

THE STUDY OF BLOOD FLOW USING
INDICATOR DILUTION TECHNIQUES

by

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This thesis is dedicated to
my wife, Nicky, for her
encouragement, understanding
and support.

ABSTRACT

An investigation has been made of the dispersion of the radioactively labelled constituents of blood during flow. In laminar flow down a long straight tube the non-cellular components are dispersed by laminar convection and indicator diffusion. It has been found that the clearance curves for labelled protein in whole blood are similar to those of plasma, except that the red blood cells speed the clearance of protein in the tail region of the curve. This is attributed to sweeping of the tube wall by the red blood cells. The dispersion characteristics of labelled red blood cells suspended in saline and plasma have provided information on velocity profile changes during flow.

A theoretical analysis of the dispersion has been made from the standpoint of the indicator dilution theory and the results suggest that previous formulations may be substantially in error. New formulae have been proposed to calculate the mean transit time and volume of the system. The validity of the analysis has been tested experimentally in the straight tube system and in a model vascular bed.

The implications of this work to the study of the mammalian circulation are discussed.

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CHAPTER IINTRODUCTIONHistorical development of the indicator dilution theory and method

The mammalian circulation is an highly complex system composed of many functionally and structurally different components. Its purpose is the maintenance of the functional integrity of the tissues and organs. Different organs and tissues have different requirements and the vascular and organ architecture and ultrastructure varies accordingly. In order that the needs of particular tissues and the possibly conflicting needs of the whole body be maintained, a complex regulatory system comprising centrally directed vasoactive nerves (vasoconstrictor and vasodilator) and local regulating mechanisms (metabolites, kinins, serotonin), co-ordinates physiological functioning.

It is not surprising then, that a vast array of methods have been developed to study the many different aspects of the circulation. One such method involves the injection of a marker substance, or indicator, into one part of the circulation and its subsequent monitoring from another point. The advantage of the method are that it requires minimal interference with the normal circulatory dynamics, flow may be measured in areas that are otherwise inaccessible and information may be obtained about different regions of the circulation by the choice

of suitable indicators.

Hering (1) is credited (2)(3) with the original application of the method, when he injected potassium ferrocyanide into a vein and collected it at timed intervals in a contralateral vein. He measured the time for the ferrocyanide to first appear after injection by using the Prussian blue reaction. He called this time the circulation time. This is now known as the appearance time and is representative only of the fastest moving indicator particles through the circulation. Hering was attempting to measure the 'velocity' of flowing blood but as Zierler (3) has pointed out, he did not appreciate that different elements of blood may take very different pathways, with different distances and times of traversal.

Stewart (4) also used the appearance time when measuring the circulation times of blood through the lungs, kidney and other organs. He injected a known amount of sodium chloride solution (1.5% - 2.5%) as an indicator and detected changes in electrical conductivity at the outflow of the organs. His detector consisted of clay and zinc sulphate electrodes in the arm of a Wheatstone bridge. A buzzer sounded when indicator was detected and reached a constant pitch at a concentration plateau. In his investigations on the factors affecting the circulation times he considered that the volume of blood in the part of the circulation under study directly determined the circulation time, provided the flow rate was constant. The mean pulmonary circulation time i.e. the mean time of the sum of the component parts of the pulmonary circulation

represented the time in which the whole of the blood in the lungs passed from the pulmonary artery to the pulmonary veins. If V is the volume of the pulmonary circulation, S the average output of the right ventricle in ml and r the number of beats of the heart per sec., then an expression for the mean pulmonary circulation time T_{circ} was given by

$$T_{\text{circ}} = \frac{V}{rS} = \frac{V}{F}$$

where F is the flow rate, or cardiac output.

In a later paper, concerned primarily with the determination of cardiac output, Steward (5) considered the possibility that the circulation time he was measuring was not representative of the elements of blood moving with the mean velocity of flow. If the initially appearing indicator traversed the circulation in the centre of the streamlines it would underestimate the true circulation time. The flow rate then calculated from the above expression would overestimate the true flow rate. To test this he conducted a series of experiments in straight and branching glass tubes, and artificial capillary systems with water and cell suspensions. He found that large errors were obtained in wide bore tubes but with a many branched capillary system (a glass tube filled with beads), approximately correct answers for flow were obtained. Further testing in the vascular system itself revealed that little additional dispersion occurred of injected saline to that produced by the injection. He concluded that the 'part of

injected substance that moves fastest does not outstrip the main body, nor does the part that moves slowest lag behind it'. He did, however, observe a 'certain thinning out' of indicator at the beginning and end, as shown by the increase and decrease in the sound of the telephone buzzer. The main purpose of this paper, however, was to develop a method to measure blood flow, or cardiac output. Injection was achieved by running sodium chloride solution from a burette, through a catheter, to the superior vena cava or heart. The amount injected after a known time interval was recorded. By dividing the rate of injection by the plateau concentration observed at outflow, a measure of the dilution of indicator by the flow and therefore flow itself was obtained. This method is now known as the continuous infusion method. He also used a sudden injection method where the indicator was introduced into the circulation instantaneously. Dividing the quantity of indicator injected by the product of the mean concentration and time of collection, enabled flow to be estimated.

The method was further improved and popularised by Hamilton and co-workers more than two decades later. They recognised that the appearance time could not give a meaningful measure of mean velocity of flow of blood (6). Instead they considered the circulation time as that time which divides the area under the concentration curve in half. This is the median time. They subsequently recognised their mistake and redefined it as the weighted mean of the concentration time graph (7) i.e. mean transit time, $\bar{t}$, given by $\bar{t} = \frac{\int t c \, dt}{\int c \, dt}$. By suddenly

injecting an easily detectable indicator, phenol-tetra iodophthalein sodium, and analysing serial samples taken every second, it was apparent that the circulatory system did produce dispersion above that caused by the time course of the injection. A 'secondary rise' due to recirculating indicator also observed, is still the largest technical problem encountered when using the method. They proposed exponential extrapolation (8) in order to complete the downslope of the curve, the extrapolation beginning where the curve becomes linear on a semi-log plot of concentration against time. The flow was then calculated from the equation $F = \frac{q}{c_{av}} \frac{60}{t_d}$, where c_{av} is the average value of the outflow concentration, q is the quantity of injected indicator and t_d is the duration of the extrapolated primary curve. In its more familiar form, $F = \frac{q}{\int c(t) dt}$, this equation is now known as the Stewart-Hamilton equation for flow rate.

Following the development work of Stewart and Hamilton was a period of consolidation where the method was compared with other methods. Many improvements were made and different applications devised. The many different aspects of the method considered at this time have been reviewed by Dow (2). Only brief reference will be made to them here, more attention being paid to the work considered most relevant to the present thesis. Conflicting results were sometimes obtained when comparing the method with the Fick method but it was felt that this reflected the inherent technical difficulties in both methods. Different indicators (dye and radioactive) and detectors were tested. Notable, was the development by MacIntyre and co-workers

(9), of the first successful continuous sampling method for the determination of the arterial concentration of radioactive albumin. The method was used to study the cardiovascular effects of pregnancy (10), changes in blood volume (11), mitral stenosis (12) and myocardial infarction (13), as well as the flow through various organs. In order to relate changes in the outflow curves to particular cardiovascular problems, some authors turned to a consideration of the basic nature of the dilution curves. Different models, including laminar flow systems (14), a combination of mixing chambers (15) and tubes packed with glass beads (16), were used to produce similar curves to the interarterial curves. Information was thus derived about possible mechanisms producing dispersion. This interest produced theoretical curves to which the arterial curves could be compared, and different extrapolation procedures to remove the effects of indicator recirculation.

Implicit in the theoretical outflow curves produced in this way, were underlying assumptions about the nature of the flow system in the models. No assumptions, however, are made about the detailed nature of the flow in the very successful stochastic approach first developed by Stephenson (17), and later used by Meier and Zierler (18), and Zierler (3). The concentration outflow curve is used to obtain the frequency function of transit times through the circulation. This frequency function is directly proportional to the concentration output curve. Provided that certain distribution and mixing criteria are satisfied, flow and mean transit time (and therefore volume) may

be obtained directly from this curve. Meier and Zierler (18) provided a mathematical rationale for the method and helped clear up a reappearing controversy regarding the volume actually being measured. Stephenson (17) and Zierler (3) also produced an elegant, if somewhat formidable, method of convolution and deconvolution to remove indicator recirculation.

Extravascular measurements

The basic indicator dilution theory of Stewart and Hamilton has had to be extended to accommodate experiments with indicators that exchange with extravascular compartments or spaces (19)(20)(21). Information can now be obtained regarding extravascular volumes of distribution and the rates of capillary exchange, or capillary permeability. It was Hamilton and Remington (22) who first, briefly, investigated indicator loss through the capillaries because it was a possible source of error in the flow determination. They found that indicators able to diffuse from the circulation had lower outflow curves and smaller recoveries than those contained wholly within for the duration of the experiment. Methods are now being developed that anticipate indicator loss from, and sometimes complete return to, the capillaries.

In order to quantify the extravascular volumes of solutes, a method of analysis based on the determination of the mean transit times was developed by Chinard and co-workers (21). An indicator that does not leave the circulation is a reference indicator (usually labelled albumin) and is used to determine the intravascular blood volume of the particular area of vascular bed under examination. The

mean transit time of a diffusible indicator gives a measure of the combined blood and extravascular volumes of distribution. By subtraction the extravascular volume may be obtained. The method is only valid for solutes that are uniformly distributed between the vascular and extravascular compartments, and all of the indicator must return to the circulation in the course of the experiment. Corrections have to be applied for indicator uptake by the red cells and plasma binding of indicators.

Another method, again using the simultaneous injection of a reference and diffusible indicator (an idea originally conceived by Chinard (23)), was developed by Lilienfield and co-workers (24) and Anthonisen and Crone (25). They calculated the extravascular volume of water in the lung of man at a point at which there was no fractional loss or gain of a diffusible indicator from the circulation. The volume of distribution of diffusible indicator in the lungs, V , at the time of equilibration was then given by $V = Q/c_e$, where Q is the total amount of indicator lost and c_e the concentration of indicator in the capillaries at equilibrium time.

The use of two indicators, one reference and one diffusible, also overcame one of the problems of quantifying the rate of loss of indicator across the capillary membrane i.e. the capillary permeability. In traversing the vascular bed a diffusible indicator is simultaneously being diluted and exchanged across the capillary wall. The degree of dilution at any time is given by the concentration of the reference

indicator. A measure of indicator loss through the capillaries is then obtained from the difference in concentration curves of diffusible and reference indicators. In an expression for the permeability of capillaries, Crone (26) used the extraction fraction, E, or fractional loss of indicator. If c_r and c_d are the concentrations of reference and diffusible indicators at a given time, then

$$E = \frac{c_r - c_d}{c_r}$$

Only indicators that do not completely leave the circulation in one transit of the capillaries may be used, as, implicit in the method, is the equivalence of the outflow curves of reference indicator and that fraction of diffusible indicator completely remaining in the circulation. The calculation has to be performed on the early part of the dilution curves because of possible indicator back diffusion into the circulation.

Chinard (23) also developed a rather crude graphical method of calculating the rate of exchange of water across the pulmonary capillaries. T-1824 labelled albumin, Na^{22} , inulin and urea were used as reference indicators and by comparing the curves obtained with those of D_2O and T_2O , the fraction of D_2O and T_2O lost was determined. Calculation of the exchange rate in this way revealed a magnitude of water exchange approaching the cardiac output.

The intravascular separation of different components of blood

Hamilton and co-workers (27) were also the first to demonstrate that it was possible for different constituents of blood to have a

different history of traversal while being completely contained within the circulation. They observed a difference in the outflow curves of T-1824 albumin and red cells while investigating the causes of the high cardiac output values they had obtained using the indicator dilution method in dogs. When radioactively labelled red cells and T-1824 albumin were injected simultaneously into the circulation, the peaks of the outflow curves for red cells were slightly ahead of the plasma curve. It was concluded that in some vessels the velocity of the red cells was faster than that of the plasma. Freis and co-workers (26), following up this investigation in the microcirculation of man, found that the arrival times for cells and plasma were the same but that the red cell curve was afterwards displaced ahead of the plasma curve. The results were found to be consistent with a model where plasma flows in a laminar manner in the microcirculation, and the red cells travel with a larger mean velocity by moving towards the centre of the streamlines. The fastest plasma and red cells would travel together in the centre streamlines and arrive simultaneously. Thereafter, the red cells would be cleared before the plasma. This concept of axial streaming was originally postulated by Fåhræus (29) to explain the reduction of haematocrit observed when blood flowed through narrow tubes. The inward motion of red cells would produce a difference in mean velocity and a reduction of the dynamic haematocrit. Other radioactive studies have shown that the red cells travel slightly faster than plasma in traversing the heart and lungs (30), the kidney (31) and the liver (32).

