BASIC TRACER KINETICS

Kinetic Principles in the Intact Organism and the Single Organs.

Jens H. Henriksen [*], Stefan Fuglsang [*] and Henrik W. B. Larsson [+]

[*] Department of Clinical Physiology/Nuclear Medicin, Hvidovre Hospital

[+] Department of Clinical Physiology/Nuclear Medicin , Copenhagen District Hospital in Glostrup

7th of February 2007

1 Introduction

Kinetics deals with the relation between time and matter. Whereas time is understood in chronometric terms, matter may encompass physical compounds (mole/L, No. of molecules, mL, etc.), electrical charges (coulomb, No. of positive, negative neutral charges), energy (joule), etc. The aim of this compendium is to review kinetics in order to describe mathematically and physically the transport, exchange, metabolism, and excretion of solutes and liquids. This occurs in and between organs, physiological compartments, or entire organisms. Kinetics is necessary for the understanding of normal physiological processes and is used to quantify these.

The mathematical terms and models applied in kinetics exist in parallel in systems other than the human organism. This manual, however, will deal with only two different classes of kinetics: non-linear and linear models.

A parent compound in the human organism is transported across biological membranes, diffuses, and is transported with the blood flow, eventually metabolised, and finally excreted from the organism. In most cases, it is not possible to conduct sufficiently precise and specific experiments involving a parent compound. In a number of experimental conditions exogenously added parent compounds will change the system to be measured. For example, infusion of insulin reduces blood glucose in attempts to study the kinetics of insulin. The key concept of a tracer is a substance, which is a small amount of a specific compound that is somehow labelled yet still behaves identically with the parent compound (tracee). In some cases, the tracer will be chemically and physically completely identical with the parent compound except for a single stable atom in the parent compound that is replaced by a corresponding radioactive atom or stable isotope. In other cases, the tracer will be identical with the parent compound, except that it contains a different radioactive-labelled compound. Most important, the tracer must have the same kinetic properties as the parent compound. Although in some cases this may be difficult to achieve in a strict sense, this is often quite well approximated.

In other settings, an indicator is used instead of a tracer. An indicator compound only needs to follow kinetically the compound or system of interest. Examples of such indicators are heat/cold, dyes, carbon monoxide, or inulin. Other indicators would include contrast agents, such as Gd DTPA. Although radioactive tracers have dominated the field, stable isotope, dyes, and other compounds useful for transduction of a signal are continually emerging.

Use the "Insert Citation" button to add citations to this document.

Historically, kinetics was developed after the era of thermodynamic and physical principles formulated in the beginning of the 20th century when the discovery of radioactive isotopes had a significant impact on biomedical science. George de Hevesy is considered to be the founder of tracer dynamics. The basic terms in kinetics are formed by the application of classical mathematics with differential equations, integration, logarithmic and exponential functions, and Fourier and Laplace transformations. Today powerful computers permit iterative procedures and numerical solutions that were not previously possible; complicated kinetic models and processes can now be imaged parametrically. Many biological systems are well described with differential equations and iterative processes whereby deterministic chaos with non-linear and non-stationary biological systems arise. This is an approach additional to classical kinetics, where the linearity is a characteristic feature. The assumption of system linearity, i.e., proportionality between input and output, has facilitated the establishment of simple use of kinetics. A consequence of linearity is that the system variables do not depend on the amount of tracer and indicator. Another basic principle is the system stationarity, which assumes that the biological system is stable within the measurement period. That is, repeated similar inputs will lead to identical outputs.

Biological systems are exceedingly complex. Their functions involve many subprocesses and a complete description of all processes involved or even just parts of them, down to the cellular level is not possible. To create a model of a biological system the different components must be considered, starting with a simplified model that is successively expanded. Often there is a trade-off between model accuracy and model complexity.

A kinetic description of biological phenomena requires at least four steps: 1) description of the biological problem; 2) registration of the associated models with a mathematical and numerical description of the problem; 3) solution of the mathematical/numerical problem (differential equation, integration, iteration, etc.); and 4) translation of the mathematical/numerical-kinetic solution into a biological meaningful explanation.

The entire process requires a combination of several disciplines, which must be carefully balanced.

This manual in basic tracer kinetics serves to illustrate commonly seen kinetic problems in relation to perfusion, organ function, and metabolism. Mathematical terms are invariably included. It is impossible to describe kinetics, even at a very basic level without the involvement of some mathematics.

2 Kinetic models

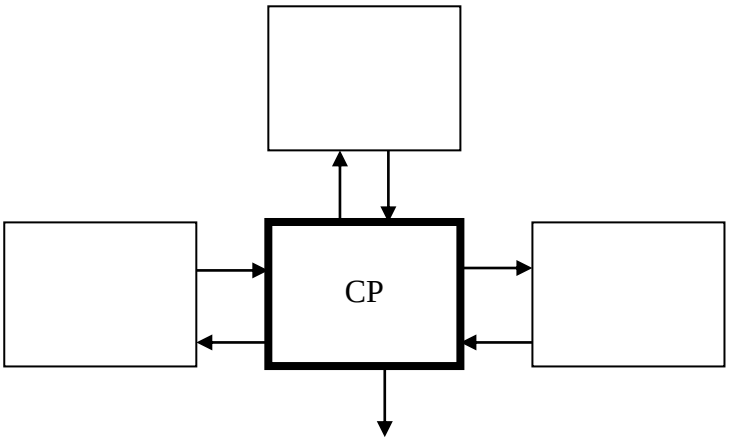
The two main types of linear kinetic design are compartmental models (or multi-compartmental) and non-compartmental (black box) models [1,3,13,17]. The models are *stationary* when it can be assumed that the parameters in the system do not change with time. With mathematical equations and computer programmes to fit the experimental data, highly sophisticated models can be built. This survey will not address all the different models, their qualifications, limitations, advantages, and defects in detail. Instead, the most important basic concepts will be examined with special reference to a series of biological problems. The survey also covers some clinically relevant examples. It is not the intention to go into details of the mathematical aspects, but the widespread use of exponential methods for both compartmental and non-compartmental problems justifies the application of some formulas. In practice exponential fitting is not difficult and utilisation of standard computer programmes has made this and other methods very popular. These procedures should often solely be reserved as practical tools for the description of curves and not necessarily to confirm some kinetic model or another [2,3].

2.1 Compartment Models

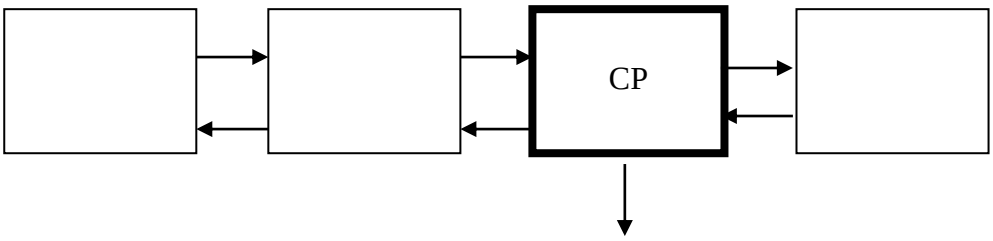
The principal behind compartment modelling is to describe a system by a certain number of rooms (pools), which are interconnected through exchange of substance. This approach requires a simplification of the processes studied. But the model chosen must be directly analogous to the particular biological system to be studied. A lack of or insufficient knowledge of the physiology of the system can mean that a wrong model is chosen (e.g. wrong number of compartments), which obviously can lead to larger or smaller fallacies[2,3,5].

One compartment (or pool) defines an area where a substance is homogenously distributed and in equilibrium, which means that the concentration of the considered substance is identical throughout the entire compartment at any time. This need not be one physical area – one physical area can consist of more compartments, where each describes a condition, e.g. two different chemical compounds of the tracer considered.

In a multi-compartmental model two or more compartments are interconnected, so that there is an exchange of substance between some or all of these compartments (see Figure 2-1). Exchange of substance can be indicators or mother-substances, which cross physiological barriers (e.g. membrane transport or blood transport) or a chemical transformation (e.g. metabolism).



Mammillary system



Catenary system

Figure 2-1: Kinetic compartmental models. The mammillary system consists of a central compartment (central pool, CP), connected to peripheral compartments, which are not connected to each other. The catenary system consists of compartments in a series.

There are two main types of compartmental models: *mammillary* consisting of a central compartment, which is connected to peripheral compartments, and a *catenary model*, where all compartments are arranged in a series with each compartment connected to its neighbour. A compartment is *open* if it is connected to the surroundings and *closed* if it is not. Compartment models of biological systems usually have one open compartment.

The mathematical aspects of compartment models normally involve writing up mass balances for certain kinetic structures, followed by quantification of the model from experimental data. The application of a physiological system involves some assumptions of which two important ones are

linearity and *stationarity*. *Linearity* or the principal of superposition means that the response from several stimuli is the sum of the separate stimulus responses. *Stationarity* means that all system parameters are constant over time. Stationarity implies that the same stimuli to the system produce the same response at all times.
The rate constant or the transport constant (k) is the fraction of the content in a compartment, which is transported to another compartment per unit of time. The distribution volume (V_D) is the equivalent volume in which a substance would be distributed with a reference concentration (often the plasma concentration), but it is far from always a true biological volume.

The substance in a compartment is per definition homogenously distributed which means that a compartment model can be a good approximation if the mixing speed inside each compartment is fast compared to transport between the compartments. If this is not true the compartment model is a poor approximation. Compartments with concentrations, which are always in a steady state relationship, can be impossible to separate. A common and practical approximation is to combine them into a single compartment (”lumped pools”), on account of the further analysis, but it cannot always be assumed that lumped pools have physiological meaning. Hence, compartmental structures may be seriously restricted by the available data, but if the model is built sensibly and interpreted with care it need not be a practical restriction.

Multi-exponential solutions to multi-compartmental models involve determination of the values of the time constants and distribution volume, which in some way best suits the experimental data. This is typically done by minimising the calculated error between data and the model function (least squares method or similar). By influencing the system with a known indicator the parameters of the model can be derived with the help of the measured response (an input-output or a residual measurement experiment). Some time constants or compartments may occassionaly be determined from independent experiments; for instance, by going through subsystems, other test materials, or from measurements on an analogous system.

One example of a resolution of a multi-compartmental model is set out below. Figure 2-2 illustrates a bicompartmental model. The rate constants k_1 and k_2 [min^{-1}] describe the fraction of the current substance, which is transferred from compartment 1 to compartment 2, per time unit, and vice versa. The rate constant k_3 describes all irreversible loss and metabolic degradation of the substance. In the present model this takes place from compartment 2. Q_0 is the amount of test substance present in compartments 1 and 2 at time 0 (i.e. $Q_1(0)=Q_0$ *and* $Q_2(0)=0$).

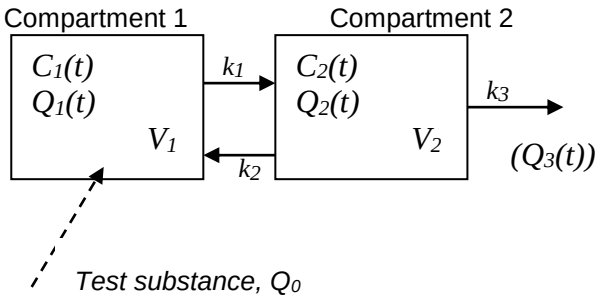


Figure 2-2: A two-compartment model with a central pool (compartment 1) and a "lumped" peripheral pool (compartment 2). Q_i, C_i and V_i are the masses, concentration and volume, respectively. k_i is the time constant.

Assuming linearity and stationarity, the following differential equations based on the principle of mass preservation can be written down.[3]

$$dQ_1 = Q_2 k_2 dt - Q_1 k_1 dt$$
$$dQ_2 = Q_1 k_1 dt - Q_2 (k_2 + k_3) dt$$

where dQ_1 is the change in indicator in compartment 1 and $k_2 dt$ is the fraction of indicator, which goes from compartment 2 to 1 in the course of a small time interval *dt.*

The above can be rewritten to the following system of differential equations (see appendix 1):

$$\frac{dQ_1}{dt} = -k_1 Q_1 + k_2 Q_2$$
$$\frac{dQ_2}{dt} = k_1 Q_1 - (k_2 + k_3) Q_2$$

The concentration in compartment 1 is $C_1 = Q_1/V_1$ and similarly the concentration in compartment 2 is $C_2 = Q_2/V_2$, where V_1 *and* V_2 are volumes of compartment 1 (often the volume of plasma) and compartment 2, respectively. The physical variables and constants, Q_1, Q_2, k_1, k_2, k_3, V_1 and V_2, are uniquely identifiable from concentration measurements in compartment 1. The solution to the differential equations is for Q_1 a biexponential function as described below.

In a system with *n* compartments the concentration-time curve from the first compartment can be described with a sum of exponential functions:

$$C_1(t) = A_1 e^{-b_1 t} + A_2 e^{-b_2 t} + \ldots + A_n e^{-b_n t}$$

or

$$C_1(t) = \sum_{i=1}^{n} A_i e^{-b_i t}$$

A range of connections between model parameters and some fundamental kinetic and mathematical terms are summed up in box 1.

In the case of multi-exponential functions of a higher order than two the solutions become much more complex. If, for example, irreversible disappearance also occurs from compartment 1 (besides that which disappears from compartment 2) a fourth time constant (k_4) must be included in the differential equations. Not all system parameters are then uniquely identifiable from the concentration–time relation in compartment 1 alone. Other details (e.g. collected disappearance from the organism, size of the distribution volume, concentration–time relation in compartment 2, etc.) must be available in order to determine the parameters of the system.

The number of exponential terms *(n)*, which can be fitted to a set of data exactly and meaningfully,

$$C(t) = A_1 e^{b_1 t} + A_2 e^{b_2 t} + \ldots + A_n e^{b_n t},$$

is one of the most limiting factors when determining the order of the compartmental model (*n*, i.e. the number of compartments) especially when only one compartment is available for measurements. The reasons for this are both mathematical and statistical. The value *n* is rarely bigger than 3 or 4 for single input-output models. This means that system compartments, which cannot be measured directly are often described as the joining of compartments with more or less identical kinetic characteristics (e.g. proportional concentrations) in relation to the compartments measured.

If $C_1 = A_1 e^{-b_1 t} + A_2 e^{-b_2 t} + \cdots + A_n e^{-b_n t} = \sum_{i=1}^{n} A_i e^{-b_i t}$ is decreasing, the b_n-values are positive and the following equations can be derived:

$$C_1(0) = \sum_{i=1}^{n} A_i$$

Area under the concentration curve: $\int_0^{\infty} C_1(t)dt = \sum_{i=1}^{n} A_i / b_i$

The slope of the curve at the time t: $\frac{dC_1}{dt} = -\sum_{i=1}^{n} A_i b_i e^{-b_i t}$

Initial slope of curve: $\left.\frac{dC_1}{dt}\right|_{t=0} = -\sum_{i=1}^{n} A_i b_i$

Mean transit time measured at residual detection:

$\bar{t} = \frac{\int_0^{\infty} C_1(t)dt}{C_1(0)} = \frac{\sum_{i=1}^{n} A_i / b_i}{\sum_{i=1}^{n} A_i}$, look at later section??.

Mean transit time measured at outflow-detection

$\bar{t} = \frac{\sum_{i=1}^{n} A_i / b_i^2}{\sum_{i=1}^{n} A_i / b_i}$, look at later section??.

If the system is two-compartmental and $C_1(0)/Q = A_1 + A_2$ is the fractional concentration of indicator (e.g. µmol/l/µmol = l^{-1}) at time 0, then the differential equations have the following solutions based on a biexponential function:

$$V_1 = \frac{Q}{C_1(0)} = \frac{1}{A_1 + A_2}$$

$$V_2 = \frac{V_1^2 (A_1 + A_2)(A_1 b_1 + A_2 b_2)^2}{A_1 A_2 (b_1 - b_2)^2 + (A_1 + A_2)^2 b_1 b_2} = \frac{V k_1}{k_2 + k_3}$$

where

$$k_1 = V_1 \ (A_1 b_1 + A_2 b_2)$$

$$k_2 = \frac{A_1 A_2 (b_1 - b_2)^2}{k_1}$$

$$k_3 = \frac{b_1 b_2}{k_1}$$

The volumes V_1 and $V = V_1 + V_2$ and the time constants k_1, k_2, and k_3 thus only depend on A_1, A_2, b_1, and b_2 and these can be determined by fitting the concentration curve from compartment 1 to the parameters in the biexponential function, either manually with the help of logarithm paper or automatically with a computer programme[3].

