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INDICATOR DILUTION ESTIMATION OF CAPILLARY ENDOTHELIAL TRANSPORT

James B. Bassingthwaighe and

Center for Bioengineering, University of Washington, Seattle, Washington 98195

Harvey V. Sparks

Michigan State University, East Lansing, Michigan 48824

INTRODUCTION

A multitude of recent findings have raised the incentive to develop quantitative approaches to studying endothelial cellular metabolism *in vivo*. Among these are the demonstration of the role of endothelial cells in regulating vascular smooth muscle tone (see review by Vanhoutte, this volume) and the large number of enzymatic and transport phenomena now known to occur on their surfaces in culture and *in vivo* (see reviews by Ryan, Betz & Goldstein, and Simionescu & Simionescu, this volume; and 31).

Our approach to quantitating endothelial transport has been to develop two techniques in parallel: (a) the formulation of a mathematically expressible, quantitative hypothesis describing the events, and (b) the definition of an experimental approach based on the multiple indicator dilution method. These go hand in hand because the hypothesis is expressed as a computer-programmed mathematical model whose behavior can be explored to aid in the design of the experiments, and the hypothesis is tested by determining whether or not the model parameters can be adjusted within realistic ranges to provide a good fit of the model solution to the data. The models should account for rapid transformation of substrate, for example, as for angiotensin-converting enzyme in the lung (30), as well as for slower events.

THE TOOLS OF THE TRADE

The Multiple Indicator Dilution Technique

The multiple indicator technique, introduced by Chinard et al (8) and developed further for studies of capillary permeability by Crone (9), is based on the principle of multiple inbuilt controls. It makes use of the principles of conservation of mass and of steady-state physiological conditions. The injection of “indicator” should not change the physiological state, so only inert substance or substances introduced in tracer, nonpharmacological amounts are considered appropriate. For studies of endothelial cells several indicators can be used simultaneously, including (a) an intravascular tracer that does not penetrate the vessel wall nor bind to its luminal surface, usually albumin or other large hydrophilic molecule; (b) an extracellular marker with characteristics similar to the test solute, such as the same molecular weight (e.g. L-glucose as the extracellular reference for D-glucose); and (c) the test solute.

The three or more tracers are injected simultaneously as a bolus into the arterial inflow to the organ, and the effluent blood is collected as a series of samples at short intervals (1 or 2 sec) for the first half minute, and then at longer intervals (4 to 10 sec) for the next few minutes, as illustrated in Figure 1. The duration of the sampling depends on the substances and the flow; it should be longer for low flows or for substances with larger volumes of

distribution. The basis of outflow detection experiments is that tracer must be returned to the outflow from any region whose characterization is needed. This outflow can be difficult or impossible to detect in the presence of recirculation in the intact animal or in an isolated organ that consumes the test solute so completely that there is no detectable return.

The same mathematical approach can be used with other techniques, for example constant tracer infusion into the inflow with outflow sampling, or brief inflow injections with recording of intraorgan concentration by external detection of positron- or gamma-emitting radioisotopes. In practice these alternative techniques are not as good as the bolus injection-outflow detection method, because the latter gives the high temporal resolution needed for accurate determination of the endothelial cell parameters. Since transport rates around or through the endothelial cells are rapid, the dilution curves must contain high frequency information with a sharply peaked outflow dilution curve to provide good resolving power for the differences between the test solute and the reference substances.

Models for Blood-Tissue Exchange

DESIGN OF TRANS ENDOTHELIAL EXCHANGE MODELS—A general approach to describing intravascular transport and exchange between capillary plasma, the interstitium, and the parenchymal cells of an organ has been outlined by Bassingthwaighe & Goresky (3). They showed that it is essential to account for flow heterogeneity as well as for exchange and chemical reaction. The new feature to be emphasized here is the role of the endothelial cell.

