11 Multiple Indicators; Capillary Permeability

Albumin

Sucrose

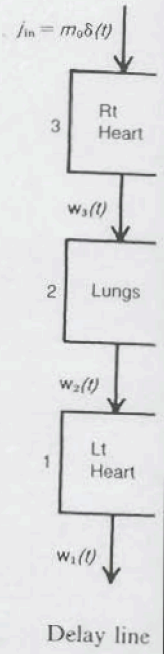
Tracer water

Nitrous oxide

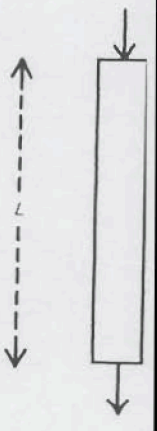
11.1 Introduction

The use of multiple indicators with different characteristics constitutes a powerful tool for dissecting the black box without entering it physically. In circulation studies three categories of tracer can be discerned: *vascular indicators* that remain in the vascular bed even in the microcirculation; *extracellular indicators* (hydrophilic) that pass the capillary wall through water-filled pores, but are unable to cross the cell membranes and hence are confined to the extracellular spaces; and *freely diffusible indicators* (lipophilic) that cross all cell membranes and hence are distributed throughout the entire tissue.

In this chapter we outline some of the possibilities afforded by these three classes of indicators in studies of the transfer of substances between blood and tissue in body organs, with particular reference to the assessment of capillary permeability.



Delay line

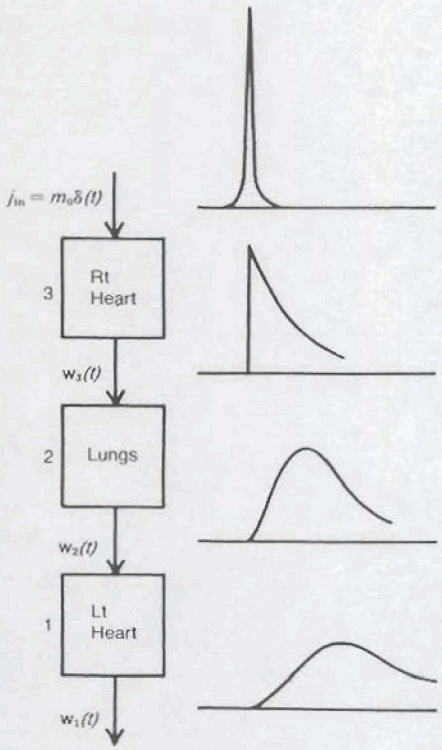


11.2 Vascular Indicators

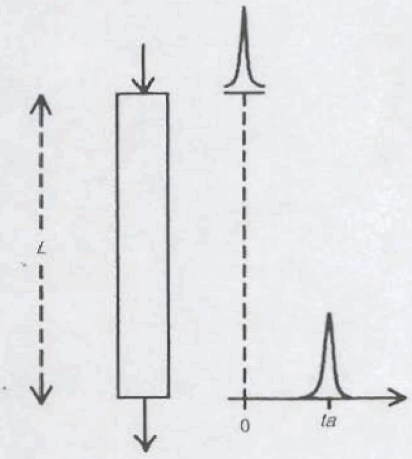
If the amount m_0 of a vascular indicator such as ^{51}Cr-labeled red cells is injected rapidly ($\simeq 0.5$ sec) into the arterial inflow to an organ, the venous curve has its characteristic shape; it starts after a *delay* of several seconds, rises *steeply* to a *maximum* value, then descends *more slowly* toward 0.

Many attempts have been made to explain this particular response—the "skew normal" or "logarithmic normal" curve shape. In general terms it can be said that the existence of a maximum point on the c_V curve corresponding to the most frequent transit time t_{max} is not surprising. If this is true then c_V *must* rise from 0 to c_{max} in the interval $0 \rightarrow t_{max}$. But in principle endless time $t_{max} \rightarrow \infty$ is available for washing indicator out of the system, hence the skewness. Otherwise expressed, a dispersing distribution sweeping past a fixed observer by convection will appear to that observer as a skew normal distribution simply because more time is available for dispersion

on the downward portion as compared with the upward portion of this curve. In the limit of infinitely fast dispersion relative to convection the downward portion represents compartmental washout. The skewness is increased by washout from "slow sites."



Delay line (length L)



Compartmental model

Suppose that the system is the central circulation consisting of the right side of the heart, the lung, and the left side of the heart. The anatomical arrangement suggests that a chain model (catenary) ① → ② → ③ be used, in which each compartment supplies the input to the next downstream compartment with no backflow. This model gives the results derived in Chapter 10:

$$\left.\begin{aligned} &\text{Define } w_i(t) \equiv c_i(t)/m_0 \\ &\text{and } k_i \equiv F/Vd_i \\ &\text{where } i = 1,2,3 \end{aligned}\right\} \qquad [11.1]$$

Then

$$w_3 = \frac{1}{F} k_3 e^{-k_3 t}$$

$$w_2 = \frac{1}{F} \frac{k_2 k_3}{(k_3 - k_2)} (e^{-k_2 t} - e^{-k_3 t}) \qquad [11.2]$$

$$w_1 = \frac{1}{F} \frac{-k_1 k_2 k_3}{(k_1 - k_2)(k_2 - k_3)(k_3 - k_1)} \times$$
$$[(k_2 - k_3)e^{-k_1 t} + (k_3 - k_1)e^{-k_2 t} + (k_1 - k_2)e^{-k_3 t}]$$

This model represents the minimum number of pools that result in a solution compatible with the shape of a vascular outlet curve. But the *delay time* is not reproduced. Hence the compartmental model could be made to include a *delay line*. This device is a tube with plug flow and of length L in which the blood flows with the velocity u such that the appearance time ta is L/u. The time t in Eqs. [11.1] and [11.2] is now regarded as

$$t = t_{obs} - ta \qquad [11.3]$$

Now the curve can be reproduced and one can by conventional curve analysis find the values for the five unknowns ta, Vd_1, Vd_2, Vd_3, and F. Noting that since F, $Vd = Vd_1 + Vd_2 + Vd_3$, and ta are readily obtained by other means, namely

$$\left.\begin{aligned} F &= m_0 / \int_0^\infty c_1(t)dt \\ \bar{t} &= \int_0^\infty t c_1(t)dt / \int_0^\infty c_1(t)dt \\ ta &= \text{read directly off curve} \\ Vd &= F(\bar{t} - ta) \end{aligned}\right\} \qquad [11.4]$$

the task of finding a solution is not formidable. Yet it is of no use because the volume estimates obtained bear no clear relationship to the actual volumes. The fitting by three exponentials and a delay line also gives too much freedom, especially because the tail part is "hidden" under the recirculation. Therefore the accuracy of the estimates is poor. The important check would be to measure $w_3(t)$ and $w_2(t)$ experimentally and thus to see if they agree with the values predicted by the model.