2.1.1 Example of a compartment model – uptake of ^{99m}Tc-pertechnetate in the thyroid gland

Pertechnetate is, like iodie, absorbed in the thyroid gland, but does not participate in the formation of thyroid hormones.

The kinetics can be described as a three-compartment model (model and data are from Hays MT 1979[140]) consisting of plasma and two compartments in the thyroid, which is assumed to represent the follicular cells and a colloidal phase, respectively.

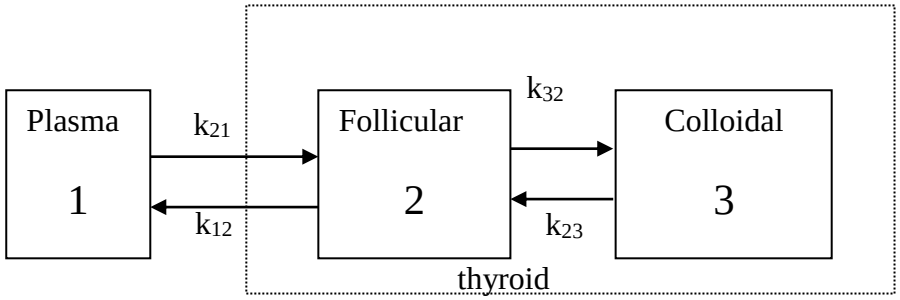


Figure 2-3: Compartment model of pertechnetate absorption in the thyroid gland.

Serial plasma tests are measured in a gamma counter and counts above the thyroid gland are measured with a gamma camera or single detector, either as a dynamic uptake or as a series of static uptakes over a period of time. In order to get comparable measured values, a standard is counted in the gamma counter and on the gamma camera/single detector, which is a test with the same radioactive concentration as the one injected into the subject.

One can set up a mathematical expression for the compartment model, similar to the one shown above, but in practice numerical methods are more usual, they are comparatively simpler to use on a computer. Some programmes allow the compartment model to be drawn direct, as in Figure 2-1 (e.g. SAAM II, a commercial programme – see http://depts.washington.edu/saam2/), others describe the differential equations for the system. Like a range of other programmes, SAAM II simulate kinetic systems, which can be emloyed when the behaviour of a system has to be investigated with different inputs and model descriptions. JSIM (can be downloaded free of charge from http://nsr.bioeng.washington.edu/PLN/Software) is a programme able to simulate very complex systems. The relatively simple model for thyroid is shown below in Figure 2-4. When the simulation

is run it allows visualization of the time activity curves for the individual compartments or, as shown in Figure 2-5, the collected concentration in the thyroid gland.

```
math Thyroid {
            // define the time axis:
            realDomain t;
            t.min=0; t.max=10; t.delta=0.1;

            // define input and concentrations in the compartments:
            extern real Cin(t);
            real p(t), f(t), k(t), thyr(t);

            // transport constants in ml/min:
            real k12=4.1, k21=0.28, k23=0.0070, k32=0.28;

            // define initial conditions:
            when (t=t.min) p=Cin;
            when (t=t.min) f=0;
            when (t=t.min) k=0;

            // differential equations:
            p:t = k12*f-k21*p+Cin;
            f:t = k21*p+k32*f-(k23+k12)*k;
            k:t=k23*f-k32*k;
            //thyroid= follicular colloidal:
            thyr=f+k;
}
```

Figure 2-4: Compartment model of the pertechnetate uptake in the thyroid gland. Described in the JSIM format.

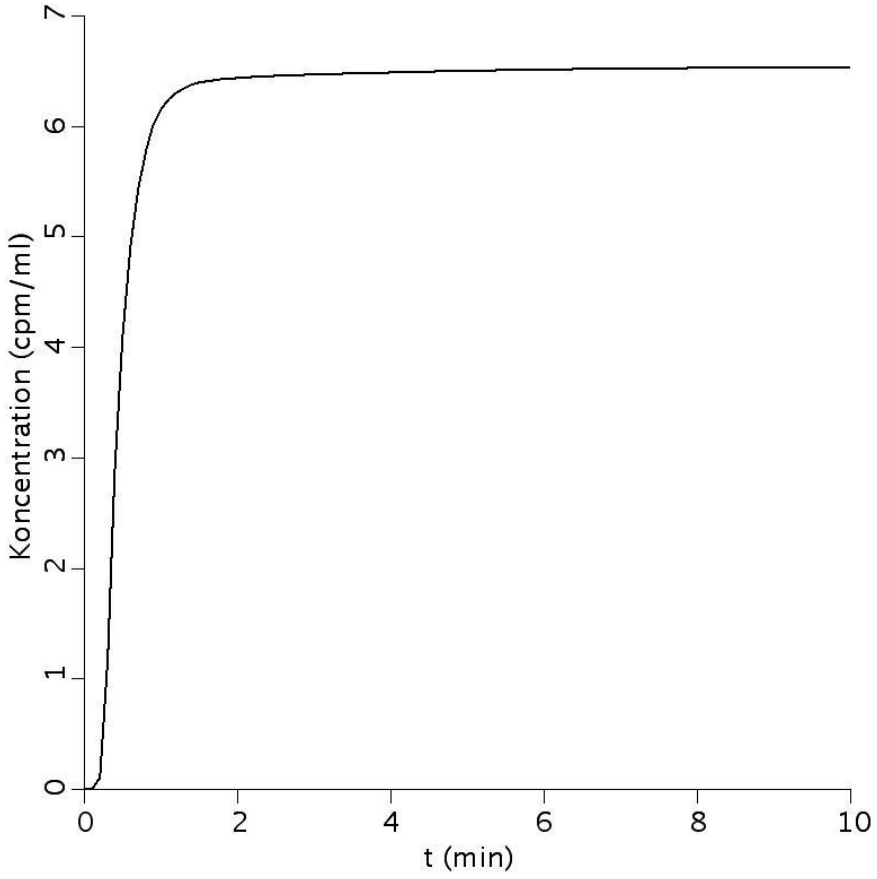


Figure 2-5: Simulation of the compartment model; graph of the concentration of pertechnetate in thyroid tissue after an intravenous bolus injection.

2.2 Non-compartmental models

The basic non-compartmental model ("black box") is illustrated in Figure 2-6. The most common model consists of a central pool connected with a certain number of peripheral pools. The inflow arrow represents the sum of all *de novo* "inflow" of substances into the central pool. Similarly, the outflow arrow represents the sum of irreversible removal of substance from the central pool, i.e. metabolism, elimination, degradation, loss to the surroundings, etc., from the system. This means that systems with a definitive structure are also fundamental for non-compartmental models[1,13]. Non-compartmental analysis is *not* independent of a model, as it sometimes wrongly has been suggested in the literature. Inflow, outflow, "sinks", recirculation, sampling, etc., are all explicitly connected to a (central) pool. The flow to the central pool is equivalent to inflows, which reflect all inflows that reach the central pool directly or indirectly: e.g. hormones generated from initial stages in peripheral tissue (indirect sources). "Sinks" can also appear at other places in the system, e.g. hormonal degradation in the tissues (indirect sinks).

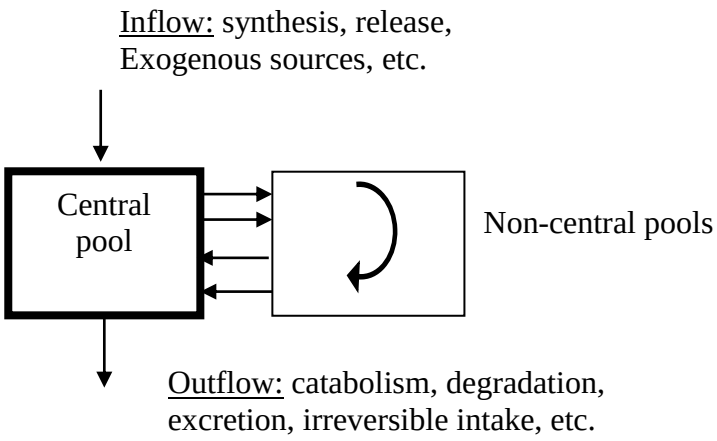


Figure 2-6: Non-compartmental ("black box") model with a central pool, inflow, outflow, and recirculation into and from non-central pools.

The greatest conceptual advantage of a non-compartmental model is that an arbitrary amount of recirculation or exchanges can occur with an arbitrary amount of non-central pools none of which need necessarily to be identified with known physiological structures. Conversely the multi-compartmental models demand more specific structures. Moreover, kinetic data can often be mathematically analysed with integral equations to a higher extent than with differential equations, which, in some settings, can be an advantage.

Non-compartmental analysis is usually employed to estimate whole body variables in steady state, plasma clearance (*Cl*), distribution space (V_D), total body-pool size, whole body mean transit time ($\bar{t}$), fractional clearance rates (*FCR*), metabolic clearance rate (*MCR*), (see the following section about whole body degradation). For determination of the above kinetic variables in non-compartmental analysis, the substance to be studied must be eliminated from the central pool into which the indicator is introduced and measured (e.g. intravenous injection and blood sampling). This means that no or relatively little disappearance can occur in or from non-central (e.g. extra vascular) pools. This condition may be difficult to meet for a range of physiological, interesting substances, which is metabolised intracellularly (e.g. hormones and neurotransmitters). In practice, the condition can be approximately met for example in those coincidences where the transcapillary exchange speed is fast compared to the collected distribution times. The degree of approximation depends both on the quantitative and on the structural relations in the system. Another condition relating to non-compartmental models is that all transport routes for the substance studied must go through the central pool, i.e. the same pool as the one where the indicator is introduced.

In short, non-compartmental models may provide a precise determination of kinetic variables for systems where all inflows and “sinks” are directly connected with (and only with) the central pool, where the measurements are made. However, plasma clearance can also be measured exactly, when non-central “sinks” are present, if only one “source” leads directly to the central pool. If these conditions are not forthcoming *Cl*, *MCR*, V_D, and the total pool size will often be underestimated and the *FCR* will be overestimated in the non-compartmental analysis.

3 Basic kinetic methods and concepts

In the 1870s Adolph Fick put forth his two diffusion laws. *Fick's first law* describes the relation between concentration, diffusive transport of substance, and lapse of time at diffusion, i.e. transport conditioned by concentration differences[10]. *Fick's second law* also involves the direction of the transport (spatial spread), besides the concentration, transport, and time. *Fick's principle,* on the other hand, does not concern diffusion, but describes the relation between convective and non-convective transport of substance from the principle of mass preservation.

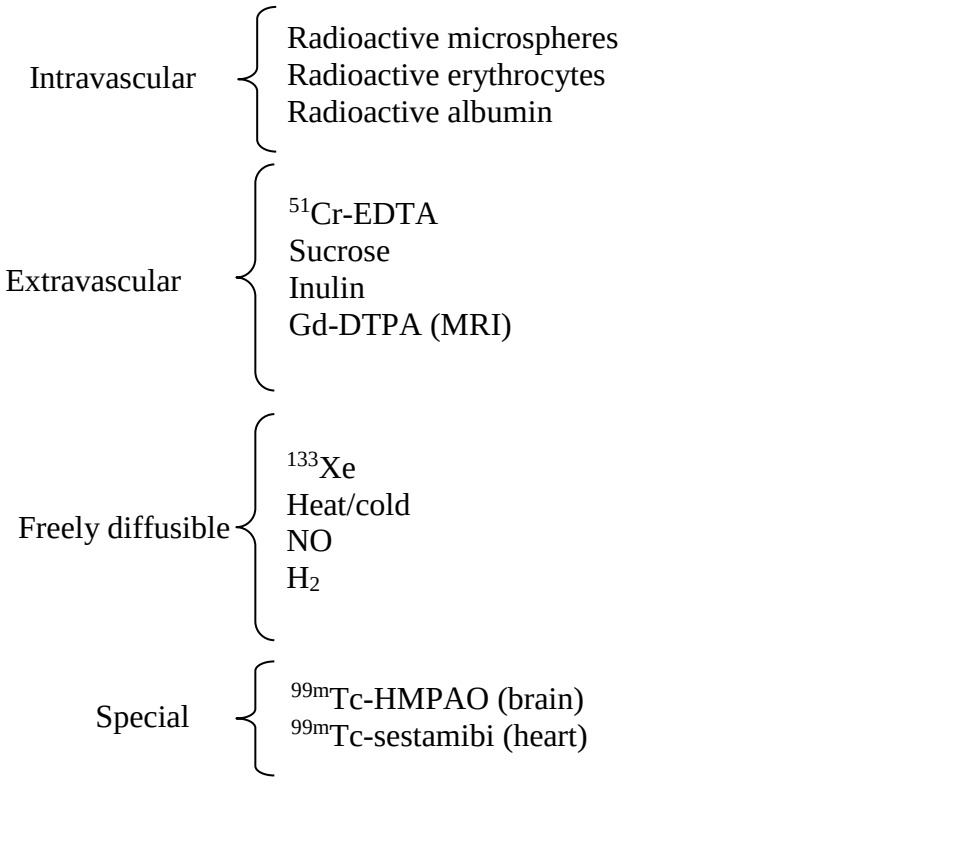
In the 1890s Ernest Starling described the filtrative transport over the capillary membrane with lymph development conditioned by the balance between hydrostatic and colloid osmotic (oncotic) pressure. Around 1915 August Krogh discovered the recruitment of capillaries as he found largely unchanged capillary mean transit time in spite of massive flow increase in skeletal musculature during work, – finding that was rewarded with the Nobel Prize in Physiology or Medicine. Prior to this George N. Stuart had described the principle of constant infusion and succeeding concentration measurement for flow determination. Valdemar Henriques described indicator dilution for measurement of blood flow in 1913 and later Hamilton reported plasma volume measurement. In the 1920s and 1930s Poul Brandt Rehberg, Eggert Møller, Donald Van Slyke, and Homer Smith [98, 99] introduced the clearance concept especially with relation to measurement of kidney function.
Subsequently general use of the clearance concept was increasingly applied. In the 1940s and '50s Poul W. Kruhøffer published kinetic studies on the spaces of the organism, and Stanley Bradley performed transit time determination by single injection and inflow/outflow monitoring, and thus could estimate the size of the splanchnic blood volume. At the same time Kety and Schmidt[100] described transit time measurement and measurement of brain perfusion by applying diffusible inert gases and inflow/outflow monitoring. In the 1950s and '60s Niels A. Lassen and David Ingvar described residual detection by applying inert diffusible radioactive gasses (^{133} Xe, ^{85}Kr) for flow measurement, especially in the brain[129]. Bertil Nosslin[90] and Reeve and Bailey[89] described the distribution kinetics of albumin from both multi-compartmental and black box models. Kenneth L. Zierler introduced the height over area method in 1965. In the 1960s Per Sejersen described extraction estimation after a single injection and residual detection[10, 100]. Eugen M. Renkin, Christian Crone, Francis Chinard, and Carl Goresky developed models to describe substance exchange between capillaries and the surrounding tissue from assumptions of flow, inflow concentration, outflow concentration, and the insertion of a transcapillary transport limitation[10]. These are functions of both the capillary condition and the size of the capillary wall surface area (the so-called permeability-

surface area product, PS). Computer based models were developed in the 1970s and ‘80s, where partly iteration and partly differential equations with different initial situations were studied. This led to the development of a range of “chaotic” models, which in some settings demonstrated linearity and in others “deterministic chaos”. From the 1990s kinetics was characterised by a combination of complex models and the use of major computer power[122-125], where the possibility of forming functional pictures and “molecular image formation” was exploited.[119-132]

3.1 Flow, flux, and perfusion – indicator dilution method

Flux (*J*), which is measured in units of amount of substance per time unit (e.g. mmol/min), is a measure for particle transport velocity, i.e. number of particles, which pass a real or imagined surface in a short time interval.
Flow (F) is similarly volume transport per time unit (e.g. ml/min). The *perfusion* (actually the *perfusions coefficient*), *f,* of a tissue area is defined as F/W, where F is the flow through the tissue and W is the weight of the tissue, and it is often stated in ml/min per 100g of tissue.

From the principle of mass preservation one can derive the following connection:
Flux [mmol/min] = flow [ml/min] · concentration [mmol/ml],
which will be exploited in the following sections.
In box 2 an example of flow indicators is shown.



