Effects of Oscillatory Mechanical Disturbance on Macromolecular Uptake by Arterial Wall

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Transport of 125I-albumin by isolated segments of canine common carotid arteries was studied in vitro at zero transmural pressure. Sinusoidal oscillatory variations in length (peak change 4%) for 15 minutes at frequencies of 5 and 10 Hz caused 40% increase in 125I-albumin uptake, and also a 30% increase in the apparent luminal surface area. Changes in the duration and frequency of oscillation indicate that the total number of oscillations (= frequency × duration) was the critical parameter in causing these effects. The increase in apparent luminal surface area was correlated with regional flattening of the internal elastic lamina and the overlying endothelial cells, as demonstrated by transmission and scanning electron microscopy. Endothelial vesicles were counted with the aid of ruthenium red as a postfixation extracellular marker. The ratio of unstained free vesicles to total vesicles averaged 0.083 in the control state and decreased slightly to 0.070 after oscillation. Although the decrease in free vesicle population indicated an acceleration of vesicle diffusion, our theoretical computations showed that the resulting increase in vesicle flux was negligible. The increase in 125I-albumin uptake by the artery following mechanical oscillation is mainly attributable to the increase in apparent luminal surface area.


There is considerable evidence that the rate of passage of macromolecules from the arterial lumen into the wall substance is affected by fluid mechanical factors. Thus, the arterial uptake of the albumin-bound blue dye T-1824 injected intravenously shows a characteristic focal pattern,1,2 which can be modified by local hemodynamic factors.3,4 Quantitative determinations of the uptake of labeled macromolecules by arterial wall in vitro have demonstrated variations of uptake with the application of fluid shearing stresses5,6 or transmural pressure.7 Studies on the effects of pressure and flow oscillations on the uptake of 125I-albumin8 and 14C-4-cholesterol8 by canine common carotid arteries suggest that transendothelial transport of these substances is influenced by the peak wall shear rate or shear stress.

It is generally accepted that the principal barrier to the passage of macromolecules from the arterial lumen into the wall substance lies at the blood-wall interface. Studies with the use of T-1824-labeled albumin9 and 125I-albumin10 have indicated that this barrier is associated with the endothelial cell layer. Electron microscopic tracer studies on the endothelium of capillaries and arteries indicate that the transendothelial motion of plasmalemmal vesicles plays an important role in the transport of macromolecules.11-13 A theoretical model has been developed to treat the steady state14 and transient15,16 diffusion and convection of plasmalemmal vesicles. In the present paper, this theory has been extended to small amplitude periodic mechanical deformations of the endothelial cell layer, in order to analyze the possible effect of such periodic disturbances on transendothelial vesicle flux and macromolecular transport.

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Caro et al. attempted to study the effect of mechanical oscillation on the uptake of albumin by the canine common carotid artery in vitro, but the data were scattered due to technical difficulties. In the present experimental investigation, an improved technique has been developed to study the effect of sinusoidal variations in vessel length on the uptake of $^{125}$-albumin by the canine common carotid artery in vitro. The in vitro preparation was used to permit a better control of the mechanical conditions. The results indicate that sinusoidal length variation at 5 or 10 Hz for 15 minutes causes a significant increase in albumin uptake. Computations based on our theoretical model and electron microscopic studies on vesicle distribution, however, indicate that mechanically enhanced vesicle diffusion cannot account for the observed increase in macromolecular uptake. An important factor turns out to be the effective surface area available for uptake.

**Methods**

This study was performed on 45 healthy mongrel dogs (body weight, 15 to 25 kg) anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Blood was drawn from the femoral artery without using anticoagulant for the preparation of the autologous serum to be used in the experiment. Following an anterior midline incision on the neck, one common carotid artery was carefully exposed over a length of approximately 10 cm. All visible vessel branches associated with the artery and bleeding points were either ligated or sealed with a cold electrocautery, which was never used near the artery itself. Meticulous care was taken to avoid any stretching or other mechanical disturbance of the artery during the dissection and all subsequent procedures. Heparin (5000 units) was injected intravenously. The artery was ligated at both ends and also in the middle; it was thus isolated into cranial and caudal halves, each of which was cannulated by the following procedure. One stainless steel cannula (i.d. 2 mm) was introduced from each end of the half, and the heparinized blood in the arterial lumen was immediately cleared with the use of autologous serum. During the cannulation procedure, care was taken to avoid sudden deflation of the lumen and collapse of the vessel. The two cannulas were fixed to a metal splint to avoid alterations in artery geometry when the cannulated arterial segment was removed from the dog.

