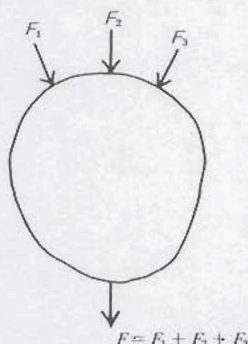
3 Flow or Flux by Bolus Injection: Outlet Mixing (The Henriques-Hamilton Principle)

3.1 The Experiment

Henriques, V. (1913): *Biochem. Zeitschr.* 56:230.

Hamilton, W. F., et al. (1928): *Am. J. Physiol.* 84:338.

A known amount (dose) m_0 of an indicator is injected as a bolus into a system having one or more inlets and a single well mixed outlet from which we can sample. The flow F of the carrier fluid through the outlet is constant while the outlet concentration $c(t)$ varies with time. The small quantity of indicator leaving the system during the short time interval dt from time t to time $t + dt$ is thus



Total IN = Total OUT

$$\begin{array}{ccccc} \text{Amount leaving} & = & \text{Volume of carrier fluid leaving} & \times & \text{Concentration} \\ dm_{\text{out}} & = & F dt & & c(t) \end{array} \qquad [3.1]$$

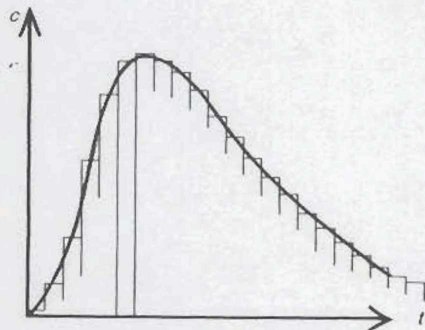
because the interval is so brief that $c(t)$ can be considered constant throughout it. Summing up individual amounts leaving the system until all indicator has left yields the mass balance equation

$$\begin{array}{ccc} \text{Total amount injected (the dose)} & = & \text{Sum of all amounts leaving} \\ m_0 & = & F\int_0^\infty c(t)dt \end{array} \qquad [3.2]$$

Solving Eq. [3.2] for the flow F gives

$$\text{Flow} = \frac{\text{Dose}}{\text{Area}}$$

$$F = \frac{m_0}{\int_0^\infty c(t)dt} \qquad [3.3]$$



Sum of all rectangles $c(t) \times dt$ is equal to

$$\int_0^\infty c(t)\, dt = \text{area}$$

In Eqs. [3.2] and [3.3] the time integral is the sum of all the products $c(t)dt$ and therefore it represents the total area under the outflow concentration curve.

This is the well known indicator dilution method first used by Henriques in 1913 and subsequently thoroughly analyzed by Hamilton and his collaborators in 1928 and in a series of subsequent publications.

If the indicator is a tracer for a mother substance whose concentration in the carrier fluid at the outlet is C then the flux J of the mother substance is the product of F (flow) and C. Hence

Remember that
$s(t) = c(t)/C$

$$\text{Flux} \qquad \frac{\text{Dose}}{\text{Area}}$$

$$J = FC = \frac{m_0 C}{\int_0^\infty c(t)dt} = \frac{m_0}{\int_0^\infty s(t)dt} \tag{3.4}$$

That is, the flux can also be calculated as the dose/area ratio; in this case the area is that under the specific activity curve.

Equation [3.4] is also valid when there is no carrier fluid flow F but when the tracer leaves the system with the mother substance in a measurable ratio, the specific activity $s(t)$. Consider for example the intravenous injection of the albumin tracer ^{131}I labeled serum albumin as a bolus. By taking daily blood samples we can measure the specific activity $s(t)$ in plasma. Assuming that this is the same as the specific activity at the breakdown site we can write an equation that is analogous to Eq. [3.1]: In the short time interval from t to $t + dt$ the amount of indicator leaving is

Because
$s(t) = dm/dM$
and
$dM + Jdt$

$$\begin{matrix} \text{Amount} \\ \text{leaving} \end{matrix} = \begin{matrix} \text{Amount of carrier} \\ \text{mother substance} \\ \text{leaving} \end{matrix} \times \begin{matrix} \text{Specific} \\ \text{activity} \\ \text{of tracer} \end{matrix} \tag{3.5}$$

$$dm_{\text{out}} = \quad Jdt \quad s(t)$$

And, by integration, one gets

$$\begin{matrix} \text{Amount} \\ \text{injected} \end{matrix} = \begin{matrix} \text{Sum of all} \\ \text{amounts leaving} \end{matrix} \tag{3.6}$$

$$m_0 = J\int_0^\infty s(t)dt$$

Hence we can obtain the result denoted in Eq. [3.4], $J = m_0/\int_0^\infty s(t)dt$, without using the concept of a flow F.

Assume that, as in the example in which albumin "turnover" = flux, we measured $s(t)$ in a fluid that contains both tracer and mother substance in the same ratio as at the outlet. This fluid may be considered the *reference fluid*. The clearance Cl (ml/min) of mother substance may then be defined as J/C where C is the concentration of mother substance in the reference fluid. Inserting this into Eq. [3.4] gives

$s(t) = c(t)/C$

$$\text{Clearance} = \frac{\text{Dose}}{\text{Area}} \tag{3.7}$$

$$Cl = \frac{J}{C} = \frac{m_0}{\int_0^\infty c(t)dt}$$

where $c(t)$ is the concentration of tracer in the reference fluid.

