Local Intimal-Medial Uptakes of $^{125}$I-Albumin, $^{125}$I-LDL, and Parenteral Evans Blue Dye Protein Complex Along the Aortas of Normocholesterolemic Minipigs as Predictors of Subsequent Hypercholesterolemic Atherogenesis

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This report describes the normalized intimal-medial uptakes [uptake (M, mg·cm$^{-2}$) ± serum concentration ($C_0$, mg·cm$^{-2}$)] of $^{125}$I-albumin, $^{125}$I-low-density lipoprotein (LDL), and in vivo Evans blue dye (EBD–albumin complex as functions of pressure (P), time (t), molecular species (i), and location (z)) along a ventral longitudinal z axis of the normal, intact, aortic endothelial surface in adult normocholesterolemic Sinclair Research Farm (SRF) minipigs and compares these uptake (M/$C_0$) measurements with atherogenesis in hypercholesterolemic cohorts. Uptakes of porcine serum $^{125}$I-albumin (n=21) and $^{125}$I-LDL (n=10) were measured in freshly excised, metabolically supported aortas using a recently developed organ-support system. In vivo intimal-medial EBD uptake vs z data were measured photometrically on opened descending aortas from another group (n=6) of normocholesterolemic, adult, SRF minipigs 18 hours after the intravenous administration of EBD. For comparison purposes, the corresponding incidence of atherosclerotic lesions along the aortic z axis was calculated using topographic data from hypercholesterolemic minipig cohorts (n=39). The results showed that uptakes varied greatly with z, and macromolecule (i) but not with P. More specifically, the value of M/$C_0$ at any location (z) rose with t, was insensitive to P, decreased with macromolecular (i) size, and varied with z in a pattern that “peaked” in the upstream region, decreased to a nadir in the downstream region, and then rose again as it approached the abdominal celiac orifice. The spatially z-averaged uptake rates for the three different labeled serum proteins were 0.31×10$^{-3}$ cm·h$^{-1}$ for $^{125}$I-albumin, 0.42×10$^{-3}$ cm·h$^{-1}$ for EBD-albumin, and 0.04×10$^{-3}$ cm·h$^{-1}$ for $^{125}$I-LDL. Nondimensionalized analysis of the individual sets of uptake data indicated that the overall uptake relationship [M(t,P,z,i)/$C_0$, cm$^{-2}$] could be characterized empirically by the simple product of two separate functions: one, a “scaling function” [m(z,i)], that described the uptake magnitude for a given i and z and appeared to be independent of t or P; the other, a “shape function” [s(t,P)], that described the shapes of the uptake vs t and P relationships and appeared to be independent of z or i. The “scaling function” [m(z,i)] vs z contour appeared to correlate well with the corresponding atherosclerotic lesion incidence vs z contour from the group of hypercholesterolemic minipig cohorts. Assuming passive transport, it was shown (“Appendix”) that m(z,i) can be interpreted physically in terms of an endothelial diffusive permeability coefficient (P, cm·s$^{-1}$). We conclude that (1) transport of albumin and LDL across the intact, normocholesterolemic, aortic endothelial surface is independent of pressure; (2) in vivo intimal-medial uptake of EBD can be used as a reasonable measure of $^{125}$I-albumin uptake; and (3) intimal-medial uptake rates of $^{125}$I-albumin, $^{125}$I-LDL, and in vivo EBD in the normocholesterolemic state correlate with the local probability of subsequent atherogenesis in the hypercholesterolemic state.

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Atherosclerotic lesions do not occur in a spatially random pattern in arteries but instead appear to have a characteristic topographic distribution of severity and frequency of occurrence (spatial incidence) along the arterial tree in humans and animals.2,3 The similarity of this topographic distribution to that of arterial “endothelial permeability” has been noted previously.2,4,5 Dr Henry McGill and associates appear to have been the first investigators to have noted a correlation between the topographic distribution of
arterial Evans blue dye (EBD) permeability and the local "vulnerability" of the vessel to subsequent intimal disease. At that time it was well known that cholesterol seemed to play an important role in atherogenesis. Accordingly, the interest of many investigators became focused on efforts to understand the possible connections between endothelial "permeability" to serum macromolecules and intimal cholesterol deposition. Briefly, the results of these studies have demonstrated that (1) the visual patterns of EBD-albumin uptake along the arterial trees of many different animal species are strikingly similar to the corresponding patterns of sudanophilic atherosclerotic lesions along the trees of their cholesterol-fed cohorts; (2) regions of increased EBD-albumin uptake also have an increased uptake of \(^{125}\)I-labeled cholesterol; (3) the intimal cholesterol deposits appear to arise primarily from transport of exogenous apolipoprotein B-containing plasma lipoproteins into the arterial wall; (4) transendothelial macromolecular transport is by passive, molecular-size-dependent processes; and (5) the short-term intimal accumulation of radioactively labeled low-density lipoprotein (LDL) in normal rabbits appears to be greatest in regions that develop fatty streak lesions in cholesterol-fed rabbits. Although the observations of the present study will be shown to be consistent with the results from all of the foregoing types of studies, the measurements of endothelial "permeability," the animal model used, questions addressed, and breadth of inferences differ from each of these prior studies in many new respects.

The term, endothelial permeability, is commonly used metaphorically to indicate the ease with which a selected type of plasma macromolecule (i) can enter the intimal-medial tissue system at a particular arterial location (z) under specified conditions such as transmural pressure (P, mm Hg), duration (t, minutes) of exposure to the macromolecule, and plasma concentration (c, mg · cm\(^{-2}\)) of the macromolecule. In recent in vitro studies using the metabolically supported adult Sinclair Research Farm (SRF) normocholesterolemic minipig aorta, intimal-medial macromolecular uptakes (M, mg · cm\(^{-2}\)) were measured as functions of t, P, type of macromolecule (i = \(^{125}\)I-albumin or \(^{125}\)I-LDL), and endothelial integrity. The purpose of the present research was to determine (1) how such \(^{125}\)I-albumin and \(^{125}\)I-LDL uptake relationships varied with location (z) along the intact endothelial surface of the descending thoracic and upper abdominal aortas of normocholesterolemic SRF minipigs, (2) how these in vitro uptake measurements compared with the in vivo uptake of EBD-albumin complex at the same locations (z) along this artery, and (3) how these in vivo and in vitro uptake measurements in normocholesterolemic arteries compared with the corresponding incidence of atherosclerosis at the same locations (z) in hypercholesterolemic cohorts.