Box 1. Examples of flow indicators

3.1.1 Measuring the time-activity curves

In principle there are three ways (direct or indirect) of measuring the time elapse for an indicator concentration in tissue: outflow detection, cumulated outflow detection and residual detection. For *outflow detection* the concentration is measured as a function of time at the outflow place from an organ or tissue area, e.g. when collecting blood samples with appropriate time intervals, perhaps with an automatic sampler. *Cumulated outflow detection* is a measurement at one spot where there is inflow of indicator but where there is no outflow, and the concentration is measured rapidly as a function of time. *Residual detection* is measured as residue (rest amount) in an organ as a function of time typically with a gamma camera or another detector placed over the organ.
Residual detection can also be done by measuring the difference between inflow and outflow detection, which is exploited in the determination of perfusion. An example of an experimental setup is shown in Figure 3-7.

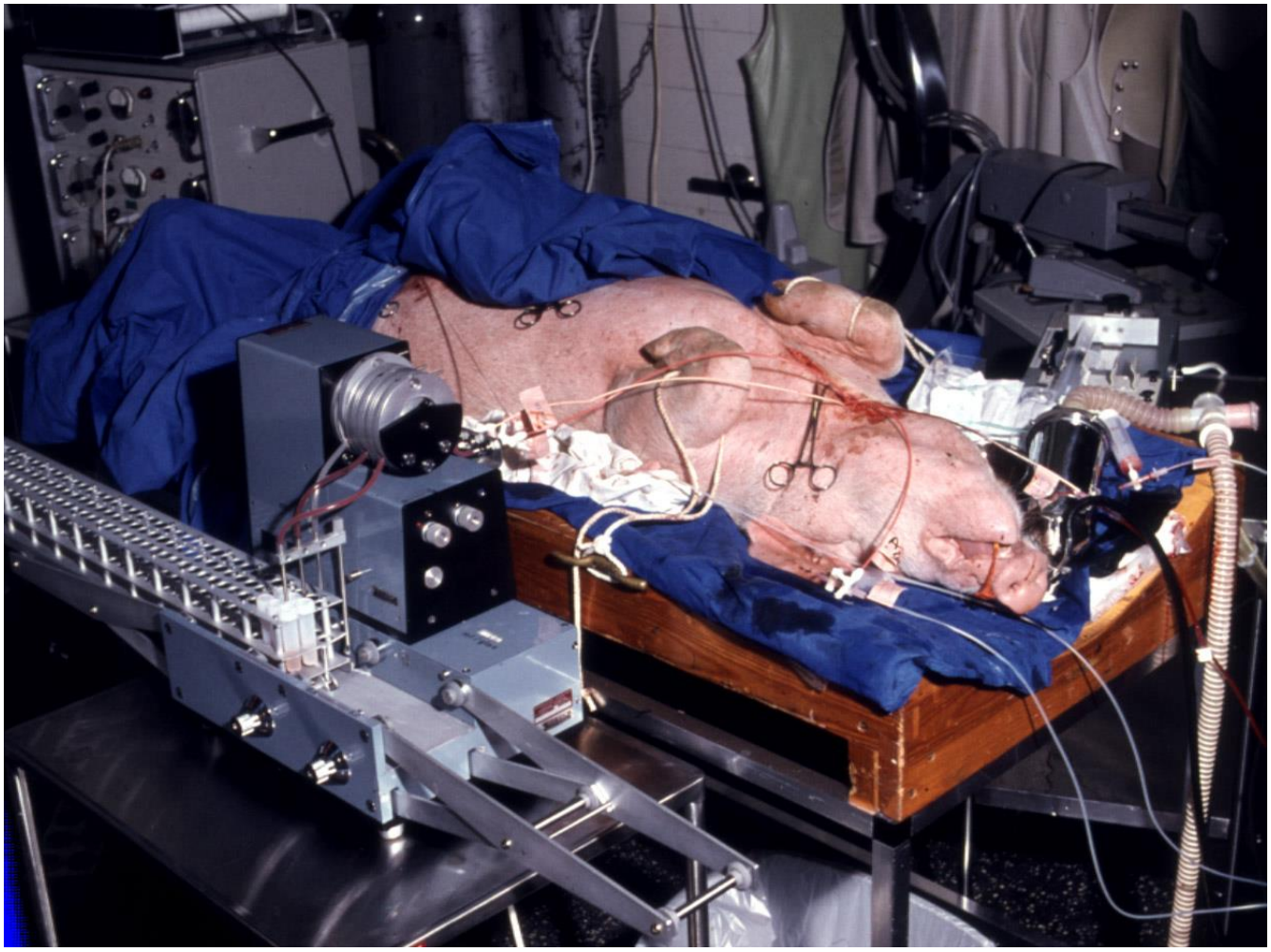


Figure 3-7: Measurement of the time-activity curves in a multi-catheterised pig.

3.1.2 Determination of cardiac output

Cardiac output can be measured by different methods, all of which involve kinetic principles and a range of assumptions. Classically, it was determined by Fick's principle (see the section on "Convective flow, non-convective flux – Fick's principle"), later by an indicator dilution method, subsequently with impedance measurement, and most recently by pulse pressure measurement and compliance estimation. In addition, stroke volume (and thus the cardiac output) can be determined by isotope cardiography, MR techniques, and echocardiography.

In *Fick's principle* the speed of oxygen uptake in the lungs and the concentration of oxygen are measured in mixed venous blood (*a. pulmonalis*) and blood from the arterial system, see Figure 3-2. With this method, measurement takes place over a sufficiently long period to obtain representative and connected values for oxygen uptake, mixed venous oxygen concentration, and arterial oxygen concentration. The cardiac output measured, is that of the right heart. It is therefore a requirement that no significant shunts are present and that the contribution from bronchial artery/veins and myocardium directly to the left atrium is minimal, see Figure 3-9. In the presence of shunts these will affect the size of the blood flow measured. By taking elective blood samples from different locations in the circulation the size of the shunt can be measured. Fick's principle is described in greater detail in section 3.1.5.

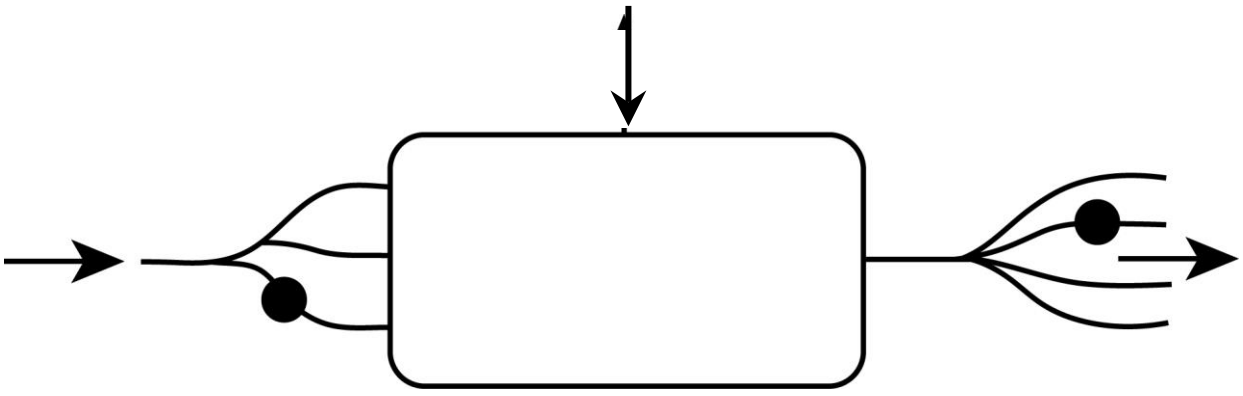


Figure 3-8: Fick's principle, where convective flow and non-convective flux are combined in the principle of mass preservation. The two sampling locations illustrate well-mixed inflows and well-mixed outflows, where inflow concentration (C_i) and outflow concentration (C_o) can be measured.

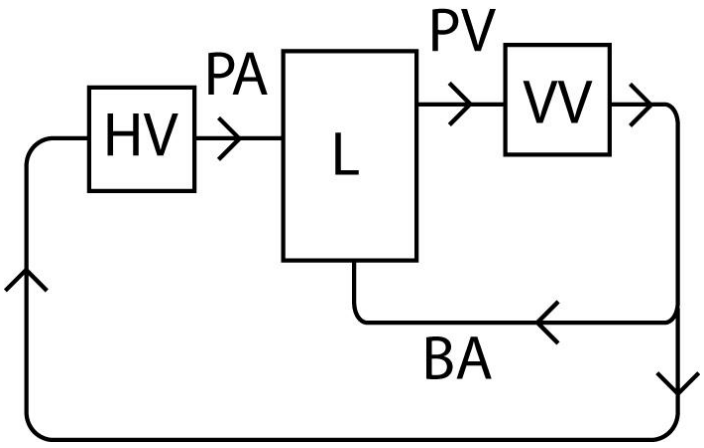


Figure 3-9: Determination of cardiac output by Fick's principle. It is seen that the bronchial arterial circuit (BA) disturbs the principle of mass preservation. At low flow this has no quantitative importance. (HV: right ventricle; VV: left ventricle; PA: pulmonal artery; PV: pulmonary vein, L: lung.)

In the *indicator dilution method* an intravascular tracer (marked albumin, protein-bound dye, marked erythrocytes) is injected as a bolus into the right atrium and serial blood samples are taken from the arterial system. The method is most often called the Stuart-Hamilton method, but should more correctly be known as the Henriques-Hamilton method, since Stuart used constant infusion. A requirement for cardiac output determination by this method is that a complete mixture occurs in at least one vascular section from the site of injection to the reception area, generally the left ventricle. The injection can thus be given in both the right atrium and *a. pulmonalis*. It is not important which arterial vessel is used for the blood sampling. After some time, the indicator will recirculate and it is essential that it is corrected for. This can (as mentioned elsewhere) be done by exponential extrapolation or fitting to a suitable curve, e.g. a gamma variate function, see Figure 3-10 and Figure 3-11. With the usual correction for recirculation, long transit times, especially in cardiac insufficiency, may be somewhat underestimated, for which reason the method can give slightly (a few per cent) increased values for the cardiac output. A requirement for the tracer to be distributed purely intravascularly is not crucial since tracer extravasation to, for instance, the pulmonary interstitium will give correct values if the tracer is freely diffusible and also diffuse backwards (e.g. pertechnetate, alcohol, ^{51}Cr-EDTA and $^{3}H_2O$). However, transit time with a pulmonary extravasation of tracer will be prolonged and cause potentially bigger problems with correction for recirculation. It is, therefore, advisable to use an intravascular tracer. The pulsating flow in the left ventricle is not critical, because more than ten sinus-like oscillations (i.e. equal negative and positive oscillations) occur during the measurement of cardiac output. The indicator method is described in more detail in section 3.1.4.

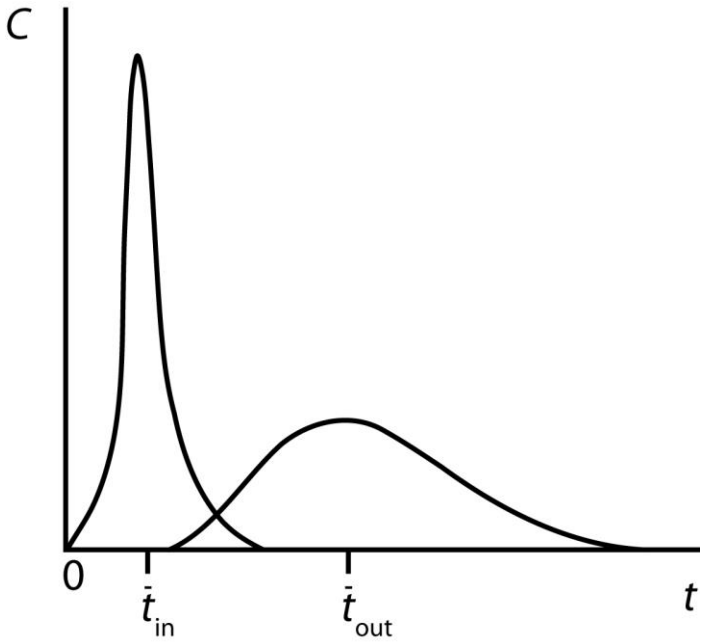


Figure 3-10: The shape of indicator dilution curves at inflow and outflow after injection of an intravascular tracer. The area under the curve is the same. (With a bolus injection the mean transit time of the bolus itself will be very short and in some cases a standard correction can be used (e.g. 1.1 second), so that the outflow curve can be taken as an approximation of the transfer function (*h(t)*). The mean transit time of the system will thus be $\bar{t} = \bar{t}_{out} - \bar{t}_{in}$, see below).

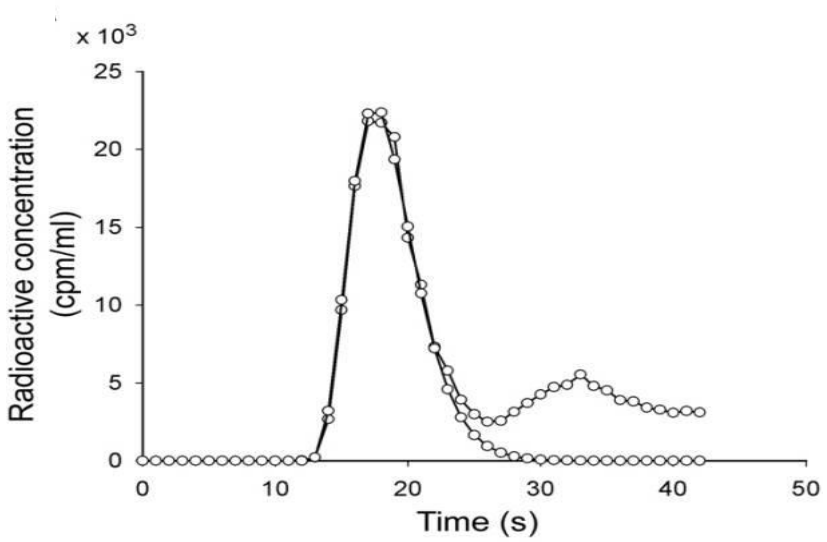


Figure 3-11: Indicator curves: Measured values, gamma variate function, fitted to the measured values, and with the recirculation correction (from Henriksen et. al. 2005[118]).

Coldness has been used as an indicator. This eliminates the recirculation problem but brings about different caloric calibrations, and besides catheter constants most thermodilution equipment will depend on a standard extrapolation of the down-slope, which is often carried out at 20% of the maximum peak. As with other indicator dilution techniques, this raises the risk of underestimating slow transit times and thus introduces certain errors in the estimation of low cardiac outputs. There is only a small loss of coldness in the lungs.

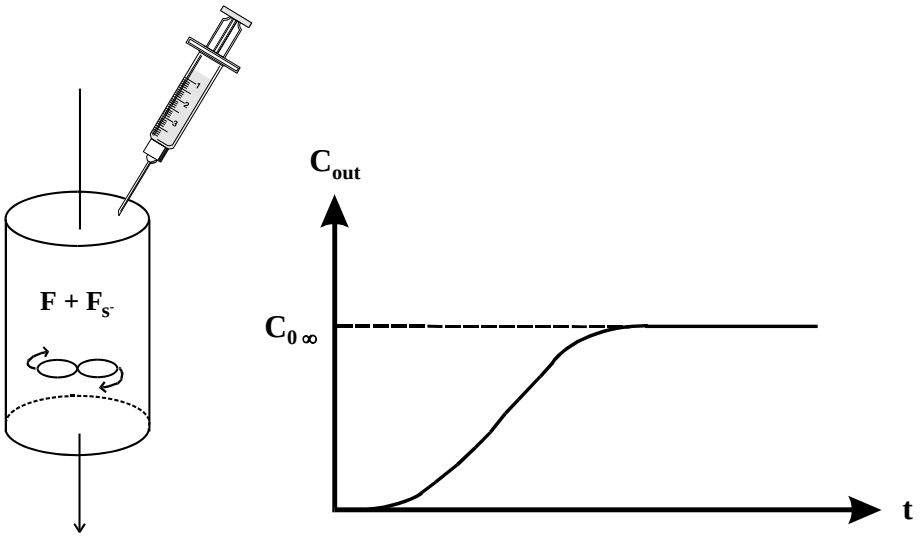
The impedance of the organism (resistance to alternating current passage through the thorax) depends upon the amount of blood standing in the heart cavities. During the systole, a volume corresponding to the stroke volume will be displaced and it has been found that the collected volume in larger arteries and veins does not change appreciably. This is why the stroke volume measurement multiplied by the heart frequency will give an approximate value for the cardiac output. The use of a standard calibration produces surprisingly correct values for the cardiac output in quite a large fraction of healthy subjects, as well as for patients with various diseases. However, individual subjects are quite often also seen with considerable over or underestimation of the cardiac output. For these reasons impedance cardiography is most often used to register *changes* in cardiac output for which the method gives acceptable values in all subjects.