Cl in Eq. [3.7] is thus an equivalent flow that is analogous to F in Eq. [3.3]. By this we mean that the outflux J corresponds to the complete clearing (rinsing) of all the mother substance in Cl ml/min of the reference fluid that has a specific activity that at any time t is the same as that at the outlet.

We can summarize the bolus injection method for measuring flow and flux as

$$\left.\begin{array}{ll}\text{Flow of} & \\ \text{carrier fluid} & F \\ \text{Flux of mother} & \\ \text{substance} & J \\ \text{Clearance of} & \\ \text{reference fluid} & Cl\end{array}\right\} = \frac{\text{Dose}}{\text{Area}} = \left\{\begin{array}{l}\text{Area under } c(t)\text{, measured} \\ \text{in carrier fluid} \\ \text{Area under } s(t)\text{, measured} \\ \text{in mother substance} \\ \text{Area under } c(t)\text{, measured} \\ \text{in reference fluid}\end{array}\right. \qquad [3.8]$$

Although the mass balance principle expressed in Eq. [3.8] is simple and clearcut, various complications arise in applications to experiments, some of which are discussed in the following sections.

3.2 Single Cumulative Sample

Suppose we could collect the entire outflow of fluid at the outlet in one single "bucket" from time 0 to such time t_n after which no further indicator emerges. That means

Mass balance in single cumulative sample technique

$$\begin{array}{ccc}\text{Amount injected} & = & \text{Amount recovered} \\ & & \overbrace{\text{Volume} \qquad \text{Concentration}} \\ m_0 & = & (Ft_n + V_i) \qquad \bar{c}\end{array} \qquad [3.9]$$

where $Ft_n + V_i$ is the total amount of fluid (carrier fluid plus injected volume) leaving the system during the sampling period of t_n sec, and $\bar{c}$ is the average outlet concentration of indicator within that period. As we shall discuss in more detail in Sec. 3.7, this concentration $\bar{c}$ would also be determined if only a constant fraction, instead of the total outflow, is collected in the bucket. Therefore $\bar{c}$ may be termed the flow-averaged concentration of a single cumulative sample from time 0 to time t_n.

If t_n is measured in seconds then F is the volume per second leaving the system; if $\bar{c}$ is measured in mass per ml of whole blood, then the unit of volume for F is ml of whole blood.

Because the injected volume V_i is much smaller than the cumulative outflow of blood (the small perturbation condition), $Ft_n + V_i \simeq Ft_n$ and hence Eq. [3.9] solved for flow becomes approximately

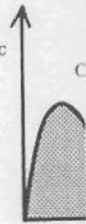
$$F = \frac{m_0}{\bar{c}t_n} \qquad [3.10]$$

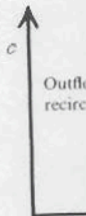
The area $\bar{c}t_n$ equals area under concentration curve Ac

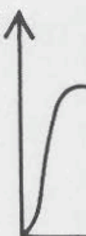
$$F = \frac{\text{Dose}}{\text{Area}}$$

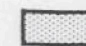
The product $\bar{c}t_n$ equals the area under the indicator concentration curve A_c. (This area divided by t_n is actually the mathematical definition of $\bar{c}$). Hence Eq. [3.1] can be expressed in the form of *flow equals dose divided by area.*

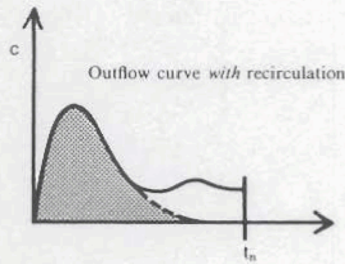
The single cumulative sample approach outlined here has

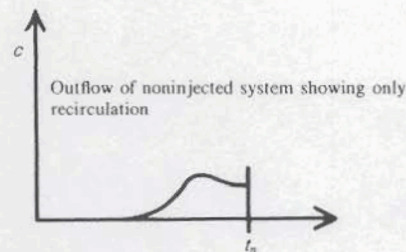


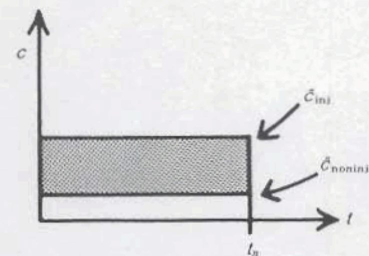




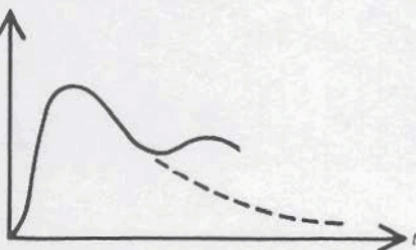


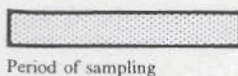






The two continuously drawn samples of injected and noninjected systems




Period of sampling

a major limitation: it is assumed that the sampling will last until complete recovery of the bolus has occurred yet without recirculation of indicator, in a situation where we have no experimental evidence that both of these conditions can be fulfilled. In circulation studies recirculation generally occurs long before complete recovery of the bolus has been achieved. As mentioned in the case of the constant infusion method, if a similar (bilaterally symmetrical) noninjected system is available for simultaneous sampling, then the correct $\bar{c}$ is obtained by subtraction as

$$\bar{c} = \bar{c}_{\text{inj}} - \bar{c}_{\text{noninj}} \qquad [3.11]$$

It may be noted that the experimental correction for recirculation [Eqs. 1.15 and 3.11] is based on outlet sampling from an assumed bilaterally symmetrical system. This is a direct approach, in contrast to inlet sampling methods for recirculation correction that are indirect because a convolution procedure is required to calculate the outlet recirculatory response (see Chapter 9). It should be noted that in many metabolic systems, such as the total albumin mass in the body, no recirculation occurs.