**Methods**

Each of the aforementioned objectives required different methodologies and protocols, which are described below. However, several features were common to all three protocols: (1) animal models, (2) anatomic region of study, and (3) the analysis and expression of uptake data in normalized and nondimensionalized forms. The animal models were adult SRF minipigs of about the same age (2.5 years old), size (80 kg), and approximately equal sex distribution. The relevance of this animal model to human atherosclerosis has been noted elsewhere (Reference 20 and its list of references). All animals were fed, housed, anesthetized, humanely treated, and euthanized in accordance with the National Institutes of Health and the Ohio State University Institutional Guidelines for Care and Treatment of Laboratory Animals.

The anatomic region of study is shown diagrammatically in Fig 1 as the shaded area approximately ±0.4 cm on either side of an axial z coordinate along the ventral descending aorta that extended longitudinally from a point at the level of the ductus scar, downstream to the center of the downstream lip of the abdominal celiac artery orifice. For purposes of anatomic comparison, all uptake data and atherosclerotic lesion incidence data were transformed to this nondimensionalized z coordinate, ie, to the ventral axis of the vessel from z = 0 at the level of the ductus scar to z = 1 at the abdominal celiac orifice as indicated in the figure.

Analysis and comparison of data from studies with different serum concentrations (c) and different molecular species (i) are greatly simplified if expressed in normalized form. Serum protein uptake appears to vary linearly with its plasma concentration; therefore, all data from the \(^{125}\)I-albumin, \(^{125}\)I-LDL, and EBD-albumin uptake studies were normalized with their corresponding serum concentrations (c) and expressed as normalized uptakes, ie, \(M/c\) cm. These normalized uptake values are presented as the group mean ± SEM at the corresponding value of the particular independent variable being studied, eg, time (t), pressure (P), or location (z) for a given labeled macromolecule (i). Conceptually, the present work explored the temporal (t), pressure (P), location (z), and macromolecular dependencies of the uptake relationships \(M(t,P,z,i)/c\) by measuring the value of \(M/c\) over an experimentally controlled range of values for the particular independent variable of interest, eg, location (z), while holding the remaining

**FIG 1.** Diagram of the z-axis coordinate system along the ventral descending aorta to which all data are referred. All locally area-averaged measurements were within the shaded strip. The bracketed regions \(z_{\text{u}}\) (upstream) and \(z_{\text{d}}\) (downstream) indicate the regions along z from which the z dependencies of the M(t) and M(P) processes were compared.

*[The quantity M(t,P,z,i) is mathematical "shorthand" (functional notation) to indicate that the value of the measured (dependent) variable M is a function of, or depends on, the experimentally controlled (independent) variables appearing within the parentheses. Variables that are experimentally held constant in subsequent considerations will either be omitted from the parenthetical quantity or appear as subscripts in such notation.]*
variables, eg, in this particular example time \( (t) \), pressure \( (P) \), and type of macromolecule \( (i) \), constant.

In an effort to separate major qualitative dependence from major qualitative dependence of the \( M(z)/c_0 \) data on each of the independent variables, the experimental values of \( M(z)/c_0 \) for a range of the independent experimental variable of interest were nondimensionalized by division with the regionally z-averaged value of the measured \( M(z)/c_0 \) values, ie, with the \( M(z)/c_0 \) (in which the overbar indicates the averaging). For example, the nondimensional uptake vs z curve was expressed as \( M(z)/M(z) \), which would represent the qualitative dependence of the \( M(z)/c_0 \) values on z, ie, the shape of the z dependence, whereas the quantitative dependence would be expressed by the z-averaged uptake value, \( M(z)/c_0 \).

Such nondimensionalized uptake data, eg, \( M(z)/M(z) \), also allowed the individual uptake vs z contours to be plotted on the same ordinate scale around an averaged value of unity for detailed comparison of the shapes (in this example, the shapes of the z dependencies) of uptake curves among different individuals and among the three different labeled proteins. In contrast, comparison of the spatially averaged values \( [M(z)/c_0] \) do not provide information on the z dependence of shape but do provide important quantitative comparisons of the magnitudes of uptake among the individuals and the three different labeled proteins. Both types of analysis will be presented.

Finally, since considerable differences will be demonstrated between the magnitudes of the upstream and the downstream temporal \( (t) \) and pressure-driven \( (P) \) uptake processes along the vessel, and since the dependence of \( M(z)/c_0 \) on \( t \) or \( P \) cannot be measured at a single point \( z \) (by the present methods), local regions of \( z \) were chosen to explore the regional z dependence of the temporal and pressure-driven transport processes. Referring to Fig 1, the in vitro (see below) \( ^{125}\)I-albumin and \( ^{125}\)I-LDL uptake vs time \( [M(t)/c_0] \) and uptake vs pressure \( [M(P)/c_0] \) relationships were each measured in a limited upstream region, ie, \( z_0 \) (representing the range \( 0.1 < z < 0.3 \)), and in a limited downstream region, \( z_5 \) (representing the range \( 0.7 < z < 0.9 \)), along the aorta as indicated by the bracketed regions in Fig 1.

In Vitro Measurement of Intimal-Medial Uptakes of \( ^{125}\)I-Albumin and \( ^{125}\)I-LDL Along the Normal Descending Minipig Aorta as Functions of \( t \), \( P \), and \( z \)

The purpose of these studies was twofold: (1) to compare the pressure-dependent and time-dependent uptake relationships \( [M(t)\text{ or } P]\) of \( ^{125}\)I-albumin and \( ^{125}\)I-LDL in the upstream \( (z_0) \) with those of the downstream \( (z_5) \) region of the aorta and (2) to compare the z dependence of uptake relationships \( [M(z)] \) of \( ^{125}\)I-albumin and \( ^{125}\)I-LDL for constant time \( (t) \) and pressure \( (P) \). To achieve the requisite experimental control for these studies, it was necessary to do them in the metabolically supported ascending aorta using an in vitro organ-support system (OSS). The strengths and limitations of this methodology have been described previously.\(^{20,27}\) Briefly, the descending aorta (thoracic plus upper abdominal) was carefully removed from the well-ventilated, fully anesthetized,* young adult, normocholesterolemic (50 to 80 mg total serum cholesterol per deciliter) SRF minipigs (n=24); the vessel was opened along the dorsal longitudinal axis, the in situ dimensions were restored slowly in a tissue-holding device as a flat sheet with the endothelial surface up, and then the sheet was captured between two matching, multichambered devices (the well assembly) that isolated the intimal and adventitial surfaces of the vessel into 18 contiguous, 1.2-cm-square regions along the z axis of the opened vessel (see Fig 1; also see Fig 1 of Reference 20). Accordingly, this system provided for local independent experimental control of transmural pressure, flow (stirring), nutrient media, etc, at each of 18 sites along the vessel. Referring to Fig 1, the mean distance from the ductus scar \( (z=0) \) to the center of well No. 1 was 2.7 cm \( (z=0.10) \), to the center of well No. 18 was 25.7 cm \( (z=0.97) \), and to the center of the downstream lip of the abdominal celiac orifice was 26.6 cm \( (z=1) \). The nutrient medium was stirred autogenous serum (AS) in the wells on the intimal surface, and a simulated interstitial fluid (25% AS in physiological porcine electrolyte-glucose solution) on the matching adventitial surfaces.\(^{27}\)

