e_L$ could be calculated for comparison with Table 6 values.
† As noted by Truskey et al, the value $e_L = 0.42$ reported in Reference 28 was from tissue inulin space measurements and therefore probably unrealistically high for low density lipoprotein (LDL). Truskey et al suggest that $e_L$ for LDL should be no greater than that for albumin. This group has used a value of 0.17 for albumin in previous work.
The intimal "permeability" parameters $h_0$ and $h$ may be expressed in more explicit physical terms as

$$h_0 = \frac{v_A H_1}{D_A(1-e^{-Pe})} \text{ cm}^{-1} \quad (8)$$

and

$$h = \frac{v_A}{2D_A} \frac{v_A H_1}{D_A e^4 (1-e^{-Pe})} \text{ cm}^{-1} \quad (9)$$

in which intimal tissue chemical degradation and binding terms are considered negligible (for the present), $e_4$ is the intimal distribution coefficient, and $Pe_4$ is the intimal Peclet number that is defined by

$$Pe_4 = \frac{v_A H_1}{2D_A} \frac{v_A}{D_A e^4} \quad (10)$$

in which $H_1$ (cm) is the intimal thickness, and $D_i$ (cm$^2$ sec$^{-1}$) is the intimal diffusion coefficient. For subsequent purposes, certain ancillary expressions from these three equations will be useful. When $v_A$ becomes zero because intimal solute (but not water) convection has been prevented by complete macromolecular sieving at the endothelial surface, then

$$h_0 \bigg|_{v_A=0} = \frac{D_A e^4 H_1}{D_A} \quad (11)$$

and

$$h \bigg|_{v_A=0} = \frac{v_A}{2D_A} \frac{D_A H_1}{D_A e^4} \quad (12)$$

in which $\Psi = D_A e^4 H_1$ is the intimal diffusive permeability (or diffusive mass-transfer) coefficient, i.e., $\Psi$ equals the ratio of the area-averaged transintimal diffusive flux divided by the (chemically active) concentration difference across the intimal layer of thickness $H_1$. However, in the situation where the transintimal convective water flux is zero (e.g., when $P=0$ or a total endothelial barrier to water convection exists), Equation 11 becomes

$$h \bigg|_{v_A=0} = \frac{\Psi}{D_A e^4} \quad (13)$$

whereas Equation 11 remains unchanged. In summary, the intimal diffusive permeability coefficient can be expressed by any of the following equivalent forms:

$$\Psi = \left( \frac{D_A H_1}{H_1} \right) \bigg|_{v_A=0} = \frac{D_A e^4 H_1}{D_A e^4} \cdot \frac{v_A e^4}{2} \quad (14)$$

Finally, an intimal-medial convection ratio ($R$) can be written as

$$R = \frac{v_A H_1}{v_A e^4} \quad (15)$$

Therefore, the Peclet number can also be expressed as

$$Pe = \frac{R(v_A e^4)}{\Psi} \quad (16)$$

As noted in the text, several limiting forms of Equation 6 might be useful in providing further insight into the significance of these data. For expedience in looking at these limits, it will be assumed that medial binding also is negligible, i.e., $\beta_0 \rightarrow 0$ (see "Appendix C" of Reference 30) and that the molecule of interest in the plasma behaves "ideally," i.e., $\gamma_0 \rightarrow 1$. Referring to Equation 7, it can also be seen that the exponential quantities on the right of Equation 6, $e^{-v_A H_1 (4DA)}$, can be expressed more simply as $e^{v_A H_1 (4DA)}$. However, since measurable levels of $^{125}$I-tyrosine (protein degradation) could not be detected in the present studies, it is also reasonable to assume that the degradation constant ($k_0$) may be neglected under the present conditions. Accordingly, it is of interest first to look at the limit of Equation 6 subject to all of the aforementioned factors and for negligible degradation, i.e., $k_0 \rightarrow 0$. This limit is given by

$$\frac{M(t,v_A(P))}{c_0} \bigg|_{\lim k_0=0} = \frac{DA h_0}{2} \cdot \frac{1}{\left[ h \sqrt{D_A + 4} / (2 \sqrt{DA}) \right] + \left[ 1 / \left[ h \sqrt{D_A - 4} / (2 \sqrt{DA}) \right] \right]} \quad (11)$$

$$\cdot \left[ 2 \sqrt{\frac{4}{16H_1^2} - \left( \frac{4}{16D_A} \right) + \left( 2 \sqrt{\frac{4}{16DA} \frac{1 + \text{erf} \left( \frac{4}{16DA} \right)}}{\sqrt{\frac{4}{16DA}} \right) \right]} \right] \quad (17)$$

Equation 17 has several interesting properties relevant to the interpretation of $M(t,c_0)$ data. The magnitude of Equation 17 rises monotonically with time, rapidly at first, and then more slowly at longer times. The initial temporal rate of change of $M/c_0$ is equal to $D_A h_0$, i.e.,

$$\left( \frac{dM}{dt} \right) \bigg|_{v_A=0} = \frac{DA h_0}{2} \quad (18)$$

which can be seen from Equation 8 (see also Equation 21 in Reference 30) to be the endothelial-intimal mass-transfer coefficient or, if $v_A=0$, the intimal diffusive endothelial permeability coefficient, as indicated in Equations 11 and 14.