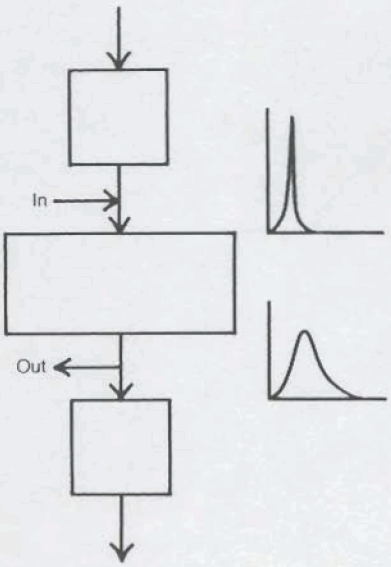
There is, nevertheless, one important aspect of the compartmental model that should be remembered. This is the catenary arrangement of a series of systems, each one feeding the next and with no backflow. In this terminology the final outlet's response is the *convolution* of all subsystem responses:

$$[w_{out}(t)]_{vasc} = g_1 * g_2 . \ . \ . \ * g_n \qquad [11.5]$$

where g_1, g_2. . . . g_n are the impulse responses of systems, 1, 2, ... n. This result can be used if one injects a diffusible cotracer such as ^{133}Xe or ^{85}Kr. Suppose the impulse response of ^{133}Xe in the lung capillary was g_{xe}. Then

$$[w_{out}(t)]_{xe} = g_1 * g_2 . \ . \ . \ * g_n * g_{xe} \qquad [11.6]$$

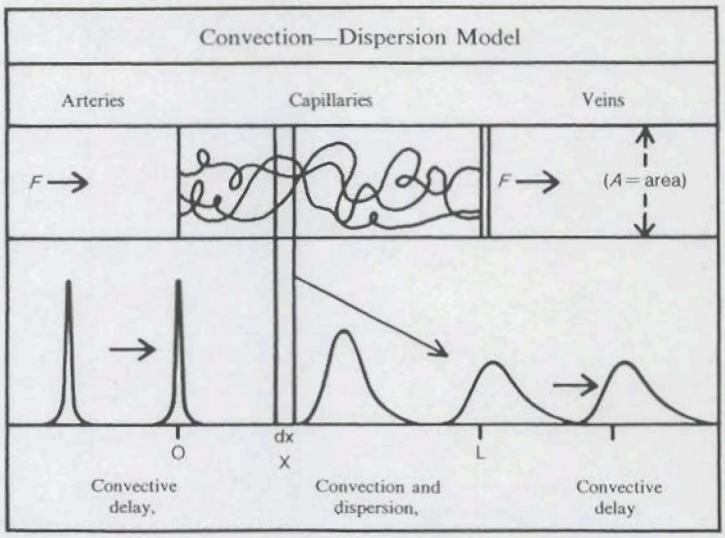
where g_{xe} is placed at the end because convolution is commutative. Now $g_1 * g_2 * g_n$ as given by the vascular cotracer as $[w_{out}(t)]_{vasc.}$. Hence $[w_{out}(t)]_{xe} = [w_{out}(t)]_{vasc.} * g_{xe}$. Conversely, one could use a deconvolution approach on the two sets of experimental data, $[w_{out}(t)]_{vasc.}$ and $[w_{out}(t)]_{xe}$, to find g_{xe}.



Convection—dispersion model

Suppose that a vascular indicator is injected as a bolus in the pulmonary artery and outflow detection is made from a pulmonary vein. An earlier appearing, more rapidly rising, and more monoexponentially decreasing curve is obtained than in the experiment in the preceding section where the right heart and left heart were also traversed. Nevertheless, the curve is still substantially different from the delayed monoexponential. This demonstrates the failure of the three-compartmental model. This suggests the use of a series of compartments to represent the lung arteries → capillaries → veins. As a limiting case we shall use an infinite series of regularly related compartments. This model is based on both *convection* and *dispersion* as expressed in the form of a partial differential equation.

The convection—dispersion model sees the black box as a labyrinth of capillaries starting from one delay line (arteries) and going to another delay line (veins). The intercapillary distance is 10 to 100 μ. At close view a randomness of connections is seen.



All statistically averaged properties are assumed constant in the two dimensions perpendicular to x but they may vary smoothly in the x-direction.

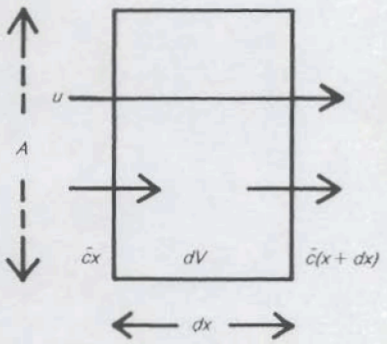
Mass balance for a volume element $dV = Adx$ with A the constant cross-sectional area and $\bar{c}$ its mean concentration, gives per unit area that

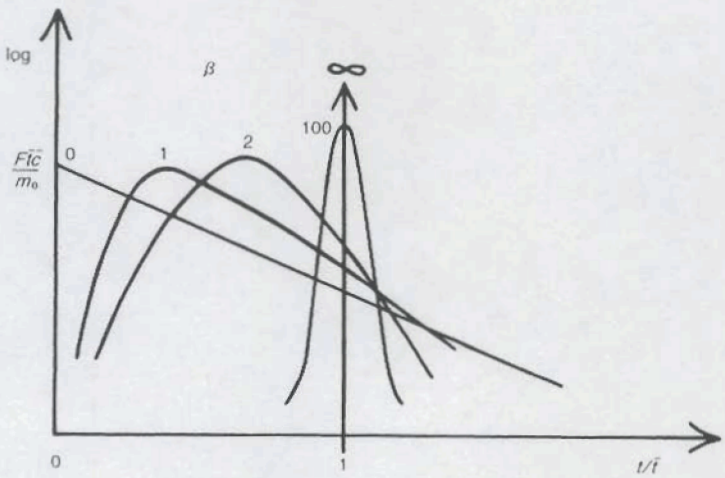
$$\frac{\partial \bar{c}}{\partial t} = D_1 \frac{\partial^2 \bar{c}}{\partial x^2} - u \frac{\partial \bar{c}}{\partial x} \qquad [11.7]$$

The right side represents the spatial changes of average indicator concentration due to ① dispersion by diffusion and by the random flow pattern in the capillary network, and ② convection due to the forwardly directed component of capillary flow. We use the symbol D_1 to indicate the dispersive process

Velocity:

$u = F/A$
$= FL/V$





similarity to simple Fick diffusion even though diffusion may be only a minor part of the dispersion effect.

For details of how to solve Eq. [11.7] see Perl, W., and Chinard, F. (1968): *Circul. Res.*, 22:273.

Solution of Eq. [11.7] gives a family of theoretical bolus injection—outflow detection curves that may in nondimensional units be expressed as a plot of $F\bar{t}\bar{c}(L,t/\bar{t})m_0$ versus $t/\bar{t}$ for various values of the Peclet parameter

Peclet Parameter

$$\beta = \frac{L^2}{D_1 \bar{t}} = \frac{L^2 F}{D_1 V} = \frac{uL}{D_1} \qquad [11.8]$$

Little work has been done to apply Eq. [11.7] to experimental vascular indicator curves. Approximate curve fits of the above illustrated curves in dog kidney yield $\beta \sim 10$. Using freely diffusible indicators of known diffusibility in tissue (and assuming that $D_1 \simeq D$ for such indicators) and Eq. [11.8], L could be estimated and compared with anatomical estimates of the probable average distance from arteriole to venule. Taking a value for L of 200 μ = 0.02 cm and a mean transit time of 4 sec yields for a vascular indicator [use Eq. (11.8)]

$$D_1 \simeq \frac{(0.02)^2}{10 \times 4} = 10^{-5}\text{cm}^2/\text{sec} \qquad [11.9]$$

That is, the intravascular pathways traversed by the tracer molecules vary enough to give (practically) all the dispersion observed.