The basic conceptual unit is a single capillary-tissue exchange region. Because intraorgan regional flows are heterogeneous (16), each is one of a set of units arrayed in parallel. For multiple solutes or for a substrate and a set of metabolites there would be a set of units for each.

Each capillary-tissue unit is axially distributed, i.e. it has the potential to vary in properties or status along its length to allow for the normal concentration gradients between inflow and outflow. Radially, there are five regions: erythrocytes, plasma, endothelial cells separated by aqueous clefts, interstitial fluid (ISF), and parenchymal cells. Metabolic reactions take place along the length of the unit in both the myocardial and endothelial cells, and in certain cases, also in the erythrocytes. The heterogeneity of the regional flows used in the modeling analysis was defined experimentally by the distributions of markers deposited in each region following arterial injection (16,24).

To account for the production of metabolites of an injected substrate or agonist and the time course of the appearance of such products in the outflow, a separate set of capillary-tissue units is required for each of the metabolites and, where necessary, for the products of their reactions as well. The input to the model for each reaction product (metabolite) is the rate of transformation of substrate; for example, the rate of adenosine removal is the sum of the rates of production of inosine and of its incorporation into adenosine monophosphate and S-adenosylhomocysteine.

FORMULATION OF MODELS FOR SOLUTION—Equations have previously been developed for plasma-ISF (two-region models) and plasma-ISF-cell (three-region) models that take into account axial concentration gradients. Analytic solutions have been obtained for models with parameter values constant along the length. Solutions for the two-region model are accurate and fast in the absence of axial diffusion using either analytic (32) or numerical solutions (1). The three-region analytic solution of Rose et al (29) loses accuracy at long solution times. Numerical solutions for a two-region model with axial diffusion are within 1 % of accurate values (1,19) and are also very fast. Numerical solutions for three-

region models with axial diffusion (18) appear accurate since they have the correct areas and mean transit times, but they await further checking when or if analytic solutions become available.

Convection-diffusion equations for four- and five-region axially distributed capillary-tissue models (erythrocytes, plasma, endothelial cells, interstitium, parenchymal cells) are very similar to those for compartmental models and can be readily defined following the fashion of Bassingthwaighe & Goresky (3). Bassingthwaighe et al (7) provide analytic and numeric solutions for a four-region model (without erythrocytes). A practical way to develop insight into the behavior of the system is to solve the equations and observe the forms of the solutions while changing parameter values. Fast execution times are of great practical importance since repetitive solutions are needed, and they have been obtained by using new numerical techniques, which can be more than a million times faster than using analytic solutions.

Flow heterogeneity is large; in the heart, a seemingly compact unifunctional organ, the regional flow per unit mass of tissue varies five- to sixfold from minimum to maximum, with a standard deviation of 30% around the mean flow (16). It is essential to take this variation into account in the analysis of the dilution curves to avoid systemic biases in the estimates of the parameters describing the exchange. A heterogeneity model is required to account properly for it, but there are many different possible forms of heterogeneous vascular networks. We are currently using a simple arrangement of parallel noninteracting units that differ only in their flows; each receives as input the same arterial concentration-time curve. Very heterogeneous networks such as skeletal muscle may need other representations, some of which have been outlined by Bassingthwaighe & Goresky (3). One model based on dispersionless transport in both large and small vessels has been used extensively (28). We prefer our approach because there is intravascular dispersion in vessels of all sizes, but their dispersionless model has the virtue of linking slow-flow large vessels with slow-flow capillaries, and fast with fast. A configuration intermediate between theirs and ours may be the most realistic.

USING MODELS TO ANALYZE DATA—Models can be used to interpret data in a variety of fashions and with varying degrees of precision. The simplest approach is to analyze by analogy. The more complex and accurate techniques involve careful fitting of the model to the observed data or the observed sets of data. In general, the more data that are obtained simultaneously and analyzed with a single composite model, the stronger the inference concerning the physiology because the degrees of freedom are lower.