The entire tissue plus well assembly plus tissue-holding device system was then placed in the chamber of the OSS for a 1- to 2-hour equilibration period with stirred AS at the prescribed pressure, \( P \) (mm Hg). The stirring intensity of the nutrient reagent in the well has been shown to abolish concentration gradients \( ^{27,28} \) and to ensure endothelial surface oxygenation and a pH of 7.4.\(^{27}\) The OSS consisted of a transparent chamber in which the temperature and ambient gas \( (O_2, CO_2,H_2O) \) partial pressures were controlled at \( P_{O_2}>200 \) mm Hg, \( P_{H_2O}=47 \) mm Hg, and a \( P_{CO_2} \) level to maintain a bicarbonate to \( P_{CO_2} \) ratio for that particular donor animal's serum and tissue at a pH of 7.4.\(^{27}\) After the equilibration period in AS under these conditions, the AS was replaced with AS containing the \( ^{125}\)I-albumin or \( ^{125}\)I-LDL for the duration \( (t) \), minutes and pressures \( (P, \text{mm Hg}) \) prescribed by the aforementioned uptake vs time \( [M(t)/c_0] \), uptake vs pressure \( [M(P)/c_0] \), or uptake vs location \( [M(z)/c_0] \) protocols. Other essential methodological details regarding the tissue preparation, nutrient media, labeled proteins, quality controls, measurement of uptake \( (M) \), etc, have been described previously.\(^{20,27}\)

Measurement of the In Vivo t=18-Hour Transendothelial Uptake of EBD-Albumin Complex Along the Descending Aorta of the Adult, Normocholesterolemic, SRF Minipig

The purpose of these studies was to examine the in vivo EBD uptake vs location \( [M(z)/c_0] \) relationship for the EBD-albumin complex and to compare these data with the corresponding in vitro uptake data from the \( ^{125}\)I-protein studies in the OSS described above. The in vivo intimal-medial accumulation of EBD from parenterally administered EBD-albumin remains densely localized in the superficial intimal-medial layers and therefore can be quantified with light reflected from the

*The animals were induced with ketamine, 40 mg/kg of body weight, plus acepromazine, 0.04 ml/kg of body weight, followed by intravenous maintenance doses of sodium pentobarbital as necessary.
opened, EBD-stained endothelial surfaces for reasons explained previously. It appeared from this earlier work that on entry to the tissue spaces, the dye "immediately" bound firmly to the connective tissue and interstitial matrix, leaving its former albumin vehicle to continue on its way, and thereby preventing significant penetration of the EBD before its tissue immobilization. Accordingly, unlike EBD-albumin, a steady-state aortic transmural distribution of EBD requires exposure durations well in excess of several days (D.L. Fry, MD, unpublished observations).

Unlike the rapid association noted above, the dissociation of the bound dye from the tissue matrix into a buffered saline elution bath (without protein) was found to be only about 3.5%/h. In view of the dense but shallow penetration of the EBD and its very slow elution into saline, it was possible to demonstrate that the intimal-medial EBD accumulation (Mₑ, nmol • cm⁻²) could be quantified from the red reflectance of the opened saline-covered vessel surface by

\[ Mₑ = 3.92\rho + 0.80\rho^2 \text{ nmol} \cdot \text{cm}^{-2} \]  

in which \( \rho \) is the negative natural logarithm of the ratio (reflectance) of reflected red light intensity (I) from the EBD-stained intimal surface to that (Iₑ) from the unstained surface. It was also shown that if \( \rho < 1 \), as was the case for the present data, then

\[ Mₑ = 3.92\rho \]  

or alternatively, in terms of optical density (OD),

\[ Mₑ = 9.026 \times OD \]

in which OD is \( -\log_{10}(I/Iₑ) \). This earlier work provided the quantitative basis for the present methodology.

Six normocholesterolemic adult minipigs were used for this study. On the evening before study the minipig was tranquilized (40 mg/kg of body weight ketamine) and administered EBD (18 mg/kg of body weight) in 250 mL of sterile saline (0.9% NaCl) intravenously over a 15- to 30-minute period to begin a t=18-hour in vivo exposure period to the EBD-albumin complex. This dosage was equivalent to an approximate initial serum EBD-albumin concentration of Cₑ₀ = 572 nmol • cm⁻³. Previous unpublished observations indicated that Cₑ₀ dropped approximately exponentially with a time constant of about 0.05 h⁻¹ (=5%/h). The integral of this exponential decay over 18 hours would suggest that the temporal mean serum EBD-albumin concentration (Cₑ) for the present studies would have been ~380 nmol • cm⁻³.

The next morning (−18 hours later) the animal was anesthetized,* euthanized (50 mg/kg of body weight of intracardiac sodium pentobarbital), and pressure rinsed at P=100 mm Hg with oxygenated phosphate-buffered saline to rinse the endothelial surfaces free of serum proteins and thereby arrest further intimal uptake or significant elution of intimal EBD. The descending aortic tree was quickly excised, dissected free of adventitial tissue, opened longitudinally, stretched to approximate the in vivo dimensions, pinned out flat on corkboards previously covered with white oilcloth, and photographed for subsequent quantitative photometric estimates of intimal-medial EBD uptake as a function of location (z) along the aorta by the following methodology.

Quantitative black-and-white (Kodak Panatomic X) photographic records of the intimal EBD uptake patterns were taken through a Wratten No. 29 (red) filter at three different, standardized exposures selected to bracket the "optimum" exposure indicated by the light-meter reading (through the filter) and the ISO rating of the film. To correct subsequent OD measurements from these negatives (due to nonuniform photographic illumination, emulsion inhomogeneities, film development processing, etc), calibrating photographs were taken on the same photograph of film immediately before and after photographing the intimal surfaces. These calibrating photographs contained a smoothly milled, white-epoxy-enameded, aluminum plate of known, uniform white reflectance to record the uniformity of the photographic field illumination. A calibrated gray-level scale (a standard Kodak gray-scale step tablet) was included in these photographs to establish the relationships of local film densities on the companion tissue photographs to these known gray-level reflectance scales and the mean unstained reflectance values along z from separate, unstained porcine aortas.