This result may be compared with the diffusion coefficient of albumin, $D \sim 10^{-7}$ cm^2/sec (albumin was the vascular tracer used, but D_1 would be almost the same for labeled red cells where $D \simeq 0$). The discrepancy suggests that the random capillary convective component dominates completely over diffusion for such indicators; the similarity of curve shape for red cells and for albumin also leads to this conclusion.

11.3. Extracellular Indicators, Their Volume of Distribution

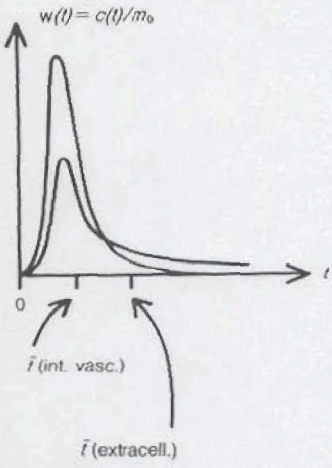
Typical extracellular indicators are sucrose and inulin. They do not cross cell membranes, or more precisely expressed, their uptake in cells is so slow that it can be neglected in the context of the experiments considered here.

Water is in most contexts "freely diffusible."

D-glucose and other metabolites cross cell membranes; they do *not* belong to the extracellular indicators.

The intravascular indicators, such as labeled red cells, labeled proteins, or labeled dextran molecules, are of course also extracellular. But similarly, in *the context* of the experiments considered they remain essentially intravascular (less than 1/1000 of albumin molecules cross the average body capillary's wall in one single passage).

A list of commonly employed extracellular indicators is given as Eq. [11.10]. They are *relatively small* and *strongly hydrophilic* molecules



Some Extracellular Indicators

Molecule	Molecular weight (approximate)
Creatinine	113
Mannitol	180
Sucrose	340
^{51}Chromium-EDTA	360
Raffinose	500
Inulin	5500

[11.10]

The extracellular indicators have a longer mean transit time $\bar{t}$ than the intravascular ones. And, correspondingly, their volume of distribution is larger as it also comprises the interstitial space.

Because the fluid in the extracellular space is a plasma ultrafiltrate one should when calculating the interstitial volume from a double indicator experiment express the flow F *as the flow of plasma water.* Let the pair of indicators be labeled albumin and sucrose. Then, using outlet detection after bolus injection with $w(t) = c(t)/m_0$:

$$\left.\begin{matrix}\bar{t}\,\text{alb.}\\ \bar{t}\,\text{sūc.}\end{matrix}\right\} = \frac{\int_0^\infty t w(t)dt}{\int_0^\infty w(t)dt} \qquad [11.11]$$

and

$$V_{\text{interst}} = [Vd]_{\text{suc.}} - [Vd]_{\text{alb.}} \qquad [11.12]$$

where both volumes are found by multiplying $\bar{t}$ by F.

Evidence is presently accumulating showing that the extracellular indicators *do not distribute in precisely the same space.* The space is largest for the smallest molecules, indicating that some passages or spaces are so narrow as to be accessible only to small molecules. The experimental evidence pointing to this conclusion has come from studies of the whole body, from muscle, and from kidney. They suggest that the volume for small molecules is 50 to 100% larger than for big ones.

Where can the excess volume available only for the small molecules be located? Two sites are being discussed: a) The extra volume is the "interior" of large but loosely knit macromolecules in the anatomical interstitial space (such as collagen or hyaluronic acid complexes). b) The extra volume is anatomically located inside the cells in organelles that communicate by means of narrow passages with the anatomical interstitial space. The electrolyte composition of these organelles would then be extracellular.

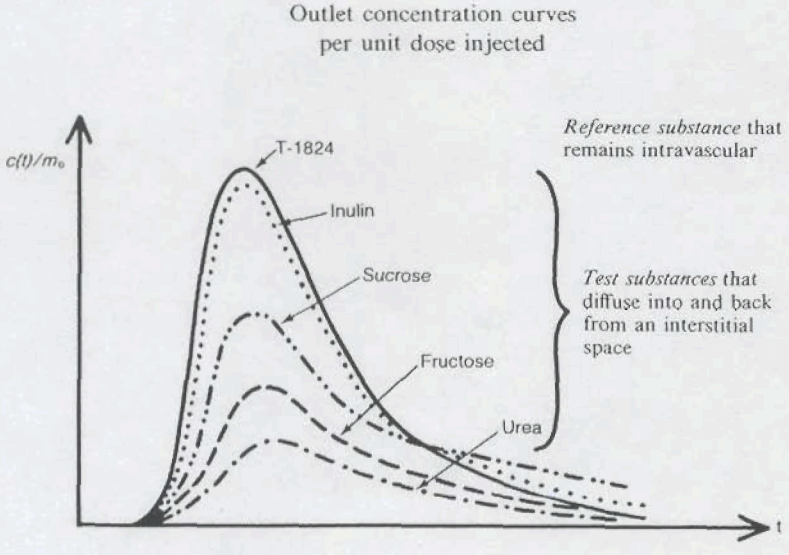
Longitudinal tubular system in skeletal muscle cells, that is the *sarcoplasmatic reticulum* of these cells

11.4. Extraction and Transmission

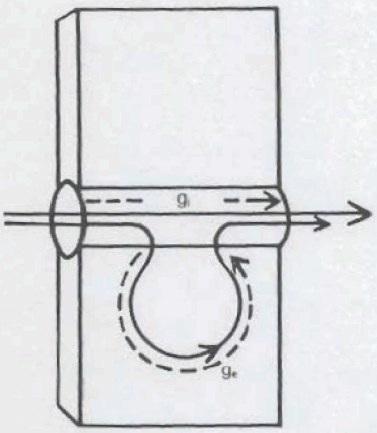
It is of fundamental importance for the analysis of the indicator curves of extracellular indicators to recognize that in many instances the passage from the blood to the interstitium is significantly impeded by *a barrier of very low permeability,* that is, a permeability several orders of magnitude less than a layer of water of equal thickness. It is often held that the most permeable part of the microcirculation is the venules but capillaries, arterioles, and even the larger vessels are all permeable but to a variable degree. What we in the following sections call capillary or blood-tissue permeability is an average of total microvascular permeability.

Chinard, F. and Enns, T. (1955): *Am. J. Physiol.*, 178:197.

How can one know that the capillary wall constitutes a barrier? Direct observation of single capillaries injected with dye has led to this knowledge. Also, the indicator curves suggest the situation first pointed out by Chinard and Enns. If one injects a mixture of a vascular indicator (usually T-1824 albumin) and various extracellular test indicators such as urea, fructose, sucrose, and inulin into the arterial inlet to skeletal muscle then the venous outlet curves all have their peak value practically *simultaneously;* the test substances however, has a lower peak height. This suggests that a fraction of the test molecules has left the vascular bed with the remaining fraction washed out in the same way as the intravascular reference indicator. In the tail part, however, the test molecules show curves that lie higher than that of the reference indicator, due to *back-diffusion* of the indicator molecules from the interstitium.



It is the practically identical shape of the initial curve part that suggests that the early part consists essentially only



of those test molecules that have stayed inside the vascular bed throughout their passage through the tissue.