With a measurement of arterial compliance (relation between volume change and pressure change) combined with a measurement of the shape of the pulse pressure (for instance with finger plethysmography) the minute volume can, in principle, be determined as curve compliance multiplied by pulse pressure. There are a number of pitfalls here, which fall outside the scope of these notes.

Crdiac output can also be determined by blood pool-marking and gamma-camera examination of the heart, MR scanning, and echocardiography. These methods include a range of assumptions and limitations, which are described in various textbooks.

3.1.3 Constant infusion – Stewart's principle

In order to estimate flow in an organ one can infuse a tracer at constant (and known) speed:



$$J_{in} = F_{st} \cdot C_{st}$$

Figure 3-12: Constant infusion.

$$J_{out} = (F + F_{st}) \cdot C_{o\infty}$$

where F_{st} is the flow of indicator and C_{st} is its concentration. $C_{o\infty}$ is the stationary outflow concentration of indicator after ("infinity" long time), i.e. at total mixing but before eventual recirculation, see Figure 3-12. A requirement is that the indicator obtains complete mixing responding to at least one "cross section" between inflow and outflow.

After some time there will be mass balance which means that the flux into the system is equal to the flux out of the system:

$$J_{in} = J_{out} \Rightarrow F_{st} \cdot C_{st} = (F + F_{st}) \cdot C_{o\infty}$$

If F_{st} can be assumed to be much smaller than F, then F can be approximated as:

$$F \approx F_{st} \cdot \frac{C_{st}}{C_{o\infty}} = \frac{J_{in}}{C_{o\infty}}$$

The flux into the system can also be written as:

$$J_{in} = \frac{q}{\Delta t} \Rightarrow F \approx \frac{q}{C_{o\infty} \Delta t}$$

where q is the amount of indicator or dose (activity) and Δt is the time it takes to infuse the indicator (Figure 3-13 illustrates determination of cardiac output by Stewart's principle).

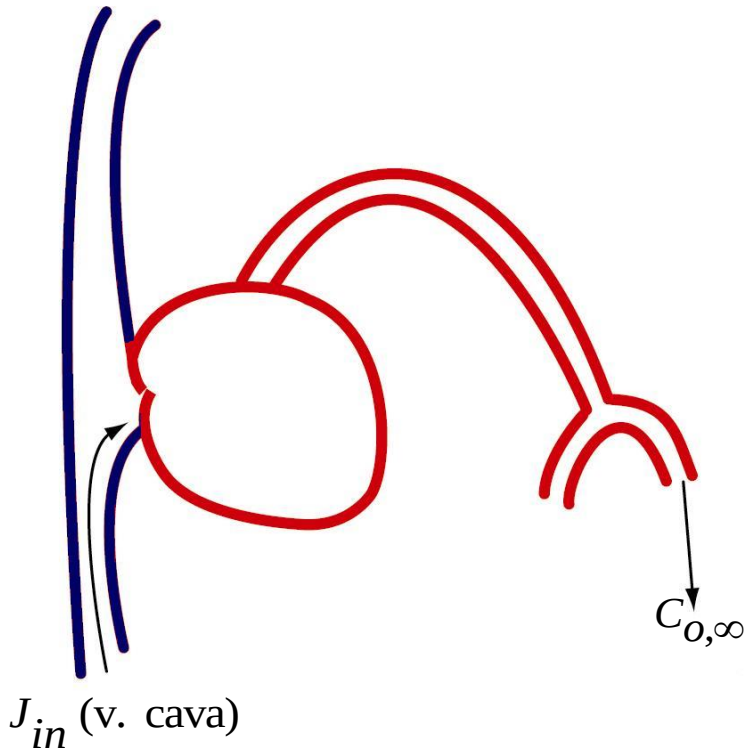
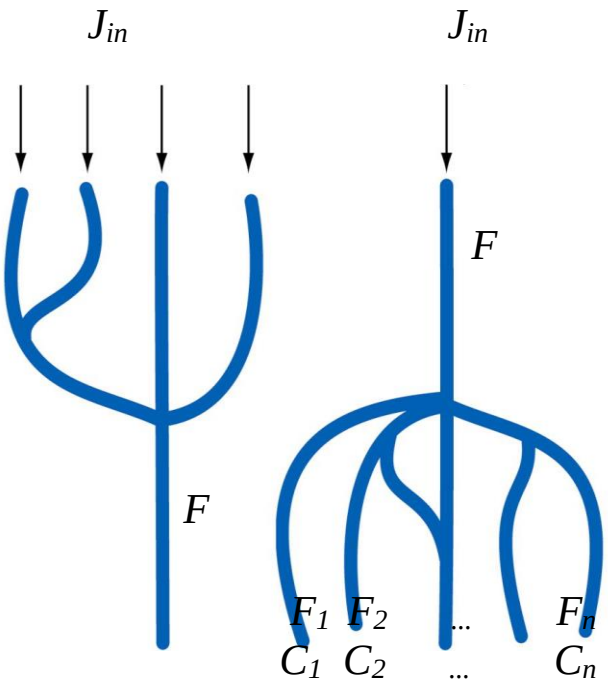


Figure 3-13. Stewart's principle: continuous infusion in vena cava through a catheter, sampling in peripheral artery.



$J_{in}=FC_o$

$$J_{in}=FC_1=FC_2=...=FC_n$$
$$= F_1C_1 + F_2C_2+ F_3C_3+... F_nC_n$$

Figure 3-14. After mixing, the concentration in each branch is similar. The sum of flux in all the branches is J_{in}

3.1.3.1 Constant infusion with recirculation

Since the indicator normally returns to the sampling area after a passage through arteries and veins (except the coincidence where the indicator is excreted, broken down or decayed very fast) one has to consider this recirculation by e.g. measuring the concentration in a vein on the "not-injected side", $C_{noninj}(t)$, which is the concentration the recirculation contributes with (Figure 3-15):

$$J_{out} = (F + F_{st}) \cdot C_o(t) = (F + F_{st}) \cdot C_{o\infty} + (F + F_{st}) \cdot C_{noninj}(t)$$
$$= (F + F_{st}) \cdot (C_{o\infty} + C_{noninj}(t))$$

$$J_{in} = J_{out} - (F + F_{st}) \cdot C_{noninj}(t) \Leftrightarrow$$
$$F_{st} \cdot C_{st} = (F + F_{st}) \cdot C_o(t) - (F + F_{st}) \cdot C_{noninj}(t)$$
$$= (F + F_{st}) \cdot (C_o(t) - C_{noninj}(t))$$

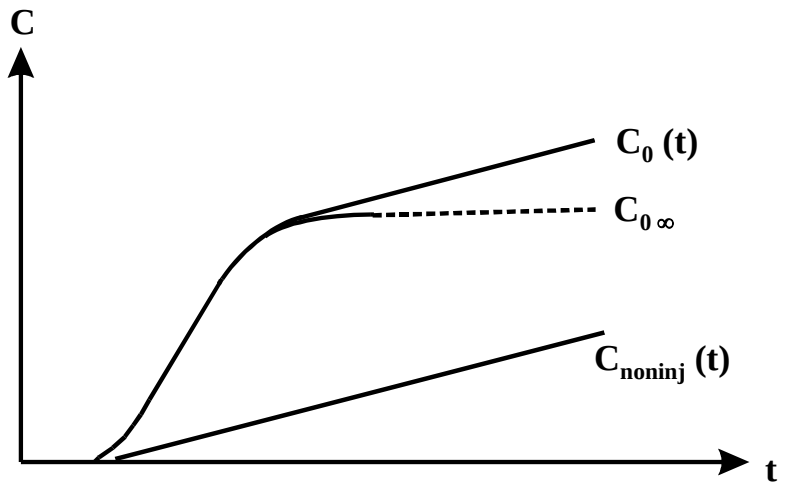


Figure 3-15: Measurement of the concentration on the injected and the opposite ("noninj") side.

One should always consider where there is mixing at inflow and outflow to the actual system, see Figure 3-16.

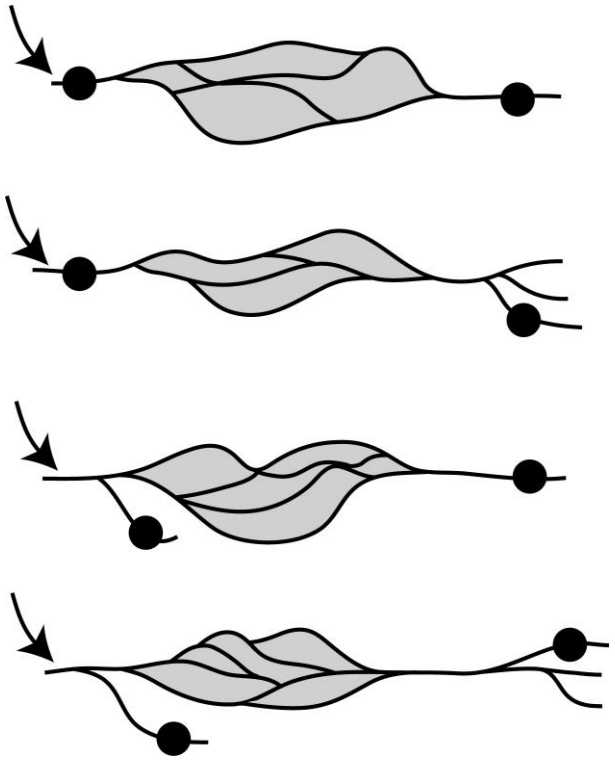


Figure 3-16. Four systems with cross-flow and mixing at those sites, where the sampling is carried out. The figure shows that the inflow concentration in certain cases can also be measured upstream of the cross-flow provided there is no exchange. This will be the case in many places in the arterial system.

3.1.4 Bolus injection - Henriques and Hamilton

The following flow determination by bolus injection, i.e. fast single injection of a known indicator amount describes Q_0.

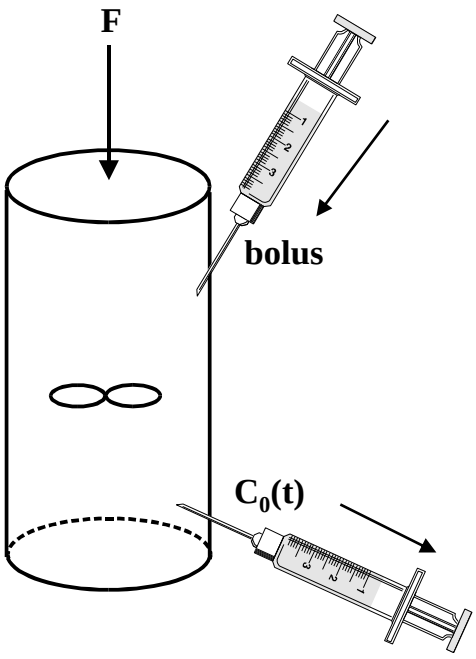


Figure 3-17: Bolus injection and sampling.

Mass balance at outflow detection (i.e. by serial sampling and measuring C_o) can be written as:

$$J_{in} = \frac{dQ(t)}{dt} = FC_o(t)$$

or

$$dQ(t) = F \cdot C_o(t)dt \Rightarrow \quad \text{the integral of dQ(t) is the total indicator amount } Q_0$$

$$Q_o = F\int_o^\infty C_o(t)dt \Leftrightarrow$$

$$F = \frac{Q_o}{\int_o^\infty C_o(t)dt} = \frac{dose}{area}$$

Assuming that the concentration curve, without recirculation, approaches an exponential function with delay τ, recirculation can be corrected for by writing the area as the sum of two integrals:

$$\int_o^\infty C_o(t)dt = \int_o^\tau C_o(t)dt + \int_\tau^\infty C(\tau)e^{-k(t-\tau)}dt =$$

$$\int_o^\tau C_o(t)dt + \frac{C(\tau)}{-k}\left[e^{-k(t-\tau)}\right]_\tau^\infty =$$

$$\int_o^\tau C_o(t)dt + \frac{C(\tau)}{k} \Rightarrow$$

$$F = \frac{Q_o}{\int_o^\tau C_o(t)dt + \frac{C(\tau)}{k}}$$

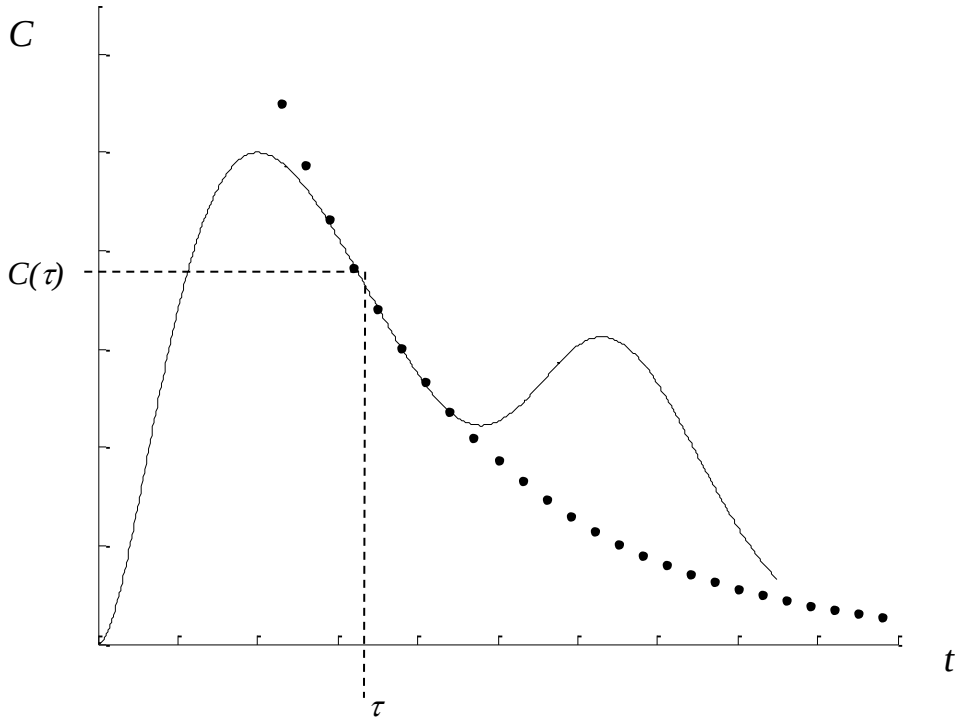


Figure 3-18: Correction for recirculation by exponential fitting.

The constant k and the concentration C (τ) should then be determined from the course of the curve before recirculation. The integral from 0 to τ can be determined by applying numerical methods.

3.1.4.1 The principle of the bolus fraction – Sapirstein's principle

In the following, an injected bolus is assumed to be mixed in the blood of heart and lungs (central mixing) and then ramified in the arteries. The amount of indicator in each "branch", *n*, is distributed according to the flow, F_n, in the branch.

$$Q_n = \frac{F_n}{F} Q_0 \text{, where } \sum_{n=1}^{N} Q_n = Q_0$$

This is called the bolus fraction principle or Sapirstein's principle.

The flow can be written as dose divided by area under the concentration curve, as above:

$$F_n = \frac{Q_n}{\int_o^\infty C_{on}(t)dt} = \frac{\frac{F_n}{F} Q_0}{\int_o^\infty C_{on}(t)dt}$$, where $C_{on}(t)$ is the concentration in branch *n.*

i.e.

$$F = \frac{Q_0}{\int_o^\infty C_{on}(t)dt},$$

It may be concluded that the same flow is determined independently of the branch where the concentration is measured, because the areas under all the time-concentration curves are identical even though the shape, maximum, and insertion of the curve may be very different, see Figure 3-19.