3.3 Continuous Monitoring

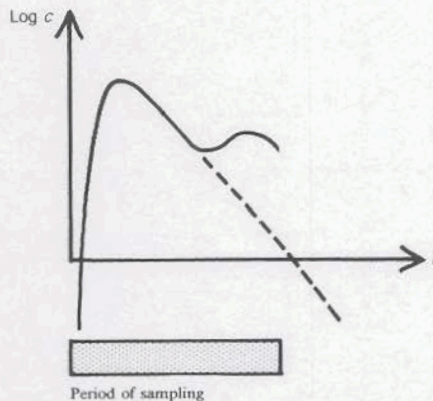
Suppose a densitometer is mounted on the sampling catheter through which blood is withdrawn at a constant rate. The densitometer is calibrated to yield the indicator concentration $c(t)$. A smooth continuous curve of $c(t)$ versus time t is thus obtained. This curve allows extrapolation to complete recovery as well as elimination of the influence of recirculation. These corrections are based on an analysis of the downslope of the curve. It has been shown for many systems that a monoexponential function can be used to approximate the lower part of the downslope if recirculation is absent. Recirculation is therefore recognized as a deviation from linearity on a semilogarithmic plot of the tail part of the curve (log c plotted against t).

It is the area under the corrected $c(t)$ curve that we must calculate. This area A_c is obtained in two sections. One integrates the actual curve from time 0 until the time t_r. From then on the curve is considered monoexponential. The remaining area, from t_r to infinity, is obtained as the product of the curve height at time t_r (that is $c(t_r)$) and the time constant $t_{1/e}$. Thus

$$A_c = \int_0^{t_r} c(t)\, dt + c(t_r)\, t_{1/e} \qquad [3.12]$$

The integral can be obtained by planimetry of the linear curve or simply by adding together curve heights measured at sufficiently brief intervals (e.g., 0.5 sec), remembering to

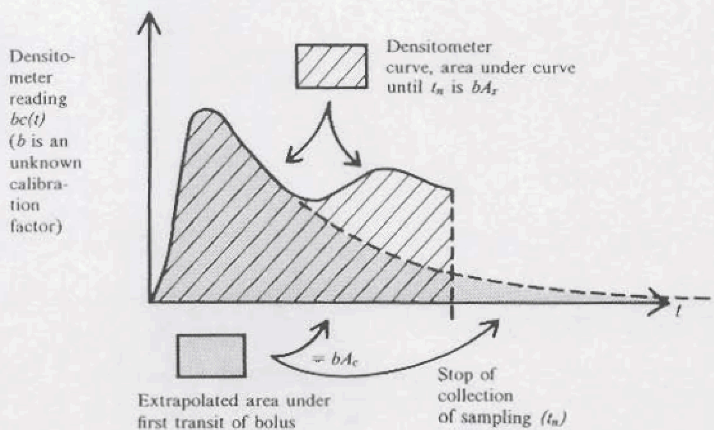
multiply by dt (1/120 min in this example). The time constant $t_{1/e}$ equals $t_{1/2}/\ln(2) \simeq t_{1/2}/0.693$. $t_{1/e}$ is the time it takes for the monoexponential curve to decrease to $1/e$ of a given value; since $1/e \simeq 0.37$, $t_{1/e}$ can be obtained as the time until the curve decreases to 37% of its initial value. $t_{1/e}$ is the reciprocal of the exponential coefficient k; that is $k = 1/t_{1/e} \simeq 0.693/t_{1/2}$. In terms of k the area under the tail part of the curve is $c(t_r)/k$.



When extrapolating remember to measure $t_{1/e}$ in the same units as Δt.

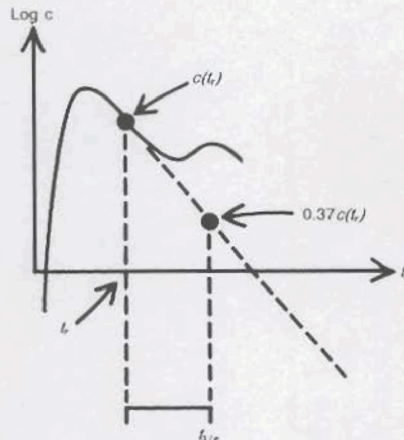
Note the inconsistency: one cannot directly compare *in situ* and bucket sampling, but in practice the error is negligible.