A model can be made of this result by assuming a capillary permeability so low that only a fraction E is extracted and the remaining fraction $1 - E = T$ is transmitted. This means that the unit response function for the test molecules is given by two parallel pathways, so that

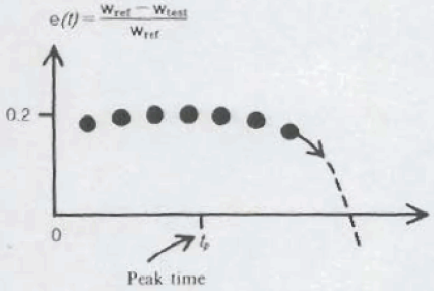
$$g_{test}(t) = \left.\begin{array}{l}(1 - E)g_{i.vasc.}(t) + \\ E\, g_{extracell.}(t)\end{array}\right\} \qquad [11.13]$$

In this situation $g_{extracell.}(t)$ is considered to be the convolution of the vascular transfer function $g_{i.vasc.}$ and the extravascular one (for that molecule) $g_{e.vasc.}$ Thus

$$\begin{aligned} g_{test} &= (1 - E)g_i + E\, g_i * g_e \\ &= Tg_i + E\, g_i * g_e \end{aligned} \qquad [11.14]$$

Equation [11.14] is the model for extraction and transmission. g_e has a mean transit time $\bar{t}_e$ several times that of g_i (the interstitial volume is several times that of the plasma volume of the vessels in the tissue); assuming that the interstitial space is well-mixed (that is, $g_e = ke^{-kt}$ with $k = 1/\bar{t}_e$), back-diffusion *is* negligible during the upslope portion of $g_{test}(t)$, for which $t << \bar{t}_e$.

But the crux of this problem cannot be solved by calculations. One cannot *a priori* state that the interstitium will function as a reasonably well-mixed space. Hence the experimental evidence of the similarity of the shapes of the early part of test and reference curves becomes essential. This experimental result is best expressed as a constant extraction ratio

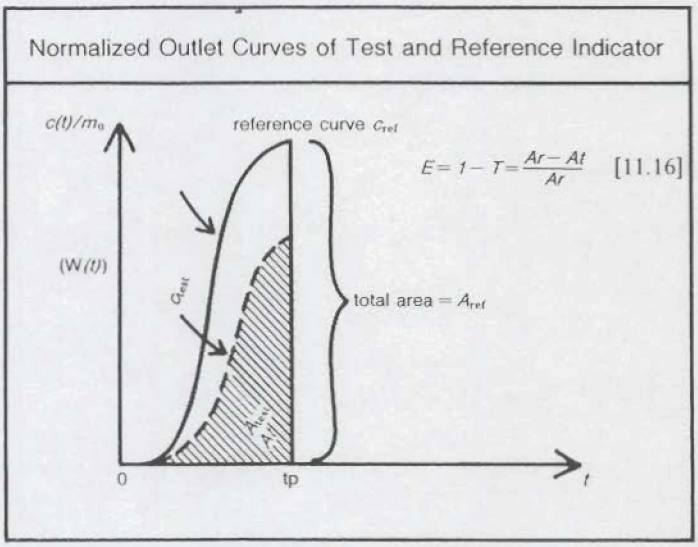


Extraction Ratio in Successive Samples

$$e(t) = \frac{W_{ref} - W_{test}}{W_{ref}} \qquad [11.15]$$

The $e(t)$ curve is practically horizontal (proportionality of c_{test} and c_{ref}). This is compatible with a) equal extraction in various transits, b) the reference curve giving a good estimate of what would have been found if no loss had taken place (equal *intravascular* transfer function of nonextracted test molecules and of all reference molecules), and c) negligible early back-diffusion.

Accepting these assumptions, one obtains a measure of the average extraction of the transcapillary transits from the fastest ones to the most frequent one (peak time) from the area ratio expressed in Eq. [11.16], and with integration symbols in Eq. [11.17].



Flow-averaged Mean Extraction

$$E = \frac{\int_0^{t_p} W_{ref} - \int_0^{t_p} W_{test}}{\int_0^{t_p} W_{ref}} \qquad [11.17]$$

Various other ways of assessing E from the upslope part of the curves have been proposed. We only give the area ratio method, which affords a flow weighted mean of all transits until the peak time t_p, which is the most frequent (vascular) transit time of the system.

It should be noted that four other methods have been described for assessing E from indicator studies:

for some references see *Capillary Permeability*, Crone, C. and Lassen, N. A., eds. Academic Press, New York, 1970.

1. The steady-state method of Renkin that in its initial part is identical to the bolus injection method of Chinard and Enns as further developed by Crone, described above. After the initial few seconds Renkin's method is, however, quite massively influenced by indicator back-diffusion.

2. The local tissue injection method of Lassen, where a freely diffusible reference indicator ^{133}Xe is injected locally into the tissue in a mixture with the test molecule. In this method the interstitial fluid volume must be guessed at and this adds a considerable element of uncertainty to the method.

3. The residue detection method of Sejrsen which takes advantage of the fact that at sufficiently low extraction fractions the transmitted and extracted moieties can be clearly seen as separate. This method is correct if the injection is rapid (so that $m_0 = m_{max}$) and if the two fractions are well-separated. It has the advantage that no blood samples are needed. The method has not yet been critically evaluated.

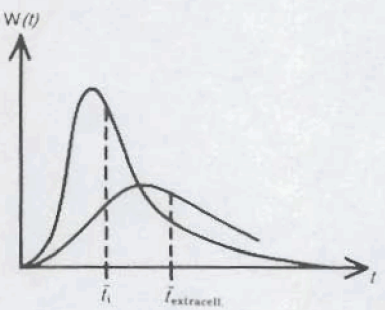
4. The tissue uptake method of Oldendorf where a bolus is injected intraarterially, comprising a *reference* substance that indicates the fractional amount of bolus to the tissue (labeled water was used by Oldendorf: microspheres are more correct in theory) and a test *molecule.* After 15 sec the tissue (the brain was studied) was sampled, that is, at a time when the transmitted fraction of test molecules has essentially left the vascular bed while extracted test molecules remain (essentially) in the tissue. For relative measurements comparing E for a series of test molecules at the same experimental situation the method is very useful.

Taylor, G. (1953): *Proc. Roy. Soc. (Lond) Ser. A,* 219:186.

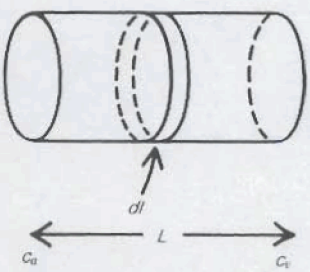
A comment on Taylor diffusion: The double indicator method is based on the use of a reference tracer to indicate how the transmitted test molecules travel. But because the reference molecule generally is larger than the test molecule, it has a lower diffusibility and does not, therefore, undergo precisely the same dispersion intravascularly as the transmitted test molecules. Except when very small extractions are studied with large differences between test and reference molecular size this effect is negligible.

Goresky, C. A. (1970): In *Capillary Permeability,* p. 415.