However, there are other possible explanations of the differences in the curves, or at least, other possible contributory factors. A difference has been observed in dilution spaces of simultaneously injected ^{131}I - fibrinogen and albumin bound T-1824 in splenectomized dogs, indicating a possible small extra-vascular volume for albumin(33). Albumin has a lower M.W. than the red cells, and this could lead to differences in transit times caused by intravascular diffusion of albumin (34). A mechanism proposed by Pappenheimer & Kinter (94) for the kidney, draws upon the intravascular separation of cells and plasma by plasma skimming. When blood traverses the microcirculation, branches in the vessels can 'skim off' more plasma than cells, leaving a reduced concentration of cells in the side branches, and a higher concentration in the other vessels. The packed red cells are then believed to take a shorter path length than the plasma.

It was in order to elucidate the mechanisms governing the dispersion of labelled constituents of whole blood through the mammalian microcirculation that Groom (35) decided to study the passage of radioactive tracers through an isolated cat gastrocnemius muscle. He considered that quantitative evaluation of the relative effects of axial streaming, plasma skimming and different extravascular volumes on concentration against time outflow curves is hampered in vivo by recirculation and anatomical considerations. The indicator dilution curves require some form of extrapolation, which is usually arbitrarily assumed to be exponential in character, in order to complete the final part of the curve that is obscured by indicator recirculation. A useful

measure of the dispersion of the outflow curves was the mean transit time of the curves. While the form of the extrapolation used did not appear to affect the calculation of flow to a great extent, the mean transit time was determined by measuring the first moment of the curve and this was thought to depend critically on the form of extrapolation used. It was assumed that the phase separation took place in the microcirculation and the 'dead space' of the large vessels in series with the microcirculation as well as the sampling techniques, could mark the differences in outflow curves and mean transit time.

While attempting to reduce the number of variables and so isolate the critical parameters involved in the phase separation, Groom, in fact, produced more variables for consideration. He proposed that by altering the flow rate and haematocrit in his experiment, a distinction could have been drawn between the effects of red cell aggregation or streaming effects, and extravascular diffusive exchange. His results did not allow any firm conclusion to be drawn on this, but they illustrated a hitherto undocumented phenomenon viz., the faster clearance of plasma than cells, and a ratio of mean transit times of plasma to cells of less than unity. This unexpected phenomenon was to a large extent dependent on haematocrit but the exact role of cell concentration was unclear.

Working parallel to Groom, Thomas et al. (36) made an artificial microcirculatory bed from a parallel arrangement of 200 μ polystyrene tubes. Here they studied the dispersion of radioactive inorganic ions

(phosphorous, ^{32}P , and Iodide, ^{131}I), a labelled protein (^{131}I - serum albumin), and labelled red cells (^{32}P). They found that differences in the dispersion of the inorganic ions and the protein could be accounted for by mechanisms first proposed by Taylor (37). He had demonstrated that the dispersion of diffusing solutes in a fluid flowing under laminar flow (in a straight tube) was due to the combined effects of molecular diffusion and laminar convection. Differences in the shape of curves could be obtained by altering the diffusion coefficients of the indicator. The differences in the outflow curves of labelled albumin and cells could not, however, be explained in these terms, although the authors did discuss the possibility of the red cells modifying the diffusion coefficients of albumin by following irregular paths during flow. The term 'shear-induced collision diffusion' was used to describe the effective diffusion coefficient of albumin because the micro-rheological properties of blood were known to be shear dependent.

Taylor's work is germinal to the present thesis and it is necessary to review in some detail the results he, and workers following up his initial work, obtained.

The dispersion of solutes in laminar flow through a straight tube

Taylor considered theoretically the two extreme cases of indicator dispersion (a) when the velocity profile alone was responsible for the dispersion (subsequently to be known as Taylor A) and (b) when the diffusion of the indicator dominated the dispersion, the velocity profile playing only a small role (to be known as Taylor B). Three types of input were considered (i) infinitesimally small bolus (ii) continuous

infusion/or washout) (iii) a finite size bolus. All three began at $t = 0$ and the dispersion was calculated at time, t , later at various distances, x , from the input site.

The effect of molecular diffusion on dispersion was investigated by considering an equation which combined terms for parabolic convection and Fick (molecular) diffusion. If D is the molecular diffusion coefficient, r the distance from the centre line of the tube, u_0 the maximum velocity of flow, a the radius of the tube and c the concentration of the solute, then the equation for solute dispersion was given by

$$D \left(\frac{\partial^2 c}{\partial t^2} + \frac{1}{r} \frac{\partial c}{\partial r} + \frac{\partial^2 c}{\partial x^2} \right) = \frac{\partial c}{\partial t} + u_0 \left(1 - \frac{r^2}{a^2} \right) \frac{\partial c}{\partial x}$$

By neglecting axial (as opposed to radial) diffusion and using the appropriate boundary conditions, theoretical solutions in the form of error functions were obtained for the mean cross section concentration in Taylor B. An analytical requirement for Taylor B to apply was obtained and given by $\frac{L}{u_0} \gg \frac{R^2}{38^2 D}$, where L is the length of section considered. It was found that with diffusion dominating the indicator would be dispersed relative to a plane moving with the mean velocity of flow with an effective diffusion coefficient given by K , where $K = \frac{a^2 u_0^2}{192D}$.

The analytical solutions were tested using a 1% potassium permanganate solution in distilled water. In order to examine laminar dispersion the solution was flowed for a short time at a fast rate so that diffusive effects did not have time to occur. Slow flow rates and long experiment times were used to examine diffusive effects. Good agreement was obtained with the theory for all the cases studied. It was found

that diffusion reduced the dispersion produced by the velocity profile. For input (iii) (the finite size bolus), with the velocity profile alone causing dispersion, when the concentration was monitored as a function of distance along the tube at a fixed time, the distribution was symmetrical about a point. This point corresponded to the distance moved by the place, moving with the mean velocity of flow, that began at the mid-point of the bolus. The curves were characterised by initial and final portions of equal and opposite sign slopes and a plateau of uniform concentration. The effect of indicator diffusion was to change the amount of longitudinal dispersion and the height of the curves. Increasing the amount of diffusion resulted in narrower and higher curves, the curves always being symmetrically distributed. Taylor considered that because different amounts of diffusion produced different curves, the experimental procedure might provide a method for measuring diffusion coefficients.

Aris (38), using a method of moments, removed the restrictions imposed on some of the parameters by Taylor's analysis and produced solutions for the general case of flow with arbitrary cross-section, flow profile and variation of diffusion coefficient. Ananthakrishnan et al. (39) presented a completed mathematical analysis of a step input that included the effects of axial diffusion. They obtained an analytical criterion for radial diffusion to dominate (Taylor B situation) that agreed with Taylor's, in that $\frac{Dt}{a^2} > 0.8$, where t is the time of flow. Rich and Goodman (40) were directly concerned with the physiological importance of Taylor's analysis and derived an

expression for mean volume concentration of indicator at the outflow of a tube as a function of time. The solutions were tested by washing indicator out of a long straight section with unlabelled solution. Using Taylor's condition for diffusion to dominate, they estimated that diffusive forces should play an important role in indicator dispersion when blood flows through the mammalian capillaries and arterioles of diameter 20-30 μ .

Bate and co-workers (41)(42) presented solutions for Taylor A, Taylor B and the region in between where dispersion was produced by both convection and diffusion. Mean cross-sectional concentration as a function of distance and time was examined when indicator was washed out of a straight section. The theoretical and experimental solutions were plotted with dimensionless parameters $X = \frac{x}{\bar{u} t_0}$ for distance and $T = \frac{\bar{u} t}{x_0}$ for time, where x_0 and t_0 are the sampling distances and times respectively, and $\bar{u}$ is the mean velocity. The curves were then found to be characterised by the parameters $A_{t_0} = \frac{a^2}{D t_0}$ and $A_{x_0} = \frac{a^2 \bar{u}}{D x_0}$ respectively. The theoretical solutions were tested using iodide ion (^{131}I) in 2N KCl solution. In a later paper (43) the significance of these results to the interpretation of multiple indicator injection studies was considered. Solutions to Taylor's differential equation were obtained for bolus injection and were confirmed experimentally. When the curves were plotted as a function of T again the shape of the curve was found to depend on the dimensionless parameter A_x i.e. the relationship between tube radius, molecular diffusion coefficient, mean flow velocity and the position

of the monitoring site, for a particular injection to monitoring length ratio. Most of their studies were performed with $x_1/x_2 = 0.893$, where x_1 and x_2 are the distances from leading and trailing edges of the bolus to the monitoring site. Because of the curve shape dependence on the dimensionless parameter it was possible to simulate the effects of different indicators (with different diffusion coefficients) by altering the flow rate of one solution with one indicator. Flowing very fast produced an outflow curve corresponding to an indicator with a very high molecular weight and low diffusion coefficient. The curve began at about $T = 0.5$, showing that the labelled solute travelled along the centre streamlines at twice the mean velocity, rose to a relatively low peak and was cleared very late. The indicator did not have enough time to diffuse out of the streamlines in which it began and, in consequence, some remained by the tube wall. At very slow flow rates diffusion dominated the dispersion. The bolus of indicator moved downstream effectively as a bolus, and produced a curve that began relatively late, rose to a high peak and was cleared early. At intermediate flow conditions curves were obtained that progressed from the laminar case to the well mixed case.

It was concluded, that in multiple indicator work it would be possible to obtain different curves of indicators completely contained within the circulation if the diffusion coefficients of the indicators were different. Of particular interest was the possibility that Groom's results in the gastrocnemius muscle for red cells and plasma could be explained by the difference in the diffusion coefficients of red cells

and plasma. The red cells, being much larger, could be expected to behave with a near zero diffusion coefficient and be finally cleared after the proteins.

A preliminary examination (44) revealed that the different flows, representing different indicators, not only gave different shaped curves but, when subjected to indicator dilution analysis, provided different results for the measurement of the dilution parameters. When the flow was slow and diffusion dominated the dispersion, good agreement was obtained with the directly determined values of flow, mean transit time and volume. As the flow rate was increased, the errors in all three parameters became progressively larger. All the experiments were performed with the experimental tube placed in a groove cut in a detecting crystal. When a small mixing device was placed just before the detector to mix the flow stream across that section of the tube, the error in flow was largely reduced. The errors in mean transit time and volume were reduced but they were still significant. The reasons for these changes in dilution parameters were not altogether clear, although it was reasoned that the flow measurements were influenced by the manner in which indicator was distributed across the tube at the detecting point.

The work of Bate and co-workers in a straight tube was seen to be potentially important to indicator dilution studies *in vivo*. The results for flow and volume, when using the method *in vivo*, depend on the measurement of the area and first moment of the

concentration outflow curves. It was implied that variations of these parameters were possible in a laminar flow situation purely by indicator diffusion across the streamlines, and by different sampling techniques. Further, different shaped curves were obtained when differing amounts of diffusion were permitted. This could affect the calculations of capillary exchange and extravascular volumes that depend on curve shape, mentioned earlier. However, these results were of a preliminary nature. Only one indicator was used and that was in inorganic solution. Also, the effects of altering the flow system with a mixer had not been thoroughly investigated.

The work in the present thesis was begun in order to provide a theoretical basis for these results and to investigate further the influence of the indicator distribution on results obtained when using the indicator dilution theory and method. It was necessary also to investigate the dispersion of the different components of plasma, blood and red cell suspensions down a straight tube and through a model vascular bed. It was felt that not only could the relevance of this work to circulatory studies then be better assessed, but information could also be obtained on the way in which interacting particulate suspensions, such as blood, behave when flowing. There has been, in recent years, a proliferation of work on the pressure/flow (or viscometric) relationships of whole blood and red cell suspensions in vivo and in vitro. It seemed desirable to relate these studies to the dispersion of the constituents in flow. With this in mind, the investigation of dispersion in the present thesis was performed with

pressure and flow being simultaneously measured in some of the experiments in order to be able to calculate the viscosity of solutions or suspensions.