Each pinned-out vessel, white plate, and gray scale were photographed while submerged under 1 cm of buffered saline (to remove the intimal surface reflections from the illuminating lights) at each selected exposure. The entire set of films from each animal study was submitted as a single batch for processing under identical developmental conditions. The processed negatives from a given animal preparation and from the corresponding reflectance calibrations were photometrically scanned (on an Eikonix 78/99 digital scanning system in the Biomedical Engineering laboratory), processed for corrections (on a VAX station 4000 model 60), and then converted to locally averaged EBD OD distributions along z, which were then converted to Mₑ(z) with Eq 3 and to Mₑ(z)/Cₑ(z) vs z contours by division with the aforementioned extrapolated mean value of Cₑ₀ (380 nmol • cm⁻³).
or “absence” of red-stained (sudanophilic), intimal atherosclerosis at each point on the intimal surface. The spatial incidence of lesions at that point was calculated as the percentage of animals having a lesion “present” at that particular location. The incidence of atherosclerotic lesions at each location along \( z \) was expressed as the laterally averaged incidence \( \pm 0.4 \) cm on either side of the ventral \( z \) axis.

**Results**

**In Vitro Arterial Uptakes of \( ^{125}I \)-Albumin and \( ^{125}I \)-LDL as Functions of Pressure and Time in the Upstream and Downstream Regions of the Minipig Aorta**

The nature of the pressure (\( P \)) and temporal (\( t \)) dependencies of the uptake processes in the proximal (\( z_0 \)) and distal (\( z_D \)) descending aorta are summarized in Figs 2 and 3. The group mean uptake (M/\( c_0 \) ±SEM) vs \( P \) data for the upstream aorta appear as the dashed line and for the downstream aorta as the solid line in Fig 2, A for albumin and in Fig 2, B for LDL. Note first that at a given site (\( z_0 \) or \( z_D \)) the albumin curves are about eight times higher than the LDL curves. Second, it can be seen that the upstream uptake vs pressure \( [M(P)] \) values for both proteins are all significantly greater than the corresponding downstream \( M(P)_D \) data. Third, none of the four albumin and LDL curves has linear regression slopes that vary significantly from zero, ie, the \( M/c_0 \) values are insensitive to pressure but do vary significantly with location (\( z \)) and molecular species.

When each set of uptake vs pressure \( [M(P)\_Alb, c_0\_Alb, M(P)\_LDL\_c_0\_LDL] \) data was nondimensionalized by division with the respective \( z \)-averaged values for the individual sets, one obtained the corresponding set of nondimensionalized \( M(P)_r, M(P)_D \) curves shown in Fig 2, C. Note that these nondimensionalized curves appear to have no consistent differences among them; ie, apart from moderate random variation, the shapes of the albumin curves do not differ from those of the LDL curves, and the shapes of the upstream (\( z_0 \)) curves cannot be distinguished from the downstream (\( z_D \)) curves. Thus, it would appear that the shapes of the nondimensionalized uptake relationships could be considered to be ostensibly independent of location (\( z \)) and macromolecule (\( i \)).

Referring now to Fig 3, the uptake vs time (\( t \)) data \( [M(t)/c_0] \) for the upstream aorta appear as the dashed lines, and the uptake for the downstream aorta appear as the solid lines in Fig 3, A for albumin and Fig 3, B for LDL. Note that the uptake vs time \( [M(t)/c_0] \) curves all increase with time (\( t \)) and that each protein curve for the upstream aorta is significantly higher than that for the downstream aorta. At a given location (\( z_0 \) or \( z_D \)) the albumin curves are again seen to be about eight times higher than the LDL curves. If each of these sets of

**FIG 2.** Line plots of aortic intimal-medial uptake (\( M/c_0, \) cm) of \( ^{125}I \)-proteins as functions of pressure (\( P \)). A, \( ^{125}I \)-albumin (Alb); B, \( ^{125}I \)-low-density lipoprotein (LDL); C, nondimensionalized data from panels A and B.

**FIG 3.** Line plots of aortic intimal-medial uptake (\( M/c_0, \) cm) of \( ^{125}I \)-proteins as functions of time (\( t \)). A, \( ^{125}I \)-albumin (Alb); B, \( ^{125}I \)-low-density lipoprotein (LDL); C, nondimensionalized data from panels A and B.
FIG 4. Line plot showing topographic comparison of the nondimensionalized uptake \([M(z)/M(z)] = F(z,i)\) of \(^{125}\)I-albumin (i=A, open circles), \(^{125}\)I-low-density lipoprotein (i=L, open squares), and in vivo Evans blue dye–albumin (i=E, open diamonds) along the \(z\) axis of the intact, normocholesterolemic aortic endothelial surface with the corresponding incidence (% on right y-axis scale, open triangles) of atherosclerotic lesions along the aortic \(z\) axis of hypercholesterolemic cohorts. U, upstream; D, downstream.

\[ M(t)_x/c_x \text{ and } M(t)_y/c_y \text{ data is nondimensionalized with the respective } z\text{-averaged uptake value for the set, one obtains the corresponding four nondimensionalized } M(t)_x \text{ and } M(t)_y \text{ curves shown in Fig 3, C. Note the virtual congruence of these nondimensionalized uptake vs time curves; there do not appear to be significant differences between the shapes of the upstream and downstream curves or between the albumin and LDL curves.} \]

As was observed above for the uptake vs pressure \([M(P)]\) data, it would appear that the shapes of the normalized uptake vs time \([M(t)]\) relationships are also virtually independent of location \((z)\) and macromolecule \((i)\). A more comprehensive examination of these spatial \((z)\) and macromolecular \((i)\) dependencies of aortic transendothelial uptake was provided by the following sets of experiments.

Regional Transendothelial Macromolecular Uptake Along the \(z\) Axis of \(^{125}\)I-Albumin, \(^{125}\)I-LDL, and EBD-Albumin in Normocholesterolemic Minipig Aortas Compared With Regional Incidence of Arteriosclerosis in Hypercholesterolemic Cohorts

These experiments encompassed all three of the aforementioned methodologies, ie, the in vitro, OSS radioactive albumin and LDL method; the in vivo parenteral EBD-albumin photometric technique; and the atherosclerotic lesion topographic analytic methods. The results of the uptake vs location \((z)\) studies are compactly presented in the aforementioned nondimensionalized uptake vs \(z\) format as shown in Fig 4 for comparison with the atherosclerosis incidence data.

The triangles connected by the solid bold lines represent the incidence (percentage on right y axis) of sudanophilic atherosclerotic lesions along \(z\) from the group of 39 hypercholesterolemic minipigs. The y-axis values represent the percentage of animals that had a lesion at each location \((z)\) along the aorta. Note that the incidence of atherosclerosis along \(z\) is low opposite to the ductus scar \((z=0)\) and then rises rapidly to about 22% at \(z=0.17\), after which it tends to decrease in a "roller-coaster" pattern to a nadir of about 3% at \(z=0.83\) and then rises rapidly again in anticipation of the abdominal celiac orifice at \((z=1.0)\).