In concluding this section it is emphasized that in many organs (e.g., liver or kidney), the capillary permeability is relative to the blood flow *so high* that for small size indicators almost all the molecules cross the capillary wall in a single transit. In this case the peak of the test-molecule curve is quite low and typically *delayed* relative to the intravascular indicator's peak. In this case the intravascular and extracellular curves are of similar shape if scaled by $t/\bar{t}$ (Goresky, 1970). Another way of expressing the limitation of the upslope method of Crone is to stress that E should not exceed 0.2 to 0.3; otherwise, early back-diffusion as well as differences of extraction in the different transits invalidate the method. However, for any organ or tissue it should in principle be possible to find a test molecule of a size that, relative to permeability and blood flow, gives a suitably small and hence measurable capillary extraction according to the dual indicator upslope extraction method.



11.5 Calculation of Capillary Permeability P from Measurement of Extraction E and Flow F



Consider that all capillaries of the organ are equal (single capillary model). Assume also that the permeability is the same per unit of capillary surface area throughout the length and that the diameter (and hence also surface area and flow) is constant.

With the length of the capillary L, at a distance l from the entrance the *relative* distance $x = l/L$. Consider then a thin disc of relative thickness $dx = dl/L$. Mass balance of permeant indicator gives

Mass Balance in Segment of Thickness dx

In through arterial end		Out through venous end		Out through vessel wall	
$F\,c(x)$	=	$F\,c(x+dx)$	+	$PS\,dx\,c(x)$	[11.18]

This is the Bohr integration: Bohr, C. (1909): *Zbl. Physiol. 23:1.*

where PS is the product of the permeability per unit surface area P and the surface area S. Thus $PS\,dx$ is that fraction of the total permeability—surface area product that is available in dx. Note that the term for the outflux through the vessel wall, $PSdxc(x)$, contains no term for the concentration in the interstitium: here the concentration is assumed to remain essentially 0 due to dilution into the large and fairly well-mixed (by diffusion) interstitial space.

Rearranging and dividing by dx gives as $c(x + dx) - c(x) = dc$ that

$$\frac{dc}{dx} = -\frac{PS}{F}\,c(x) \qquad [11.19]$$

This simple differential equation was encountered in Chapter 10. Hence we can write the solution without intermediary steps

$$c(x) = c(0)e^{-\frac{PS}{F}x} \qquad [11.20]$$

This shows that the concentration inside the capillary falls monoexponentially along the length of the capillary. At the *venous* end, that is for $x = 1$, the concentration is

$$c_v = c_a e^{-\frac{PS}{F}} \qquad [11.21]$$

Krogh, A. (1919): *J. Physiol. (Lond),* 52:409.

The absence of the distance variable x in the final result, Eq. [11.21], of the preceding analysis suggests the possibility that this result follows from more general geometries of the blood-tissue exchange region than the Krogh cylinder. The "bare bones" basis of the preceding derivation is solute balance in the form

$$F\,dc + Pc\,dS = 0 \qquad [11.22]$$

The solute concentration c is here regarded as a function of the cumulative exchange area S that varies from $S = 0$ at the "sources" (arterioles or prearteriolar vessels) to $S = s$ at the "sinks" (venules or postvenular vessels) of the exchange region (the same terminology s for the variable and its final value at the "sinks" is used for simplicity of notation). Instead of a Krogh cylinder as the basis of Eq. [11.22] one might visualize a "Krogh sphere," in which the blood flow F flows radially outward from an arteriolar source to concentrically situated venular sinks. The capillary connections between sources and sinks need not be straight or regular but might be tortuous (a bowl of spaghetti with "source meatballs" and "sink meatballs" stuck in here and there). In this case the quantities occurring in Eq. [11.22] are to be regarded as statistical averages over

local distance and short time interval, multiplied by correlation coefficients if any two variables in a product are correlated either in space or time. Equation [11.22] is prepared for integration by writing it as

$$\int_{c_a}^{c_v} \frac{dc}{c} = \int_0^S \frac{PdS}{F} \qquad [11.23]$$

in which the limits of integration of the left side are "source" (arterial) concentration c_a and "sink" (venous) concentration c_v. The corresponding limits of integration on exchange area are 0 and S. The permeability P is in general a function of S; thus $P = P(S)$ in Eq. [11.23]. The flow F, however, is constant by assumption in Eq. [11.22] (for variable F see the corresponding osmotic exchange formulation in Chapter 12). Equation [11.23] may thus be integrated as

$$\ln \frac{c_v}{c_a} = -\frac{\int_0^S PdS}{F} = -\frac{\bar{P}S}{F} \qquad [11.24]$$

where

$$\bar{P} \equiv \frac{\int_0^S PdS}{S} \qquad [11.25]$$

is the "exchange area averaged" permeability. It will be seen that Eq. [11.24] is essentially the same as Eq. [11.21].

If the tissue concentration c_t is not 0 during the passage of solute from sources to sinks "in a single pass," the solute balance equation becomes

$$F\,dc + P\,(c - c_t)\,dS = 0 \qquad [11.26]$$

and Eq. [11.21] is replaced by

$$\frac{c_v - c_t}{c_a - c_t} = e^{-\frac{PS}{F}} = T = 1 - E \qquad [11.27]$$

In deriving Eq. [11.27] or [11.21] all concentrations are regarded as constant in time; that is, the solute balance Eqs. [11.18], [11.22], or [11.26] are regarded as indicator steady-state equations. This represents an approximation since the experimental situation is that of an indicator transient; for example, with bolus injection, $c_a(t)$, $c_v(t)$, and $c_t(t)$ are all time-dependent. The justification for steady-state solute balance, if this justification exists, is that the events during the indicator transient can be split into two types, fast and slow. The fast event is the passage of indicator from source to sink. The slow events are the variation with time of indicator concentration at the source $c_a(t)$ (the rate of arrival of the dispersed bolus), the variation with time of the indicator concentration in the tissue $c_t(t)$ (tissue washin or washout) and the variation with time of the outflow concentration $c_v(t)$, controlled by $c_a(t)$ and $c_t(t)$. The slow events are pictured as so slow as to be temporarily "frozen" or unchanging while the fast event is taking place. The fast event, passage of indicator from source to sink, is

pictured as occurring so fast that a steady state, Eq. [11.27], is established with the then existing values of $c_a(t)$, $c_v(t)$, and $c_t(t)$.

The mathematical formulation of the preceding approximation, well known in physics as the "adiabatic" approximation, is to start from the rigorous time-dependent solute balance in the vascular volume, which in one dimension (Krogh cylinder) is

Vascular Local Mass Balance

Partical differential equation model = $p - d -$ e model

$$\begin{matrix}\text{Time} \\ \text{derivative}\end{matrix} = \text{Convection} \quad \text{Permeability}$$

$$\frac{\partial c(x,t)}{\partial t} = -\frac{F}{A}\frac{\partial\, c(x,t)}{\partial x} - \frac{PdS}{A\,dx}[c(x,t) - c_t(t)] \qquad [11.28]$$

The time derivative term on the left is then assumed negligible, which yields Eq. [11.26] and thence Eq. [11.27]. Equation [11.28] is another example of the partial differential equation, or continuum, approach to formulating models for the interior of the black box. In Eq. [11.28] diffusion terms are neglected. Obviously all terms may be retained for a very general formulation of an inside-the-black-box model from the partial differential equation point of view. The slow time completion of the fast time solution, Eq. [11.27] may be obtained by substituting Eq. [11.27] into the global mass balance equation [time-dependent Fick Eq. (11.28)] which is obtainable by spatial integration from the partial differential equation formulation. The result, for example, for a constant step function source concentration $c_a(t) = c_a =$ constant is

$$\frac{c_a - c_v(t)}{c_a} = Ee^{-kt} \qquad [11.29]$$

where

$$k = \frac{F}{Vd} \qquad [11.30]$$

and Vd can be approximated as the steady-state volume of distribution of the indicator. Equation [11.29] is the basis of the Renkin plateau method of measuring E with correction (e^{-kt}) for back-diffusion from the extravascular volume.