CHAPTER 2THEORY

Consider a liquid undergoing flow in the laminar regime down a long uniform and impermeable tube. It is wished to determine the flow parameters from the concentration against time outflow curves of an injected indicator. The indicator may be subject to a variety of states of mixing due to interlaminae diffusion and by formed elements, if any, in the liquid. An equation to cover all situations is too complex for a general solution and so the approach will be to consider the extreme situations of a well mixed system and a purely laminar system with no lateral mixing.

Well mixed flow

Consider a bolus of labelled solution injected into a tube and contained between two planes as represented in Fig. 2.1. It is assumed that during flow the bolus is efficiently mixed all the way down the tube to the observation point at O . The mixing may be due to turbulence or to indicator diffusion dominating the dispersion in a manner described by Taylor (37). What is required is to determine the flow rate and vessel volume from the recorded changes in indicator concentration with time when monitoring at O . By definition the flow, F , is given by

$$F = \pi a^2 \bar{u} \dots\dots\dots (1)$$

where a is the radius of the tube and $\bar{u}$ the mean velocity. Also,

$$q = \pi a^2 c_0 (x_2 - x_1)$$

where q is the total quantity of indicator injected, c_0 is the initial

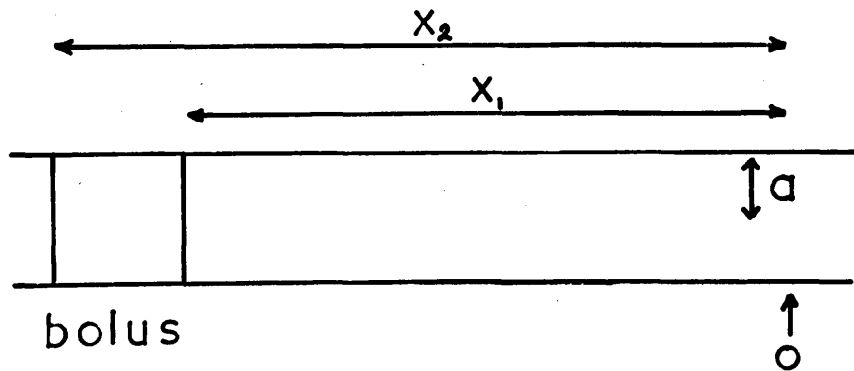


Fig. 2.1: Initial distribution of labelled solution injected as a bolus.

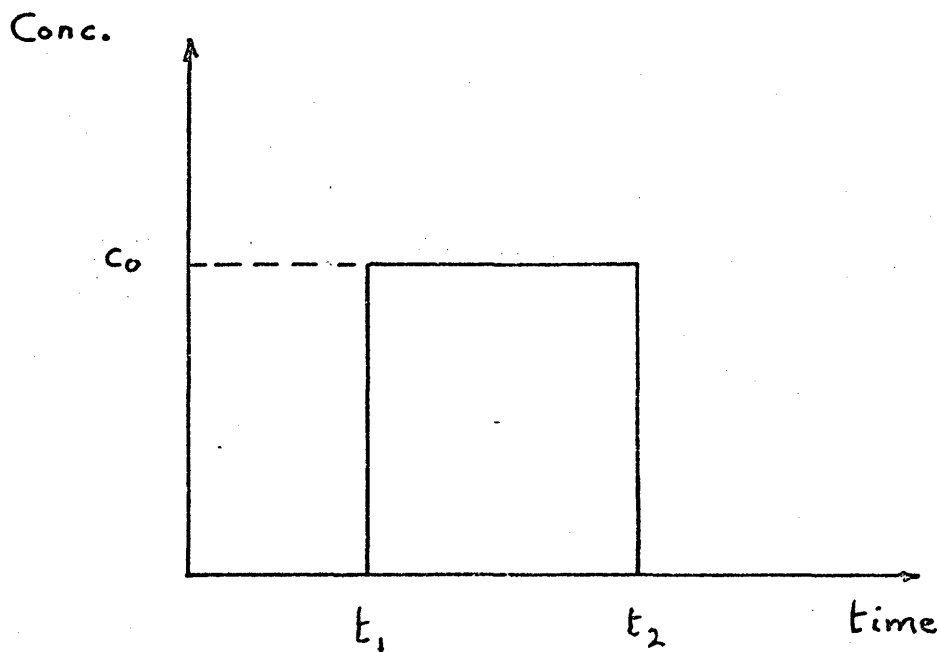


Fig. 2.2: Outflow characteristics during well mixed flow.

concentration of indicator, and x_2 and x_1 are the distances of trailing and leading edges of the bolus to the sampling point, respectively. The rate at which injected indicator flows past the monitoring point at 0 is given by

$$\frac{dq}{dt} = F c(t)$$

where $c(t)$ is the concentration of indicator at time t .

So,

$$dq = F c(t) dt \quad \dots\dots\dots (2)$$

$$\text{and} \quad q = \int F c(t) dt \quad \dots\dots\dots (3)$$

Therefore,

$$F = \frac{q}{\int c(t) dt} \quad \dots\dots\dots (4)$$

$$= \frac{\pi a^2 c_0 (x_2 - x_1)}{c_0 (t_2 - t_1)} \quad \dots\dots\dots (4a)$$

where t_1 is the arrival time of the leading edge of the bolus and t_2 is that of the trailing edge. Equation (4) is the well known formula for indicator dilution theory (3). It is assumed here that there is no spreading of the edges of the bolus as they move down the tube and the outflow characteristics appear as in fig. 2.2. So from equation (4a)

$$F = \pi a^2 \bar{u}$$

as in equation (1). As the denominator in equation (4) can be determined by calculating the area under the outflow curve and the numerator, the quantity of indicator injected, is known, the flow rate may be quickly calculated.

The mean transit time of an indicator has been defined in several ways but the definition adopted here is the one commonly accepted for indicator dilution work (3) and is given by

$$\bar{t} = \frac{n_1 t_1 + n_2 t_2 + n_3 t_3 + \dots}{n_1 + n_2 + n_3 + \dots} \quad (5)$$

where n_1 is the number of particles (in the fluid dynamic sense) of indicator arriving at t_1 , and so on. For the purposes here, a continuous rather than discrete distribution will give

$$\begin{aligned} \bar{t} &= \frac{\int F c(t) t dt}{\int F c(t) dt} \\ &= \frac{\int t c(t) dt}{\int c(t) dt} \quad (6) \end{aligned}$$

which for the well mixed system becomes

$$\bar{t} = \frac{c_o(t_2 - t_1)(t_1 + t_2)}{2 c_o(t_2 - t_1)} = \frac{(t_1 + t_2)}{2} \quad (7)$$

The volume of the system can be calculated from the formula (3)

$$V = F \bar{t} \quad (8)$$

using $t_1 = \frac{x_1}{\bar{u}}$ and $t_2 = \frac{x_2}{\bar{u}}$

$$V = \pi a^2 \bar{u} \frac{(x_1 + x_2)}{2\bar{u}} = \frac{\pi a^2}{2} (x_1 + x_2) \quad (9)$$

It should be noted that the volume measured is that from the mid point of the bolus to the mid point of the detector.

A new definition of mean transit time considered later, is given

by

$$\bar{t}_n = \frac{\int_{t_1}^{t_2} \frac{c(t)}{t} dt}{\int_{t_1}^{t_2} \frac{c(t)}{t} dt} \dots\dots\dots (10)$$

With the well mixed system equation (10) becomes

$$\bar{t}_n = \frac{\int_{t_1}^{t_2} dt}{\int_{t_1}^{t_2} \frac{dt}{t}} = \frac{(t_2 - t_1)}{\ln \frac{t_2}{t_1}} \dots\dots\dots (11)$$

If the bolus is much smaller than the distance from the bolus to the sampling point i.e. $(t_2 - t_1) \ll t_1$, putting $\Delta t = (t_2 - t_1)$, then

$$\bar{t}_n = \frac{\frac{\Delta t}{t}}{\frac{\Delta t}{t} - \frac{(\Delta t)^2}{2t_1^2}} = \frac{t_1}{1 - \frac{\Delta t}{2t_1}} \dots\dots\dots (12)$$

Expanding the denominator and ignoring high orders of $\frac{\Delta t}{2t_1}$, equation (12) becomes

$$\bar{t}_n = \frac{(t_1 + t_2)}{2}$$

the same as equation (7). Using

$$v_n = F \bar{t}_n \dots\dots\dots (8a)$$

the correct answer for volume can be obtained. It is clear, then, that use of either equation (6) or equation (10) for mean transit time with equation (4) for flow gives the correct value for volume provided the bolus is small.

System with a parabolic velocity profile

The way in which an indicator is distributed through a laminar

flow system has been treated in several ways. In general, these studies have been concerned with how catheters that are used to inject and sample indicators modify the shape of the concentration against time outflow curves from vascular beds. It has been shown that the shape of the catheter outflow curve depends on the type of injection used and on the sampling method used (14)(16). The bolus injection method will be considered here because it is felt that this method is the most important as far as circulation studies are concerned. The two sampling methods considered by Sheppard et al. (16) will also be used. They first pointed out the important difference between mean concentration and mean flux of indicator at outflow of a tube containing a liquid under laminar flow. By transillumination or by external monitoring of the indicator, the mean concentration of indicator taken across the tube is recorded. When the outflow is collected into a series of sampling containers, the velocity and concentration of indicator determines the rate at which indicator is delivered to the containers; the mean flux of indicator is then recorded. Mean flux sampling results in a higher peak and faster clearance of the outflow curve than mean concentration sampling. These two types of sampling were called mean cross sectional concentration and mean flow proportional concentration by Gonzalez-Fernandez (45). The analysis used here follows the mathematical form and method of this paper. An alternative derivation of the results is given elsewhere (46).

Consider a bolus of indicator dispersed by the parabolic velocity in a manner illustrated in Fig. 2.3. Consider also, a surface s that

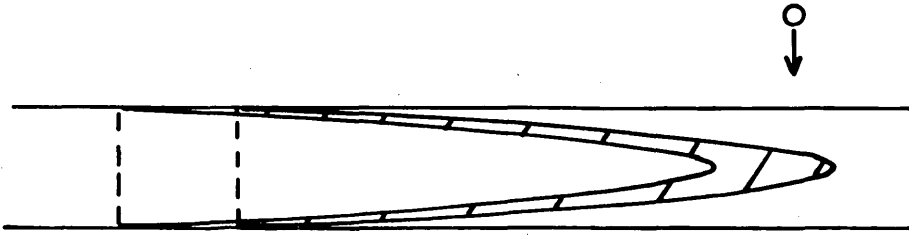


Fig. 2.3: Distribution of bolus during laminar flow.

has an area dA , a point distance r from its centre, a concentration $c(r,t)$ of indicator and a local liquid velocity $v(r,t)$ through the surface. The tube radius is a . The mean cross sectional concentration, $\bar{c}$, is then given by

$$\bar{c}(t) = \frac{\int_S c(r,t) dA}{\int_S dA} \dots\dots\dots (13)$$

and if $dA = 2\pi r dr$

$$\bar{c}(t) = \frac{2}{a^2} \int_0^a r c(r,t) dr \dots\dots\dots (14)$$

The mean flow proportional concentration, $\bar{c}^*$, is given by

$$\begin{aligned} \bar{c}^*(t) &= \frac{\int_S c(r,t) v(r,t) dA}{\int_S v(r,t) dA} = \frac{\int_S c(r,t) v(r,t) dA}{F(t)} \\ &= \frac{2\pi}{F} \int_0^a r c(r,t) v(r) dr \dots\dots\dots (15) \end{aligned}$$

Assuming the flow is laminar the velocity profile will be parabolic and is given by

$$v(r) = \frac{2F}{\pi R^4} (a^2 - r^2) \text{ for } (0 \leq r \leq a) \dots (16)$$

so that

$$r = \left[a^2 - \frac{v\pi R^4}{2F} \right]^{\frac{1}{2}}$$

Mean cross sectional concentration sampling

The mean cross sectional concentration at $x = 0$ is given by

$$\bar{c}(t) = \begin{cases} 0 & \text{..... for } 0 \leq t \leq t_1 \\ \frac{2}{a^2} \int_0^{r_1} r c(r, t) dr & \text{.... for } t_1 \leq t \leq t_2 \\ \frac{2}{a^2} \int_{r_2}^{r_1} r c(r, t) dr & \text{.... for } t_2 < t \end{cases}$$

$$\text{where } r_1 = \left[a^2 - \frac{x_1 \pi a^4}{2Ft} \right]^{\frac{1}{2}}, \quad r_2 = \left[a^2 - \frac{x_2 \pi a^4}{2Ft} \right]^{\frac{1}{2}},$$

$$t_1 = \frac{x_1}{v(0)} \quad \text{and} \quad t_2 = \frac{x_2}{v(0)}.$$

$v(0)$ is the velocity at $r = 0$, see equation (16).