The other three curves in Fig 4 represent plots of the nondimensionalized uptake vs location \((z)\) contours \([M(z)/M(z)], \text{symbolized by } F(z,i)\) for (1) in vivo EBD-albumin (E, diamonds), (2) in vitro native \(^{125}\)I-LDL (L, squares), and (3) in vitro \(^{125}\)I-albumin (A, circles). Note that the \(F(z,i)\) ordinate has been truncated from below to facilitate comparison of the \(F(z,i)\) with the lesion incidence (%) curve. Within the limits of measurement error, the three different \(F(z,i)\) curves appear to be very similar in shape and magnitude. Moreover, their shapes are also similar to the shape of the lesion incidence curve in the figure. These data have a number of implications that will be discussed subsequently.

The corresponding regionally averaged uptake values \((M(z)/c_z)\) are also of interest, since these values reflect the spatially averaged, absolute magnitudes of the uptake of each type of macromolecule \((i)\) for the specified durations of exposure \((t)\). The group mean (±SEM) \(t=18\text{-hour in vivo EBD-albumin (E, diamonds), (2) in vitro native }^{125}\text{-LDL (L, squares), and (3) in vitro }^{125}\text{-albumin (A, circles).} \] [Note that the \(F(z,i)\) ordinate has been truncated from below to facilitate comparison of the \(F(z,i)\) with the lesion incidence (%) curve. Within the limits of measurement error, the three different \(F(z,i)\) curves appear to be very similar in shape and magnitude. Moreover, their shapes are also similar to the shape of the lesion incidence curve in the figure. These data have a number of implications that will be discussed subsequently.

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be used to rescale the respective nondimensional uptakes, $F(z,i)$ in Fig 4, to show the actual $(M(z)/c_0)$ uptake values along $z$ for each type of molecule. More importantly, if the three mean $(M(z)/c_0)$ values are normalized with respect to time, i.e., $(M(z)/c_0)$, one obtains the mean rates of uptake for each molecule: $(0.424\pm0.033)\times10^{-3}$ cm h$^{-1}$ for in vivo EBD-albumin, $(0.314\pm0.014)\times10^{-3}$ cm h$^{-1}$ for in vitro $^{125}$I-albumin, and $(0.040\pm0.003)\times10^{-3}$ cm h$^{-1}$ for in vitro $^{125}$I-LDL. Note that the in vitro albumin and in vivo EBD-albumin uptake rates are approximately the same,* whereas both of these are almost an order of magnitude (nine times) larger than the LDL uptake rate.

**Discussion**

The present research examined the relationship of transendothelial macromolecular transport along the normocholesterolemic SRF minipig aorta to the local development of sudanophilic atheromatous lesions along the corresponding vessel in hypercholesterolemic cohorts. It was shown that the intimal-medial macromolecular uptake $(M_{ic})$ across the intact endothelial surface varied greatly with time $(t)$, location $(z)$, and molecular species $(i)$ but was insensitive to pressure $(P)$. It was also shown that the shapes of the macromolecular uptake vs location $(z)$ contours for $^{125}$I-albumin, $^{125}$I-LDL, and in vivo EBD-albumin were similar. When these uptake vs location data were nondimensionalized, the uptake vs contour for the three different molecules tended to become surprisingly congruent.

The following is an effort to provide a somewhat more global view of the data than as is presented in the “Results.” More specifically, when the $M(t)$ and $M(t)$ data shown in Figs 2 and 3 are viewed together, it appears that the overall $M(t)$, $(t)$ contours for $(t)$-albumin, $^{125}$I-albumin, $^{125}$I-LDL, and in vivo EBD-albumin were similar. If these three different contours are in fact congruent, it would imply that the molecular dependence $(i)$ in the $m(z,i)$ of Eqs 4 and 5 must be constant with $z$, i.e., $m(z,i)=K_i \cdot m(z)$, so that the $K_i$ in the ratio of $m(z,i)$ to its integral would also cancel, like $c_0$ and $s(t)$ in Eq 5, to give

$$m(z,i) = K_i \cdot m(z) \quad m(z)$$

in which the function of $z$ only. Thus, the formulation in Eq 4 (based on the data in Figs 2 and 3) implies the congruence of the three $M(z)/M(z)$ curves in Fig 4. By inference, one concludes that $F(z)$ must represent an independent set of tissue properties that, from the data in Fig 2, appear to be purely diffusive in nature (although an active transport mechanism is not ruled out) and from the data in Figs 2, 3, and 4 to be independent of $t$, $P$, and $i$. Assuming that transport is in fact passive, it is shown in the “Appendix” that $F(z)$ can be interpreted physically as the nondimensionalized endothelial diffusive permeability coefficient, $P(z)$.

*Note that the interpretation of uptake rate as a measure of transmural transport in all of the present experiments assumes that one is considering durations of uptake of the labeled solute that are short compared with the times required to approach steady-state transport. In the case of EBD-albumin transport, the transport of the albumin “vehicle” molecules (that have been shorn of the EBD ligand by the intimal and superficial medial tissue layers) has approached steady state by 18 hours; however, the accumulation rate of intimal-medial EBD continues to be a function of time and, to a lesser extent, pressure; and are determined primarily by a functional notation by $s(t)$ or, in view of the data in Fig 2, more simply by $s(t)$.† As can also be seen in Figs 2 and 3, the scales of the magnitudes of the $M(t,P,z)/c_0$ relationships appear to be determined completely by a function of location $(z)$ and the type of macromolecular solute $(i)$; accordingly, this observation can be symbolized in functional notation by $m(z,i)$. Thus, the overall temporal $(t)$, pressure $(P)$, location $(z)$, and macromolecular $(i)$ dependencies of the $M_{ic}$ data can be characterized compactly in simple functional notation as the product of $m(z,i)$ and $s(t)$, i.e.,

$$M(t,P,z) = \frac{1}{c_0} \cdot [m(z,i) \cdot [s(t)]] \quad \text{cm}$$

Moreover, if Eq 4 is integrated to obtain a representation of the regionally averaged value of the data $[M(t,P,z)/c_0]$, $s(t)$ will act as a constant in this integration so that as $c_0$ will cancel out in the ratio of Eq 4 (representing the data at $z$) to its regionally averaged integral (representing the $z$-averaged data). Thus,

$$\frac{[M(t,P,z)]/c_0}{[M(t,P,z)]/c_0} = [m(z,i)]/\equiv F(z)$$

in which the nondimensionalized data can be seen to depend only on the location $(z)$ and macromolecule $(i)$ as symbolized by $F(z,i)$.