At later times, the rate of change of $M/c_0$ with respect to $t$ approaches a constant, i.e., $M/c_0$ increases approximately linearly with time. If, on the other hand, convection is negligible ($v_A=0$), the temporal rate of change of $M/c_0$ becomes constant with respect to $t^{12}$, i.e., $M/c_0$ for large times ($t$) increases approximately linearly with the square root of time ($t^{12}$). The present protocols were designed to acquire $M(t)/c_0$ data during windows of time that could correspond approximately to the limits of Equation 17 for "large times." Four hypothetical cases are considered below.

**Case 1**

Hypothesis is that the normal endothelial surface provides a complete convective barrier to explain the insensitivity of $M/c_0$ to pressure (Figure 5). In this case, water and solute convection are negligible ($v_A=0$) and therefore, the limit of Equation 17 is given by

$$\left( \frac{dM}{dt} \right) \bigg|_{v_A=0} = DA h_0$$

While this assumption is reasonable for the case of albumin transport, it is questionable for LDL transport analysis, in which case the fitted parameters for the media may be interpreted as the "true" apparent parameter modified by the "binding" factor, $(1+\beta_0)$, as noted in Appendix C of Reference 30. An analogous binding factor ($\beta_i$) for the intimal layer may also be required for LDL.
\[ M(t) = \frac{\epsilon_F}{\epsilon_G} \left[ \frac{\epsilon_F}{\epsilon_G} \cdot \text{erfc} \left( \sqrt{\frac{D_{AT}}{h}} t \right) \right] \text{ cm} \]

(19)

where \( \epsilon_F \) is the medial distribution coefficient since

\[ \lim_{\epsilon_F \to 0} \left( \frac{\epsilon_F}{\epsilon_G} \cdot \text{erfc} \left( \sqrt{\frac{D_{AT}}{h}} t \right) \right) = 0 \]

as indicated by the ratio of Equation 11 to Equation 13. For increases in \( t \), the bracketed quantity on the far right of Equation 19 rapidly approaches zero. Accordingly, for large values of \( t \), the limit of Equation 19 approximates the following simple linear relation:

\[ M(t) = A_0 + B_0 t \text{ cm} \]

(21)

in which

\[ A_0 = -\frac{\epsilon_F}{h_{DA}} \]

and

\[ B_0 = 2\sqrt{D_{AT}/\pi} \text{ cm sec}^{-1} \]

(22)

Thus, the linear regression of \( M/c_0 \) versus \( t^2 \) data (for sufficiently large \( t \)) provides \( A_0 \), the value of \( y_4 \) and \( B_0 \), the value of \( \beta_0 \), from which the intimal permeability coefficient \( (2P) \) and the medial diffusion parameter group \( (DA) \) may be calculated as represented in the model by

\[ D_{AT} = \frac{\pi h_0^2}{4} \text{ cm}^2 \text{ sec}^{-1} \]

(24)

and

\[ \beta_0 = \frac{\pi h_0^2}{4A_0} \text{ cm sec}^{-1} \]

(25)

Case 2

Hypotheses are presented for a combined diffusion and convection model to explain \( M(c_0) \) data. If both diffusion and convection are significant \( (vAeF > 0) \) and if, as above, we consider time \( (t) \) frames sufficiently "long" (i.e., long enough for convection to have reduced the concentration gradient of the radiolabeled molecules at the intimal-medial interface to a negligible value), then the limit of Equation 17 approaches the linear relation,

\[ M(t) = A_d + B_d t \text{ cm} \]

where the \( A_d \) and \( B_d \) are defined by

\[ A_d = \frac{\left( h_0 D_A \right) \cdot \left( \frac{1}{2} \frac{h_0 D_A}{(vA^2/2)} \right)}{\left( h_0 D_A + (vA^2/2) \right)} \text{ cm} \]

(27)

and

\[ B_d = \frac{2\left( h_0 D_A \right) \cdot \left( vA^2/2 \right)}{\left( h_0 D_A + (vA^2/2) \right)} \text{ cm sec}^{-1} \]

(28)

If Equations 8 (for \( h_0, \text{cm}^{-1} \)) and 9 (for \( h, \text{cm}^{-1} \)) are substituted into Equations 27 and 28, one obtains

\[ A_d = D_A e_{1/3} \frac{1}{\epsilon_F} \frac{1}{\epsilon_F} \left[ \frac{1-e_{1/3}}{v(\epsilon_F(1-e_{1/3})-v(\epsilon_F(1-e_{1/3})-v_1e_1)} \right] \]