Therefore,

$$\bar{c}(t) = \begin{cases} 0 & \text{..... for } 0 \leq t \leq t_1 \\ c_0 \left[1 - \frac{x_1 \pi a^2}{2Ft} \right] & \text{..... for } t_1 \leq t \leq t_2 \\ c_0 (x_2 - x_1) \frac{\pi a^2}{2FT} & \text{..... for } t_2 < t \end{cases}$$

where c_0 is the original concentration of indicator. The area under the outflow curve then becomes

$$\begin{aligned} \int \bar{c}(t) dt &= c_0 \int_{t_1}^{t_2} \left(1 - \frac{t_1}{t} \right) dt + c_0 \int_{t_2}^{\infty} (t_2 - t_1) \frac{dt}{t} \quad (17) \\ &= c_0 \left[t - t_1 \ln t \right]_{t_1}^{t_2} + (t_2 - t_1) \left[\ln t \right]_{t_2}^{\infty} \\ &= \infty \end{aligned}$$

so that substituting into the Stewart-Hamilton equation for flow,

(4), a zero value for flow is obtained. The mean transit time defined by equation (6) will be infinity, as multiplication of equation (17) by t does nothing to prevent it going to infinity. This situation arises physically because the indicator contained in laminae near to the wall is cleared very slowly, the laminae adjacent to the wall never being cleared, and no allowance is made for the difference in clearance of indicator in the sampling formula. Volume and flow cannot be calculated then, using these formulae.

Instead, if the alternative equation, equation 10, for mean transit time is used, volume can still be calculated.

$$\begin{aligned}
 V_n &= F \bar{t}_n \dots\dots\dots (18) \\
 &= \frac{q}{\int \bar{c}(t) dt} \cdot \frac{\int \bar{c}(t) dt}{\int \frac{\bar{c}(t)}{t} dt}
 \end{aligned}$$

Now, using equation (17),

$$\begin{aligned}
 \int \frac{\bar{c}(t) dt}{t} &= c_0 \int_{t_1}^{t_2} \left(1 - \frac{t_1}{t}\right) \frac{dt}{t} + c_0 \int_{t_2}^{\infty} (t_2 - t_1) \frac{dt}{t^2} \\
 &= c_0 \left[\log t + \frac{t_1}{t} \right]_{t_1}^{t_2} + c_0 (t_2 - t_1) \left[\frac{1}{t} \right]_{t_2}^{\infty} \\
 &= c_0 (\log t_2 - \log t_1) \\
 &= c_0 \log \frac{t_2}{t_1} = \log 1 + \frac{\Delta t}{t_1} \\
 &= c_0 \frac{(t_2 - t_1)}{t_1}
 \end{aligned}$$

Substituting for t_1 and t_2

$$\int \frac{\bar{c}(t)}{t} dt = \frac{c_0 (x_2 - x_1)}{x_1}$$

so that using equation (8a)

$$V_n = \frac{c_0 \pi a^2 (x_2 - x_1) x_1}{c_0 (x_2 - x_1)}$$

$$V_n = \pi a^2 x_1$$

Of course, the actual volume when measured by the bolus method is given by

$$V = c_0 \pi a^2 \frac{(x_1 + x_2)}{2}$$

but provided that the bolus length is small compared with the sampling distance, the error incurred is small. To summarise, although flow and $\bar{c}_{\text{new}}$ are in error because of the $\int \bar{c}(t)dt$ term, when they are multiplied to calculate the volume, approximately the right answer is obtained because the troublesome terms cancel out. Dividing $\bar{c}(t)$ by t has the effect of changing a diverging integral to a converging one.

Mean flow proportional concentration sampling

At $x = 0$,

$$0 \dots\dots\dots \text{for } 0 \leq t \leq t_1$$

$$\bar{c}^*(t) = \frac{c_0}{F} \int_0^{x_1} 2\pi r v(r) dr \dots\dots \text{for } t_1 \leq t \leq t_2$$

$$\frac{c_0}{F} \int_{x_2}^{x_1} 2\pi r v(r) dr \dots\dots \text{for } t_2 < t$$

$$\begin{aligned}
 & 0 \quad \dots\dots\dots \text{for } 0 \leq t \leq t_1 \\
 \bar{c}(t) &= c_0 \left[1 - \frac{x_2^2 \pi^2 a^4}{4F^2 t^2} \right] \dots\dots\dots \text{for } t_1 \leq t \leq t_2 \\
 & \frac{c_0 \pi^2 a^2}{4F^2 t^2} (x_2^2 - x_1^2) \dots\dots \text{for } t_2 < t
 \end{aligned}$$

The area under the flow sampled curve is given by

$$\begin{aligned}
 \int \bar{c}(t) dt &= c_0 \int_{t_1}^{t_2} \left(1 - \frac{t_1^2}{t^2} \right) dt + c_0 \int_{t_2}^{\infty} (t_2^2 - t_1^2) \frac{dt}{t^2} \\
 &= c_0 \left[t + \frac{t_1^2}{t} \right]_{t_1}^{t_2} - c_0 (t_2^2 - t_1^2) \left[\frac{1}{t} \right]_{t_2}^{\infty} \\
 &= 2 c_0 (t_1 - t_2) \\
 &= \frac{c_0 \pi a^2}{F} (x_1 - x_2)
 \end{aligned}$$

so that $F = \frac{q}{\int \bar{c}(t) dt}$ which is the Stewart-Hamilton equation.

For the volume measurement, again, $\int t \bar{c}(t) dt \rightarrow \infty$ (as first indicated by Gonzalez-Fernandez (45)). This is because the $1/t$ term when integrated becomes $\log t$, which $\rightarrow \infty$ as $t \rightarrow \infty$. Instead, consider the formula

$$\begin{aligned}
 \bar{t}_n &= \frac{\int \bar{c}(t) dt}{\int \frac{\bar{c}(t)}{t} dt} \\
 \int \frac{\bar{c}(t)}{t} dt &= c_0 \int_{t_1}^{t_2} \left(\frac{1}{t} - \frac{t_1^2}{t^3} \right) dt + c_0 \int_{t_2}^{\infty} (t_2^2 - t_1^2) \frac{1}{t^3} dt \\
 &= c_0 (\log t_2 - \log t_1)
 \end{aligned}$$

so that,

$$\begin{aligned}
 \bar{t}_n &= \frac{c_o (2t_2 - 2t_1)}{c_o (\log t_2 - \log t_1)} \\
 &= \frac{2(t_2 - t_1)}{\log t_2/t_1} \\
 &= \frac{2 \Delta t}{\log \frac{t_1 + \Delta t}{t_1}} = \frac{2 \Delta t}{\log(1 + \frac{\Delta t}{t_1})} \\
 &\approx 2t_1 (1 + \frac{\Delta t}{2t_1}) \\
 &= 2t_1 + \Delta t \\
 &= t_1 + t_2 \\
 &= \frac{x_1 + x_2}{2} \cdot \frac{\pi a^2}{F}
 \end{aligned}$$

Using this in equation (8a), provides the correct answer for volume.

To summarise, the theoretical analysis shows that in the case of well mixed flow along the section, flow and volume can be calculated using the well known equations of Stewart and Hamilton. Volume may also be calculated using a newly defined mean transit time. For the parabolic flow system with bolus injection the results depend on the method of sampling. When using mean cross-sectional concentration sampling the Stewart-Hamilton formulae for flow and volume do not give correct estimates of those parameters. However, volume may still be calculated if the new mean transit time is used in conjunction with the Stewart-Hamilton formula for flow. With mean flow proportional concentration sampling the correct answer for flow will be obtained but

again, the new mean transit time has to be used in order to calculate the true volume.

Practical considerations

The two extreme dispersion situations have been analysed from the standpoint of the indicator dilution theory and the results presented in a readily examinable form. However, in the experimental investigation that follows the flow regime will be such that, usually, neither of these two extreme situations applies. When there are no formed elements present in the solutions, indicator diffusion alone will contribute to mixing (at Reynold's number much less than 1000). The contribution of indicator diffusion to mixing can be assessed, in part, using the criterion of Ananthakrishnan et al. (39). Their analytical requirement for diffusion to completely dominate the dispersion, the Taylor B situation, is given $\hat{\tau} > 0.8$, where $\hat{\tau} = \frac{Dt}{a^2}$ (see Introduction). This applied to flow after a fixed time but may be used when monitoring as a function of time by considering the value of $\hat{\tau}$ for the indicator first arriving at the detector (44). If $\hat{\tau} > 0.8$ for these indicator particles it can be assumed that diffusion has dominated the dispersion.

The value of the diffusion coefficient of iodide in 2N KCl, at 25°C, has been discussed by Bate et al. (43). They found that theoretical dispersion curves gave the best fit to their experimental results when a value of D equal to $2.39 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ was used. Using this value and assuming that the arrival time of indicator is determined by twice the mean velocity of flow (clearly an approximation when diffusion removes

indicator from the centre streamlines) flow rates of less than 0.9×10^{-2} ml/sec are required for diffusion to dominate the dispersion. Because of the approximation regarding arrival times this value for flow rate will tend to be too large by about 20%. Nevertheless, it will serve as a simple guide to show where diffusion dominated dispersion is to be expected with the low molecular weight indicators that are to be considered.

Experimental results using a high molecular weight indicator, human serum albumin, will also be presented. An accurate value of the diffusion coefficient in these experiments is not known. However, Bate (41) has arrived at a value of approximately 10^{-6} cm² sec⁻¹ from a consideration of diffusion data for proteins in aqueous solutions and a review of the theoretical concepts of indicator diffusion. The critical flow rate for diffusion dominance with serum albumin is therefore approximately 0.3×10^{-3} ml/sec.

From the dimensionless controlling parameter, $\frac{a^2 \bar{u}}{Dx_0}$, Bate et al. (43), it can be seen that with increasing flow rate the contribution of diffusion to dispersion will decrease. However, there is not a single analytical criterion for assessing when the effect of diffusion is negligible and this will not be attempted. It is clear, though, that fast flow rates may be obtained at which the dispersion will be predominantly produced by the velocity profile, diffusion playing only a small role.

The previous considerations can only be applied a priori to dispersion in inorganic solutions, though it may be safely assumed that dispersion in plasma will also be produced by the same mechanisms. With

whole blood it must be expected that the presence of the red and white cells will modify the dispersive mechanisms in some unknown manner. Because of the higher concentrations of red cells ($5 \times 10^6/\text{mm}^3$ c.f. $5 \times 10^3/\text{mm}$ for white cells) and their known effects on the viscometric properties of blood, they are likely to have the largest influence on the concentration against time outflow curves. In order to assess the relative effects of diffusion, velocity profile and red cells, some of the experiments of Bate et al. with ^{131}I in 2N KCl were first repeated, and extended. The dispersion of solutes in plasma and in whole blood were then examined. Finally, the red cells themselves were labelled to determine which factors are involved in their dispersion.

For each solution or suspension, experiments were performed over a wide range of flow rates and the indicator dilution parameters derived from the outflow dispersion curves, i.e. the concentration against time curves. The equations calculated in the previous two sections that applied to both well mixed and parabolic flow systems can be expected to apply over the whole flow range and be invariant to the degree of mixing produced either by diffusion, or red cell induced mixing. Such equations are those for F , $\bar{t}_n$ and V_n when mean flow proportional sampling, and V_n when mean cross sectional sampling is used.