† Although $M(P)$ was not shown to depend significantly on pressure, $P$ would be expected to influence the shape of the $M(t,P)$ relationship, particularly the $M(t)$ curve, depending on the pressure-induced changes in wall constitutive properties, etc (also see the footnote on p 1201). Measurements of $M(t)$ at different pressures to evaluate this dependence exceeded the scope of this research. In the interest of simplicity, the presumably small $P$ dependence in the shape function will be ignored until future data to the contrary emerge.
FIG 5. Topographic comparison of the nondimensionalized intimal permeability function $F(z)$ on left y-axis scale, solid circles along the z axis for normocholesterolemic SRF minipig aortas with the corresponding incidence [%] right y-axis scale, open triangles of atherosclerotic lesions along the aortic z axis of hypercholesterolemic cohorts.

Relationship of Uptake Data to Arteriosclerosis

In view of the apparent congruence of the uptake vs $z$ contours in Fig 4, the y-axis values of all three sets of $M(z)/M_\infty(z)$ data were averaged at each $z$ to obtain a better estimate of the putative underlying tissue property, the $F(z)$ relationship. This average contour is shown in Fig 5 as the dashed curve $F(z)$, left y-axis scale. For comparison purposes, the lesion incidence data [% right y-axis scale] was included as the solid curve. The degree of similarity between the $F(z)$ and percentage lesion incidence curves in Fig 4 provides the first convincingly discrete, quantitative, topographic evidence that (1) there is an underlying "diffusive" tissue permeability property of the normocholesterolemic aortic endothelial system that determines local intimal access of macromolecules and, more importantly, (2) these local properties correlate reasonably well with the subsequent development of atherosclerotic lesions at that site in the hypercholesterolemic artery. The sudanophilic lesion incidence curve (Fig 5) did not distinguish between sudanophilic fatty streak lesions and raised atheromatous plaques, which are the potentially lethal lesions of atherosclerosis. The upstream "high-risk" region ($0.1<z<0.6$ in Fig 5) in hypercholesterolemic minipigs usually demonstrates classic fatty streak lesions that seldom progress into significant raised, atheromatous plaques. The downstream ($0.85<z<1.0$) "high-risk" region of these same arteries also develops a ventral, fatty streak lesion; however, this streak lesion almost always shows a spatially (and temporally?) progressive evolution into a classic raised, linear, fibrocellular, atheromatous plaque in its downstream portion, which extends to and includes the upstream lip of the abdominal celiac artery orifice. This latter portion is also the location of non-sudanophilic intimal fibromuscular hyperplasia in normocholesterolemic minipigs (D.L. Fry, MD, unpublished observations).

These sets of observations are consistent with earlier human studies showing that raised atheromatous plaques appeared to develop at sites of preexisting sudanophilic streak lesions that were located at certain characteristic sites along the arterial tree, whereas streak lesions located at sites other than these characteristic sites appear to be stable or even regress. These important pioneering observations would suggest that in human disease, intimal sudanophilia would appear to be an antecedent (but not sufficient) condition for development of subsequent, clinically significant, local atheromatous plaque formation.

A second set of equally important observations has shown that local regions of the arterial tree with normal physiologically induced or pathologically related simple intimal fibromuscular hyperplasia are also sites of predilection for subsequent development of raised atheromatous plaques. If these interesting observations are to be consistent with the aforementioned correlation of atheromata with antecedent fatty streaks, we must infer that these regions of initially nonsudanophilic intimal thickening would also coincide with regions of antecedent increased "diffusive" permeability as represented by the $F(z)$ curve in Fig 5.

In either case the present observations suggest that sites of increased endothelial diffusive permeability in the normal physiological state are vulnerable to the subsequent development of intimal sudanophilia, non-
sudanophilic fibrocellular hyperplasia, and, most importantly, the potentially dangerous, raised, atheromatous plaque. Although detailed speculation regarding mechanisms connecting this "vulnerability" to plaque development would be premature, it is of interest to note several points relevant to the present data. For example, an increased diffusive transport of various growth factors across the normal surface may be part of a physiological remodeling response to reestablish stable blood flow patterns and as such may be the reason for normally occurring regions of intimal fibromuscular hyperplasia. Such areas of increased permeability are also vulnerable to pathological changes. For example, small perpendicular concentration gradients must exist in the boundary layer of blood flow at the arterial luminal surface, particularly in hemodynamically low-shear regions and/or in regions of large transmural water flux with macromolecular sieving at the endothelial surface. If such gradients were of potent chemotactic agents, these could act as "homing beacons" for activated blood cells, e.g., monocytes in the adjacent boundary layer of blood flow. Moreover, it is reasonable that sites of increased diffusive permeability would also be sites of increased intimal exposure to blood-borne biological mediators, pathogens, toxins, etc, that could compromise normal intimal metabolism and disrupt the normal endothelial diffusive barrier. Such disruption would allow a further increase in the intimal diffusive influx and perhaps more importantly, could also allow transendothelial convection of various plasma substances such as LDL, chemotactic agents, growth factors, etc, into the intimal space. If there are macromolecular sieving structures in the subjacent intimal layer at such sites, the associated convective tides can result in very high intimal atherogenic chemical activities and activity gradients, particularly in areas of intimal thickening. This scenario suggests only one of several plausible ensembles of events that could relate the observed phenotypically determined normocholesterolenic pattern of endothelial diffusive permeability [F(z) contour] to the development of intimal sudanophilic lesions, fibrocellular intimal thickening, and the transition of these benign intimal changes into raised atheromatous lesions.

Insight into why F(z) along the thoracic and upper abdominal aorta has the characteristic shape shown in Fig 5 will require inquiry into the nature of local genetic determinants, various physiological localizing forces such as adjacent hemodynamic events and the cellular response mechanisms driven by such localizing forces. The present work has provided important new information regarding normal arterial transport processes that were shown to be intimately related to atherogenesis. These observations can help shape the design of future studies to clarify the aforementioned structural and functional tissue interactions that constitute the local risk of fatty streaking, intimal fibrocellular hyperplasia, and the transition of these into the more dangerous, atheromatous, raised lesions at preferential sites in the arterial tree.

Appendix

The overall set of uptake data \[M(t,P,z,i/c_0)\] that has been presented in the body of this report cannot be translated directly into explicitly defined physical quantities. A model is required for this purpose. Although transmural transport, particularly across deendothelialized arterial surfaces, appears to occur by passive-diffusive and convective processes, the present observations of transport across the intact aortic endothelial surface could also be explained by active transendothelial transport mechanisms. This Appendix examines the extent to which passive transendothelial transport can explain the present set of observations. To do this it examines the behavior of a simplified one-dimensional mathematical model of arterial intimal-medial transmural passive transport of radiolabeled macromolecules. Based on the assumption of passive transport, this exercise is designed to provide the reader with (1) further insight into the nature of the assumptions that are required to explain the ensemble of observations presented in the body of this report and, (2) subject to the aforementioned assumptions, a physical definition of the F(z) contour in terms of an endothelial diffusive permeability coefficient.