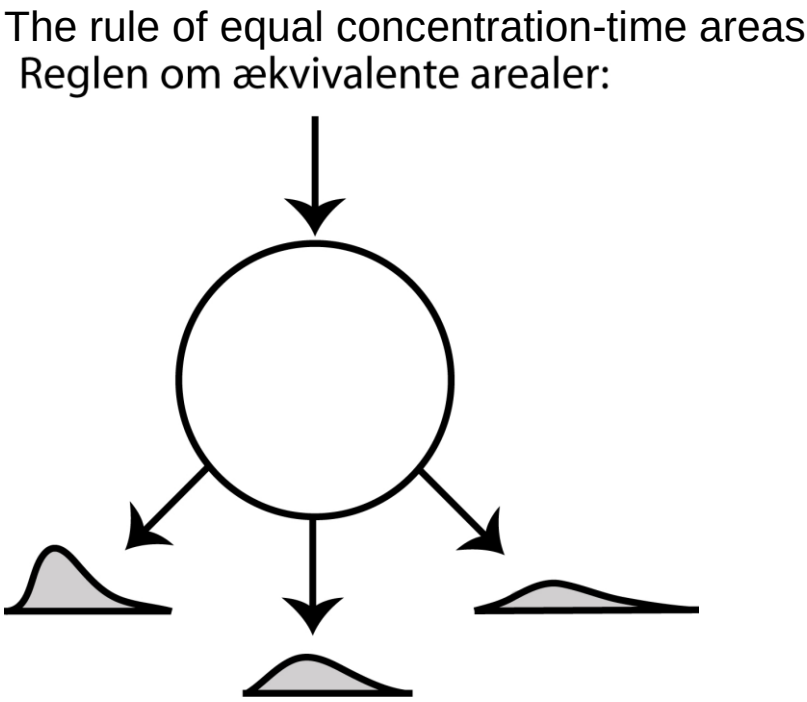


Figure 3-19: Illustration of the rule of the equivalent areas at different sampling sites. The shape and mean transit time of the curves can be different, but the areas are identical. This is, among other things, used when sampling at different places in the arterial system when determining cardiac output after indicator injection.

3.1.5 Convective flow, non-convective flux – Fick's principle

Fick's principle is applied when there is a combination of convective flow (particles which flow with the blood) and non-convective flux (particles which are removed from or added to the blood). As shown in the following, the principle can be used to determine the cardiac output.

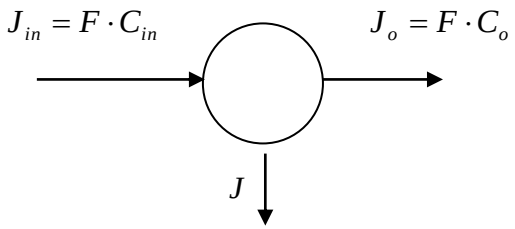


Figure 3-20: Fick's principle. *F* is the convective flow, *J* is the non-convective flux.

Input and output are represented by the suffixes *in* and *o*, respectively. *J* is the non-convective flux. Once again, we apply the principle of mass preservation, see Figure 3-20:

$$J_{in} = J + J_o \Rightarrow$$
$$F \cdot C_{in} = J + F \cdot C_o$$
$$F(C_{in} - C_o) = J$$
$$F = \frac{J}{C_{in} - C_o},$$

or written as an equilibrium equation:

$$F = \frac{J_\infty}{C_{in\infty} - C_{o\infty}}$$

where flux and concentration are included in the steady-state in the latter equation.

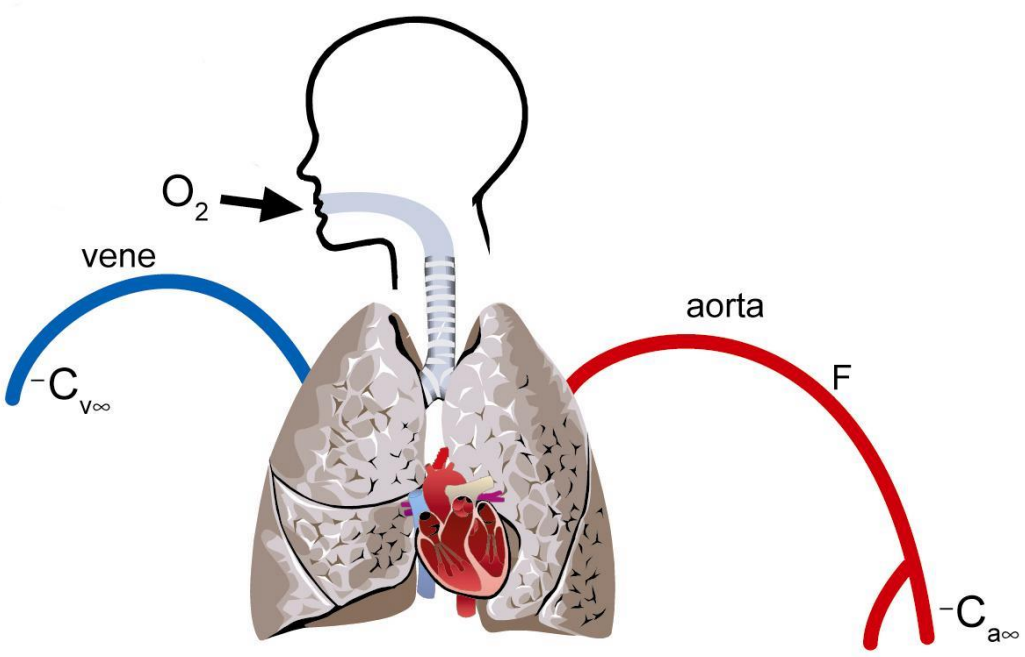


Figure 3-21: Fick's principle used to measure the cardiac output.

By measuring the oxygen uptake in the lungs ($J = J_{O_2}$), the oxygen concentration in arterial blood ($C_o = C_{a\infty}$), and the oxygen concentration in mixed venous blood ($C_{in} = C_{v\infty}$, arteria pulmonalis), the cardiac output (F) can be determined, see Figure 3-21.

$$J_a = J_{O_2} + J_v$$
$$F \cdot C_{a\infty} = J_{O_2} + F \cdot C_{v\infty} \Rightarrow$$
$$F = \frac{J_{O_2}}{C_{a\infty} - C_{v\infty}}$$

3.1.6 Tissue perfusion – Kety-Schmidt's tissue saturation method

In order to determine perfusion in a tissue area, the concentrations on the inflow and outflow sides of the tissue area are measured by a suitable indifferent indicator. The index t (*tissue*) specifies concentrations and doses in tissue and should not be confused with the *t* in parentheses, such as *C(t)*, which indicates that the size is a time-dependent variable (Figure 3-22).

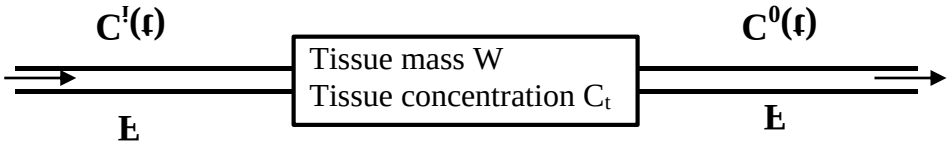


Figure 3-22: Tissue perfusion.

We again apply the principle of mass preservation:

$\frac{dQ_t(t)}{dt} = FC_i(t) - FC_o(t)$ is the change per unit of time in the amount of indicator in the tissue studied, dQ_t is the difference in amount of indicator on the outflow side and inflow side at any time. The partition coefficient, λ, is defined as the ratio between the tissue and plasma concentration of indicator in equilibrium at constant infusion, i.e. $C_{t\infty} = \lambda C_{o\infty}$.

The equilibrium concentration, C_∞, is the value that the inflow and outflow concentrations approach after a "long" time, as shown in Figure 3-23.

The indicator amount $Q_{t\infty}$ in the tissue during equilibrium is:

$$\left.\begin{aligned} Q_{t\infty} &= C_{t\infty}W = \lambda C_{0\infty}W \\ Q_{t\infty} &= \int_0^\infty \frac{dQ_t(t)}{dt}dt = F\int_0^\infty [C_i(t) - C_o(t)]dt \end{aligned}\right\} \Rightarrow$$

$$\lambda C_{0\infty}W = F\int_0^\infty [C_i(t) - C_o(t)]dt$$

From the equation we then get:

$$\frac{F}{W} = \frac{\lambda C_\infty}{\int_0^\infty [C_i(t) - C_o(t)]dt}$$

$$\frac{F}{W} = f = \frac{\lambda C_\infty}{\int_0^\infty [C_i(t) - C_o(t)]dt} \Leftrightarrow$$

$$\frac{f}{\lambda} = \frac{C_\infty}{\int_0^\infty [C_i(t) - C_o(t)]dt}$$

where *f* is the perfusion coefficient (most often just called *perfusion*), defined as flow per tissue mass $[\frac{\text{ml/min}}{100\text{g}}]$. In addition to measurements of the concentrations on the inflow and outflow sides, determination of perfusion in a tissue area demands a knowledge of the partition coefficient, perhaps by using data from the literature.

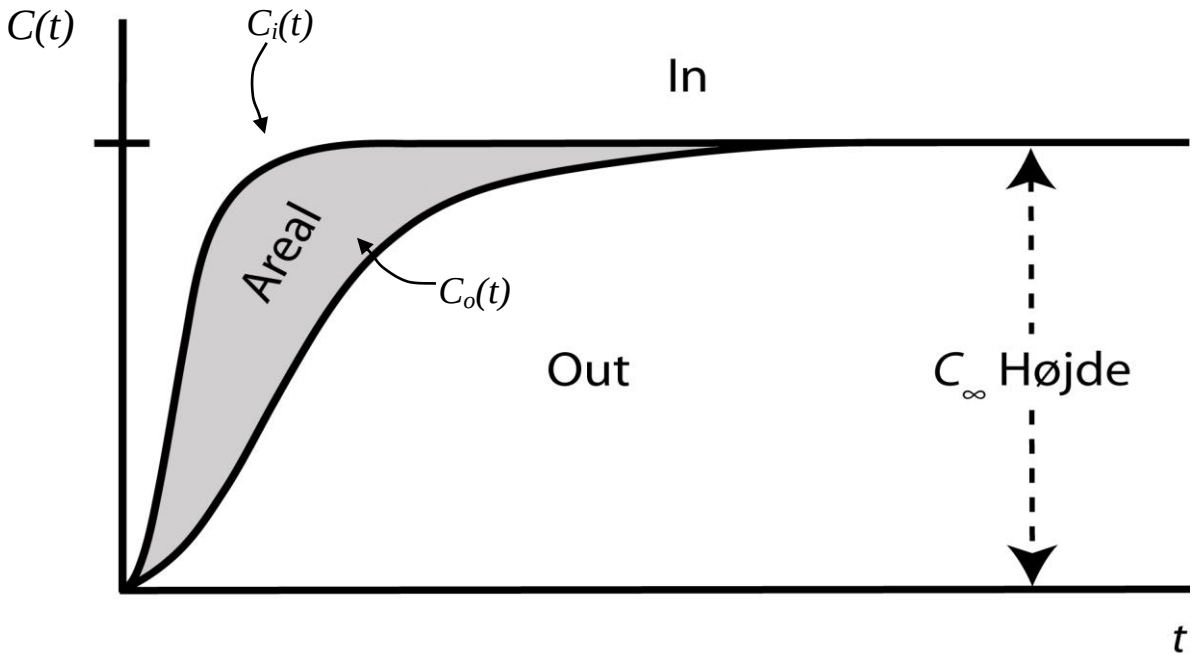


Figure 3-23: Kety-Schmidt's method under ideal conditions: *f/λ=height/area.*

3.2 Extraction

The extraction fraction, E, is the share of the incoming flux, that is absorbed or withheld in the tissue:

$$E = \frac{J_{ret}}{J_{in}} = \frac{F \cdot C_{in\infty} - F \cdot C_{o\infty}}{F \cdot C_{in\infty}} = \frac{C_{in\infty} - C_{o\infty}}{C_{in\infty}} = 1 - \frac{C_{o\infty}}{C_{in\infty}}$$

where J_{ret} is the retention flux[1,10], i.e. the amount absorbed by the tissue per time unit, see Figure 3-24.

The sum of extracted and transmitted fraction is, of course, always 1.

Transmitted fraction $T = 1\text{-}E =$

$$1 - \frac{J_{ret}}{J_{in}} = \frac{J_{in} - J_{ret}}{J_{in}} = \frac{J_o}{J_{in}} = \frac{C_{o\infty}}{C_{in\infty}}$$

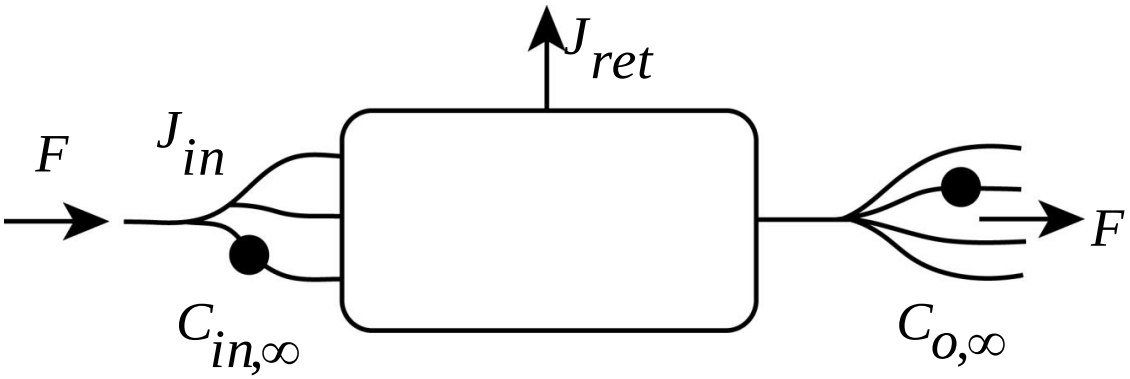


Figure 3-24. Flow through tissue area with retention flux J_{ret}

3.3 Clearance

Clearance is the speed with which the system removes a particular substance from a reference fluid measured as a fictive or real volume flow of the reference fluid.

Clearance is defined as flux divided by the concentration of a reference fluid.

$$Cl = \frac{J_{ret}}{C_{ref\infty}} = \frac{F(C_{in\infty} - C_{o\infty})}{C_{ref\infty}} \left[ml / s\right]$$

The unit is volume per unit of time and since clearance depends on both the flow in the reference fluid and the treatment of the substance in the system, the interpretation of clearance is interpreted according to the particular substance and reference fluid used in a given connection, see below. The following is valid:

$$E = \frac{J_{ret}}{J_{in}} = \frac{J_{ret}}{F \cdot C_{in}}$$

and if the inflowing fluid is taken as a reference, we get:

$$E \cdot F = \frac{J_{ret}}{C_{in}}, \; i.e. \; Cl = E \cdot F$$

If the indicator is completely extracted ($E = 1$), clearance is a measure of flow. If extraction is less than 1, clearance is proportional to the flow.

Clearance is a general kinetic term, but is especially useful to describe the function of the kidney. The renal plasma clearance of a substance is (as $C_{ref} = C_p$) $Cl = \frac{J_{ret}}{C_{ref}} = \frac{C_u}{C_p}\frac{dU}{dt} = \frac{C_u\dot{U}}{C_p}$, since the indicator is transported from plasma to the urine with a retention flux, which is equal to the urine concentration (C_u) multiplied by the urine flow $(\dot{U} = \frac{dU}{dt})$.

That amount of indicator per time unit, $-\frac{dQ}{dt}$, which is transported from plasma to urine, can be determined from clearance and plasma concentration or from urinary flow and urinary concentration:

$$-\frac{dQ}{dt} = Cl \cdot C_p = \dot{U} \cdot C_u$$

If an indicator is filtered across the glomerulus membrane with the same concentration as in the plasma water and is not secreted or reabsorbed in the kidney (e.g. ^{51}Cr-EDTA, Gd-EDTA, or inulin) the following equation is valid:

$GFR \cdot (C_{in} - C_{out}) = GFR \cdot C_p = C_u\dot{U}$, since $C_{in} - C_{out} = C_{in} = C_p$

or

$GFR = \frac{C_u\dot{U}}{C_p} = Cl$, i.e. the clearance principle can be used to measure GFR.

The conditions for GFR determination are then that the indicator is freely filterable through the glomerulus membrane, that the substance is not bound to plasma colloids, and that the substance is not secreted or reabsorbed in the tubular walls. An example of determination of GFR is given in Figure 3-25.

Note that the clearance of a substance does not need to be a physical flow, but it describes the ability of the kidneys to remove the substance and can be used as a measure of kidney function[1]. Clearance of creatinine and urea is respectively larger and smaller than the GFR, see later sections about determination of the kidney function.