The cranial and caudal halves of the artery on one side were mounted on two separate rigs for the determination of $^{125}$-albumin uptake, as described below. One half was used to study the effect of oscillatory variations in vessel length on protein uptake, and the other half served as a control (no oscillation). The common carotid artery on the other side was dissected and isolated into cranial and caudal halves in the same manner. The matching between the location of the arterial segments (cranial or caudal) and the experimental condition (control or oscillated) was reversed between the two sides, with the purpose of avoiding any systematic error due to possible differences in the transport behavior between the cranial and caudal halves.

The rig for studying macromolecular uptake by arterial segments (figure 1) was modified from that described by Caro and Nerem. The apparatus consisted mainly of a stainless steel trough with two vertical end walls, which were milled for mounting the stainless steel cannulae attached
to the isolated arterial segment. The base on one of the end walls was attached to the trough through a cam which was coupled to a variable speed motor via a gear system. Rotation of the motor resulted in a periodic sliding motion of the coupled end wall and an oscillatory variation in length of the mounted arterial segment. The frequency of oscillation was varied by controlling the motor speed, and the wave form and amplitude could be altered by changing the cam and its eccentricity. In the present investigation, the frequency was varied from 1 to 10 Hz, the amplitude was 2 mm (approximately 4% of vessel length), and the wave form was sinusoidal. The entire system, including the arterial segment, was immersed in a 0.9 percent NaCl bath, with the temperature maintained at 37.0 ± 0.2°C.

After the arterial segment had been mounted at its in vivo length in the rig by fixing its cannulas in the milled end walls, the metal splint was removed. The lumen of the arterial segment was filled with autologous serum (37°C) containing approximately 4 μCi/ml of pyrogen free 125I-albumin (Mallinkrodt, St. Louis, Missouri) which had been passed through an ion exchange resin column immediately before the experiment to remove free 125I. The rate of filling was approximately 10 ml/min, which allowed the filling to complete within a short period while keeping the attendant wall shear stress under 50 dyn/cm² (calculated on the basis of laminar flow), a value significantly below that known to cause endothelial damage.9 The pressure generated in the arterial segment during the filling process was less than 5 mm Hg. The volume of the serum used in the filling process was 1–2 ml. In segments to be subdivided from a given experimental segment generally agreed within ± 10% of the mean, except in cases where endothelial damage led to a marked enhancement of 125I-albumin uptake. Such endothelial damage, in the form of endothelial denudation or holes, was demonstrable by scanning electron microscopy. Experiments with evidence of endothelial damage (found in three arterial subsegments) were discarded. The Rₚ values for the subsegments were averaged for each experiment.

For scanning electron microscopic studies, the fixed arterial segments were silt open along their longitudinal axis, subjected to critical point drying, coated with gold palladium in a Hummer I sputterer, and examined in a Jeolco scanning electron microscope (model JSM 25) at 15 kV.

For transmission electron microscopic studies, the fixed arterial segments were post-fixed in 2% OsO₄ in 0.15 M cacodylate buffer, dehydrated in ascending ethanol series, placed in propylene oxide and embedded in Spurr's resin. In some experiments 0.1% ruthenium red (m.w. 786.5, purchased from Ventron Corporation, Danvers, Massachusetts as ammoniated ruthenium III chloride) was added to the OsO₄ and dehydrating solutions for the purpose of identifying endothelial vesicles attached to the plasmalemma. Thin sections were cut along the cross section of the artery and contrasted with 6% uranyl acetate and lead citrate. A Zeiss EM9 electron microscope was used to view sections at 60 kV. It was necessary to use the postfixation ruthenium staining for the determination of vesicle attachment because vesicles without a visible stalk may

The 125I-activity determined in each arterial sample (Qᵢ in cpm) was divided by the sample weight (Wᵢ in g) and the 125I-albumin activity per unit volume of the labeled serum (Cₛ in cpm/ml) to yield the tissue uptake in terms of a tissue/serum activity ratio (Rᵢ, in ml/g or μl/g over a 15-minute period):

\[ Rᵢ = \frac{Qᵢ}{WᵢCₛ} \] (1)