The physical properties of the arterial wall are determined by the interaction of myriad heterogeneous microscopic, cellular, and connective-tissue elements that comprise histologically distinguishable layers of composite materials. This microscopic architecture creates highly tortuous transmural flux paths for the labeled molecules. Thus, a microscopically realistic mathematical model of transmural transport must be three-dimensional and must have an enormous number of constitutive parameters that present intractable mathematical problems and serious difficulties in experimental verification. If the aforementioned heterogeneous tissue layers can be treated as if they were homogeneous, the mathematical description of mass transport across the multilayered wall can be simplified and rendered more amenable to experimental testing. The first useful model of this sort was for one-dimensional diffusive-convective transport across a single layered, homogeneous surface, which was described with verifying experimental data by the group at the Massachusetts Institute of Technology. The usefulness of this simple model for data analysis has been well established. Another effort to model this complex system resulted in a simplified two-dimensional model consisting of diffusive transmural transport through a mosaic of identical, contiguous, homogeneous wall units, each of which is exposed to the plasma mostly through a single, central "leaky" pore. This "leaky" pore model has been updated recently to include convection. Except for the important observation that there are apparently significant circumferential and longitudinal components to subendothelial transport from local sites of endothelial replacement, the "pores" in the two-dimensional model, the model does not appear to have been fitted to experimental mass transport data.

The one-dimensional model to be described below evolved from carefully considered simplifications of a relatively realis-
practical analytic tool (one-dimensional with fewest parameters) to relate experimentally measurable (locally volume-averaged) tissue concentrations of labeled macromolecules to local atherogenesis as well as to experimentally imposed variables (serum composition, flow, pressure, etc) relevant to atherogenesis. This model deals with the aforementioned problems of tortuosity, heterogeneity, multiple tissue layers, etc, in an explicit set of assumptions, the plausibility and limitations of which have been discussed in detail in the texts and appendices of References 7, 20, 39, and 43. A list of these assumptions is reproduced below for the reader's critical review.

The model describes arterial one-dimensional (x direction normal to intimal surface) transmural transport of a labeled macromolecule (i) of interest as a function of time (t, seconds), transmural pressure-driven (P) solute convective velocity (v, cm·s⁻¹), and plasma concentration (cᵢ, mg·cm⁻³) as derived previously. The model prescribes the locally volume-averaged transmural tissue concentration distribution c(x,t,P) (mg·cm⁻³) and its integral with respect to x, which represents the associated arterial uptake [M(t,P), mg·cm⁻³] as measured in the present research. Cardinal assumptions of this model are that (1) the intimal and the medial tissue layers may each be treated as statistically homogeneous layers, ie, concentrations are locally volume-averaged and fluxes are locally area-averaged values; diffusive and convective fluxes perpendicular to x are sufficiently rapid that local, transverse, steady-state transport and chemical quasi-equilibrium is achieved in times that are short compared with (2) pressure-driven transmural water flux is always at steady state; (3) the times to achieve intimal steady-state solute transport are negligible compared with t; (4) all reversible chemical reactions, such as EBD and LDL, solute tissue-binding reactions, are rapid enough that virtual chemical equilibrium exists at all points across the intima and media; and (5) solute concentration and its gradient remain negligible at the adventitial-medial interface, ie, t is small compared with the times to achieve medially steady-state transport. For present considerations it will also be assumed that the intimal accumulation (Mi) can be ignored compared with the medial accumulation (Mag) in the normal young arteries (very thin intimal layer) that were relevant to the present work.

Recent in vitro OSS data for normal intimal-surface arteries have provided two additional (simplifying) constraints for this model: (6) Observations from the present and from earlier work demonstrated that uptake across the normocholesterolemic, intact, endothelial surface was insensitive to pressure. This implies that intimal solute convection (but not necessarily transintimal water convection or medial solute or water convection) is prevented by sieving of the macromolecular solute by the normal endothelial tissue system (glycocalyx-base membrane system), so that transintimal solute transport is by diffusion only. (7) Moreover, measurable levels of tyrosine generation (a measure of the rate of protein degradation) were not found. This allows one to assume that chemical degradation parameters for the solute are not major determinants of macromolecular transport under the present experimental conditions, ie, short-term uptake studies in the normal artery. Based on these two added observations and the aforementioned five assumptions, the normalized medial uptake (Mᵢ(t,νᵢ(P))/ρᵢ; cm⁻¹) at a given location (z) along the vessel and for a particular labeled macromolecule (i) can be expressed mathematically by

\[
\Phi \left( \left[ \frac{1}{2(2V + p)} + \frac{\left( v_{i} \right)^{\frac{1}{2}}}{\pi^{\frac{1}{2}}} e^{-\left( v_{i} \right)^{\frac{1}{2}} + V \cdot t \cdot (1 + \text{erf} \left( V t^{\frac{1}{2}} \right))} \right] \right) = \frac{\text{erf} \left( V t^{\frac{1}{2}} \right)}{2Vp} + \frac{\text{erf} \left( V t^{\frac{1}{2}} \right)}{p(2V + p)^{\frac{1}{2}}} \frac{c_{i}(x,t,P)}{\rho_{i}}
\]

in which \( \gamma \) is the serum chemical activity of \( i \); \( \Phi \) (cm·s⁻¹) is the endothelial-intimal diffusive permeability coefficient as defined by \( \Phi = D_{i} / (H_{i} (c_{i} \cdot s^{-1})) \), in which \( D_{i} (c_{i} \cdot s^{-1}) \), \( e_{i} \), and \( H_{i} (cm) \) are the intimal diffusion coefficient, distribution coefficient, and thickness, respectively; \( V \) is a pressure-driven, convective parameter group for the medial solute as defined by

\[
V = \frac{\nu_{i}(P)}{2D_{i}^{\frac{3}{2}} \left( 1 + \beta_{i} \right)} \frac{1}{s^{\frac{1}{2}}}
\]

in which \( \nu_{i}(P) (cm \cdot s^{-1}) \) is the transmural, pressure-driven solute convective velocity across the media, \( D_{i} (c_{i} \cdot s^{-1}) \) is the solute diffusion coefficient for the media, and \( \beta_{i} \) is a medial equilibrium binding constant for the EBD or LDL tissue-binding reaction (\( \beta_{i} = 0 \) for no tissue binding) that depends on the chemical nature of the solute (i) and receptor-macromolecule site molecules; \( p \) is a permeability parameter group defined by

\[
p = \frac{\epsilon_{i} D_{H}^{\frac{3}{2}} (1 + \beta_{i})}{s^{\frac{1}{2}}}
\]

in which \( \epsilon_{i} \) is the median distribution coefficient for the solute; and the other primary parameters are defined as above. Finally, the notation "erf" and "erfc" are the error function and complementary error function, respectively, of the quantities (arguments) appearing immediately following each notation, and \( e \) is the base of natural logarithms.