The *transmission* T is the complement of E; i.e., $T = 1 - E$

We define the *extraction* as the fractional transcapillary loss of test indicator in one passage through the capillary. Thus use of Eq. [11.21] gives

$$E = \frac{c_a - c_v}{c_a} = 1 - e^{-\frac{PS}{F}} \qquad [11.31]$$

This means that we can solve Eq. [11.31] to obtain the permeability – surface area product PS as

$$\left.\begin{aligned} 1-E &= e^{-\frac{PS}{F}} \\ \ln(1-E) &= -\frac{PS}{F} \\ \text{or} \\ PS &= -F\ln(1-E) \end{aligned}\right\} \qquad [11.32]$$

As calculated here PS has the same unit as F. It could be in milliliters of blood per minute and per 100 g of tissue. It is more reasonable to measure PS and F in *milliliters of plasma water* [*i.e., plasma ultrafiltrate* per minute (or second) and per 100 g (or 1 g)]. In this way one is paying attention to the actual situation, namely that the transcapillary transport appears for the smaller hydrophilic (extracellular) molecules to be diffusion through water-filled channels (pores) traversing the vessel wall, channels filled with a fluid with a low concentration of plasma proteins. To express F and hence PS in plasma water units one must multiply F by (1 − Htc)—to obtain plasma flow, and then multiply by the fractional water content of plasma (an additional correction of a few percent may be made in the case of charged indicators due to the Donnan equilibrium). Having thus essentially expressed F in the same units of flow of plasma water and with the total capillary surface area S being constant, we can as indicated by Crone use Eq. [11.32] to obtain the ratio of the capillary permeability of two permeant indicators, 1 and 2

Crone, C. (1963): *Acta Physiol. Scand.*, 58:292.

$$\frac{P_1}{P_2} = \frac{\ln(1-E_1)}{\ln(1-E_2)} \qquad [11.33]$$

where E_1 and E_2 are obtained from the upslopes of two simultaneously measured indicator curves. As $d\ln(x)/dx = 1$ for $x = 1$ it follows that for small values of E $\ln(1-E) \simeq -E$. This means that in conditions of high blood flow F when the extraction is quite small Eq. [11.32] becomes

$$PS = FE$$

But, FE is the clearance Cl of permeant tracer (see Chapter 2). Hence it follows *that PS can be considered as the clearance across the capillary wall in the case where the flow had been made so high that the arterial concentration* c_a *did not materially* change [small E means $c_v \simeq c_a$].

Results of measurement of capillary permeability P in skeletal muscle have shown that P decreases with increase in molecular size *more* than the free diffusion coefficient in water. This result is interpreted to indicate that in this organ the transcapillary passages are quite narrow.

Pappenheimer J. R., et al. (1951): *Am. J. Physiol.*, 167:13.

This was the conclusion also reached by Pappenheimer, Renkin, and Borrero (1951). These investigators performed the "isogravimetric permeation" experiment, in which the quantity

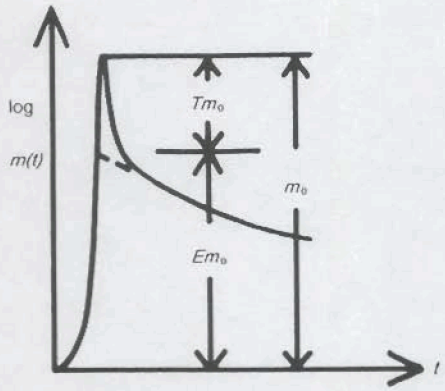
Perl, W. (1971): *Microvasc. Res.*, 3:233.

measured was not permeability P but rather P/σ_d where σ_d is the osmotic reflection coefficient (see Chapter 12). It is possible, by assuming a geometry for the interendothelial channels, to "disentangle" the Pappenheimer et al. values of P/σ_d for his series of hydrophilic solutes in cat hind limb muscle capillaries into P and σd values (see Perl, 1971). The results agree with the above-mentioned measurements of tracer permeability. Thus, a coherent model of transcapillary passive transport of hydrophilic molecules with restricted diffusion through narrow slits or other continuous aqueous channels ("fused vesicles") seems to be emerging.

11.6 Freely Diffusible (i.e., Intracellular) Indicators

This class of indicators comprises lipophilic molecules that readily diffuse across the lipoidic cell membranes. All gases belong to this class, as does antipyrine and a number of other fat-soluble organic molecules. A gradual transition from lipophobic to very lipophilic indicators may be discerned on the basis of the olive oil/water partition coefficient. An exceptional molecule is water. Although hydrophilic, it is so small that it crosses the capillary barrier by both pathways, cells and clefts (pores). It can be estimated that tracer water diffusion across the skeletal muscle capillary wall is only 3% via the pores and 97% via the cells (Ex. 1, Chapter 11). Despite the high diffusibility and small size, of the water molecule, its tracer permeability, although large, is not "infinitely" large and can be "seen" in certain experiments. Thus, bolus injection of the amount m_0 tracer water ($H_2\,{}^{15}O$) into the brain arterial inflow at sufficiently high blood flow exhibits a residue curve (external gamma detection of ^{15}O) with an initial "spike." This result suggests a transmitted fraction $(1 - E)$ and an extracted fraction E of the injected bolus m_0 with a permeability interpretation by Eq. [11.32].

Eichling, J. O., et al. (1974): *Circulat. Res.*, 35:358.

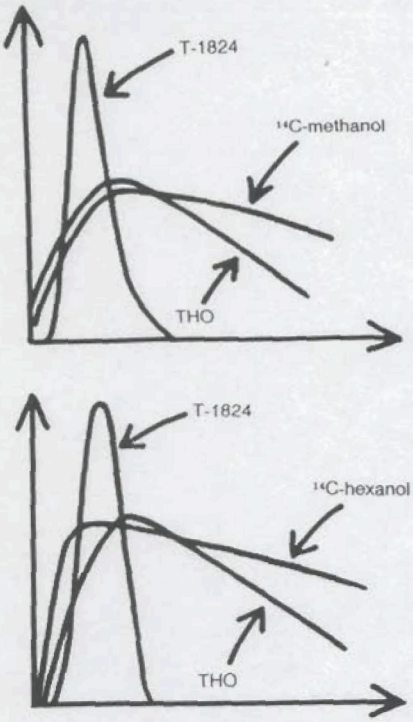


Chinard, F. P., et al. (1969): *Circ. Res.*, 25:343.

$\frac{\text{Olive oil}}{\text{Water}}$ Partition coefficient of hexanol at 37° C = 10.9

Chinard, F. P., et al. (1971): In *Central Hemodynamics and Gas Exchange*, Giuntini, C., ed. Minerva Medica, Italy, p. 191.