Sometimes it is practical to combine the continuous monitoring approach with the single cumulative sample approach. Let the densitometer yielding the outlet concentration curve be mounted immediately in front of a collection syringe by which we take one cumulative sample at a constant rate to obtain $\bar{c}$ by chemical analysis. In this case the densitometer need not be calibrated in absolute units (the calibration factor might depend on individual experimental conditions such as the blood hematocrit). It is necessary only that the densitometer deflection is proportional to $c(t)$:



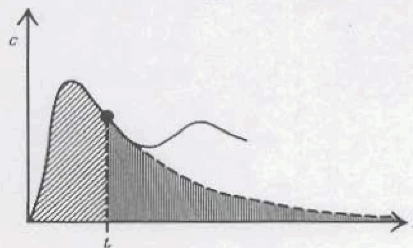
We can now obtain the ratio of the extrapolated area bA_c to the actual area bA_x:

$$\frac{bA_c}{bA_x} = \frac{A_c}{A_x} \tag{3.13}$$



In the sample collected the measurement of $\bar{c}$ is uncorrected for recirculation and for incompleteness of recovery. Multiplying by the known sampling time t_n gives $A_x = \bar{c}t_n$. Inserting this into Eq. [3.13] yields the corrected and extrapolated area A_c

$$A_c = A_x \frac{bA_c}{bA_x} = \bar{c}t_n \frac{bA_c}{bA_x}$$

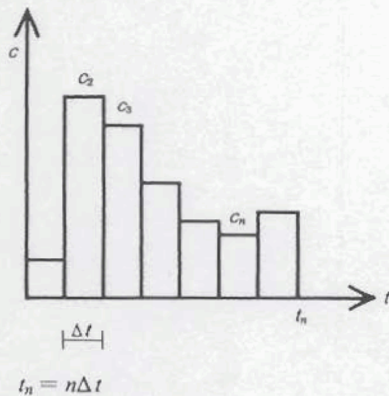


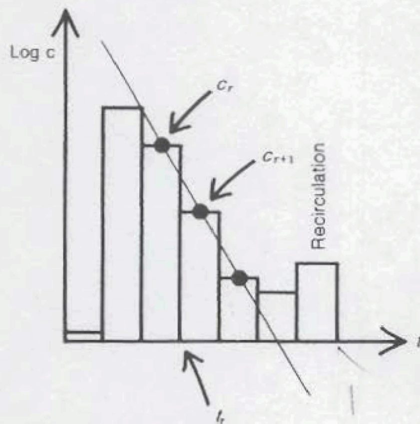
The two areas can be illustrated only on the linear curve.

that together with Eq. [3.12] gives the flow equation

$$F = \frac{m_0}{\bar{c}t_n[bA_c/bA_x]} \tag{3.14}$$

Successive cumulative samples





where the ratio bA_c/bA_x is the correction that changes the measured area A_x to the correct area A_c.

3.4 Successive Cumulative Samples

This sampling procedure is widely used because it allows extrapolation without the necessity of special instrumentation ["on-line" densitometry of dye in whole blood can present problems of linearity whereas conventional spectrophotometric analysis of dye in samples of blood or plasma together with a suitable standard dilution avoids (corrects for) such problems].

Usually a series of n samples is collected at the outlet over successive time intervals of equal duration Δt (e.g., 1 sec). *Thus one obtains a succession of cumulative samples in the* n *time intervals from 0 to time* t_n. Consequently $t_n = n\Delta t$. The outlet concentrations in the samples numbered 1, 2, ... n are denoted $c_1, c_2, \ldots . c_n$.

The curve is drawn as a "staircase" on semilogarithmic paper and extrapolated to infinity by a line intersecting the middle of the steps. Let the first step that fits on this monoexponential curve be step number r; the area A_c is then obtained in two parts as

$$A_c = \sum_{i=1}^{i=r} c_i \Delta t + c(t_r) t_{1/e} \qquad [3.15]$$

This indicates that the individual areas of all the r rectangles that comprise the area are summed up to time t_r. Then $c(t_r)$ is read off the semilogarithmic plot midway between c_r and c_{r+1}. The time constant $t_{1/e}$ is, as previously discussed, the time that it takes for the curve to decrease to 0.37 of its initial value $c(t_r)$. One can also just take the half-time $t_{1/2}$ and then calculate $t_{1/e} \approx t_{1/2}/0.693$.

3.5 Comments on the Measurement of Flow by the Bolus Injection Method

It is important to consider extrapolation to infinity. A practical way of assessing the validity of extrapolation consists of checking the calculated flow with flow determined by a method not requiring extrapolation. Usually the initial downslope of the curve is employed in the extrapolation. But not uncommonly a careful correction for the recirculation will show that this initial part of the downslope is not the correct exponential to be used for the final part of the washout process. The error involved will probably in most cases be an underestimation of the true area under $c(t)$ because slower outwash rates of the tail are masked by the recirculation. In instances with which the authors are familiar (Evans Blue T-1824 in human brain and in exercising human forearm) an underestimation

of approximately 10% of the $c(t)$ area results from using the conventional mode of extrapolation plotting the initial part of the downslope of $c(t)$ on semilogarithmic paper.

The recovery of the indicator bolus at the outlet that we sample from may be incomplete for reasons other than errors in extrapolation or in correcting for recirculation. We may, for instance, be wrong in our assumption that the indicator used is leaving the system solely via the well mixed outlet where sampling is made. That is, losses through other outlets could occur at sites designated as sinks (remember that all systems are by definition *conservative;* neither breakdown nor permanent retention sequestration occur inside the system), through evaporation through skin, lymph drainage, and so forth. If several indicators are injected simultaneously one indicator may be completely recovered whereas others may not. It is convenient in such cases to replace m_0 in the dose divided by area equation by Rm_0 where the recovery R is the fraction of the injected amount m_0 that emerges via the outlet from which recordings are taken. Eq. (3.3 thus may be written

In a *conservative* system the total recovery through all outlets is by *definition* 100%!

$$F_{out} = \frac{R}{\int_0^\infty [c(t)/m_0]dt} = \frac{R}{\int_0^\infty w(t)dt} \qquad [3.16]$$

where F_{out} is the flow through that outlet and $w(t) = c(t)/m_0$ is the concentration per unit dose at the same site. The concentration per unit dose is a quantity especially useful when comparing results for different injection doses and for different indicators.