(29)

and

\[ B_d = \frac{v_1e_1Pe}{\left( v(\epsilon_F(1-e_{1/3})-v(\epsilon_F(1-e_{1/3})-v_1e_1) \right)} \text{ cm} \]

(30)

in which the explicit convective as well as diffusive parameters of the model are related to the two coefficients, \( A_0 \) and \( B_0 \). Substitution of Equations 15 and 16 into the above may be used to obtain further simplification as given by

\[ A_d = \frac{D_A e_{1/3} B_d^2}{\left( v(\epsilon_F(1-e_{1/3})-v(\epsilon_F(1-e_{1/3})-v_1e_1) \right)} \text{ cm} \]

(31)

and

\[ B_d = \frac{R(\epsilon_F(1-e_{1/3})-v(\epsilon_F(1-e_{1/3})-v_1e_1))}{\left( R-1+e_{1/3} \right)} \text{ cm sec}^{-1} \]

(32)

These equations contain four variable parameter groups and only two known values \( (A_0 \) and \( B_0 \)). Thus, only two parameter groups may be determined by the data, and values for the other two must be assumed or known from other sources. Three such hypothetical cases of combined convection and diffusion relevant to the present data will be considered as follows.

Hypothesis 2A is an alternate explanation of the normal surface \( M(t) \) data in Figure 6. The normal endothelial surface acts like a perfect macromolecular sieve, i.e., \( vAeF = 0 \), \( R = 0 \), \( vAeF > 0 \). Assume \( D_A e_{1/3} \) from Case 1, and solve Equation 31 for \( vAeF \) and the limit (as \( R \to 0 \)) of Equation 32 for \( \beta_0 \) to give

\[ B_d = \frac{v_1e_1Pe}{\left( v(\epsilon_F(1-e_{1/3})-v(\epsilon_F(1-e_{1/3})-v_1e_1) \right)} \text{ cm sec}^{-1} \]

(33)

Hypothesis 2B is same as 2A, but unlike the previous cases in which uptake by the thin intimal layer has been ignored, Case 2B assumes that avid, rapid, reversible uptake and/or binding of the radioactively labeled solute by the intimal layer has occurred. In this situation the mean linear regression intercept \( (A) \) in Table 6 for Case 2A would now represent the sum of a large intimal uptake component \( (Ad) \) as well as a linearly extrapolated medial component \( (\beta_0 \) cm). Thus, at \( t = 0 \), \( M(c_0) = A_d + B_d \) or, for use in Equation 33,

\[ A_d = A_{d\text{med}} = A - A_{d\text{med}} \]

(35)

If \( A_{d\text{med}} \) is large so that \( A_d \) becomes small in Equation 33, then \( vAeF \) becomes large. Referring to Equation 34, if \( vAeF \) becomes much larger than \( B_d \), then \( \beta_0 \) approaches \( B_d \) and Equation 34 reduces approximately to

\[ \beta_0 = B_d \text{ cm sec}^{-1} \]

(36)

This result can also be argued from "first principles": if there is a significant transintimal convective water flux, then the associated medial solute convection \( (vAeF) \) at the intimal-medial interface will tend to "wash away" any appreciable solute accumulation in the media, so that the concentration ("chemical activity") difference to drive the diffusive flux across the intimal barrier will remain at approximately \( c_0 \) and, therefore,

\[ \frac{dM}{dt} = -B_d \text{ cm} \]

(37)

Hypothesis 2C can be used to explain the injured endothelial surface-albumin data in Figures 3 and 4. Assume combined diffusion and convection but, for simplicity, no solute rejection at the intimal-medial interface, i.e., \( R = 1 \) or \( vAeF = v_1e_1 \). Also assume a value of \( \beta_0 = 0.64 \times 10^{-5} \) using Equation 14 and data from Table 1 of Reference 30 (canine injured...
surface, P = 0, serum reagent, D_a and h_0 values). Solve Equations 31 and 32 for \( \nu_{ep} \) and \( D_{ep} \) to give

\[
\nu_{ep} = \frac{B_0}{A} \text{ cm} \cdot \text{sec}^{-1} \tag{38}
\]

\[
D_{ep} = \frac{A D_0 (\nu_{ep})^2}{4 \gamma (\nu_{ep})} \text{ cm}^2 \cdot \text{sec}^{-1} \tag{39}
\]

Equations 5, 14, 24, 25, and 33–39 were used to calculate the approximate parameter values in Table 6 from the fitted A and B values as noted in the table.

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References


*See "Corrigenda," Am J Physiol 1985;248(Heart Circ Physiol 17):end of issue 6 for corrections of editor's and/or printer's errors in Reference 30, Equations 14a, A11a, C6, C7, and C9. These errors have been corrected in the reprints of Reference 30.
Effect of endothelial integrity, transmural pressure, and time on the intimal-medial uptake of serum 125I-albumin and 125I-LDL in an in vitro porcine arterial organ-support system.
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