The Rᵢ values for the three to five subsegments subdivided from a given experimental segment to yield a marked enhancement of 125I-albumin uptake. For transmission electron microscopic studies, the fixed arterial segments were post-fixed in 2% OsO₄ in 0.15 M cacodylate buffer, dehydrated in ascending ethanol series, placed in propylene oxide and embedded in Spurr's resin. In some experiments 0.1% ruthenium red (m.w. 786.5, purchased from Ventron Corporation, Danvers, Massachusetts as ammoniated ruthenium III chloride) was added to the OsO₄ and dehydrating solutions for the purpose of identifying endothelial vesicles attached to the plasmalemma. Thin sections were cut along the cross section of the artery and contrasted with 6% uranyl acetate and lead citrate. A Zeiss EM9 electron microscope was used to view sections at 60 kV. It was necessary to use the postfixation ruthenium staining for the determination of vesicle attachment because vesicles without a visible stalk may
be attached to the plasmalemma out of the plane of the section. Determinations of attached and free vesicles were made in the peripheral zone of those endothelial cells which were stained by ruthenium red on both the luminal and abluminal fronts. The vesicles stained by ruthenium red were considered attached, whereas the unstained ones were counted as free vesicles.

**Theory**

The experiments described in this report were motivated by the suggestion from an earlier theoretical study\(^\text{14}\) that transendothelial transport of macromolecules may be enhanced by periodic mechanical disturbances. To test this possibility, the present investigation was designed to perform parallel experimental measurements and theoretical analysis. The aim of the theoretical analysis is to determine whether the magnitude of the effect of mechanical oscillation on macromolecular uptake can be explained on the basis of an enhancement of vesicle diffusion.

In this analysis, it is assumed that the mechanical oscillations introduced in our experiments do not alter the residence time of an attached vesicle \(t_p\) but can shorten the transendothelial vesicle diffusion time \(t_D\). This assumption is probably not unreasonable considering the high speed of propagation of an elastic wave through an attached vesicle neck during vesicle detachment\(^\text{15}\) and the slow transendothelial diffusion velocity of the vesicle,\(^\text{19,20}\) in comparison to the order of magnitude of the velocity of the cell membrane relative to the intracellular fluid in our experiments. Under this assumption, we shall derive an expression of the influence of a shortening of \(t_D\) on the enhancement of vesicle transport and macromolecule permeability in the endothelial cell.

As shown in Weinbaum and Caro\(^\text{14}\), the net transport of macromolecules across the endothelial cell layer due to vesicle diffusion, assuming that the vesicle fluxes toward the luminal and abluminal fronts are equal, is given by:

\[
J = \phi_R V [C_p - C(0)]
\]  
(2)

where \(J\) is the vesicle flux/cm\(^2\), \(V\) is the available interior volume of each vesicle, \(\phi_R\) is the number of vesicles/cm\(^2\)-sec that cross the cell once released, and \(C_p\) and \(C(0)\) are the macromolecular concentrations at the luminal and abluminal surfaces of the endothelial cell, respectively. The duration of our oscillatory experiments (15 minutes) is long compared to the time required for the vesicle flux to achieve steady state conditions, which has been found to be 10 to 60 seconds for capillary endothelium,\(^\text{19,20}\) but is shorter than the time required to achieve steady state in the underlying tissue.\(^\text{21}\) The value of \(C(0)\) is small compared to \(C_p\), since the major part of the total wall resistance to macromolecule transport lies across the endothelial cell layer.\(^\text{9,10}\) Thus, from Equation (2), \(J\) is nearly proportional to \(\phi_R\).