The remaining equations give correct answers for the well mixed case, where diffusion dominates the dispersion, but tend to infinity when the flow is completely dominated by a parabolic velocity profile. These equations are those for $\bar{t}$ and V for mean flow proportional sampling and F , $\bar{t}$, $\bar{t}_n$ and V for mean cross sectional sampling. It is likely,

therefore, that at slow flows, where diffusion dominates the dispersion, these parameters will give values within the experimental error. As the flow rate is increased and the velocity profile becomes more dominant, larger and larger percentage errors in these parameters are to be expected.

CHAPTER 3

APPARATUS, METHODS & MATERIALS

Straight tube system

In order to investigate the theoretical predictions, an apparatus simulating a bolus injection system has been developed. A diagram of the apparatus is shown in Fig. 3.1. and a photograph in plate 1. It is basically similar to that used by Bate (41) but the method of pressure regulation has been adapted for increased accuracy. In all of the straight tube experiments the bolus length was 12.2 cms and the length from the leading edge of the bolus to the mid point of the sampling crystal was 101.8 cms. The ratio of lengths from the leading and trailing edges of the bolus to the crystal was therefore 0.893, the same as that used by Bate et al. (43).

Reservoir

The reservoir was made of perspex and was cylindrical with a volume of approximately 300 ml. There was a hole near the base into which the experimental tube was inserted, and an O-ring and detachable perspex disc were used to ensure a leak-proof fit. An O-ring was also used to make an air-tight fit between the top of the reservoir and the lid. A hole was drilled through the lid and a tight fitting bung with a long 1 mm bore glass tube inserted (see Fig. 3.1). This kept a reduced pressure above the liquid and so enabled a constant pressure head to be maintained throughout the course of an experiment. A

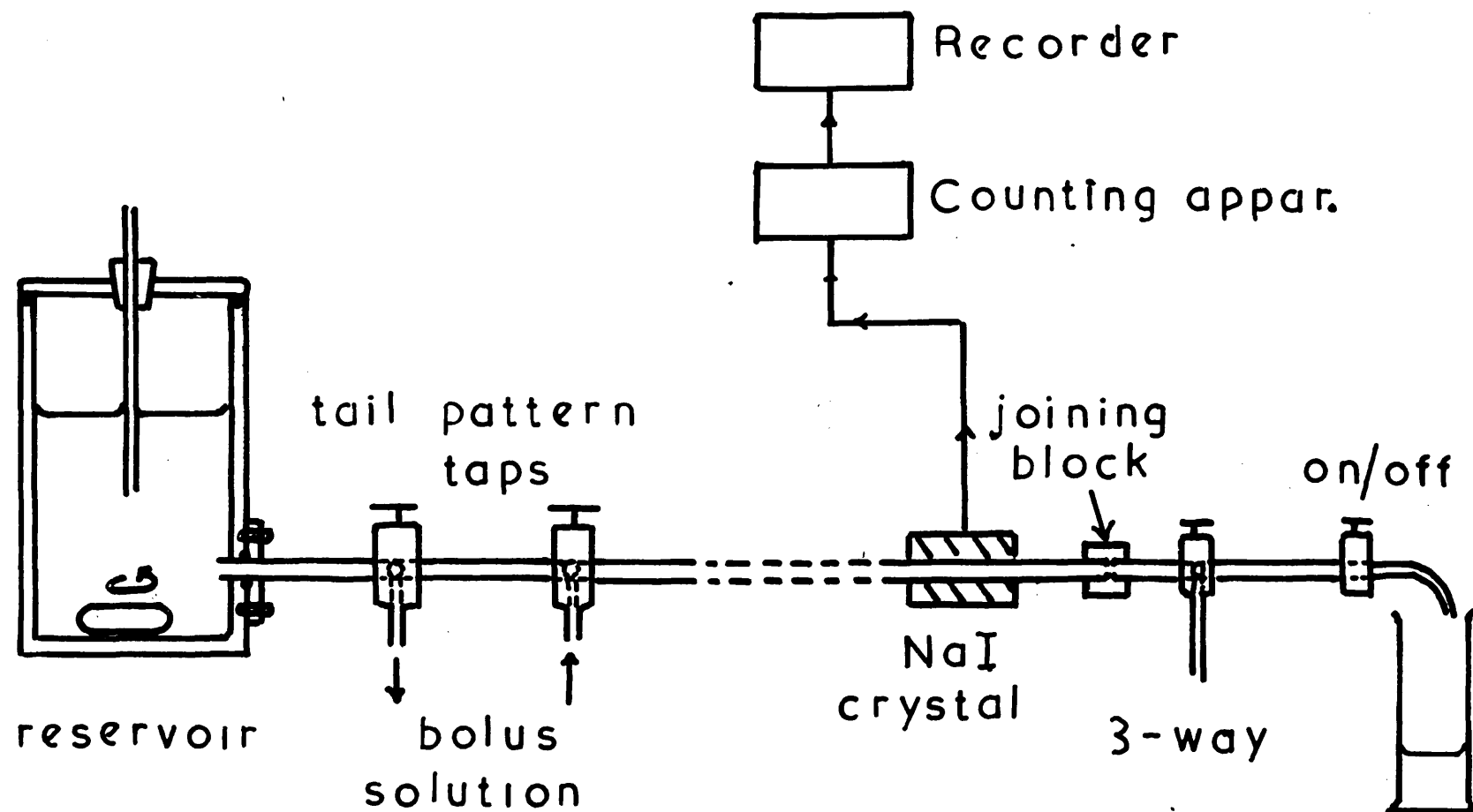


Fig. 3.1: Experimental apparatus.

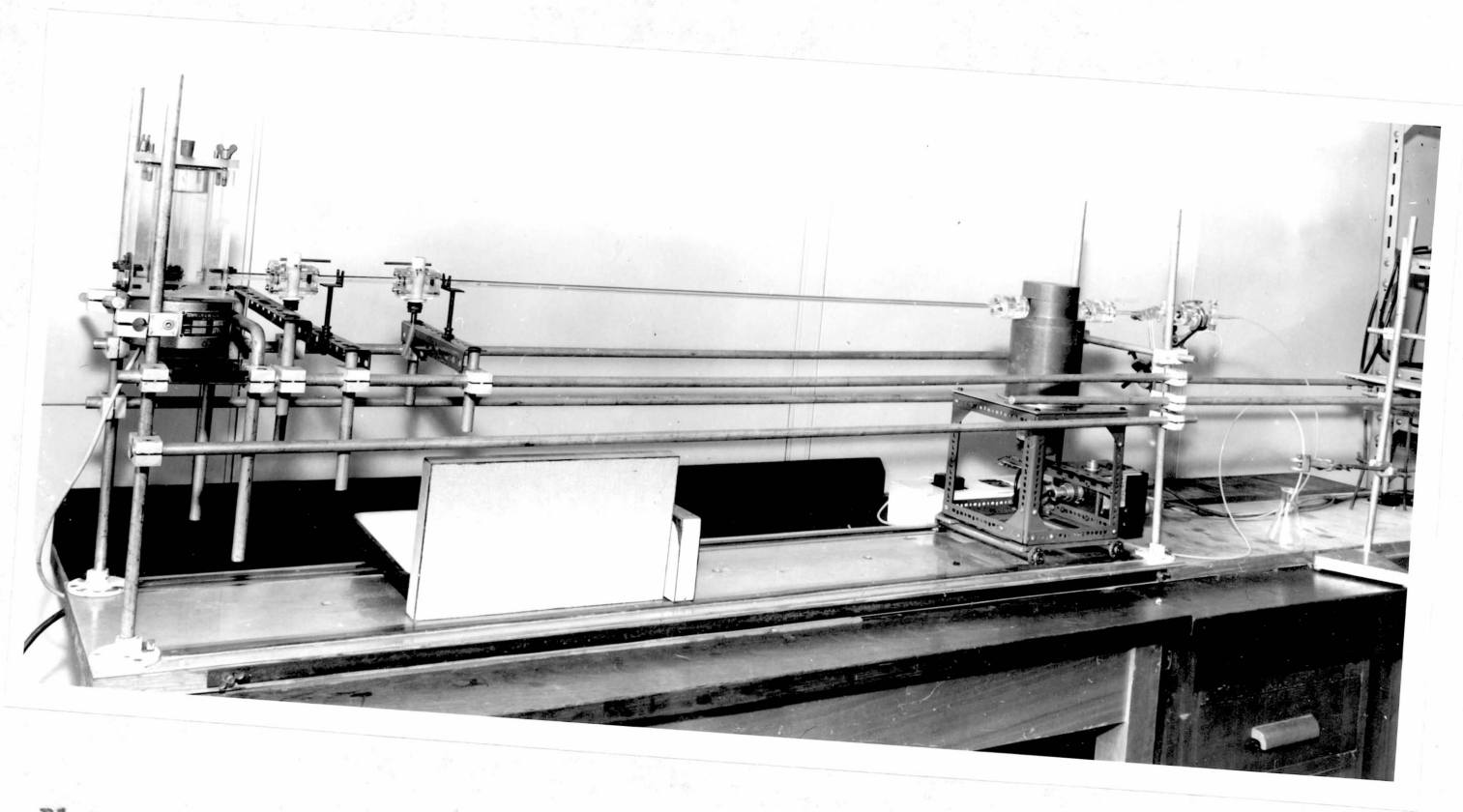


Plate 1: Photograph of the Straight Tube System.

magnetic stirrer was used in the reservoir to prevent red cell sedimentation.

Experimental tube

The tube through which the dispersion of indicators was studied was a 120 cm high precision bore tube (bore 2.50 mm). The manufacturers specified not greater than 1% variation in bore and this was confirmed by measuring the variation in length of a mercury thread with a traveling microscope at different points in the tube. At the downstream end of the tube a perspex block (fig. 3.2) was used to join the tube to the service and controlling glass tap system. The tube ends were ground flat and held in a light tight seal with O-rings and perspex discs. The latter section containing the glass taps was not of uniform bore but this was only of consequence to the viscometric data (see later) as all other measurements were concerned with dispersion in the straight tube section.

Tail pattern taps

The tail pattern taps used for introducing the radioactive solutions as a bolus injection are illustrated in fig. 3.3. Essentially they consisted of a spring loaded close fitting, teflon centre in a perspex block. A small amount of grease was applied for smooth operation. The holes through the taps were drilled to correspond as closely as possible to the bore of the experimental tube. In one position liquid could be injected from the underneath of one tap, into the region between the taps, and out of the bottom of the other tap. By turning

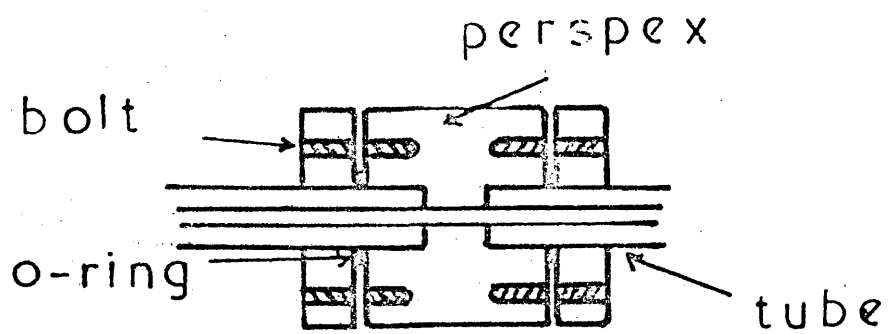


Fig. 3.2: Perspex joining block.