Analyzing by analogy is usually done by using the data in a calculation based on a model, but the model often can be fitted only to a part of the data. An example is the use of the Crone-Renkin equation $PS_c = -F_s \log_e (1-E)$, where PS_c is the capillary permeability surface area product, F_s is the flow of solute-containing fluid, and E is a chosen apparent extraction that represents the normalized difference between an intravascular reference tracer and the permeating tracer at a time point following a bolus injection. This model assumes that tracer that has escaped from the capillary never returns. The analogy is acceptable when the extraction is low, i.e. PS_c is only mildly underestimated when E is calculated from the upslope of the outflow indicator dilution curves. But the value for E changes as a function of time, and diminishes to zero during the downslope phase of the curve as tracer returns from the ISF to the capillary. The inconstancy of E illustrates that the Crone-Renkin expression is an incomplete capillary-tissue exchange model.

Fitting of all of the data is the best test of a model; it tests how precisely the model accounts for the experimentally observed data. In indicator-dilution experiments the data that must be

fitted include: the direct measurement of flow; the observed set of indicator dilution curves; the regional heterogeneity of flows [estimated using deposited tracer such as microspheres, or highly retained markers, such as desmethylinipramine (23)]; and observations of the steady-state volumes of distribution for the vascular, interstitial, and total water space in the organ. Ideally it is not merely the indicator dilution curves that are fitted by the model, but all of these other observations as well.

Optimization of the model solutions to fit the data is achieved using automated routines that adjust the parameters until good fits are obtained. The procedures can take into account all of the data listed above. Sensitivity functions, which measure the degree to which each of the parameters influence the fit of the experimental data, can be obtained from the model solutions in order to determine whether a parameter can be reliably determined from the data (2). These functions can also be used in the optimization technique outlined by Levin et al (20). The method of optimization is not as important as the precision of the fit with the model solutions over the full extent of the data sets. If there are systematic deviations of the model solutions from the data, assuming the data are accurate, the model is either incorrect or incomplete. Such systematic biases are the incentive to continue to refine one's hypothesis concerning the physiological system.

The development of a model as a statement of a hypothesis concerning the function of the microcirculation involves acquiring data, fitting the data with the models, developing the models further, and improving the experiment designs. Any model of the microcirculation is necessarily a simplification of the actual situation. Despite this it is important that the model not only predict the available data but also have a structure that is physiologically valid, i.e. that agrees with observations made using a variety of techniques. For example, although no practical model can represent the biological variability of a vascular bed, it should represent a reasonable statistical abstraction of that heterogeneity. Model development should continue as long as new information that can serve as a test of the model becomes available. The ultimate goal is a model that represents all of the functionally significant elements of the microcirculation in such a way as to accurately predict, i.e. fit, all of the available data.

MECHANISMS OF TRANSPORT ACROSS THE CAPILLARY ENDOTHELIAL BARRIER

There are two routes for solute escape from the capillary blood into the interstitium. Tracers injected into the arterial inflow can escape through the clefts between endothelial cells or enter endothelial cells by traversing the cell membrane. Those tracers that bind to the cell membrane can also be removed from the blood without any transport. For many substrates there are facilitating transporters on the endothelial cell membranes (see Reference 7 for a discussion of the relationship between *PS* and parameters of facilitated transport.) For adenosine in the heart there is a special transporter that can be blocked by dipyridamole. Passive diffusional transport through the clefts is unaffected by competitors or blockers (6,14). In experiments such as that shown in Figure 1, ^{14}C -adenosine, ^3H -AraH (an inert, nontransported adenosine analog), and ^{125}I -albumin were injected simultaneously into the inflow to isolated perfused hearts and skeletal muscle. Despite the fact that adenosine and AraH have the same molecular weight, the extraction of adenosine was more than twice that of AraH. Since AraH traverses only the water-filled clefts between endothelial cells, this indicates that adenosine is transported across the plasmalemma of the endothelial cell as well as through the aqueous channels between cells. The blocking effect of dipyridamole showed that the cellular entry was carrier-mediated. When dipyridamole was administered the adenosine curve had the same shape as that of AraH; both behaved as inert hydrophilic solutes traversing only the clefts.