The release rate of vesicles at the luminal plasmalemma per cm\(^2\)-sec is given by \(\phi = N_a/t_p\), where \(N_a\) is the number density (per cm\(^2\)) of attached vesicles at the luminal membrane. The probability that a vesicle will cross to the abluminal front of the cell without first reattaching to the luminal front from which it was released is \(\phi_R/\phi\). This probability is a function of the vesicle radius, a transendothelial diffuse distance \(L\), location of the plane of vesicle release \(y\), and the effective distance of approach \(\epsilon\) at which molecular level forces between membranes become the dominant factor. In the theoretical model, the diffusion of a spherical particle is treated between the opposing membrane boundaries, which have been idealized as locally parallel walls. An approximate expression for \(\phi_R/\phi\)\(^\text{16}\), taking into account the hydrodynamic resistance law derived in Ganatos et al.\(^\text{21}\) in the central region of the cell and the steric hindrance of attached vesicles near each plasmalemma, is:

\[
\frac{\phi_R}{\phi} = \frac{\beta(y - \epsilon) + \theta a \ln \frac{L - \epsilon}{L - y}}{\beta(L - 2\epsilon) + 2\theta a \ln \frac{L - \epsilon}{\epsilon}} = G(a, y, \epsilon, L)
\]  
(3)

\(\beta\) and \(\theta\) are correction factors that can be obtained by curve fitting the exact solutions of Ganatos et al.\(^\text{21}\) near the center of the cell and the additional fluid resistance near each boundary plasmalemma as determined by scaled laboratory model experiments described in Arminski et al.\(^\text{16}\).

The vesicle flux \(\phi_R\) can be written in terms of the probability function \(G(a, y, \epsilon, L)\) defined in Equation (3) as:

\[
\phi_R = G \frac{N_a}{t_p}
\]  
(4)

While \(N_a\) and the number density (per cm\(^2\)) of free vesicles \((N_f, \text{per cm}^2)\) will vary as the ratio of \(t_p/t_D\) is changed, the total vesicle density \((N = 2N_a + N_f, \text{per cm}^2)\) is assumed to be essentially constant over short times characteristic of the experiments described herein. Equation (4) rewritten in terms of \(N\) and \(N_a\) is:

\[
\phi_R = G \left[ \frac{N}{2t_a} \right] \left[ 1 - \frac{N_a}{N} \right]
\]  
(5)

From Arminski et al.\(^\text{15}\) an expression for \(N_a/N\) can be derived as:
where $N^*_1$ is a dimensionless free vesicle concentration given by:

$$N^*_1 = \frac{1}{2L^2} \left[ \beta(y - \epsilon) + \theta a \ln \frac{y(L - \epsilon)}{(L - y) \epsilon} \right] \times$$

$$\left[ \beta(L - y - \epsilon) + \theta a \ln \frac{(L - \epsilon)(L - y)}{\epsilon y} \right]$$

and $D_o$ is the Stokes–Einstein diffusion coefficient for a vesicle in an infinite medium with viscosity $\mu_o$. $L^2/D_o$ is the characteristic vesicle diffusion time $t_D$ introduced earlier. $N^*_1$, like the function $G$, depends only on the vesicle-cell geometry, the spatial variation of fluid resistance and $\epsilon$. When the foregoing results and the definition of $t_D$ are substituted in Equation (5) one obtains:

$$\phi_R = \frac{G N}{2t_s} \left[ \frac{1}{1 + (N^*_1 t_D/2t_s)} \right]$$

We next inquire how Equation (8) might be modified by a small amplitude deformation of the cell. Both the functions $G$ and $N^*_1$ would not be expected to change significantly since the relative dimensions of the vesicle and the cell vary, at most, a few percent and the molecular level force range $\epsilon$ is not affected. Also, by previous reasoning $t_s$ should be unaffected, and the principal modification should occur in $t_D$. The random motions of the cell boundary may produce an intracellular mixing that is equivalent to adding an eddy diffusivity to the molecular diffusivity that is present when intracellular fluid motions are absent. The reduction in $t_D$ can, therefore, be thought of as an increase in the effective diffusion coefficient $D_o$ in the cell interior.