Another suggestion of the finite permeability of water comes from multiple indicator experiments on kidney in which the series of monohydric alcohols, from ^{14}C-methanol to ^{14}C-hexanol were injected (bolus injection in renal artery, outflow detection in renal vein). Each bolus contained a ^{14}C-alcohol, tritiated water (THO) and a vascular indicator (T-1824 albumin). The successively higher alcohols appeared increasingly sooner than THO, which in turn appeared somewhat sooner than the vascular indicator T-1824. These results were interpreted as indicating "diffusion bypass" or direct "arteriole-to-venule" diffusion of the THO and alcohol indicators through kidney cortical tissue. It was interpreted that the alcohols had a higher diffusion coefficient than THO because their lipid solubility increases their permeability through the lipoid barriers of the tissue cell membranes. Conversely, THO experiences a greater resistance to passage through the cell membranes; that



Perl, W. (1971): *Microvasc. Res.*, 3:233.

is, the permeability of THO across the cell membrane is much less than that of, say, ^{14}C-hexanol (see Ex. 2). Similar evidence for the finite tracer permeability of water is seen from comparing the upslopes of the THO and ^{125}I-antipyrine indicator curves measured in dog lung. The upslope of the THO curve lies slightly above the upslope of the iodoantipyrine. A smaller blood-tissue barrier (capillary wall and/or cell wall) permeability of THO than of the lipid soluble iodoantipyrine is suggested. Quantitative evaluation is possible by a generalization of the upslope formulas, Eqs. [11.17] and [11.32].

EXERCISES

11.1 The parallel pathway model of the capillary wall blood-tissue barrier consists of the endothelial cells (cell pathway) in parallel with the interendothelial clefts (pore pathway). Tracer water crosses the barrier in response to a blood-tissue difference of tracer concentration. Estimate the division of tracer water flux between the pore pathway and the cell pathway. Assume the data:

$$\frac{S^p}{S^c} = \frac{\text{exchange area of pores}}{\text{exchange area of cells}} = 3 \times 10^{-5} \text{ (Perl, 1971)}$$

D_w = free diffusion coefficient of tracer water in "filtrate" aqueous solution = 2.4×10^{-5} cm^2/sec.

P_m = permeability coefficient of tracer water for a cell membrane = 4×10^{-3} cm/sec.

Solution to Ex. 11.1: The ratio r of tracer fluxes by the two pathways equals the ratio of the PS products in the two pathways. Thus

$$r = \frac{P^p S^p}{P^c S^c} = 3 \times 10^{-5} \frac{P^p}{P^c}$$

$$P^p = \frac{D_w}{\Delta x d} = \frac{2.4 \times 10^{-5}}{(200 + \frac{1}{5} \times 5000) \times 10^{-8}} = 2 \text{ cm/sec}$$

Assuming two endothelial cell membranes in series gives

$$P^c = \frac{Pm}{2} = \frac{4 \times 10^{-3}}{2} = 2 \times 10^{-3} \text{ cm/sec.}$$

Hence

$$r = 3 \times 10^{-5} \times \frac{2}{2 \times 10^{-3}} = 0.03 = \frac{\text{Pore pathway flux}}{\text{Cell pathway flux}}$$

11.2 Estimate the permeability coefficient of tritiated water (THO) for the kidney cortical cell membrane from the experimental deduction

$$\frac{D\,(\text{Cortex, THO, 37°C})}{D\,(\text{Water, THO, 37°C})} = 0.2$$

Assume a kidney cortical intracellular membrane to membrane distance of 10×10^{-4} cm,

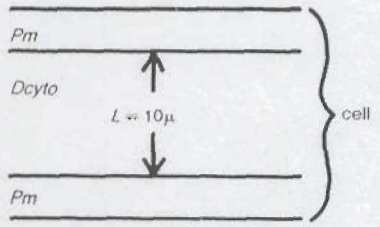
$$J = DS\frac{\Delta c}{\Delta x}$$
$$= PS\Delta c$$
$$P = \frac{D}{\Delta x}$$

$\Delta x d$ = effective diffusion length taken to be $200 + 1/5 \times 5000$. For details see Perl, 1971.

$$D(\text{water, THO, } 37°\text{C}) = 3 \times 10^{-5}\ \text{cm}^2/\text{sec} = Dw$$

$$D(\text{cytoplasm, THO, } 37°\text{C}) = 1.5 \times 10^{-5}\ \text{cm}^2/\text{sec} = Dcyto$$

Neglect extracellular pathways for diffusional flux.



Solution to Ex. 11.2: Assuming a series model of two membranes each of permeability Pm and an intervening cytoplasm of thickness $L = 10\mu$ and adding the series resistances gives the overall resistance in terms of the cortical diffusion coefficient, as

$$\frac{1}{Dcort.} = \frac{2}{L\,Pm} + \frac{1}{Dcyto.}$$

or

$$\frac{1}{Pm} = \frac{L}{2}\left(\frac{Dw}{Dcort.} - \frac{Dw}{Dcyto.}\right)\frac{1}{Dw}$$

$$= \frac{10 \times 10^{-4}}{2}(5-2)\frac{1}{3 \times 10^{-5}} = \frac{100}{2}$$

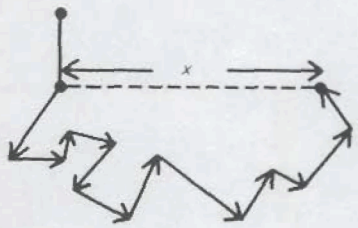
$$Pm = \frac{2}{100} = 20 \times 10^{-3}\ \text{cm/sec.}$$

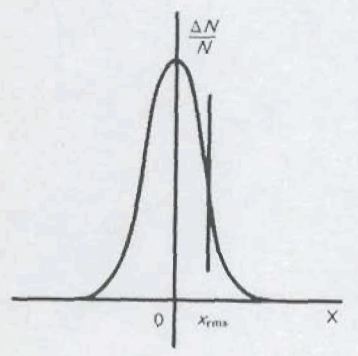
This result is reasonable in comparison with the value of Pm (THO) in red cell at 23°C where $Pm = 3 \times 10^{-3}$ cm/sec.

11.7 Note on Diffusion

Kinetic theory of gases

$\frac{1}{2}\,mv^2 = \frac{3}{2}\,kT$





$$\bar{x}^2 = \frac{\Sigma(x-0)^2 \Delta N}{N}$$

The Brownian motion or stochastic model of diffusion pictures a molecule as moving with a constant speed proportional to its temperature, but changing course at random because of collisions with other molecules. If the molecule is tagged in some way so as to be observable, and the x-component of its distance from a fixed point is measured, the frequency function of the distribution x is found to be Gaussian in shape with a mean value of x equal to 0 and a variance or mean square value $\overline{x^2}$ given by

$$\overline{x^2} = 2\ Dt \qquad [11.34]$$

The proportionality constant D (cm²/sec) is denoted the free diffusion coefficient of the observed molecule in the medium in which it is diffusing.