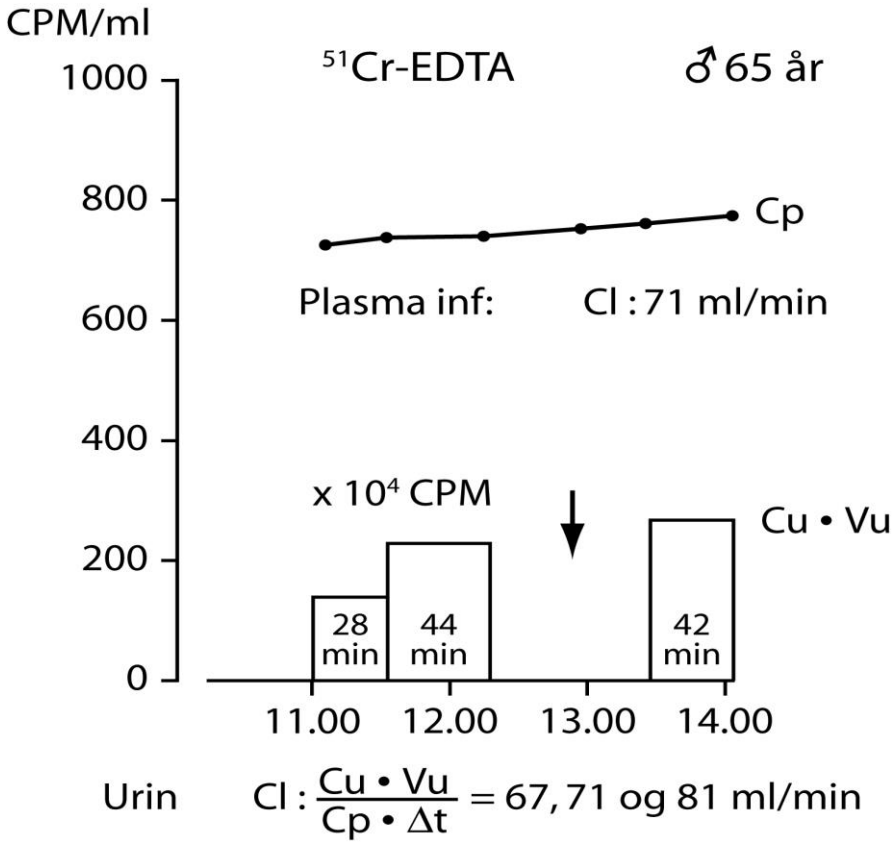


Figure 3-25. A 65-year-old male patient is given a GFR determination with continuous infusion of ^{51}Cr-EDTA. Renal plasma clearance is determined from the infusion speed, the change in plasma concentration of ^{51}Cr-EDTA (C_p), and the urine collection. The separated amount of indicator ($C_u \cdot V_u$) in the urine is shown in three collection periods of 28, 44, and 42 minutes' duration. At the mark (arrow) blood pressure manipulating treatment is carried out. It can be seen that there is good agreement between clearance determined by urine collection (67 and 71 ml/min) and infusion (71 ml/min).

The perhaps slightly increasing clearance in the last period (81 ml/min) has not yet resulted in a decreasing plasma concentration of indicator, because of the large distribution volume of the indicator.

Another example of application of the clearance concept is in connection with the metabolism of hormones. After constant infusion of a radioactive-labelled hormone, a blood sample is taken when the steady state is reached, i.e. the speed of infusion J_{in} is equal to the flux from plasma $J_{o.}$

$Cl = \frac{J_{in}}{C_p} = \frac{J_o}{C_p}$, where J_{in} is the known speed of infusion in cps/s and C_p is the measured plasma concentration in cps/ml.

If the plasma concentration of the endogenous hormone, $C_{h,}$ (ng/ml) is known the secretion (appearance) rate R, can be determined as:

$$R = ClC_h = \frac{J_{in}}{C_p} C_h$$

The clearance concept can also be used generally when looking at problems with the transport of various substances, including pharmaceuticals and their break-down products[120].

3.3.1 Clearance and flux determined at constant infusion and single injection

At the times $t_1, t_2 ...$ the following apply:

$$Cl(t_1) = \frac{F(C_{in}(t_1) - C_o(t_1))}{C_{in}(t_1)} = \frac{\Delta Q(t_1)}{\Delta t C_{in}(t_1)},$$

$$Cl(t_2) = \frac{F(C_{in}(t_2) - C_o(t_2))}{C_{in}(t_2)} = \frac{\Delta Q(t_2)}{\Delta t C_{in}(t_2)},$$

...

But since $Cl(t_1) = Cl(t_2) = Cl$ (applies to linear and time invariant systems), we get

$$\Delta Q(t_i) = Cl C_{in}(t_i)\Delta t$$

and

$$Q_0 = \sum_i \Delta Q(t_i) = \sum_i Cl C_{in}(t_i)\Delta t = Cl \sum_i C_{in}(t_i)\ \Delta t \to Cl \int_0^\infty C_{in}(t)dt$$, that is

$$Cl = \frac{Q_0}{\int_0^\infty C_{in}(t)dt}$$

This dose-area formula is analogous to the formula for cardiac output[102]. In addition, see Figure 3-26 and Figure 3-27.

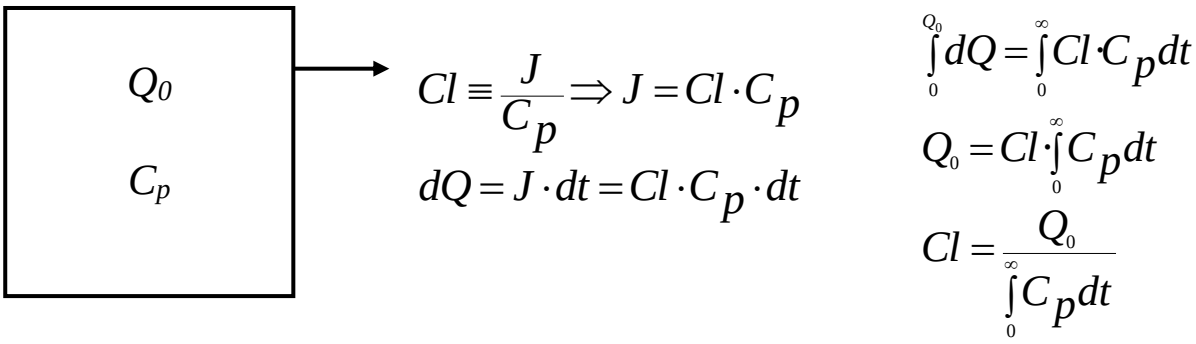


Figure 3-26. Clearance is determined as the amount of indicator in proportion to the area under the plasma-concentration curve.

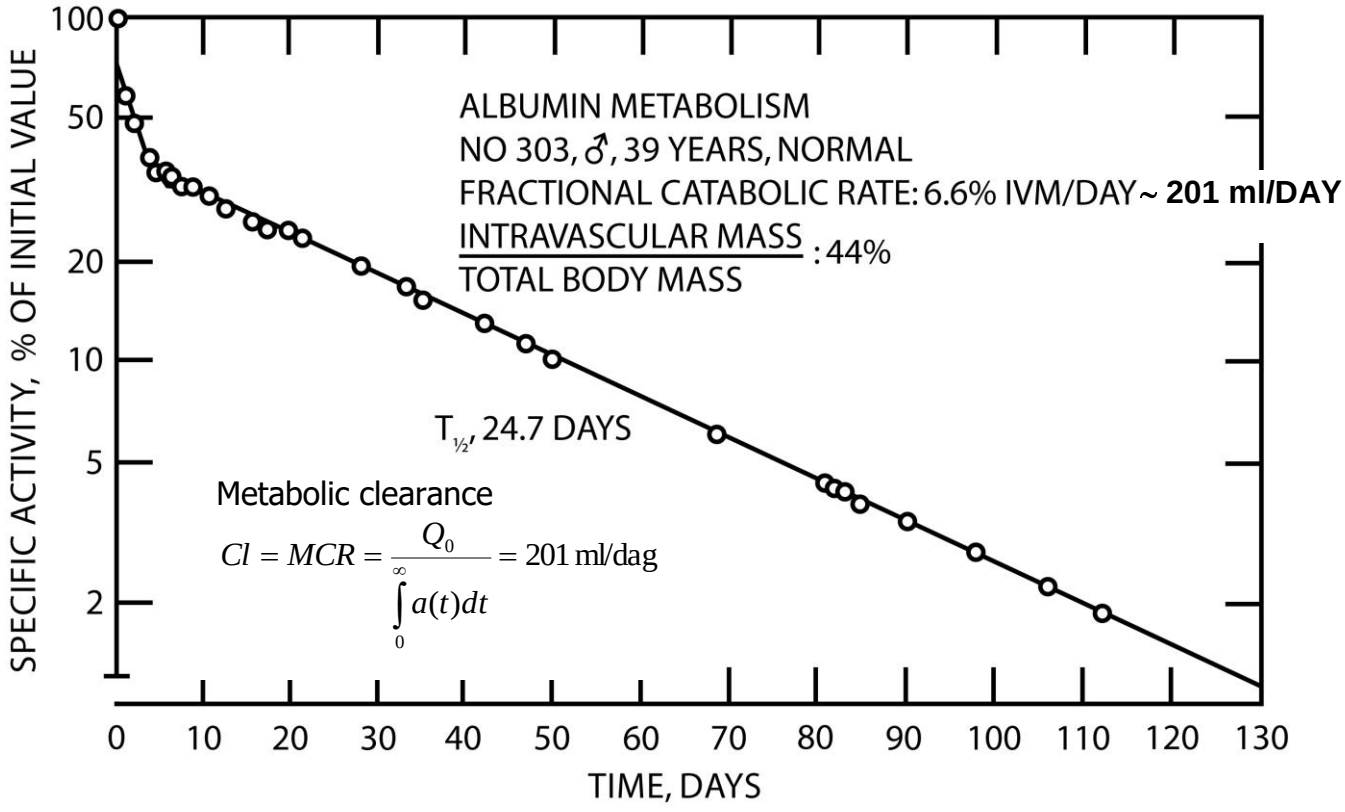


Figure 3-27. Plasma concentration of ^{125}I-serum albumin (expressed as specific activity in per cent of the initial value on the y-axis) shown as a function of time. Plasma samples are taken over 3 to 4 months. The linear course in a semilogarithmic plot shows that it takes a real monoexponential course. From Rossing N (1971)[90].

3.4 Example of curve fit - Clearance of ^{99m}Tc-sestamibi from plasma.

As an example of a calculation of clearance, where it is assumed that a particular model can describe the concentration-time curve, the plasma clearance of Sestamibi is examined.
Sestamibi is injected at time 0 and blood samples are taken at the following times.

Q_0 = 1,24·10^9 cpm

Time **min**	**Concentration** **cpm/ml**
0	477000
31	8531
61	6678
107	5260
163	3987
193	3654
221	3457

The concentration at time 0 is estimated as the injected dose per unit of plasma volume (cpm=counts per minute) and the remaining concentrations are those from the plasma samples. The samples are measured in a gamma counter and corrected for decay (half-time 6 hours = 360 min, $A_{corr}=A_m e^{ln2/360 \cdot t}$)

The detailed kinetics of Sestamibi in plasma is sparsely described in the literature, but if we draw a number of concentration curves on semilogarithmic paper (or use a spreadsheet) we can see that the curves have two phases corresponding to the two exponents
k_1 and k_2 in the expression $C(t) = C_1 e^{-k_1 t} + C_2 e^{-k_2 t}$, see Figure 3-28. This implies that the model is two-compartmental model (where more than two compartments could be pooled).

It is possible to estimate k_1, k_2, C_1 og C_2 from the sketched curve but it is faster and more accurate to use a programme to fit a non-linear curve (of commercial programme packs available the following can be mentioned: SAS, MatLab, SigmaPlot, and GraphPad Prism).

A two exponential curve fit on the above data (by GraphPad Prism) gives the following values: C_1 = 8604 cpm/ml, k_1 = 0.004423 min^{-1}, C_2 = 468396 cpm/ml and k_2 = 0.1974 min^{-1}. The measured data and the fitted curve are shown in Figure 3-28 (logarithmic y-axis).

To determine the plasma clearance of Sestamibi we apply the principle from the chapter, *Bolus injection - Henriques and Hamilton*. We have measured the amount of Sestamibi before injection, Q_0, and can now calculate the area under the fitted concentration curve:

$$Cl = \frac{Injected\,amount}{Area} = \frac{Q_0}{\int_0^\infty (C_1 e^{-k_1 t} + C_2 e^{-k_2 t})dt} = \frac{Q_0}{\frac{C_1}{k_1} + \frac{C_2}{k_2}} =$$

$$\frac{1{,}24 \cdot 10^{9}\ \text{cpm}}{\frac{8604\ \text{cpm/ml}}{0{,}004423\ \text{min}^{-1}} + \frac{46396\ \text{cpm/ml}}{0{,}1974\ \text{min}^{-1}}} = \underline{\underline{287\ \text{ml/min}}}$$

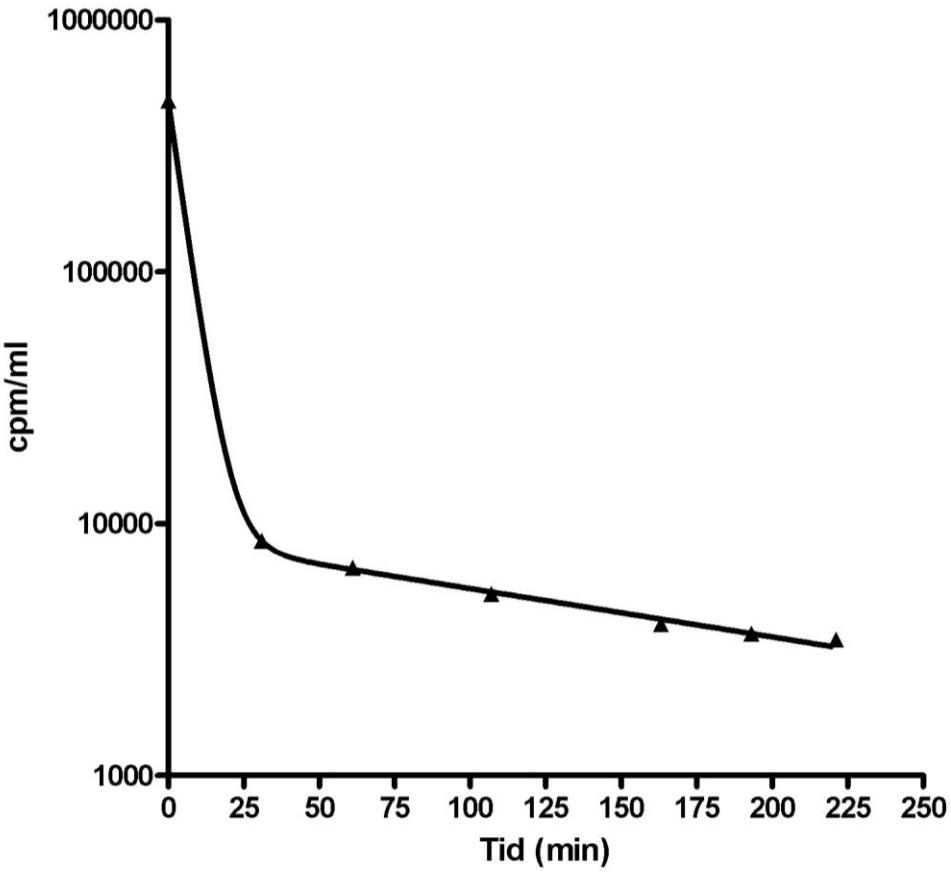


Figure 3-28: Plasma time-activity curve for ^{99m}Tc-sestamibi after intravenous bolus injection.

3.5 Mean transit time

When particles are transported through a system, they will disperse (spread) during the transport time, because they are exposed to various influences from the system.

Examples of such influences are:

1) different rates of flow (e.g. faster in the middle of the vessel than in the periphery)
2) different distances (e.g. through shunts)
3) mixing in the transport medium of volume of distribution (e.g. in the plasma in the heart cavities)
4) transports across barriers or through media with limited transport capacity.

The mean transit time ($\bar{t}$, *retention time, mean sojourn time*) is the designation for the average time it takes all the molecules, particles, etc. of a given sort to pass through a specified system[1].

As an illustration one can regard a number of horses (particles) running in a race (the system), see Figure 3-29. The "measurement" is a residual one as we make up the number of horses on the track each time until they pass the finishing post. If we plot the number of horses as a function of time, we get a graph looking like the one in Figure 3-29. The curve is divided in blocks, where each block corresponds to one or more horses, which pass the finishing post at time t_i.