The following properties of Eqs 7 through 9 are noteworthy: 1) The normalized medial uptake of a macromolecule (i) at a given location (z) is determined by the independent, experimentally imposed variables, time (t, seconds), pressure \( [v_{i}(P), cm^{-1}] \), type of solute (i), and by the four parameters, \( \gamma, \beta_{i}, V, (Eq 5) \), and \( p \) (Eq 9). 2) Although \( \beta_{i} \) probably varies mostly with the type of macromolecule (i), the other primary transport parameters, \( \gamma, \nu_{i}, D_{H}, \) and \( \epsilon_{i} \) will each vary not only with the type of solute molecule (i) but also with the location (z) in relation to the changing tissue properties along the z axis of the vessel. 3) The shapes of the \( M_{i}^{\left( \gamma, \nu_{i}(P) / \gamma \right)} / \rho_{i} \) relationship are determined by the variables and parameters within the braces in Eq 7, whereas the magnitudes are determined also by the multiplier \( \Phi \) at the left brace.

If this formulation is to be consistent with the observations that were summarized in the phenomenological format expressed by Eq 4, one would be forced to equate \( \Phi \) in Eq 7 with the observed scale factor \( m(z) \) and to equate the complicated functions of \( t, P, i, \) and parameters within the braces to the aforementioned shape factor \( l_{i}(P) \). This shape factor must be
independent of \( z \) and \( i \) if Eq 7 is to be consistent with the observed data. Referring to Eqs 8 and 9, this would require that \( f_{th} \) depended only on the chemical nature of the binding reaction and not on the location \( (z) \) and if (despite the likely \( z \) dependence of \( D_A, e_A, v_A, etc \)) the ratios \( V \) (Eq 8) and \( p \) (Eq 9) did not vary significantly with location \( (z) \). Although plausible arguments could be made for this, a simpler explanation would be that certain of the medial parameters, such as diffusivity \( (D_A e_F) \) or convection \( (v_A e_F) \), have values very large relative to intimal permeability, so that transport rate becomes determined predominantly by the (normal) intimal layer. Accordingly, it is of interest to examine the limit of Eq 7 for these two cases: Case 1, the limit for \( v_A e_F \gg \theta \), \( (v_A e_F) \) and Case 2, the limit for \( D_A e_F >> \theta \), \( (D_A e_F) \) and \( v_A e_F << \theta \).

It turns out that both of these limits approach the same value, i.e.,

\[
M_{th}[v_A e_F(P), z] \gamma c_0 \approx [\Phi(z,i) + t \cdot cm]
\]

which states that the uptake for either of these cases varies linearly with time \( (t) \) and that the uptake rate is the intimal diffusive permeability coefficient \( \theta(z,i) \).

The corresponding physical interpretation of Eq 10 for Case 1 would be that the macromolecular solute \( (i) \) is completely fixed at the plasma-endothelial interface, allowing only the convective water flux to proceed through the intima into the media. The subintimal solute accumulation from the transintimal solute diffusive flux would be continuously "washed away" by the "(large" \( v_A e_F \) ) transintimal water flux such that the chemical driving force for solute diffusion across the intimal layer remains virtually constant at \( \gamma c_0 \).

Parenthetically, we note that this scenario suggests that the media is being continuously purged by protein-poor serum electrolyte solution. Moreover, for sufficiently elevated pressure, the luminal surface of the endothelial layer will be exposed to a protein concentration in excess of that \( (c_0) \) in the bulk phase of the blood, particularly in "low" shear regions where the sieved solute is not rapidly washed away by the blood flow. A boundary layer accumulation of various "toxic" solutes by such a mechanism may contribute to the endothelial "vulnerability" mentioned in the "Discussion" and Reference 4.

The physical interpretation of Eq 10 for Case 2 would be that transmedial diffusivity \( (D_A e_F) \) is so large relative to \( \theta \) that the transmedial chemical activity gradient is very small compared with the intimal gradient. Accordingly, the subintimal chemical activity remains virtually constant at \( \gamma c_0 \). Parenthetically, we note that this scenario suggests that the media is being continuously purged by protein-poor serum electrolyte solution. Moreover, for sufficiently elevated pressure, the luminal surface of the endothelial layer will be exposed to a protein concentration in excess of that \( (c_0) \) in the bulk phase of the blood, particularly in "low" shear regions where the sieved solute is not rapidly washed away by the blood flow. A boundary layer accumulation of various "toxic" solutes by such a mechanism may contribute to the endothelial "vulnerability" mentioned in the "Discussion" and Reference 4.

In accordance with Eq 5, if Eq 10 is divided by its integral with respect to \( z \), one obtains

\[
M_{th}[v_A e_F(P), z] \gamma c_0 \approx [\Phi(z,i) + t \cdot cm]
\]

or, in view of the approximate congruence of the \( F(z,i) \) contours (see Eq 6),

\[
\frac{\Phi(z)}{\Phi(z)} \approx F(z)
\]

Therefore, in each of these extreme situations (Cases 1 or 2), the chemical activity of the labeled solute at the intimal-medial interface is held virtually constant so that the transintimal diffusive driving force also remains approximately constant with time, thus explaining an approximately "linear" uptake with time. As a result, the contour of the ratio \( M_{th}(z)/M_{th}(z) \) represents the distribution of the local, nondimensionalized, intimal diffusive permeability coefficient of the intact, normocholesterolemic, endothelial surface as indicated in Eqs 11 and 12. More importantly, the data in Fig 5 indicate that the \( \Phi(z)/\Phi(z) \) also appear to correlate topographically with the local tissue risk of subsequent atherogenesis in the hypercholesterolemic state as discussed more fully in the body of this report.

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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 39, Eqs 14a, A10a, C6, C7, and C9. These errors have been corrected in the reprints of Reference 39. In rechecking the derivations for the present report, the author discovered a minor error that apparently survived the exhaustive earlier review process. The multiplier (1 + β') for the right hand sides of Eq 29 and Eq 31 in Reference 39 is an algebraic error and should be deleted from these two equations only.
Local intimal-medial uptakes of 125I-albumin, 125I-LDL, and parenteral Evans blue dye protein complex along the aortas of normocholesterolemic minipigs as predictors of subsequent hypercholesterolemic atherogenesis.

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