Analyses of adenosine indicator dilution curves from heart and skeletal muscle indicate that under normal circumstances the flux via the endothelial luminal transporter is about twice that through the clefts between the cells. This high flux is still not high enough to explain completely the magnitude of the accumulation of tracer adenosine in endothelial cells observed by Nees et al (26). However, it is sufficiently high to explain the very rapid appearance of adenosine metabolites in the outflow under conditions in which no deamination occurs in the vascular space (14).

In skeletal muscle studies Gorman et al (14) observed that after tracer-labeled adenosine was injected into the inflow the peaks of the outflow dilution curves for tracer-labeled metabolites, inosine and hypoxanthine, were almost coincident with the peak of that for tracer adenosine. In the case of inosine, an ectodeaminase may be present on the luminal plasmalemma of the endothelial cell; the formation of hypoxanthine cannot be so readily explained since there has been no suggestion that an extracellular form of the enzyme could be involved. The appearance of these metabolites therefore suggests that the metabolism is intracellular. The time it takes for metabolites to emerge from an extravascular volume should have caused some delay, but very little was observed, which is in accord with the small volume of the endothelial cells. (The volume is estimated by multiplying the capillary surface area and the endothelial cell thickness. In skeletal muscle this value is about $50 \text{ cm}^2/\text{g} \times 0.2 \times 10^{-4} \text{ cm}$, or about 0.1 % of the muscle volume. In heart the value is ten times larger, i.e. about 1 %.) The rapid appearance of metabolites also suggests that there is no significant binding of either inosine or hypoxanthine within the cells, which would have resulted in a larger delay. The magnitude of the outflow curves for inosine and hypoxanthine provides a measure of what fraction of the adenosine was deaminated rather than being incorporated into adenine nucleotide. In the first few seconds about 10–15% of the tracer emerges on inosine and another 2–4% as hypoxanthine.

The modeling analysis can account for the chemical reactions occurring within endothelial and parenchymal cells. Model solutions with permeabilities that fit adenosine curves show very early emergence of inosine and hypoxanthine, with the peaks of their curves lagging only a small fraction of a second behind the peak of the adenosine curve. This result shows that the rapid appearance of products does not require an ectodeaminase on the luminal surface, but that endothelial cell uptake, intracellular deamination to inosine and hydrolysis to hypoxanthine, followed by outward transport of these two metabolites into the effluent plasmas are fast enough to explain quantitatively the appearance of both metabolites.

The form of the effluent curves for inosine and hypoxanthine show early peaks like adenosine. This observation implies that their concentrations inside the cell have transient peaks, and that the production slacks off quickly. This differs from the lung data of Hellewell & Pearson (15), which showed a prolonged increase in the release of both metabolites. Our modeling did account for adenosine phosphorylation to AMP. The rapid incorporation of adenosine into nucleotide, which reduces intraendothelial free adenosine levels, explains the brief peaking of the inosine and hypoxanthine curves. [Incorporation of adenosine into adenine-nucleotide was observed by Nees et al (26) and by Pearson et al (27) in monolayer endothelial cell cultures.] Single pass adenosine extraction was about 75%, while the extraction of AraH was only about 25% (Figure 1); given that 25% of adenosine traverses the clefts along with the AraH, the 50% difference is accounted for by adenosine entering the endothelial cells via the luminal surface.