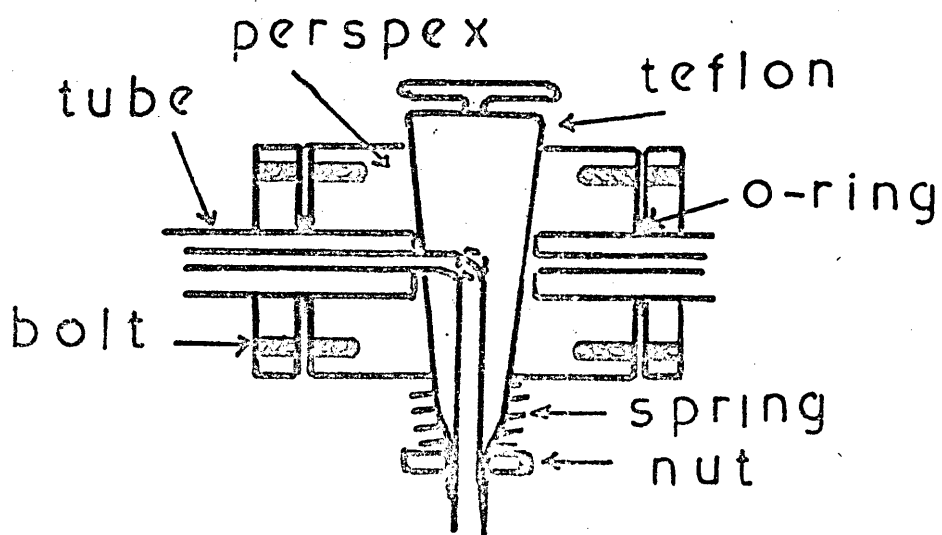


Fig. 3.3: Tail pattern taps.

the taps through right angles, the bolus was ready for delivery into the flow stream. The tubes on each side of the taps were held flush against the perspex, in line with the 2.50 mm holes, with O-rings and perspex plates.

Pressure regulation and determination

For the viscometric determinations it was required to accurately measure and control the driving pressure across the apparatus. The height of the reservoir alone was insufficient to supply the range of pressures and flows required, and so a negative pressure was applied by varying the height of a 2.5 mm bore, 2 metres long, catheter attached to the outflow end of the apparatus. The effective pressure difference in cms of solution could then be measured directly with a metre rule. Knowing the specific gravity (determined with a hydrometer) the effective hydrostatic pressure could be calculated. This method provided a convenient and relatively accurate method of measuring and controlling the pressure, the accuracy being determined by the accuracy to which the rule could be read.

Direct flow rate, Q , and volume, V_d , determination

An accurate measurement for the flow rate, Q , through the system was obtained for each experiment by collecting the liquid displaced in a pre-weighed conical flask or beaker. The volume flow rate was then given by dividing the weight difference by the product of the specific gravity and duration of the experiment. The duration of the experiment was determined from the recorder charts, the chart speed

being known and an event recorder signalling the beginning and end of the experiment. The actual volume, V_a , that the indicator dilution method measures is that between the mid-points of bolus and detecting crystal (see theory). The distance between the mid points could be measured to less than 1% error with a metre rule, and by multiplying by the cross sectional area the volume could be accurately determined. For all of the straight tube experiments in this thesis this volume was 5.30 ml.

Viscometric determinations

The straight tube system was not of uniform bore throughout its length. This precluded the direct calculation of viscosity from Poiseuilles equation. The approach used was therefore to first calibrate the apparatus by determining the (physiologically normal) saline pressure to flow ratio, R_s . Similar ratios for plasma, R_p , and red cell suspensions, R_b , were then determined over a range of flow rates. The relative viscosities, r , of plasma and red cell suspensions were then given by the ratios $\frac{R_p}{R_s}$ and $\frac{R_b}{R_s}$ respectively.

Model vascular bed system

The particular model used was a glass cylinder packed with different diameter glass beads, see fig. 3.4. The two ends of a 25 ml glass pipette were cut off at approximately 6 cms from the centre bulb. The entrance on one side of the bulb was then heated and narrowed such that a 3 mm glass bead could not pass out of that side. 3 mm beads were then poured into the bulb from the open side so that approximately 1/3 of the volume

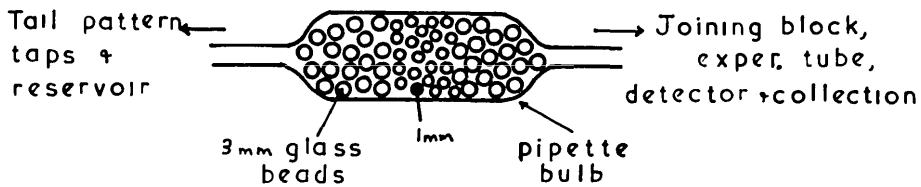


Fig. 3.4: Model vascular bed.

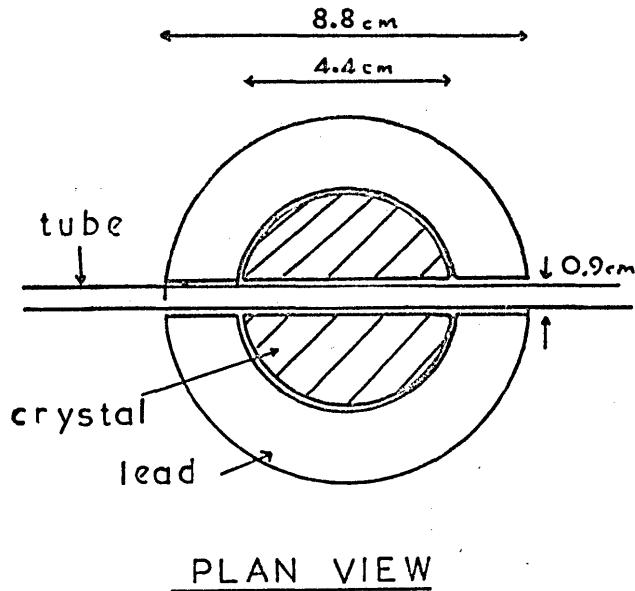


Fig. 3.5: NaI detecting crystal.

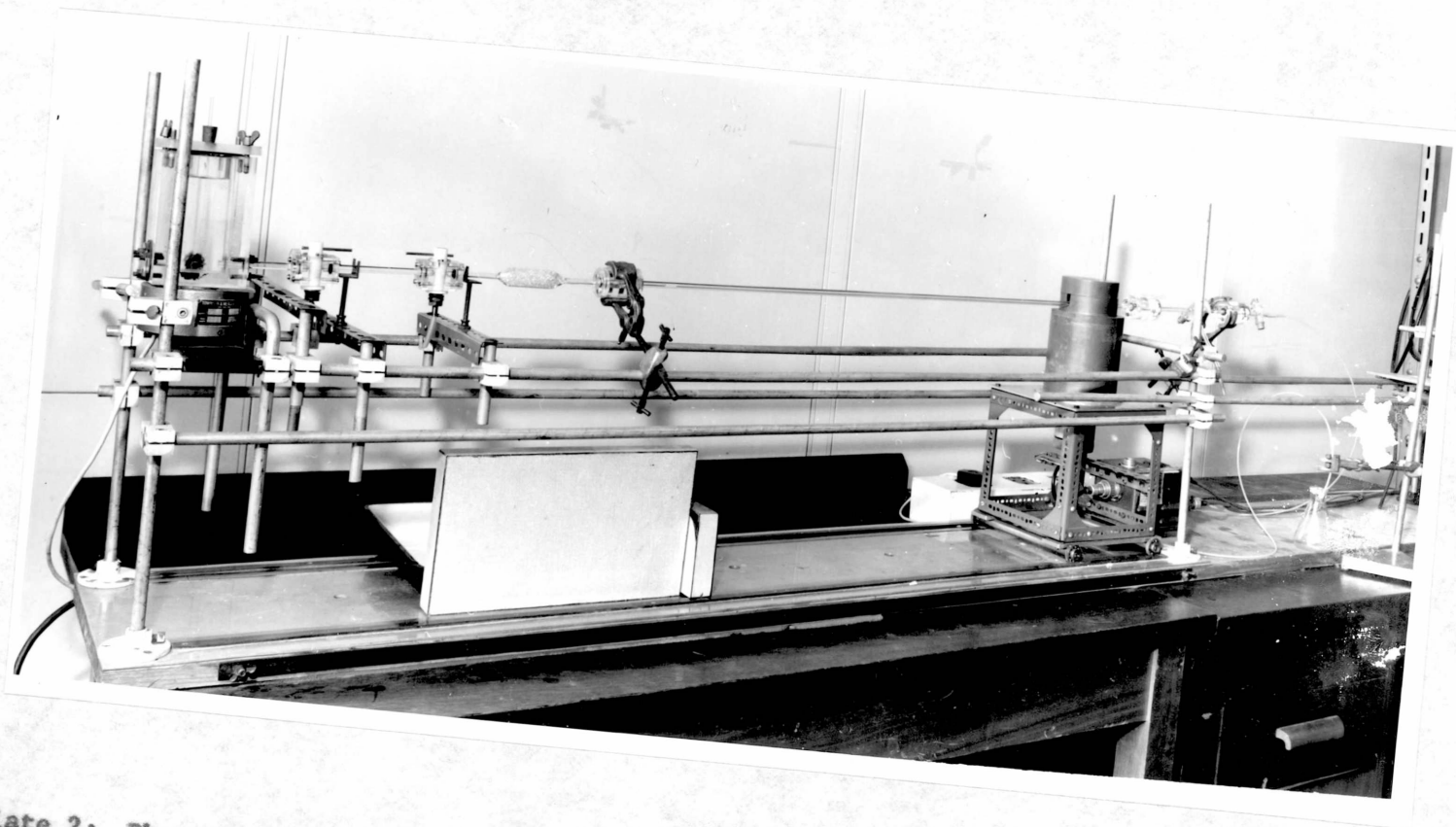


Plate 2: Photograph of the Model Vascular Bed System.

was occupied. 1.5 mm beads were then poured on top of these so that 2/3 of the bulb was full. The remaining volume was then filled with 3 mm beads and the end narrowed to contain all the beads within the bulb. Subsequently, the bulb filled with glass beads will be referred to as the model bed section. This section was then joined to the bolus injection taps at one side and the experimental tube at the other with perspex blocks and O-rings, as previously described^(see Plate 2). The bore of the pipette tube did not exactly mate with that of the high precision glass tube, but this was considered inconsequential for the present experiments. Inconsistencies at these joints could only minimize the effects that were to be examined by inducing mixing across the tube section at the joints. The length of precision bore tube at the detection end of the apparatus was 82.2 cms (measured to the mid point of the detecting crystal). This represents the longest length that could conveniently be used.

By adding the volume of the high precision tube sections to that of the glass bead filled bulb, the volume of the whole model could be found. The volume of the tube sections were obtained by multiplying the lengths used by the cross sectional area and was calculated to be 4.33 ml. The volume of the bulb was found by weighing the volume of water required to completely fill it. This was done five times and the mean volume so found was 9.09 ± 0.18 ($\pm$ S.D.). The total volume of the model system was therefore calculated as 13.4 ml.

All experiments with the model vascular bed were done without a mixer before the detector.

Detection, counting & recording apparatus

Fig. 3.5 is a diagram illustrating the geometry of the NaI scintillation crystal that was used to monitor the radiation within the experimental tube. The diameter of the crystal was 4.45 cms and the depth 2.4 cms. There was a rectangular channel 0.9 cms wide and 1.7 cms deep for accurate and reproducible positioning of the experimental tube. The crystal was surrounded by 2.2 cm thick lead shielding to increase its sensitivity to radiation from within the tube.

The scintillation crystal was attached to an EMI photomultiplier (IDL 663A), the output of which was fed to a pulse discriminating and counting apparatus, fig. 3.6. Most of the experiments were performed with a Nuclear Enterprises stabilised EHT supply (NE 5307), non-overloading linear pulse amplifier (NE 5202), single channel differential pulse height selector (NE 5102) and ratemeter (NE 5401). In some experiments, however, the crystal and photomultiplier used were connected to a J & P Engineering rate meter unit (MS 310). All recording was done with a Leeds Northrup high speed potentiometric pen recorder (Speedomax G) which had a response time of 0.25 secs for 90% of the full scale deflection.