Recoveries apparently differing from unity for a single outlet system could also result from analytical errors in measuring $c(t)$ and/or m_0.

Nonstationarity of systemic parameters is another problem. The outflow is, of course, never exactly constant. The act of injecting the bolus alone must disturb F if only to eliminate the amount of fluid injected. Conservation of mass still holds and for a small time interval dt, the eliminated amount is

$$dm_{out} = F(t)\, c(t)dt \qquad [3.17]$$

Integrating and defining a mean flow $\bar{F}$ according to the dose divided by area concept

$$m_{out} = m_0 = \int_0^\infty F(t)\, c(t)dt = \bar{F}\int_0^\infty c(t)dt \qquad [3.18]$$

Therefore, when the flow situation is varied the dose/area ratio yields a weighted mean flow defined by Eq. [3.18].

Many authors studied the influence of the nonsteadiness of F in the circulation. Consider, for example, the cardiac output. At the aortic valves blood flows only during the systole. The cardiac output measured as dose/area is found to yield the correct average flow with very little error because the cardiac cycle (approximate 1/sec) is brief relative to the mean transit time of a bolus (10 to 15 sec).

3.6 The Bolus

The dose m_0 of indicator that is injected must be measured accurately. It is trivial to remark that any amount remaining in the injection system (catheter, stopcock) must not be included. Yet, the need to be certain about the amount entering the system does pose some practical problems. In some cases it is most practical to fill the entire injection system with the injectate prior to injecting the bolus and then to determine the volume injected V_i as the weight loss of the syringe divided by the specific gravity of the solution.

The concentration of the injectate c_i may be determined by diluting a known volume ΔV_i (e.g., 0.100 ml) by a known volume ΔV_b of blank carrier fluid (e.g., 10 ml) collected from the outlet just prior to the study. This diluted injectate is termed the standard and its concentration c_s is measured in the same way as all the samples collected during the study are measured. The use of a standard that is diluted to about the same indicator concentration as that of the samples tends to correct for nonlinearity of the analytical procedure. The dose of indicator is calculated by

$$\text{Dose} = m_0 = c_i V_i = c_s \frac{\Delta V_b + \Delta V_i}{\Delta V_i} V_i \qquad [3.19]$$

$$B = \frac{\Delta V_b + \Delta V_i}{\Delta V_i}$$

is the dilution factor.

$m_0 = c_s V_s$

Compare to Eq. [3.19]. Remember that $\Delta V_b \gg \Delta V_i$.

When the carrier fluid is a multiphase fluid such as blood it may be convenient to consider the dilution factor $B = (\Delta V_b + \Delta V_i)/\Delta V_i$ as a conversion factor for the injected volume V_i. In this way the experiment can be considered to consist of the injection of V_s milliliters of carrier fluid containing indicator at concentration c_s, with $V_s = [(\Delta V_b + \Delta V_i)/\Delta V_i] V_i$. This mode of viewing the experiment may help to clarify that the flow F determined as dose/area is the flow of carrier fluid (blood) used to make up the standard regardless of how the concentrations are actually measured. The point discussed in detail in Chapter 1, Sec. 1.8 has been repeated here because of the confusion often resulting when, for example, $c(t)$ and c_s are actually measured in plasma while F is obtained in milliliters of whole blood per unit of time.

But the volume injected V_i must be small.

The bolus can be injected rapidly or slowly and at constant or variable speed. The time and the site of injection are not important. All we must know is that the amount m_0 has entered. However, in practice it is important to deliver the bolus rapidly and close to the mixing site in order to minimize recirculation problems.

3.7 Convection and Diffusion

In Sec. 3.1 we derived the dose divided by area equation on the basis of the equations

$$dm_{\text{out}} = \begin{cases} Fc(t)dt \\ Js(t)dt \end{cases} \qquad [3.20]$$

Membrane "pumps" and pinocytosis are also bulk transport processes in that carrier and indicator (or tracer and mother substance) move together.

These equations state that the amount of tracer crossing the outlet boundary surface during dt is given by the amount of carrier substance multiplied by the amount of indicator per unit amount of carrier. Both equations may be termed bulk transport equations in that carrier and indicator move together. A typical form of bulk transport is convective flux, that is, bulk transport by fluid flow.

In addition to the bulk transport, diffusion may transport indicator out of the system without a corresponding (net) transport of carrier substance. This means that the correct equation for the amount leaving in dt is

$$dm_{\text{out}} = \begin{cases} Fc(t)dt - DS(\partial c/\partial x)dt \\ Js(t)dt - D^*S(\partial s/\partial x)dt \end{cases} \qquad [3.21]$$

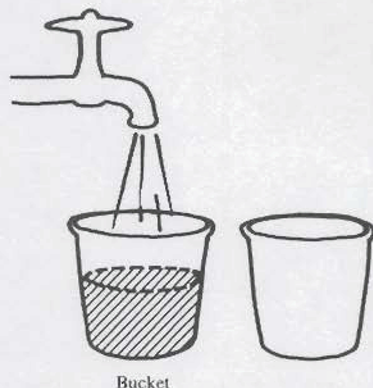
Fick's diffusion equation
Fick, A. (1855): *Pogg. Ann. Physik.*, 94:59.

where D (cm^2/sec) is the diffusion coefficient in the fluid and D^* is the corresponding coefficient for interdiffusion of tracer in the mother substance, and S is the cross sectional area of the outlet.