Figure 1 shows that the four-region axially distributed model can fit the dilution curves for adenosine, even with the constraints imposed by the need to fit the albumin and AraR dilution curves. The combination of the microsphere data, defining flow heterogeneity, and the albumin curve delimits the vascular dispersion of the adenosine and AraH. The form of

the AraH curve further constrains the interpretation of the adenosine curve by fixing the estimates of the permeability of the aqueous clefts between endothelial cells and of the interstitial volume of distribution for adenosine. However, not all the curves fit so well: A recurring problem is that the tails of model curves are often above the observed adenosine curves, which indicates that the model did not adequately account for the adenosine uptake and retention in the slower transit time pathways. The tail is almost insensitive to the luminal endothelial PS (2), and the close fit of the upslope and peak provides assurance that the relative importance of diffusion via the paracellular pathway and transport into endothelial cells is reasonably well evaluated. Even so, such misfitting demonstrates incompleteness or incorrectness of the model, and demands that the model be developed further. Better heterogeneity models (for regional volumes and PS 's as well as for flows) are needed, as well as better geometric descriptions of the microvasculature and, in this particular case where a constant PS_c was used, a concentration-dependent PS_c in accord with a facilitated transport mechanism.

ESTIMATION OF TRANSPORTER KINETICS WITH INDICATOR DILUTION

The multiple indicator dilution technique was designed for use in steady-state situations. The formal requirements are for stationary (steady flow and distribution) and linear (transport rates unaffected by changes in concentration) conditions. High frequency fluctuations in flow are not a significant problem for estimating membrane transport rates of tracers; Bassingthwaighe et al (4) found that so long as flow oscillations were so frequent that at least several occurred during the time of passage of the main part of the bolus (usually 8–20 sec), the estimates of flows and mean transit times had little error. Explorations with a blood-tissue exchange model that allowed fluctuating flows suggested that for substances with permeabilities similar to that of sucrose in the heart the rate of equilibration between blood and tissue was unaffected by fluctuations in flow or even intermittent emptying of the capillaries (5).

The other condition, linearity, raises more difficult issues. There is no likelihood of an artifactual change in PS when one uses radioactive tracer techniques in which the specific activity of the tracer is very high and the chemical concentration is constant. However, there are two situations in which problems occur: (a) when the chemical levels are changed by the injection of tracer, and (b) when one wishes to characterize saturable transport kinetics using the indicator dilution approach. In the first instance, an observed rate constant for exchange will be less than in the steady state because of selfcompetition (between additional nontracer and tracer). In the second instance, when the relationship between transport rate and concentration over the whole range is needed, a set of experiments can be designed to elicit the desired information.

The Steady-State Tracer Bolus Technique

The determination of the endothelial luminal surface conductance (PS_{ec1}) over a range of substrate concentrations can be accomplished by a series of experiments each of which fulfills the requirement of stationary, linear conditions. The technique involves the use of tracer-labeled substrates with high specific activity in the multiple indicator dilution experiment in the presence of a set of different fixed concentration levels of the nontracer substrate. At low concentrations of the nontracer mother substance, tracer access to the transporter is unimpeded and PS_{ec1} is maximal. At higher concentrations of mother substance the competition reduces the observed PS_{ec1} for tracer. In order to determine the form of the relationship the investigator must perform indicator dilution studies at concentrations well below and well above the K_m . Transport via other mechanisms must be either identified or eliminated from consideration. Such experiments provide the “raw data” for another level of analysis, namely, that at which we determine whether an observed PS -

concentration relationship can be well fitted by a model for transporter kinetics. Higher-order binding will result in steeper slopes in the mid-portion of the curve. Information about the effects of competitors and inhibitors is also necessary for more detailed evaluation, and may reveal further complexities such as sidedness or asymmetry of the transporter (e.g. 17). Many transporters exhibit countertransport, in which a rise in concentration on one side of the membrane increases flux from the opposite side toward the first side. While first order Michaelis-Menten kinetics have been considered (12,21), no blood-tissue exchange model that accounts for bidirectional, saturable transport with countertransport has been published.