If we denote the steady state control without oscillations by the subscript zero and take the ratio of the vesicle flux in the mechanically disturbed state (i.e., Equation (8) with $t_D/t_s < t_{Do}/t_s$) to the flux in the control (i.e., Equation (5) with $N^*_1 = N_{o1}$) we have:

$$\frac{\phi_R}{\phi_{Ro}} = \frac{1}{[1 - (N_{o1}/N)] [1 + (N^*_1 t_D/2t_s)]}$$

From electron microscopic measurements, typical values for the geometric parameters in the expression for $N^*_1$, Equation (7), are: $a = 35$ nm, $L = 400$ nm, and $y = 20$ nm. For these values, a good approximation for the variation in hydrodynamic resistance across the cell is obtained when $\beta = 0.941$ and $\theta = 1.050$. The parameter for which there is a large measure of uncertainty is $\epsilon$. Reasonable estimates of this effective molecular force distance vary from 2.5 to 10 nm. In figure 2 we have plotted the flux ratio $\phi_R/\phi_{Ro}$ as a function of $t_D/t_s$ for several representative values of $N_{o1}/N$ and two values of $\epsilon$ (2.5 and 5.0 nm). The maximum value of $t_D/t_s$ occurs when $t_D$ is equal to $t_{Do}$, as given in Equation (10). The maximum possible enhancement of the vesicle flux occurs as $t_D \to 0$ where $\phi_R/\phi_{Ro}$ approaches the limiting value $1/[1 - (N_{o1}/N)]$.

A comparison between the results for $\epsilon = 2.5$ and 5.0 nm shows that $\epsilon$ affects $\phi_R/\phi_{Ro}$ when the diffusion time is rate controlling, but not the limiting value when $t_D \to 0$.

Figure 2. Theoretical computation of the ratio of transendothelial vesicle flux resulting from enhanced vesicle diffusion ($\phi_R$) to that in the control state ($\phi_{Ro}$) as a function of the ratio of vesicle diffusion time ($t_D$) to vesicle attachment time ($t_s$). Solid lines are for an effective range of molecular interaction force $\epsilon$ of 5.0 nm, and broken lines are for $\epsilon$ of 2.5 nm. For each $\epsilon$ value, a family of curves are shown for different fractional free vesicle densities in the control state ($N_{o1}/N$) from 0.1 to 0.4.
Results

125I-Albumin Uptake

To assess the effect of oscillation on macromolecular uptake by the carotid arterial segment, the $R_t$ value for the oscillated segment ($R$) was divided by that for the corresponding control segment ($R_0$). This ratio ($R/R_0$) is plotted in figure 3 against the frequency of oscillation. In some experiments, both cranial and caudal segments of a given artery were not subjected to oscillation, and the ratio of $R_t$ value for the cranial segment to that for the caudal segment is shown as $R/R_0$ at zero frequency. With an increase in frequency of oscillation, $R/R_0$ rose to approach a plateau value of approximately 1.4 with frequencies above 5 Hz. The increases were statistically significant at 5 Hz ($p < 0.05$) and 10 Hz ($p < 0.01$), but not at the lower frequencies.

The use of the ratio $R/R_0$ in individual arteries was intended to reduce individual variations and to enhance the sensitivity of the experimental test. Even when the values at 10 Hz of different arteries were combined (Mean $\pm$ SEM = 3.04 $\pm$ 0.29 $\mu$/g/15 min), $R$ was still significantly ($p < 0.05$) higher than $R_0$ (2.27 $\pm$ 0.12 $\mu$/g/15 min).

Vessel Geometry

When cross sections of thin rings of arterial segments were examined after photographic enlargement, the segments which had been subjected to 5 or 10 Hz oscillations showed increases in their internal and external diameters ($D_i$ and $D_e$, respectively) with a reduction in wall thickness, $(D_e - D_i)/2$, as compared to their corresponding controls obtained from the same artery (figure 4). The apparent luminal circumference ($C_i = $ apparent luminal surface area per unit vessel length) and the wall cross section area ($A_w = $ wall volume per unit vessel length) were calculated as:

$$C_i = \pi D_i \quad (12)$$

$$A_w = \pi (D_e^2 - D_i^2)/4 \quad (13)$$

The ratio of apparent luminal surface areas between the oscillated and control segments ($C_i/C_{i0}$) increased with an increase in frequency to reach a plateau level of approximately 1.3 at frequencies of 5 and 10 Hz (figure 5). The reliabili-
ity of the geometric measurements is supported by the agreement in the $A_w$ values for the oscillated and control segments (i.e., the conservation of wall volume).