Thus it is apparent that dm_{out} depends not only on the concentration of indicator at the outlet but also on the concentration gradient at the outlet in the direction of the flow or flux. In deriving the dose divided by area equation the diffusion transport term was neglected. This is permissible as the effects of diffusion cancel out, as will become apparent from the analysis given below.

Bucket sampling

Bucket sampling



Bucket

In the usual sampling procedure we collect fluid (respectively, mother substance) as it leaves the outlet. Each sample is collected in a "bucket" (test tube, syringe, etc.). This assures that if we collected *the total amount* dm_{out} of indicator leaving the system in intervals of dt sec then

$$dm_{\text{out}} = \begin{cases} Fc \text{ bucket } (t)dt \\ Js \text{ bucket } (t)dt \end{cases} \qquad [3.22]$$

In other words, because the buckets accumulate *all* the effluent carrier fluid (respectively, carrier mother substance) in sequential samples we can use the simple bulk transport equation for obtaining dm_{out}. The same is true if our bucket collects a constant fraction of the effluent carrier fluid.

Two important conclusions are reached. First, because of diffusive flux at the outlet the concentration in the bucket is not identical to the outlet concentration in the same dt interval. Precisely expressed, from Eqs. [3.21] and [3.22] it can be seen that

Concentration in sample collected	=	Outlet concentration	Diffusive term

$$\left.\begin{matrix} c_{bucket}(t) \\ s_{bucket}(t) \end{matrix}\right\} = \begin{cases} c(t) - DS/F(\partial c/\partial x) \\ s(t) - D^{*}S/J(\partial s/\partial x) \end{cases} \qquad [3.23]$$

Second, we see that by integrating Eq. [3.22] we obtain the dose divided by area equation for flow or flux. Hence it is apparent that the bucket sampling procedure used converted a convective–diffusive indicator transport [Eq. 3.21] into a convective transport [Eq. 3.22]. Diffusion is thus automatically taken into account for flow or flux determination by the bolus technique because the bucket sampling procedure includes this transport and converts it into what we might term an *equivalent convective transport.*

In situ monitoring

Spalding, D. B. (1958): *Engng. Sci.*, 26:549.

Suppose a continuously monitoring device such as a densitometer is mounted on the outlet. The device might "look" across the stream or consist of a catheter tip sensitive to the indicator. Thus the outlet concentration $c(t)$ can be recorded. This concentration does not allow us to deduce the amount of indicator leaving the system in dt. To obtain dm_{out} a correction term for diffusion must be applied.

The magnitude of this correction, that is the relative role of diffusion, may be assessed as follows. Let Eq. [3.23] for the indicator–carrier fluid case be written

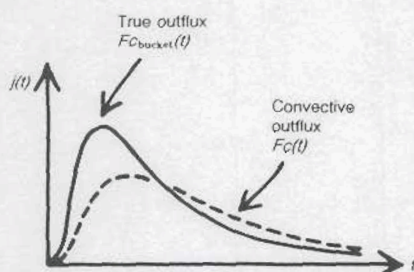
$$c_{bucket}(t) = c(t)[1 - DS/F[(1/c(t))\partial c/\partial x]] \qquad [3.24]$$

The diffusion coefficient D of an indicator molecule of order mol. wt. 100 in blood is approximately 10^{-5} cm²/sec. For an outflow velocity F/S of order 1 cm/sec and a fractional indicator concentration gradient $[1/c(t)]\,\partial c/\partial x$ of order 1 cm^{-1} the correction term in Eq. [3.24] is of order 10^{-5} which is completely negligible. For heat (or cold) as indicator the "diffusion coefficient" [thermal conductivity/(specific heat × density)] is approximately 10^{-3} cm²/sec and the correction term might become appreciable. Of course if the outflow velocity becomes sufficiently low then the diffusive term can predominate over the convective term for any indicator.

As already mentioned, *in situ* monitoring of $c(t)$ does not, after multiplication by Fdt, yield dm_{out}. Nevertheless, the correct flow is obtained by using the area under the $c(t)$ curve regardless of the relative magnitude of the diffusive component. This may seem surprising. It is derived from the equal area rule presented in Chapter 4. We can here comment on this result by stating that the flow equation is obtained from the integral of Eq. [3.21]; that is, for m_0 we have

Flow calculation from *in situ* measurement of outlet concentration

$$\text{Dose} = m_0 = F\int_0^\infty c(t)dt - DS\int_0^\infty (\partial c/\partial x)dt \qquad [3.25]$$



where the net effect of diffusion, the integral of the diffusive flux (second term on the right side) is 0. This may perhaps be intuitively accepted; the net forward diffusive flux on the upslope part of the $c(t)$ curve is precisely counterbalanced by the net retrograde flux on the downslope. The two curves $c_{\text{bucket}}(t)$ and $c(t)$ thus have the same area.