If a substance is consumed in the tissue, then at steady-state the nontracer substrate level will diminish along the capillary. In this circumstance one must account for the rise in effective PS between inflow and outflow. Goresky et al (12) developed expressions suitable for galactose uptake in the liver (11), where there is no capillary barrier. Their expressions are suitable for a first-order Michaelis-Menten transporter in the special case in which the complexed and uncomplexed transporter have equal permeabilities, there is passive transport in parallel, and there is no return flux. Goresky et al (13) applied this model to a kinetically similar situation, namely ethanol consumption within the liver. There is no effective barrier at the hepatocyte plasmalemma for ethanol, and the first rate-limiting reaction within the cell is a first-order process, which means that it is analogous to a saturable transporter at the membrane. At present, in order to account for the transporter characteristics in the analysis of each dilution curve we must search for two parameters, the K_m and V_{max} , instead of one, the PS . Although this appears to be a disadvantage, it can be offset by acquiring additional data (the concentrations of the substrate in the inflow and outflow) which are often needed anyway for the interpretation.

Transients in Tracer and Mother Substance

Another approach has been taken by Linehan & Dawson (21). They worked from the same basic premises as Goresky (i.e. a Michaelis-Menten transporter acting across a single barrier with no back diffusion of tracer from the extravascular region), which is equivalent to modeling an infinitely large extravascular space that dilutes away the tracer entering the space. Tracer-labeled and nontracer prostaglandin were simultaneously injected into the pulmonary artery, along with a reference (tracer-labeled albumin), to collect a sequence of outflow samples in the usual fashion. This is known colloquially as the “bolus sweep” technique; the concentration of mother substance is low at the initial portion of the curve, high at the peak, then low again at the tail, so the effective PS of the transporter changes from moment to moment as the bolus sweeps through a wide range of concentrations while passing through the capillary.

In the early applications of this technique (10,21,22) the concentrations of the nontracer mother substrate were not measured, so the accuracy of the estimates of K_m and V_{max} were questioned because the actual degree of dilution between inflow and outflow was unknown. Malcorps et al (24) measured the chemical and tracer concentrations, and found almost a twofold difference in K_m between the bolus sweep and the steady state technique described above. The bolus sweep technique has the virtues of providing estimates of K_m and V_{max} with each intraarterial injection of a set of tracers, and of avoiding the unphysiologic effects of prolonged infusion at concentrations above the K_m . In their studies of PGE_1 and serotonin their model solutions fit only the first 4–10 sec of the dilution curves, namely the upslope portions, since back diffusion of tracer from tissue into the blood was not accounted for. In their most recent study, Linehan et al (21a) showed that the model solution did fit the whole of pulmonary outflow dilution curves for tracer-labeled BPAP, an angiotensin analog that is hydrolyzed at the endothelial surface by angiotensin-converting enzyme and whose products are immediately released into the blood. Linehan et al model flow heterogeneity assuming a dispersed input function and random coupling between large vessels and capillaries, as we

do, and estimate the capillary transit time distribution from the dilution curves, as did Rose & Goresky (28). Since hydrolysis is a nonlinear, concentration-dependent process, this result is persuasive that the approach is a good one. To document the method completely we would recommend measuring the flow heterogeneity as well as the chemical concentrations in each sample.

Use of Small Reference Solutes

Yudilevich & de Rose (33) explored the rates of transport of glucose and amino acids across the blood-brain barrier. In that situation the intravascular reference tracer can be any inert hydrophilic solute for which there is not a special transporter. The blood-brain barrier is composed of endothelial cells joined together circumferentially by tight junctions that allow no passage of hydrophilic molecules by passive diffusion between cells. Thus the size of the reference molecule is of little consequence, and sucrose and albumin serve equally well. The instantaneous extraction of a transported solute gives a measure of the endothelial transport alone. These investigators did not fit the model to the data, but used the historically interesting technique of Martin & Yudilevich (25) in which correction for back-diffusion is attempted by a “back-extrapolation” to estimate extraction.