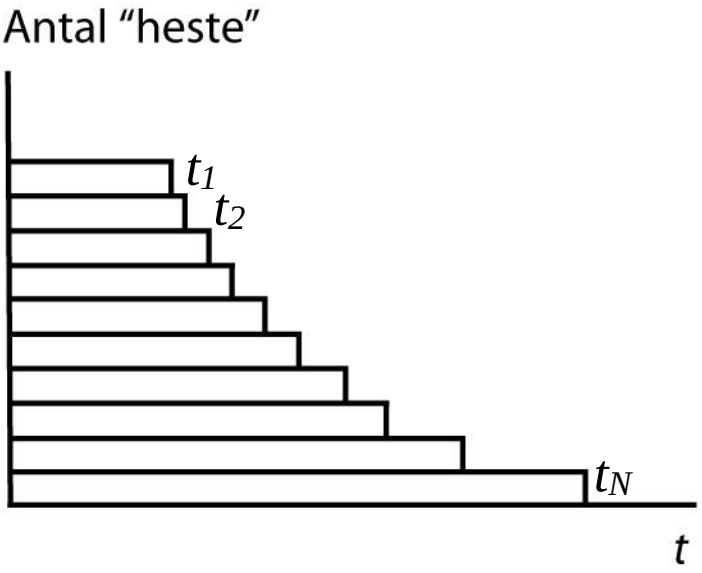


Figure 3-29: Illustration of the transit time and the mean transit time at residual measurement. The analogue is a number of horses running in race. The graph shows the number of horses. Each block ends when one or more pass the finishing post, so that the top block corresponds to the fastest horses and the bottom one to the slowest.
The mean transit time is the average time of the race.

If a final number of indicator particles is introduced into the system, the particles will run through the system at individual times: $t_1, t_2, \ldots t_N$. Then, the mean transit time $\bar{t}$ expresses the average run through time through the system:

$$\bar{t} = \frac{\sum_{i=1}^{N} t_i}{N}$$

The mean transit time can be determined by outflow detection (e.g. serial blood samples) or residual detection (e.g. measurement over the tissue area with a gamma camera), as specified in the following section.

3.5.1 Probability density function of transit times

3.5.1.1 Outflow detection

The probability density function for transit times, *h(t)*, for single outflow systems can be defined as the relative outflow speed of indicator, after a bolus dose of the size Q_0:

$$h(t) = \frac{d(Q(t)/Q_0)}{dt} = \frac{1}{Q_0}\frac{dQ(t)}{dt}\left[s^{-1}\right],$$

To understand that *h(t)* is a probability density function for transit times, we can look at the approximation $\frac{\Delta Q(t_i)}{Q_0} = h(t_i)\Delta t$, where $h(t_i)\Delta t$ is seen to be the relative amount of particles which have disappeared out of the system between t_i and $t_i+\Delta t$. This means that $h(t_i)\Delta t$ can be taken as the probability that a randomly chosen particle has passsed through the system between t_i and $t_i+\Delta t$.

From the theory of probability, we know that the integral $\int_0^\infty th(t)dt$ expresses the *expected value* or the mean value of the stochastic variable *t*. That this is exactly the mean transit time for the system is shown below.

h(t) can be found from the connection:

$$h(t)dt = \frac{dQ(t)}{Q_0} = \frac{F \cdot C_{out}(t)dt}{F\int_0^\infty C_{out}(t)dt} = \frac{C_{out}(t)dt}{\int_0^\infty C_{out}(t)dt} \Rightarrow$$

$$h(t) = \frac{C_{out}(t)}{\int_0^\infty C_{out}(t)dt}$$

The integral in the denominator is a normalisation constant, since the collected area under the probability density function must be 1:

$$\int_0^\infty h(t)dt = \int_0^\infty \frac{C_{out}(t)}{\int_0^\infty C_{out}(t)dt}dt \frac{1}{\int_0^\infty C_{out}(t)dt} \cdot \int_0^\infty C_{out}(t)dt = 1.$$

In order to get the expression for the mean transit time, we consider a system with a final number of particles, N, where there is assumed to be *i* possible run-through times. The mean transit time can be written as:

$\bar{t} = \frac{1}{N}\left[n_1t_1 + n_2t_2 + n_3t_3 + ... + n_it_i\right] = \sum_i \frac{n_it_i}{N}$, since n_i is the number of particles with flow time t_i. $\frac{n_i}{N}$ is then the fraction of particles with transit time t_i.

If a large amount of indicator, Q_0, is considered instead, the expression above should be replaced by an integral, where the fraction $\frac{n_i}{N}$ with transit time t_i is replaced by the fraction $\frac{dQ}{Q_0}$, with transit time t*:*

$$\bar{t} = \int_0^\infty t\frac{dQ}{Q_0} = \frac{\int_0^\infty tdQ}{Q_0}$$

The size tdQ is illustrated in Figure 3-30 and Figure 3-31.

If $\frac{dQ(t)}{Q_0} = h(t)dt$ is inserted in the expression for mean transit time, one gets:

$$\bar{t} = \int_0^\infty th(t)dt$$

or stated from the concentration curve: $\bar{t} = \frac{\int_0^\infty tC_{out}(t)dt}{\int_0^\infty C_{out}(t)dt}$.

The probability density function for transit times is also called the transfer function or the impulse response function of the system. These aspects are discussed further in the Appendix.

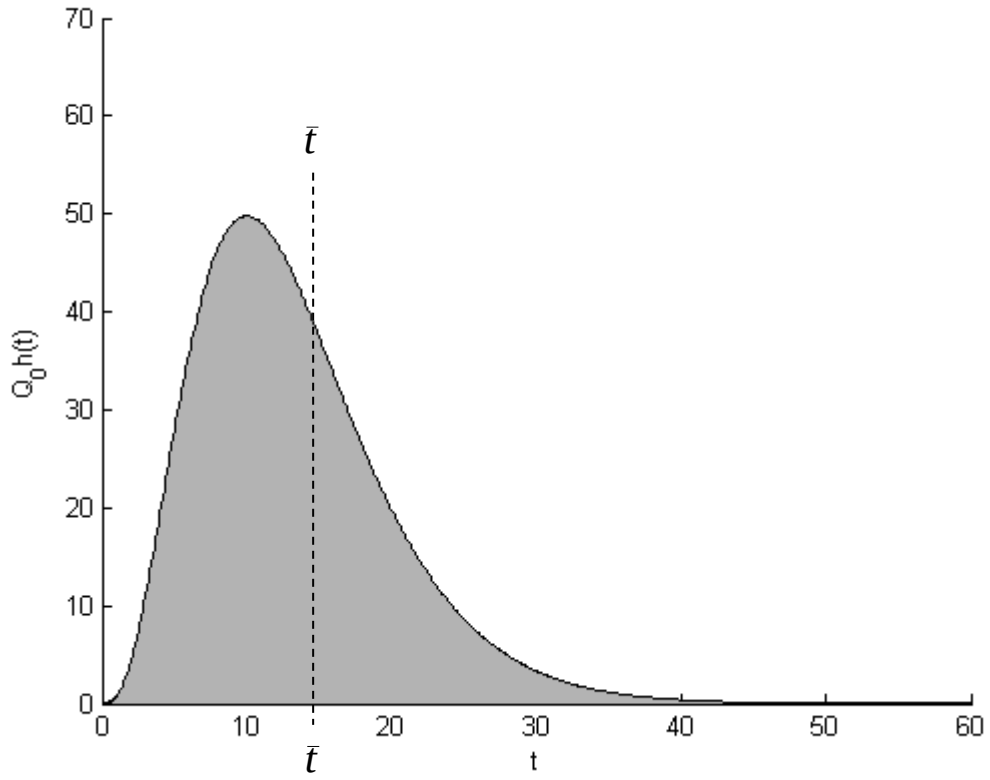


Figure 3-30: Time-activity curve with a specification of the mean transit time (from the broken line). Since the mean transit time is the central point of the curve on the time axis, the areas multiplied by the distance to $\bar{t}$ on each side of the line are equal.

The cumulated out flux *H(t), the transit time distribution,* is

$$H(t) = \int_0^t h(\tau) d\tau = \frac{1}{Q_0} \int_0^t dQ(\tau)$$ [+]

The residual function or *the residual fraction* is defined as $R(t) = 1 - H(t) = 1 - \int_0^t h(\tau) d\tau$ [+]

[+] τ is used as an integration variable instead of *t*, to avoid confusion with time *t*, which here forms the upper integration limit.

3.5.1.2 Residual detection

If an indicator is introduced at time 0 with the mass Q_0, and $Q_i = Q(t_i)$ is the *remaining* amount of indicator at all times, $\bar{t}$ can be expressed as:

$$\bar{t} = t_1(\frac{Q_0 - Q_1}{Q_0}) + t_2(\frac{Q_1 - Q_2}{Q_0}) + t_3(\frac{Q_2 - Q_3}{Q_0}) + ...$$

$$= \frac{Q_0}{Q_0}(t_1 - 0) + \frac{Q_1}{Q_0}(t_2 - t_1) + \frac{Q_2}{Q_0}(t_3 - t_2) + ...$$

$$= \sum_i \frac{Q_i}{Q_0}\Delta t \underset{for \Delta t \to 0}{\rightarrow} \frac{\int_0^\infty Q(t)dt}{Q_0}$$

From this we can see that when residuals are used for measuring $\bar{t}$, the value is determined as the area under the residual curve per mass unit of indicator. On the residual curve, Q_0 can be read as the point corresponding to $t=0$, see Figure 3-31.

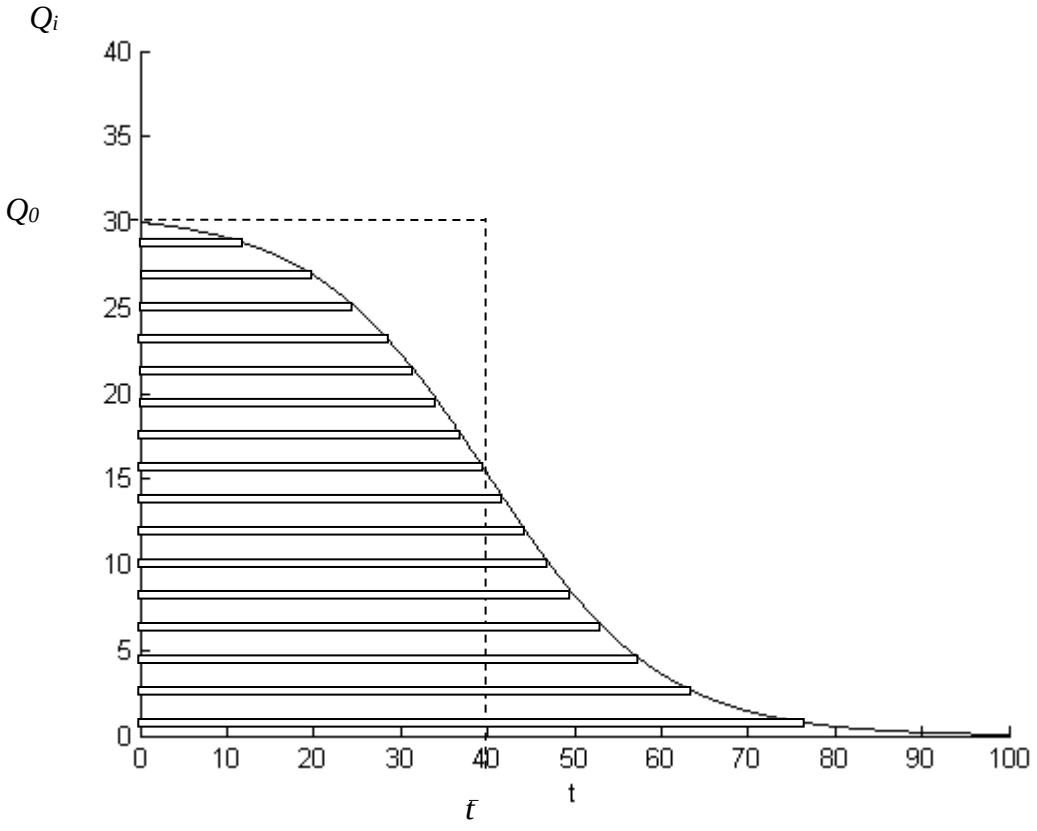


Figure 3-31: Example of mean transit time measured by residual detection. The area under the broken line, which has the height Q_0 and the "width" $\bar{t}$, is the same as the area under the residual curve. The horizontal lines express single transit times and give an expression of their dispersion.

3.6 Distribution volume

The distribution volume (V_D*) is a virtual space* (or pool), which is the (imaginary) volume in which an indicator amount is distributed after total mixing*.

V_D can be defined by using the mass conservation concept on an indicator amount, Q_0, or an endogenous substance, *M*, relative to its concentration in a reference fluid (plasma) after complete mixing:

$$V_D = \frac{Q_0}{C_\infty} \text{ or } V_D = \frac{M(t)}{C(t)}$$

where C_∞ is the indicator concentration at the steady state and *C(t)* the concentration of the endogenous substance (Figure 3-32).

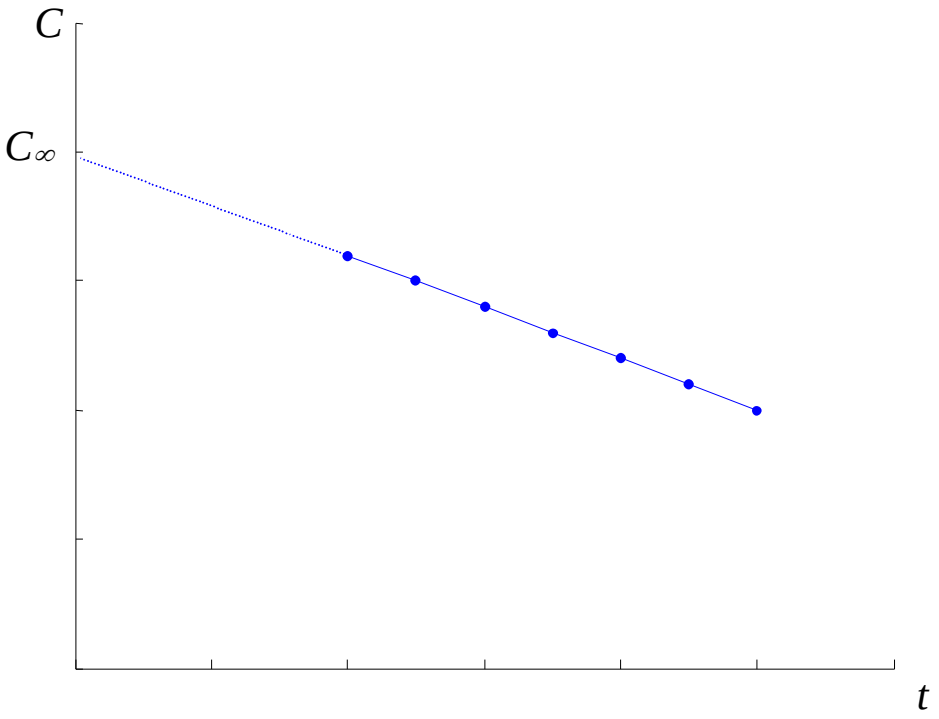


Figure 3-32: The steady-state concentration (momentary homogeneous mixture) is read as the intersection of the concentration curve with the y-axis after a single injection.

* Note that we have here a volume with the usual volume unit. PET studies show dimensionless distribution volumes. This is the same as the partition coefficient for tissue and plasma (i.e. the relation between the concentrations in tissue and plasma at equilibrium).

There is a general relation between volume (*V*), mean transit time $\bar{t}$, and flow (*F*) through a system: $V = \bar{t} \cdot F$ (see Figure 3-33).

The distribution volume (V_D) is in a similar way related to clearance and mean transit time:
$V_D = \bar{t} \cdot Cl$

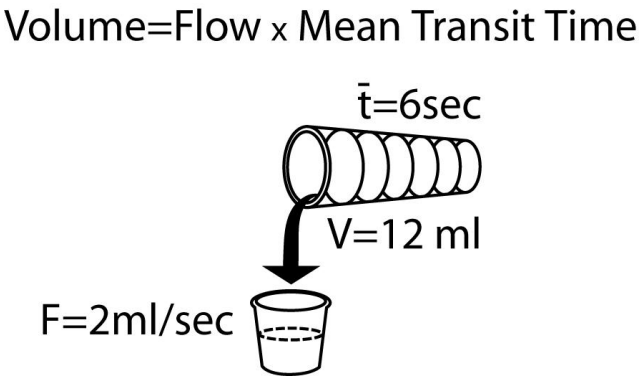


Figure 3-33: The relations between volume, flow, and mean transit time.