Calibration of monitoring apparatus

The calibration of the apparatus was performed using a pulse generator and the digital counting system in the apparatus, and by measurement of different dilutions of radioactive solutions in the experimental tube. The J & P Engineering ratemeter unit was found

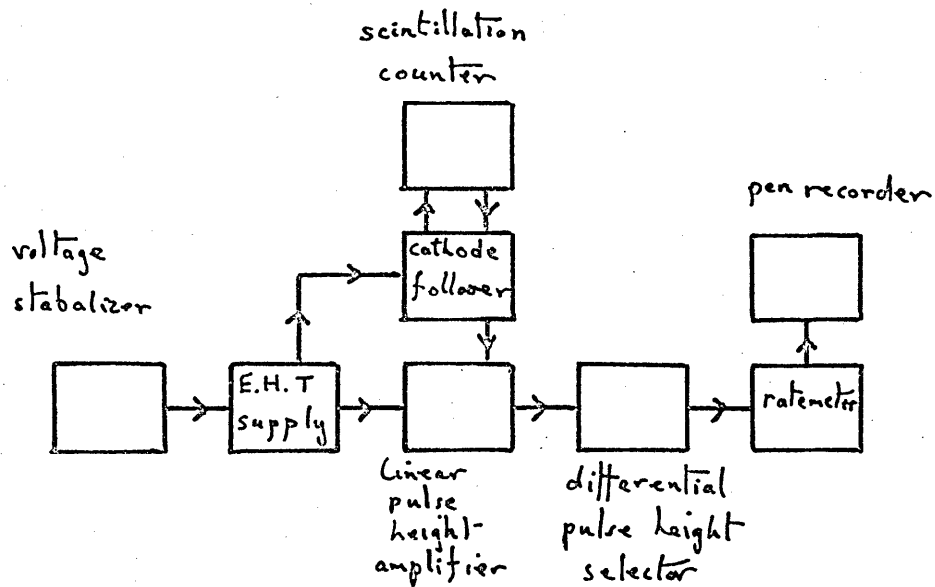


Fig. 3.6: Block diagram of counting apparatus.

to be linear on all ranges used and already accurately calibrated. However, the Nuclear Enterprises system, while being linear within each range, required the use of conversion factors when switching from range to range. For 3K $\rightarrow$ 1K c/s the factor was 2.9; from 10K $\rightarrow$ 3K it was 11.9. All the measurements were made within these ranges, which represented a convenient balance between the opposing needs of radiological safety and statistical precision.

Sampling systems

When the experimental tube was positioned in the detecting crystal the sampling system was equivalent to mean cross-sectional concentration sampling (see Theory). It was also desired to have a sampling system that monitored mean flow proportional concentration sampling. The usual method of doing this is to collect the outflow as serial samples in cups on a rotating drum. This method was considered unsuitable for the present apparatus and instead a mixer was inserted just before the detecting crystal to mix the indicator across the cross section of the tube at detection. That these two methods give the same results can be seen by inspection of the theory and reference (46). Basically, the rotating drum method mixes the samples after collection and the mixer ^{does so} at detection. The type of mixer adopted consisted of an approximately 1.5 cm spiral of plastic tubing that was positioned in the tube with the spiral extending along the line of the flow. Other types of mixer were tried, such as split tube mixing chambers, but these were found unsuitable because

they either did not mix properly or they interfered with the flow system too drastically.

Temperature control

The temperature of the solutions was always determined by the room temperature, which was controlled using an electric convector. It was found that in this way the temperature could be maintained at $\pm 1^{\circ}\text{C.}$ of the desired temperature throughout the course of the experiments. The experiments using potassium chloride solution were carried out at 25°C. and all the others between 18°C. and 21°C.

Experimental procedure

After washing the apparatus with suitable detergents and distilled water (with red cell suspensions and plasma, normal saline was also used), a final wash was performed with the liquid under examination. An initial concentration reading was then taken of the radioactively labelled solution in the experimental tube. The apparatus was again washed out with the solution. The reservoir was then filled up and solution drawn through the whole apparatus, care being taken to eliminate bubbles. After the solution had been flowing for several minutes the flow was stopped and the section of apparatus between the tail pattern taps was evacuated. Radioactively labelled solution was then introduced into this section with care again being taken to eliminate bubbles. The tail pattern taps were then turned through 45° so that a bolus of radioactive solution was trapped between the taps. The recorder was switched on, an appropriate time constant selected, the

Illustrations. The blocks of the illustrations may be obtained from The University Press, Cambridge, who will quote the price on application.

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THE DISPERSION OF SOLUTES DURING BLOOD FLOW THROUGH A STRAIGHT TUBE

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SUMMARY

1. The dispersion of solutes in plasma and blood has been studied by observing the clearance at a point down-stream of a bolus of labelled solution by the steady flow of unlabelled solution.

2. With a low molecular weight solute the clearance curve is qualitatively similar in 2N-KCl solution, plasma and whole blood.

3. The dispersion of a high molecular weight protein, [¹³¹I]HSA, in plasma is dominated by the velocity profile of the flow.

4. The clearance curves for HSA in whole blood are similar to those of plasma, except that the red blood cells speed the clearance of protein in the tail region of the curve.

5. An analysis of the data suggests that the dispersion of solutes during blood flow through a straight tube can be attributed to the combined effects of solute diffusion and laminar convection. There is no evidence that induced motion of the erythrocytes produces significant mixing, in the bulk of the flow.

INTRODUCTION

While there have been many studies of the factors which determine the rate of blood flow in the mammalian circulation, less attention has been given to the problem of how solutes are dispersed in blood during flow. This is a factor in the supply of metabolites, the deposition of solutes and thrombus formation at vessel walls, and in the practical problem of measuring flow and vessel volumes by the indicator dilution technique.

The simplest situation is to consider solute dispersion to be due to the combined effects of solute diffusion and laminar convection by a parabolic velocity profile. Bate, Rowlands & Sirs (1973), extending the analysis first proposed by Taylor (1953), showed that the dispersion during flow through a tube was determined by a parameter $a^2\bar{u}/Dx$, where D is the diffusion coefficient, a the tube radius, $\bar{u}$ the mean velocity and x the distance

between injection and sampling points. Different distributions of solute concentrations across and along the tube are obtained with changes of any of these factors. Various investigators have utilized these dispersive mechanisms to explain *in vivo* observations (Lassen & Crone, 1970; Korner, 1961; Norwich & Zelin, 1970; Baines, Gottschalk & Lassiter, 1968).

It is surprising, however, to find that all the analytical work on combined laminar and diffusive dispersion in straight tubes have been made with simple inorganic solutions. The implications of the results have been extrapolated to whole blood, despite the non-Newtonian and heterogeneous nature of blood flow. In this paper it is proposed to re-examine the model of Bate *et al.* (1973) for various solutes in plasma and whole blood during flow down a straight tube. Particularly, it seemed necessary to examine the possibility of mixing induced by the rolling and tumbling of the erythrocytes (Bloch, 1962; Wells & Schmid-Schönbein, 1969; Goldsmith & Marlow, 1972). This study also provides further information on the nature of the velocity profile in blood flow.

METHODS

The dispersion of solutes in various solutions during flow down a tube has been studied using the technique of Bate, Lane & Sirs (1972). The principle is the same as the measurement of flow by the indicator-dilution method, in which a bolus of well mixed indicator is rapidly formed by injection and the clearance of the bolus observed at a point downstream. The shape and nature of these clearance curves is dependent on the factors involved in dispersing the indicator during flow, as discussed by Bate *et al.* (1972). The sudden-injection bolus was simulated by introducing solutions, containing radioactively labelled solutes, into a length of precision bore tubing between two tail-pattern taps. The remainder of the flow system, comprising a supply reservoir and flow-tube, were filled with a similar solution without the radioactive label. Flow was started by opening the taps and the clearance of radioactive indicator past the observation point was monitored with a NaI scintillation detector. The output of the detector was taken to a ratemeter and the clearance curves, of indicator concentration with time, monitored on a fast potentiometric recorder. Precision bore glass tubing, of 2.5 mm diameter, was utilized for the observation tube. The length of the bolus was 12.2 cm and the distance from the mid-point of the bolus to the observation point was 108 cm. The flow rate was varied by altering the driving pressure and by placing a constriction just before the tube outlet. The flow rate was accurately measured by collecting the solution flowing from the outlet over a measured time interval. The range of flow rates used was such that the Reynold's number did not exceed 120. A value of the order of 1000 would be required for turbulence.

A variety of solutes and solutions have been studied. The situation of diffusion superimposed on convective flow was first studied using a solution of 2 N-KCl containing KI 1 mg/ml. The labelled solution, at exactly the same concentrations, contained ^{131}I . Similar experiments were then made with ^{86}Rb and ^{131}I HSA in plasma. The radioactive labels were obtained from the Radiochemical Centre, Amersham. The plasma was separated from fresh pig's blood by spinning in a centrifuge at 1000 g for 45 min. The pig's blood was collected from an abattoir using 6 i.u. heparin

per ml. blood to prevent coagulation. Finally measurements were undertaken using whole pig's blood with ^{86}Rb and ^{131}I HSA. Less than 0.2 ml. of the labelled solution was added to 60 ml. blood or plasma. Both the plasma and whole blood were used within 24 hr of collection, and were stored at 4° C between the time of collection and the experiment. All the experiments were made at room temperature, between 18 and 25 °C. A microscopic examination of the whole blood was made before and at the completion of each experiment to ensure the absence of crenation.

RESULTS

Preliminary experiments were undertaken of the dispersion of iodide ion (^{131}I) in 2N-KCl, and results obtained were in accordance with the data of Bate *et al.* (1973). These were followed by a study of the dispersion of ^{86}Rb in plasma. The output curves of ^{86}Rb in plasma are illustrated in

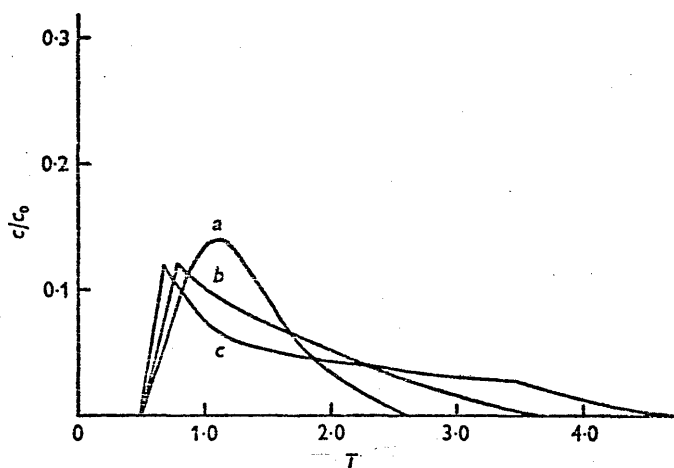


Fig. 1. Output curves of $^{86}\text{RbCl}$ in pig plasma. The continuously recorded concentration is divided by the initial bolus concentration on the ordinate. On the abscissa a dimensionless parameter $T = \bar{u}/\bar{x} t$ is used. Measurements were made at a temperature of 22° C at flow rates (a) 2.39×10^{-2} ml./sec, (b) 8.79×10^{-2} ml./sec, (c) 23.0×10^{-2} ml./sec.

Fig. 1. The observed concentration, c , is divided by the initial concentration of the bolus, c_0 , and plotted against T , where T is $\bar{u}t/\bar{x}$, t the time elapsed from the onset of flow and $\bar{x}$ the distance from the middle of the bolus to the observation point. The curves in Fig. 1 are qualitatively similar to those of ^{131}I in 2 N-KCl. The small differences from the data published by Bate *et al.* for ^{131}I in 2 N-KCl can be attributed to the ^{86}Rb ion having a higher diffusion coefficient than ^{131}I , though this was compensated to some extent by the plasma experiments being made at 18–20° C, compared to 25° C with the KCl solution. For slow flow rates, where diffusion dominates the dispersion, the initial slope is slower, the

curves rise to a high peak and are cleared early. They are centred about the point $T = 1$. As the flow is increased the curves become more dispersed along the T axis. At fast flow rates the first indicator arrived at $T \simeq 0.5$, after travelling at twice the mean velocity, rises to a relatively low peak and is cleared late. Here the velocity profile of the flow is dominating the dispersion of indicator.

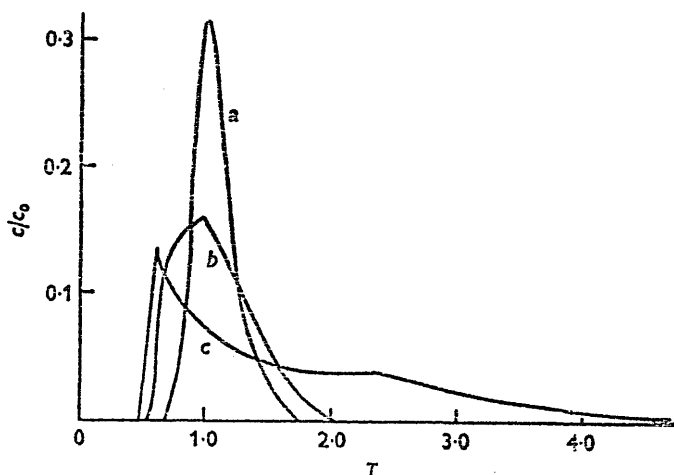


Fig. 2. The clearance curve of $^{86}\text{RbCl}$ in whole pig's blood at a haematocrit of 42%. Temperature 18°C and flow rates (a) 0.33×10^{-2} ml./sec, (b) 1.3×10^{-2} ml./sec, (c) 25.2×10^{-2} ml./sec.