The relationship Eq. [11.34] or "root mean square," diffusion distance $(\overline{x^2})^{1/2} \equiv x_{rms}$ to diffusion time t is illustrated for a small molecule in aqueous solution for which, typically, $D \approx 1 \times 10^{-5}$ cm²/sec. Equation [11.34] gives

$$t = \frac{\overline{x^2}}{2D} = \frac{x_{rms}^2}{2D} \qquad [11.35]$$

For

$x_{rms} = 10^{-4}\text{cm} = 1\mu$, $\quad t = 0.5 \times 10^{-3}$ sec

$x_{rms} = 10^{-3}\text{cm} = 10\mu$, $\quad t = 0.05$ sec

$x_{rms} = 10^{-2}\text{cm} = 100\mu$, $\quad t = 5$ sec

$x_{rms} = 10^{-1}\text{cm} = 1$ mm, $\quad t = 5 \times 10^2$ sec $= 8.3$ min

$x_{rms} = 1$ cm	$t = 5 \times 10^4$ sec $= 14$ hr
$x_{rms} = 10$ cm	$t = 5 \times 10^6$ sec $= 58$ days
$x_{rms} = 100$ cm	$t = 5 \times 10^8$ sec $= 16$ years

These diffusion times indicate why increasingly rapid convection occurs within (streaming motions), between (interstitial flow), and past (microcirculation) cells and through the body as a whole (large vessel flow) as the distance scale rises with growth. This table shows that molecular diffusion from regions with poor or absent circulation can constitute very "bad sites" giving long tails on indicator curves.

The value $D = 1 \times 10^{-5}$ cm^2/sec used to illustrate [Eq. 11.35] is typical for a small molecule of molecular weight ~ 100d in water at body temperature. Diffusion coefficients in liquids vary inversely with the square root of the molecular weight for small molecules and with the cube root for big molecules. Thus the diffusion coefficient of albumin (mw 70,000) in aqueous solution is $\sim 10^{-7}$ cm^2/sec. Diffusion coefficients in living tissues are lower than in free solution. Although it is thought by some that the diffusion coefficient of a small molecule in cell cytoplasm is many orders of magnitude lower than in free solution, the present authors share the view of Krogh, Kety, and many others that current evidence points to a diffusion coefficient of small molecules through living tissue (including cell cytoplasm) of the order 0.2 to 0.5 of their values in free solution at the same temperature.

Equation [11.34] was actually derived many years before its Brownian motion interpretation was given. It was obtained as a solution of the Fick model of diffusion processes in the form of the partial differential equation, in this case for one-dimensional diffusion

Continuous model of diffusion

Fick second diffusion equation

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \qquad [11.36]$$

where $c(x,t)$ mole/cm^3 is the concentration of the diffusing solute at distance x and time t. Equation [11.36] is derived by solute mass balance in a slab of thickness dx:
The diffusive flux

Fick first diffusion equation

$$j_s(x,t) = -DS\frac{\partial c}{\partial x} \qquad [11.37]$$

entering the slab across an exchange area S at distance χ and time t, minus the diffusive flux

Taylor expansion, higher elements negligible

$$j_s(x,t+dx,t) = -DS\left[\frac{\partial c}{\partial x} + \frac{\partial}{\partial x}\left(\frac{\partial c}{\partial x}\right)dx + \ldots.\right] \qquad [11.38]$$

$$= -DS\left[\frac{\partial c}{\partial x} + \frac{\partial^2 c}{\partial x^2}dx\right] \qquad [11.39]$$

leaving the slab at distance $x + dx$ and time t must equal the time rate of increase of solute mass in the slab $Sdx\ \partial c/\partial t$. Dividing by Sdx gives Eq. [11.36]. The partial differential equation formulation is completed by specifying initial conditions and boundary conditions on $c(x,t)$. The Fick partial differential

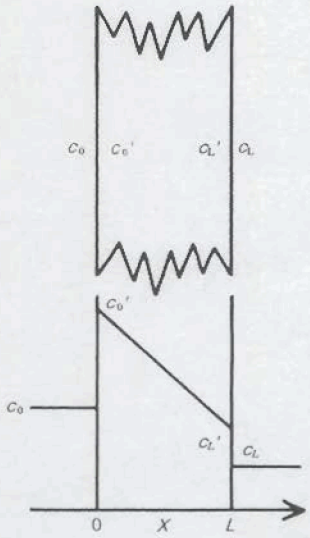
Fournier, 1822

equation for diffusion was actually formulated in analogy with the Fournier equation for heat conduction

$$\frac{\partial\theta}{\partial t} = x\frac{\partial^2\theta}{\partial x^2} \qquad [11.40]$$

in which θ is temperature (°C) and χ (cm²/sec) is the thermometric diffusitity. Furthermore

$$x = \frac{k}{\rho c_p} \qquad [11.41]$$



where k is the thermal conductivity, ρ the density and cp the specific heat at constant pressure of the medium that is conducting the heat. For watery tissue, or aqueous solution, $k \approx 10^{-3}$ cal/cm sec °C, $\rho \approx 1$ g/cm³ and $c_p \approx 1$ cal/g °C, giving $x \approx 10^{-3}$ cm²/sec. Equations [11.34] or [11.35] can be used to estimate heat conduction distances or times, respectively. Thus the diffusion times given after Eq. [11.35] should be decreased by a factor of 100 for heat, $x = D \approx 10^{-3}$ cm²/sec and increased by a factor of 100 for a macromolecule for which $D = 10^{-7}$ cm²/sec.

It is often assumed that the molecular mechanism of permeability is diffusion. For this reason the steady-state solution for one-dimensional diffusion through a slab (the barrier) will be given. Consider a slab between two reservoirs. The indicator concentration is maintained at c_0 in the left reservoir and c_L in the right reservoir. Assume a homogeneous slab and the same solvent in both reservoirs. Then the indicator concentration just inside the slab at $x = 0$ will be c_0' and just inside the slab at $x = L$ will be c_L', where

$$\frac{c_0'}{c_0} = \frac{c_L'}{c_L} = \lambda \qquad [11.42]$$

is the partition coefficient of the indicator between the slab medium and the reservoir solution. The indicator steady-state solution is given by Eq. [11.36] with $\partial c/\partial t = 0$ as the linear variation

$$c(x) = c_0' + (c_L' - c_0')\frac{x}{L} \qquad [11.43]$$

The concentration gradient dc/dx is then constant and substituting into Eq. [11.37] gives the diffusional flux j_s through the slab; thus

$$j_s = \frac{DS}{L}(c_0' - c_L') \qquad [11.44]$$

$$= \frac{\lambda DS}{L}(c_0 - c_L) \qquad [11.45]$$

Comparing Eq. [11.45] with the defining equation [12.3] for indicator permeability gives

$$P_d = \frac{\lambda D}{L} \qquad [11.46]$$

Equation [11.45] can be put in the form of Ohm's Law by writing it in the form

$$j_s = P_d S \Delta c \qquad [11.47]$$

$$= \Delta c / R_d \qquad [11.48]$$

where $P_d S$ is denoted the (diffusive) conductance of the slab and R_d the (diffusive) resistance of the slab. Series and parallel combinations of slabs or diffusion pathways may be analyzed in the indicator steady state in complete analogy with direct current networks. Thus, slabs in series in the indicator diffusion steady state are described by Eq. [11.48] where

$$R_d = R_{d1} + R_{d2} + \ldots . \qquad [11.49]$$

and

$$R_{di} = \frac{L_i}{\lambda_i D_i S_i} \qquad [11.50]$$

is the diffusional resistance of the i^{th} slab. Similarly, diffusion pathways in parallel are described by Eq. [11.47] where

$$P_d S = P_{d1} S_1 + P_{d2} S_2 + \ldots . \qquad [11.51]$$

and

$$P_{di} S_i = \frac{L_i}{\lambda_i D_i S_i} \qquad [11.52]$$

is the diffusional conductance of the i^{th} parallel pathway.