The equation can also appear as:
$M = \bar{t} \cdot J$,
where *M* is mass or quantity of material (e.g. µmol) and J is flux (e.g. µmol·min^{-1}).
The mean transit time can be determined by bolus injection and outflow detection[107-111] or by continuous infusion either with residual detection or with inflow/outflow detection, see Figure 3-34.

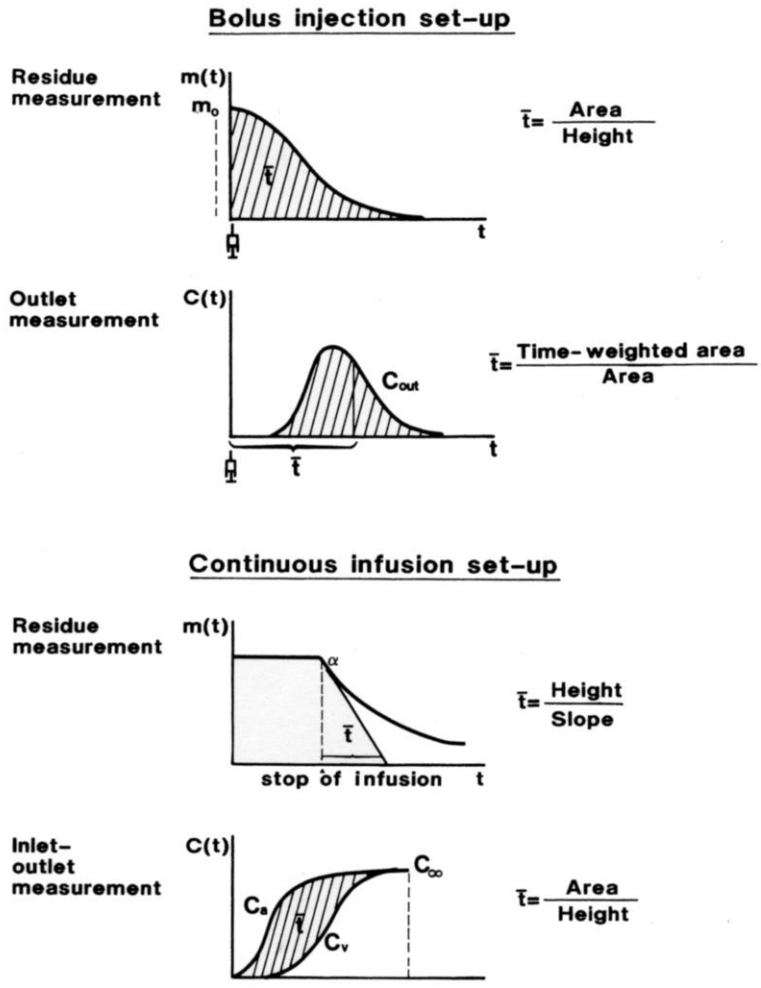


FIGURE 4. Different types of kinetic set-ups. t = mean transit time, m(t) and c(t) = amount and concentration of test substance to time t, respectively (see text).

Figure 3-34: Determination of mean transit time by bolus injection and continuous infusion. From JH Henriksen 1991[13].

3.7 Transcapillary transport

Exchange of substance between the capillaries and the surrounding tissue can be described from assumptions about flow, inflow concentration, outflow concentration, and the introduction of a transcapillary transport limitation.

The concentration on the outflow side of the capillary, C_0, can then be described by the following equation

$$C_o = C_{in} \cdot e^{-\frac{PS}{F}},$$

where C_{in} is the concentration on the inflow side, F is the flow through the capillary and PS is the permeability surface area product, often just called the PS product (permeability-surface area product), the product of the surface area of the vessel, and its permeability for the indicator in question[10].

PS can be defined as the relation between the flux and the difference in the concentration between each side of a membrane $J_{1\to 2} = PS\left(C_1 - C_2\right)$, where 1 and 2 refer to the two sides of the membrane. Thus, PS has the dimensions of a flow, for example with the unit ml/min. A high value for PS means that only a small difference in concentration is necessary to carry a large flux from capillary to the interstitial space.

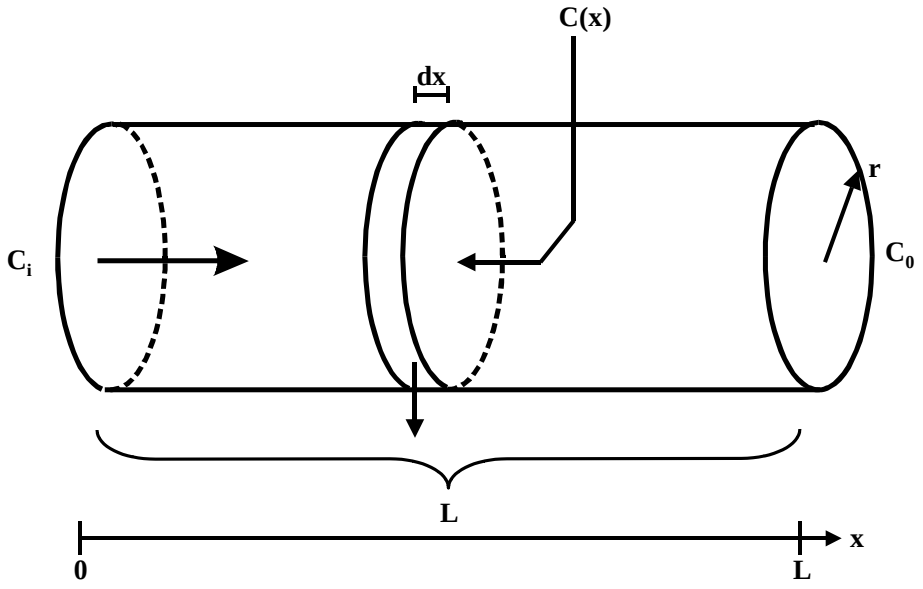


Figure 3-35: Idealised section of capillary. V is the total volume, C(x) the concentration of indicator along the capillary and x goes from 0 to the length of the capillary L.

The substance amount, *dQ*, which passes through the samll volume element *dV* with the length *dx*, is, according to the principle of mass preservation and the definition of surface permeability, see Figure 3-35:

$$dQ = VdJ = PS\left(C(x) - C_{extravas}(x)\right)\cdot dV \Rightarrow$$

$$dJ = \frac{1}{V}PS\left(C(x) - C_{extravas}(x)\right)dV = \frac{1}{L}PS\cdot C(x)dx\,,$$

where the relation between the volume: $\frac{dV}{V} = \frac{2\pi r^2 dx}{2\pi r^2 L} = \frac{dx}{L}$ is exploited, and the extra vascular concentration, $C_{extravas}(x)$, of the indicator is assumed to be disappearingly small.

At the same time $dJ = -F \cdot dC(x)$ holds true, which follows from the definition of flux and thus

$$-F\,dC(x) = \frac{1}{L} PS \cdot C(x)dx \text{ or}$$

$$\frac{1}{C(x)} dC(x) = -\frac{1}{LF} PSdx$$

By integration on both sides:

$\ln(x)\big|_{C_{in}}^{Co} = -\frac{1}{LF} PS\,x\big|_0^L$, that is

$\ln(C_o / C_{in}) = -\frac{1}{LF} PS \cdot L$, which can be rewritten $C_o = C_{in} e^{-PS/F}$ or $\frac{C_o}{C_{in}} = e^{-PS/F}$

Since the extraction, *E*, is

$$E = \frac{C_{in} - C_o}{C_{in}} = 1 - \frac{C_o}{C_{in}}, \quad E = 1 - e^{-PS/F} \textit{ is valid}$$

Similarly we can write the equation for clearance:

$\mathrm{Cl} = \mathrm{E} \cdot \mathrm{F} = F(1 - e^{-PS/F})$

The outflow concentration compared to the inflow concentration as a function of flow appears from Figure 3-36.

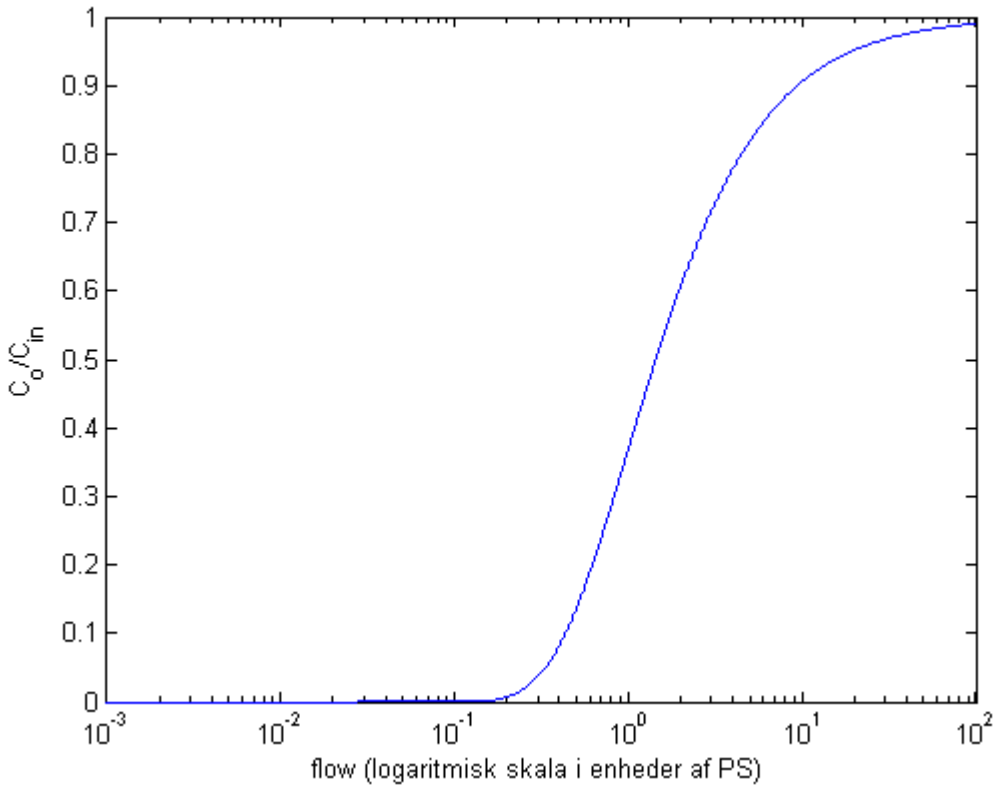


Figure 3-36: The connection between relative concentration and flow. Note the unit.

Example: We wish to determine the flow, *F*, of a system and have thus chosen an indicator, which is flow-limited. We have been informed that the extraction is *E* = 0.90, measured at a rest-flow of

F=50 ml/100 g min,

That is:

$0.90 = 1 - e^{\frac{-PS}{50}} \Rightarrow PS = -\ln(0.1) \cdot 50\,\text{ml}/100\,\text{g min} = 115\,\text{ml}/100\,\text{g min}$.

In the table below PS is kept constant, while connected values of flow and clearance are determined:

F mg/100 g min	PS mg/100 g min	PS/F	E	Cl mg/100 g min
10	115.00	11.50	1.00	10.00
25	115.00	4.60	0.99	24.75
30	114.90	3.83	0.98	29.40
50	115.00	2.30	0.90	45.00
75	114.75	1.53	0.78	58.50
100	115.00	1.15	0.68	68.00
200	116.00	0.58	0.44	88.00
300	114.00	0.38	0.32	96.00

The graph shows (Figure 3-37) that clearance and flow are equal for low flow values, whereas the curve approaches PS for very high flow values.

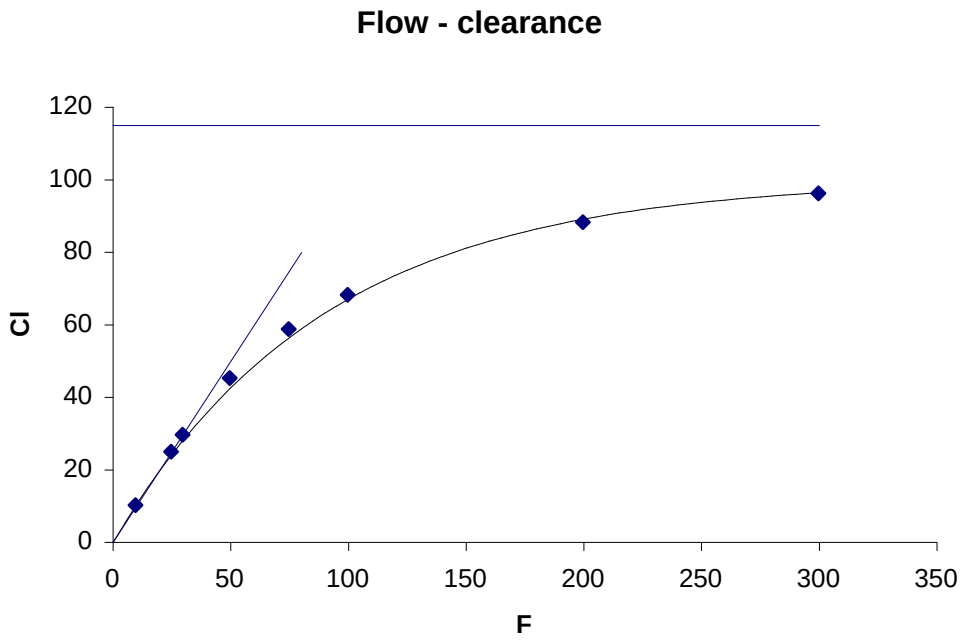


Figure 3-37: The relation between clearance and flow (from the example)

As mentioned in the section on clearance, clearance is a measure of flow, when the extraction is close to 1:

$Cl = FE \approx F$, when $E = 1 - C_o / C_{in} = 1 - e^{-PS/F} \approx 1$, i.e. when the flow F is low compared to PS (see Figure 3-37). When this is met the indicator is said to be *flow-limited*.

For low PS values compared to the flow (or a high flow compared to PS):

$Cl = FE = F(1 - e^{-PS/F}) \approx F \cdot PS / F = PS$, which follows from the fact that e^{-x} for small values of x can be approximated by $1 - x$ (Taylor row expansion, see the mathematic appendix). For these low PS values the indicator is *diffusion-limited.*

Thus, for flow-limited indicators, clearance is a measure of flow and for diffusion-limited indicators clearance is a measure of the capillaries' permeability expressed by the permeability surface area product, PS.

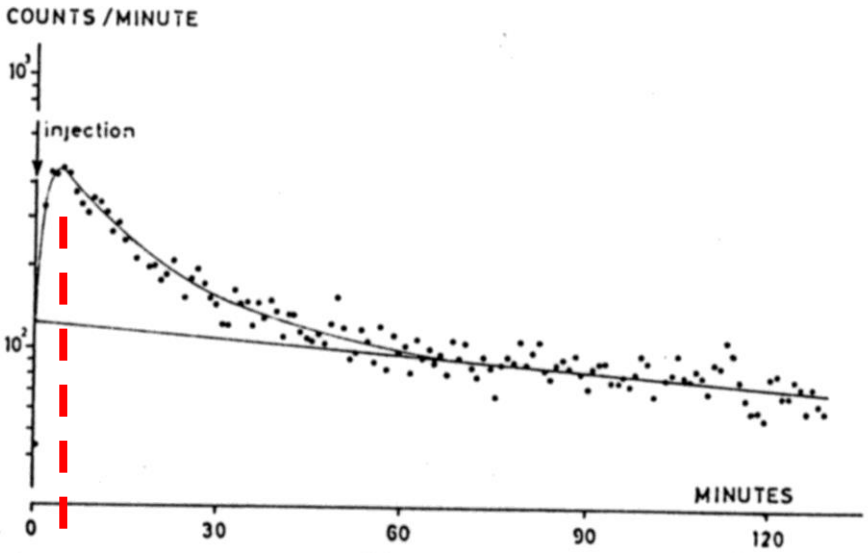


Figure 6.

Washout curve for krypton-85 after the injection of a bolus of the gas dissolved in saline into the femoral artery, during moderate vasodilation of the skin. The β-radiation was recorded over the lateral surface of the crus.

Figure 3-38: Wash-out curve for Kr-85, measured on the surface of the lower limb (crus). From Sejersen P 1971[135]

Per Sejersen[135] has shown that, with external detection (residual detection) after intra-arterial injection of an appropriate indicator, it is possible to perceive the ratio between the maximum of the curve and a retropolated value corresponding to the time of the curve maximum as identical with the transcapillary extraction of the indicator, see Figure 3-38.