The treatment given in this section has demonstrated that diffusion can be disregarded. For this reason the suffix in $c_{\text{bucket}}(t)$ may be dropped and the measured outlet concentration may be denoted by $c(t)$ regardless of whether "in bucket" or "*in situ*" measurement is used.

3.8 The Sampling Catheter

In the single inlet-multiple outlet system described in Chapter 4 the catheter tip must not move.

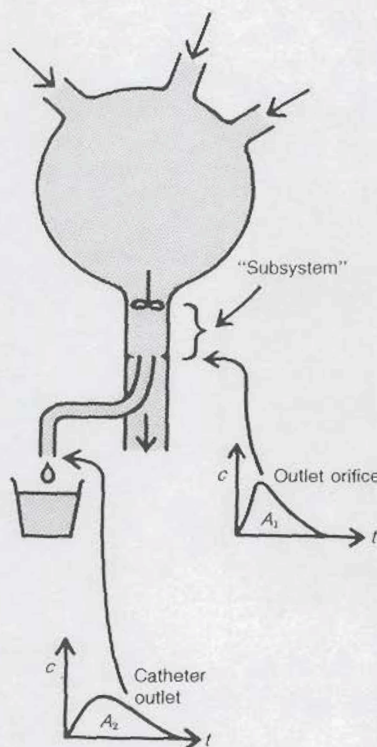
The system analyzed in this chapter has only a single outlet. Hence we may sample from any site in the outlet aperture and this sampling site (e.g., the location of the tip of the sampling catheter) need not be the same throughout the experiment.

The entire conduit connecting the outlet orifice to the "bucket" of collection will be termed the sampling catheter: needle + catheter + stopcock + nozzle of collecting syringe (or whatever else is used in a particular experiment).

The influence of the sampling catheter is simple to describe in the case where we collect a constant fraction α of the outflow F and where the linear velocity in the catheter equals that of the outlet stream (plug flow at velocity F/S). In this case the catheter sampling is precisely equivalent to the "bucket" sampling procedure outlined in Sec. 3.7. Hence mass balance relative to the catheter gives

Total amount passing catheter	=	Total amount entering catheter	=	Total amount leaving catheter

$$\alpha m_0 = \alpha F\int_0^\infty c_{(\text{into cath.})}(t)dt = \alpha F\int_0^\infty c_{(\text{out from cath.})}(t)dt \quad [3.26]$$



In general the sampling catheter gives a different curve shape, but $A_1 = A_2$. The curve shape is maintained only in instances of plug flow in the catheter.

Thus it is apparent that the area that should have been measured, that is, that under the outlet's concentration curve, equals that at the sampling end of the catheter regardless of how long it is.

But in most cases we do not collect at the same linear velocity as that of the fluid leaving the outlet orifice. Thus the local concentration pattern in the outlet [$c(t)$ as well as $\partial c/\partial x$] is deranged. In this situation it is convenient to consider the catheter as part of the system. Then, provided the cross-stream mixing at the original outlet also holds immediately upstream thereof, we can take this upstream site as a well-mixed inlet to a multi-outlet subsystem where the catheter's free end (that in the bucket) is one outlet. According to the analysis given in Chapter 4 the equal area rule states that the

area under this outlet curve is the same as that at any point in the outlet orifice.

In conclusion, the sampling catheter poses no problem. The only fairly obvious point to make is to be sure to start the sampling before the indicator reaches the systemic outlet and to continue sampling until all indicator has passed this location *and* until all indicator in the catheter has reached the collection bucket (respectively, until the time from which a valid extrapolation can be made).

3.9 Flux and Clearance

The preceding sections have been concerned mainly with flow determination by the bolus technique. But, as derived in Sec. 3.1, the same principles apply to determination of flux and clearance.

Thus, if the flux J of a systemic substance occurs via a convective flow F of carrier fluid, then in a bolus indicator experiment

Flux equals dose/area.

$$J = FC = \frac{m_0}{1/C \int_0^\infty c(t)dt} = \frac{m_0}{\int_0^\infty s(t)dt} \qquad [3.27]$$

where $s(t)$ is the ratio of the concentration of indicator $c(t)$ and of the systemic substance of interest C at the outlet.

If the indicator is a tracer for a systemic substance, Eq. [3.27] is obtained directly by the "transformation" explained in Chapter 1: $F \rightarrow J$ and $c \rightarrow s$, which changes $F = m_0/A_c$ into $J = m_0/A_s$. We can also derive Eq. [3.27] on the basis of the bulk-transport equation for mass balance, that is from $dm_{out} = Js(t)dt$ where Jdt is the amount of mother substance leaving the system in the interval from t to $t + dt$ and $s(t)$ is the specific activity of tracer of that time interval. Integration yields Eq. [3.27]. As discussed in Sec. 3.7, we are allowed to derive the flux equation as is done here, that is, by considering only bulk transport (tracer and mother substance going together). The independent flux of tracer due to interdiffusion in the carrier mother substance does not contribute to the total area under the specific activity curve.

As an example of a nonconvective flux consider the measurement of total body albumin flux J_{alb} by the use of ^{131}I-labeled albumin injected intravenously. The plasma specific activity curve $s(t)$ is followed for 14 days by daily sampling and $\int_0^\infty s(t)dt$ is determined using conventional monoexponential extrapolation. Assuming that the specific activity of tracer albumin at the true outlet equals that in the plasma at the same time permits the use of the dose/area Eq. [3.27] for calculating J_{alb}.