This experimental technique was employed by Hellewell & Pearson (15) in their study on adenosine uptake by pulmonary endothelium. They used bolus injections of tracer sucrose and adenosine because sucrose remains extracellular and the endothelial uptake of adenosine can thus be measured by comparison with it. The problem is that both sucrose and adenosine escape through the clefts between the cells; the endothelial permeability-surface area (PS_{ecf}) for adenosine cannot be calculated directly from its extraction relative to sucrose unless the two tracers have the same cleft permeability. Sucrose ($M_r = 342$) would be expected to permeate the clefts between endothelial cells less readily than does adenosine ($M_r = 287$), so the difference between the two extractions would represent endothelial uptake plus some excess escape of adenosine into the interstitium via the clefts. However, Hellewell & Pearson (15) found that high concentrations of dipyridamole resulted in the adenosine curves that were superimposed on the sucrose curves. This suggests that diffusion coefficients for sucrose and adenosine are nearly identical, in which case they could indeed estimate PS_{ecf} . In their particular studies error due to failure to use a precisely analogous reference solute does not appear to be great, but such errors are potentially large whenever the reference and test solute do not have identical conductances through the aqueous channels between cells.

This “uptake” technique was extended by Yudilevich & colleagues (34,35), who used only a small inert extracellular solute (usually sucrose) as a reference marker and no intravascular reference in an attempt to measure uptake by parenchymal cells. The advantages of reducing the number of radioactive tracers used simultaneously are offset by the restriction this places on the conditions under which one can accurately estimate parameter values. In the brain sucrose is restricted to the intravascular space, but it is not in tissues with open clefts between endothelial cells. When applied to the estimation of permeability-surface area products of parenchymal cells (PS_{pc}) the method gives correct answers if the permeability of the capillary wall is extremely high. If it is not, PS_{pc} is underestimated, usually to a considerable extent. Our calculations with a two-barrier model show that the capillary permeability-surface area product should be higher than $100 \text{ ml g}^{-1} \text{ min}^{-1}$ to have less than a few percent error in PS_{pc} . Even when passive capillary PS is as high as $10 \text{ ml g}^{-1} \text{ min}^{-1}$ their technique gives a severalfold error in PS_{pc} . Therefore, the values of PS_{pc} obtained by Yudilevich & Mann (34) are probably underestimated significantly, but by exactly how much is not known since estimates of PS_{g} were not obtained.

SUMMARY

Mechanisms of transport of substrates and small solutes across the endothelial lining of the capillaries include passive diffusion (through clefts between cells or across the plasmalemma) and transporter-mediated flux across the plasmalemma. Because the transport rates are typically high, the multiple indicator dilution technique is usually the method of choice, as it provides the high temporal resolution required. In the simplest version of this technique, a test solute is injected into the inflow simultaneously with reference solutes that are restricted to intravascular and extracellular space. Interpretation of the resulting data requires models; the most precise approach is to fit the model solutions to the data. When appropriate combinations of indicators and sufficiently complex models (those that account for flow heterogeneity, arteriovenous gradients, passive and saturable transport, reaction, and diffusion in multicomponent systems) are used the transporters can be characterized. Features such as the rapidity of intracellular reaction can also be revealed by this technique.

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Figure 1.

Outflow dilution curves from dog hindlimb skeletal muscle following injection into the femoral arterial inflow. The ordinate is the fraction of the injected dose emerging per second. The indicators were ^{125}I -albumin (intravascular reference), ^3H -araH (arabinofuranosylhypoxanthine, extracellular reference) and ^{14}C -adenosine (the “test solute”). The symbols are the data; ^{14}C -labelled metabolites of adenosine have been separated in each sample, but are not shown. The smooth curves are model solutions, using a model composed of an aggregate of 4-region units (capillary, endothelial cell, ISF, parenchymal cell) in parallel to account for the measured distribution of regional flows.