**Effects of Duration of Oscillation**

In order to further analyze the factors causing the increase in apparent luminal surface area ($C_l$) following oscillation, experiments were performed on arterial segments subjected to different durations of oscillation. It was found that oscillation at the low frequency of 1 Hz for 150 minutes caused $C_l$ to increase to a similar extent as 10 Hz oscillation for 15 minutes, whereas 10 Hz oscillation for 2 minutes or less did not cause a significant increase in $C_l$. The oscillation-induced increase in $^{125}$I-albumin uptake was likewise dependent on the total number of oscillations.

**Vessel Ultrastructure**

The ultrastructural basis of the increase in apparent luminal surface area following oscillation was studied by scanning electron microscopy. As reported previously, the luminal surface of unoscillated carotid artery segments at zero transmural pressure revealed rather regularly spaced longitudinal ridges running parallel to the direction of blood flow. The arterial segments subjected to 5 or 10 Hz oscillation for 15 minutes, however, showed the presence of many flattened areas in addition to areas containing ridges and folds (figure 6).

The wavy nature of the surface of arteries subjected to zero transmural pressure that was seen in scanning electron microscopy is associated with corresponding undulations of the internal elastic lamina as observed in transmission electron microscopy (figure 7 A), and portions of some endothelial cells are squeezed in the valley without being exposed to the vessel lumen. Following oscillation at 5 or 10 Hz for 15 minutes, some areas of the artery showed a flattening of the internal elastic lamina and the overlying endothelial cells (figure 7 B).

**Distribution of Free and Attached Vesicles**

With the use of ruthenium red as a postfixation extracellular marker, we were able to distinguish between vesicles attached to the plasmalemma

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**Figure 6.** Scanning electron micrograph of luminal surface of canine common carotid artery showing regional flattening of the luminal surface after oscillation at 10 Hz for 15 minutes, with a local loss of longitudinal foldings characteristic of normal arteries at zero transmural pressure. Tilt angle = 30°. Bar = 10 μm.
Figure 7. Transmission electron micrographs of canine common carotid arteries in the control state (A) and following 15 minutes of oscillation at 10 Hz (B). e = endothelial cell; N = nucleus of endothelial cell; IEL = internal elastic lamina; SM = smooth muscle cell. Bars = 10 μm.
Figure 8. Transmission electron micrographs of canine common carotid artery in the control state, showing the filling of attached vesicles by ruthenium red after fixation. Note that some of the vesicles (asterisks) without visible stalks are attached as evidenced by the presence of ruthenium red staining in the lumen. Bar = 0.1 μm.

(containing ruthenium red) and those free in the endothelial cytoplasm (no ruthenium red) (figure 8). In control segments without oscillation, the ratio of free vesicle density to total vesicle density (N_f/N) in the peripheral zone of the endothelial cell averaged 0.083. There was a slight decrease of N_f/N from this control level to 0.070 following oscillations at 10 Hz for 15 minutes (figure 9).

Figure 9. The fractional density of free vesicles (N_f/N) in the peripheral zone of canine common carotid artery as a function of duration of oscillation at 10 Hz. The free vesicles are identified on transmission electron micrographs as those vesicles which do not contain ruthenium red introduced as an extracellular marker following fixation. The control data (duration = 0; mean ± SEM) were obtained from five experiments. The other data points represent individual experiments.