The clearance Cl (ml/sec) has been defined for a systemic

substance as the ratio of the efflux J_{out} (g/sec) to the concentration of the substance in a suitable reference fluid C_{ref} (g/ml)

$$Cl = \frac{J_{out}}{C_{ref}} \text{ (ml/sec)} \quad [3.28]$$

This clearance could be determined by direct measurement of the amount of systemic substance leaving the system per unit time (e.g., the urinary creatinine excretion rate) and of the appropriate reference fluid concentration (the serum creatinine). We could also employ an indicator constant infusion technique (such as inulin) and measure the same parameters in the indicator steady state.

Can the clearance of a systemic substance defined in Eq. [3.28] be determined from a bolus injection experiment? Yes, it can if the system has a single outlet because substitution of Eq. [3.27] into [3.28] gives

$s_{out}(t) = c_{out}(t)/C_{out}$

$$Cl = \frac{C_{out}}{C_{ref}} \cdot \frac{m_0}{\int_0^\infty c_{out}(t)dt} \quad [3.29]$$

Thus, by using the outlet fluid as reference fluid

dose/area

We derived this equation in Sec 3.1 [Eq. 3.7]. It is derived again here to show another mode of explaining this important relationship.

$$Cl = \frac{m_0}{\int_0^\infty c_{out}(t)dt} \quad [3.30]$$

As an example we may take the urinary clearance of inulin or ^{51}Cr-labeled EDTA (ethylenediaminetetraacetate, EDTA) as determined with plasma as the reference fluid. The area is the area under the plasma disappearance curve extrapolated to infinity. Because these substances are excreted solely in the kidney and because glomerular filtration is the mode of excretion, the dose-over-plasma-curve area is the glomerular filtration rate.

Inulin and ^{51}Cr-labeled EDTA do not cross the tubular epithelium distal of the glomerulus; there is no tubular reabsorption and no tubular secretion.

Just as in the case of the flux of plasma proteins it is advantageous to employ a bi or a tri exponential representation of the plasma curve. This has the sole purpose of facilitating calculation of the area. In Chapter 10, where the exponential function is presented in detail, it can be seen that if

$ae^{-\alpha t}$ is written as a $\exp.(-\alpha t)$

$$c(t) = a \exp.(-\alpha t) + b \exp.(-\beta t)$$

then

$$\int_0^\infty c(t)dt = A_c = a/\alpha + b/\beta$$

and hence

$$Cl = \frac{m_0}{a/\alpha + b/\beta} \text{ ml/min} \quad [3.31]$$

(For urinary clearance the time unit is usually minutes and this unit must also be employed when obtaining α and β.)

EXERCISES

Background has been subtracted.

3.1 Radioactively labeled microspheres are injected in the left atrium as a bolus and continuous sampling of blood is made from the femoral artery at the constant rate of 0.5 ml/sec for 30 sec (i.e., until bolus has passed sampling site). In a fixed counting geometry the injected amount was 3×10^7 cpm and the whole blood concentration 10^4 cpm/ml.

Bolus fractionation principle of L. A. Sapirstein (1958): *Am. J. Physiol.*, 193:161.

Calculate cardiac output using Eq. [3.10]. Now think of the experiment in terms of the bolus fractionation principle:

$$\frac{\text{Cardiac output}}{\text{Flow rate through needle}} = \frac{\text{Total dose}}{\text{Counts through needle}}$$

That is, the fraction of the cardiac output sampled through the needle equals the fraction of the microspheres collected.

In the kidneys the microspheres (10 to 15 μm in diameter) are totally retained. If 1g of homogenized kidney tissue has a counting rate of 6×10^6 cpm, use the bolus fractionation principle to calculate renal blood flow.

All samples are counted for 200 sec.

3.2 ^{131}I-labeled human serum albumin in sterile saline containing carrier albumin is injected via the femoral vein using nonlabeled saline to flush the catheter. The injected amount is determined by weighing 0.8483 g, specific gravity 1.030. A series of blood samples is taken from the femoral artery starting c. 5 sec before and continuing after the injection with an interval of 1.20 sec. Whole blood (0.5 ml per sample) is counted as well as 0.5 ml of a mixture of 50 μl injectate and 5 ml of whole blank blood. The observed counts as accumulated over 200 sec were (standards in the first two holes): 563472, 558687, 739, 738, 730, 728, 735, 813, 1043, 2451, 9719, 23754, 40131, 46914, 43978, 35107, 24784, 16917, 11262, 7558, 5159, 3907, 3241, 2974, 3389, 4059.

Plot a graph of the curve both on linear and on semilogarithmic graph paper. Calculate cardiac output of whole blood and of plasma (the hematocrit is 0.40).

3.3 A bolus of inulin is injected intravenously (i.v.) and a plasma curve is observed over 5 hr that can be well approximated by

$$w(t) = 0.0005\ e^{-0.05\,t} + 0.0001\ e^{-0.01\,t}$$

where $w(t)$ is the concentration as measured in fractions of injected dose per ml of plasma, and t is measured in minutes.

Calculate Cl_{in} in ml/min and calculate the initial volume of dilution that should equal the plasma volume. Also calculate the total volume of dilution that is a measure of the interstitial space (for this calculation see Chapter 7).