Discussion

In the present experiments, we developed a splinted cannula technique by which an arterial segment can be cannulated and removed from the dog with the vessel length maintained at its in vivo value at all times. This method and the meticulous care taken during the surgical procedures allowed us to obtain isolated arterial segments for in vitro studies without signs of endothelial damage as studied by scanning electron microscopy. Although the data on 125I-albumin uptake (figure 3) were obtained after 15 minutes of incubation, studies on unoscillated control segments indicated that uptake was linear with time over a 2-hour period. The uptake of 125I-albumin by control segments averaged 2.27 ± 0.12 μg/g/15 min; by using the measured luminal diameter, this uptake rate can be converted into a permeability value of 1.3 × 10^-7 cm/sec, which is considerably lower than the value of 4.8 × 10^-7 cm/sec reported by Siflinger et al.10 at the same temperature of 37°C. In our preliminary experiments performed prior to the use of the splint system, the albumin permeability values obtained were more comparable to those of Siflinger et al.,10 and there was a high incidence of endothelial damage as a result of sudden changes in length of the vessel when it was removed without a splint.
Theoretical modeling of the enhancement of vesicle transport by intracellular fluid motions generated by periodic mechanical deformation of the endothelial cell indicates that the maximum possible enhancement is a function of the fraction of free vesicle in the endothelial cell under control conditions. Therefore, we performed transmission electron microscopic studies on arterial endothelium with the use of ruthenium red as a postfixation, extracellular marker to label the attached vesicles. This is necessary because of the three-dimensional nature of the arterial endothelial cell; a vesicle which appears to be free in a given thin section may actually be attached to the endothelial plasmalemma at a different plane. If we had not used ruthenium red, the fraction of free vesicles in the control state (Np/N) would have been taken as nearly 0.4 (i.e., approximately 40% of the vesicles had no visible stalk attachment). According to our theoretical computation (Equation 11, figure 2), this Np/N value would make it possible to have a maximum enhancement of vesicle transport by 67%. The use of the ruthenium red, however, shows that the true value of Np/N is only 0.083, and the maximum enhancement of vesicle transport starting from this low control Np/N value is only about 9% (Equation 11). In order to achieve this 9% enhancement in vesicle transport, the diffusion time must be so short that N, approaches zero. With mechanical oscillation, Np/N did decrease, but only slightly (figure 8), and the associated enhancement in vesicle transport must be considerably less than the maximum limit of 9%. Therefore, it appears that there is negligible enhancement of vesicle diffusion by periodic deformation of arterial endothelial cells.

Measurements on 125I-albumin uptake indicate that sinusoidal oscillations in arterial length (peak change of 4%) for 15 minutes at 5 and 10 Hz caused a significant increase in uptake by 40% over the control value. Simultaneous determinations of vessel geometry show that this increase in macromolecular uptake on per unit tissue weight basis was accompanied by a widening of the vessel lumen and an increase in the surface area available for macromolecular transport. Following 15 minutes of oscillation at 5 and 10 Hz, this increase in available surface area was approximately 30%, which is not significantly different from the 40% increase found for 125I-albumin uptake. The albumin permeability calculated on the basis of the available surface area did not change significantly from the control value of 1.3 $\times 10^{-7}$ cm/sec to the post-oscillation value of 1.4 $\times 10^{-7}$ cm/sec. Therefore, the oscillation induced enhancement of macromolecular transport can be explained by the increase in available surface area. Ultrastructural studies show that this increase in apparent surface area is associated with the change of the internal elastic lamina and the overlying endothelial cells in some regions of the aorta from a wavy nature to a flattened shape, thus making more endothelial surface available for macromolecular transport. This local flattening of the internal elastic lamina may represent a fatigue phenomenon (e.g., a reduction in smooth muscle tone) as a result of the repetitive length change. The finding that the total number of oscillation (frequency $\times$ duration) is more important than the frequency or duration alone in determining the geometry change supports this explanation.

In summary, the effects of oscillatory variations in vessel length on macromolecular transport by arterial endothelium have been studied by a combination of uptake determination, morphological measurement, electron microscopic examination, vesicle counting, and theoretical modeling. The results indicate that the oscillation induced increase in macromolecular uptake was primarily the result of an increase of surface area available for transport, probably as a result of material fatigue. There were no significant changes in albumin permeability or vesicle diffusion.

One of the limitations of the present investigation was that the studies were carried out on arterial segments under zero transmural pressure. Although the results provide some information relevant to the primary aim of the study (i.e., to investigate the effects of periodic endothelial deformation on vesicle transport and macromolecular permeability) one must be cautious in extrapolating these findings to physiological conditions where the transmural pressure is approximately 100 mm Hg. Experiments are in progress to study the effects of pressure variations on vesicle distribution and macromolecular transport in arterial endothelium.

The present investigation serves to point out the value of structure-function correlation. If we had studied only 125I-albumin uptake without examining the gross morphology and ultrastructure of the artery, and if we had used the Np/N values without ruthenium red in deriving the theoretical value of enhanced vesicle diffusion, we would have come to the erroneous conclusion that oscillations caused an increase in albumin transport due to an enhancement of vesicle diffusion. The present conclusion was made possible by combining physiological, ultrastructural, and theoretical approaches in analyzing the role of fluid mechanical factors in arterial transport.

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