Fig. 2 illustrates the dispersion of ^{86}Rb in whole pig's blood of haematocrit 42%. The packed cell haematocrit was measured in a microhaematocrit centrifuge operated at $13,000 g$ for 4 min. The experiments were all completed within 40 min of adding ^{86}Rb label to the cell suspension, so that a negligible amount of ^{86}Rb ion was taken up by the erythrocytes. The same qualitative features, as were found with KCl and plasma solutions, are present. It appears that with low molecular weight solutes, having a high diffusion coefficient, the dispersion in whole blood is in accord with the results of Bate *et al.* (1972).

The outflow curves for labelled protein, $[^{131}\text{I}]\text{HSA}$, in plasma are shown in Fig. 3. The scale of both the ordinate and abscissa are changed. The curves correspond to the fast flow curves of ^{86}Rb , because the protein has such a high molecular weight (67,000) and correspondingly low diffusion coefficient, in that the convection dominates in determining the dispersion. The indicator first arrives at $T \simeq 0.5$, consistent with a parabolic velocity profile; the curve then rises to a relatively low peak and the indicator is cleared much later than with ^{86}Rb . The curves still show some dependence

on the velocity of flow in the tail region of the curve. Thus, even with a high molecular weight protein, diffusion can still influence the dispersion processes.

The output curves for $[^{131}\text{I}]\text{HSA}$ in whole blood are illustrated in Fig. 4. Again the curves correspond to the fast flow curves of ^{86}Rb . The curves start at $T \approx 0.5$, indicating no large deviation from a parabolic velocity profile, are widely dispersed along the T axis and are cleared relatively late. There is again some dependence of the dispersion on the velocity of flow in the tail region of the curves.

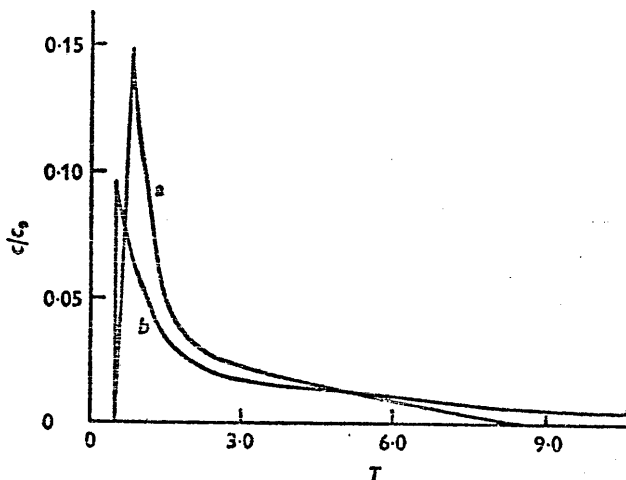


Fig. 3. Dispersion of $[^{131}\text{I}]\text{HSA}$ in pig plasma. Note change of scale on ordinate and abscissa. Temperature 19°C ; flow rate (a) 4.7×10^{-2} ml./sec, (b) 42.2×10^{-2} ml./sec.

A direct comparison of the experimental curves for inorganic solutions, plasma and whole blood, using the theoretical analysis of Bate *et al.*, is limited by the lack of accurate data for the diffusion coefficients of the indicators under the experimental conditions used. An alternative method of comparing these results is to use the mean transit time, $\bar{t}$, defined by

$$\bar{t} = \frac{\int_0^\infty ct \, dt}{\int_0^\infty c \, dt}$$

as used in indicator dilution studies. This parameter is dependent on the degree of dispersion and shape of the outflow curves. Under well mixed conditions, such as pertain when diffusion predominates in determining the dispersion, the value of $\bar{t}$ is $\bar{x}/\bar{u}$. As laminar convection progressively becomes the determining factor, $\bar{t}$ becomes larger (Lane & Sirs, 1974).

The mean transit times of the low molecular weight solutes were determined directly with a planimeter. This method did not prove sufficiently accurate however, for concentration against time curves, when the dispersion time was long. In these circumstances, an accurate value of $\bar{t}$ was

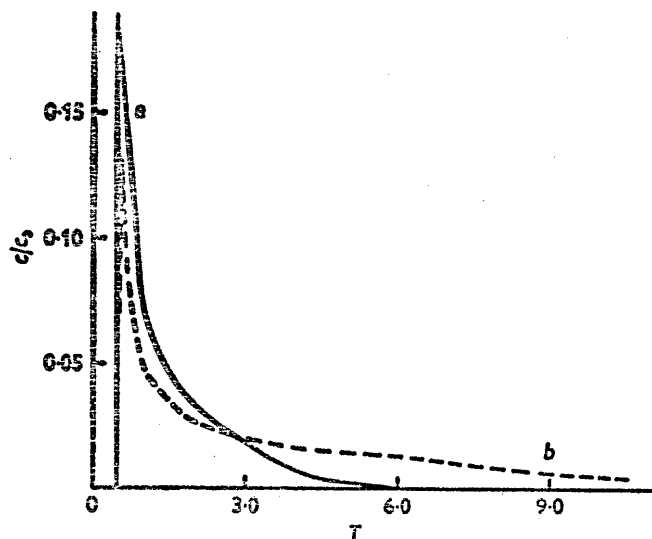


Fig. 4. The clearance of $[^{131}\text{I}]\text{HSA}$ in whole pig's blood at a haematocrit of 45 %. Temperature 20°C ; flowrate (a) 2.54×10^{-2} ml./sec, (b) 31.8×10^{-2} ml./sec.

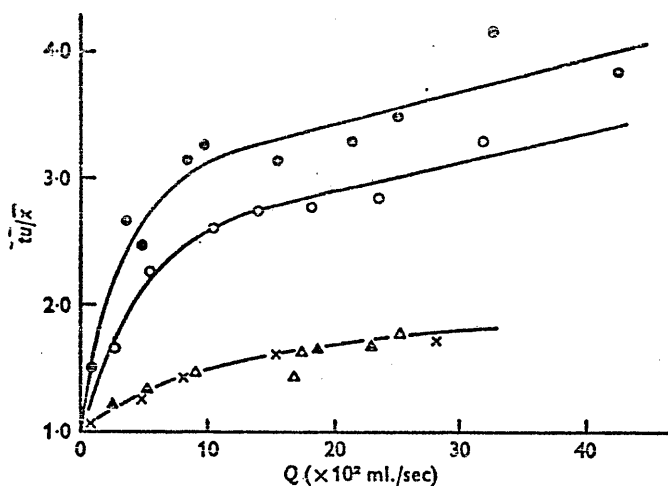


Fig. 5. Variation of mean transit time with flow rate, Q . The mean transit time is expressed in units of $\bar{t}/\bar{x}$. $\bullet$ $[^{131}\text{I}]\text{HSA}$ in plasma; $\circ$ $[^{131}\text{I}]\text{HSA}$ in whole blood, haematocrit 45 %; $\times$ ^{131}I in 2N-KCl; Δ $^{86}\text{RbCl}$ in plasma; $\triangle$ $^{86}\text{RbCl}$ in whole blood, haematocrit 42 %.

measured by using curves of $(c/c_0) T$ against T . The values of $\bar{t}$, determined for all the different combinations of solute and solvent, at different flow rates are shown in Fig. 5. The mean transit times are plotted in terms of $\bar{t}\bar{u}/\bar{x}$, in order to facilitate a comparison of the different combinations. The crosses represent the mean transit times of the iodide (^{131}I) ion in 2 N-KCl. The slower the flow rate the more closely the points approach $\bar{t}\bar{u}/\bar{x} = 1$, and as the flow increases, the less influence diffusion has on the dispersion, the larger the mean transit time becomes. The filled and open triangles represent the mean transit times of ^{86}Rb in whole blood and plasma respectively. It can be seen that there is no difference between the dispersion of low molecular weight solutes in inorganic solution, plasma and whole blood. For clarity, not all the points have been included. The presence of red blood cells does not influence the dispersion in these circumstances.

The filled circles and top line are the results for the mean transit times using [^{131}I]HSA in plasma. Again, the flow velocity dependence of the dispersion of a protein is reflected in the curve. There is an initial large change in the mean transit time with flow rate, which then becomes more gradual. The mean transit times measured with a protein thus reflect the shape of the outflow curves.

The open circles and middle curve in Fig. 5 are the values of mean transit times obtained at different flow rates using [^{131}I]HSA in whole blood of haematocrit 42 %. In this case the presence of the red blood cells appears to increase the clearance of the protein, as indicated by lower values of $\bar{t}$ relative to plasma, at all flow rates. The difference between the clearance by red cells of high and low molecular weight solutes is due to the normally very slow diffusion of proteins to and from the stagnant fluid layer near the vessel wall. It is this effect which gives rise to the extremely long tail of the outflow curves of HSA in plasma. Low molecular weight solutes are able to diffuse faster from this stagnant layer region, as shown by the short tails of the ^{86}Rb curve, and so the presence of cells has a negligible effect on the clearance curve.

To obtain further evidence on the mechanism of this effect, an investigation was made of the influence of the haematocrit on the dispersion of labelled protein. The red blood cells were separated from plasma by centrifuging for 1 hr at 1000 *g*. The dispersion of [^{131}I]HSA in the separated plasma was measured at a flow rate of 1.5×10^{-1} ml./sec (shear rate $\approx 100 \text{ sec}^{-1}$). Red blood cells were then added to the plasma and the experiment repeated at the same flow rate at different haematocrits. For haematocrits of 0, 12, 23.5, and 51.5 %, mean transit times of 3.53, 3.12, 2.90, and 2.16 (in terms of $\bar{t}\bar{u}/\bar{x}$) respectively, were obtained. Up to a haematocrit of 23.5 % the concentration curves were characterized by a

progressive increase in clearance in the tail region. There is also an increase in the height of the curve, at high haematocrits, as indicated by the curve at 51.5% in Fig. 6. Comparing this curve with the ^{86}Rb curves, where indicator is mainly dispersed by diffusion, it can be seen that any possible contribution of the red blood cells to mixing in blood flow is still very small.

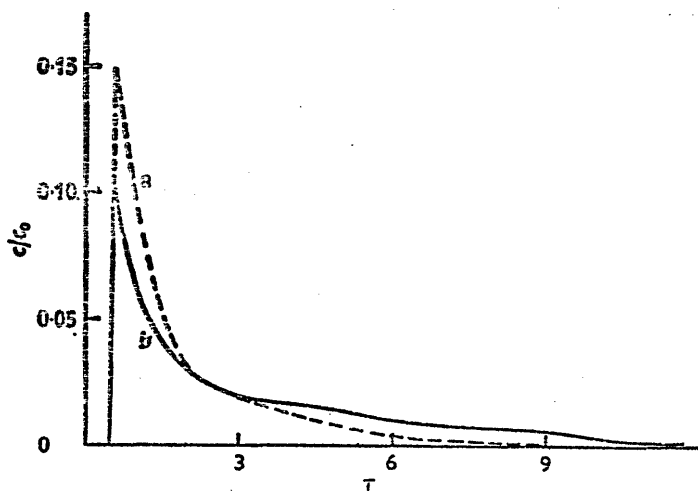


Fig. 6. Output curves of ^{131}I HSA in blood at haematocrits and flow rates of (a) 51.5% at 16.6×10^{-2} ml./sec, (b) 12% at 15.2×10^{-2} ml./sec. Temperature 21°C .

DISCUSSION

The results for both high and low molecular weight indicators provide additional evidence for the model of dispersion of solutes in fluid flow presented by Bate *et al.* (1972). With high molecular weight solutes in blood flow, however, the dispersion is modified in the tail region of the curves, there being an increased clearance of HSA relative to that of plasma. This may be due to an enhanced clearance of protein from the vessel wall or an alteration of the velocity profile, particularly at low shear rates. If the profile were blunted there should be a delay in arrival times of HSA in blood (Fig. 6), the delay increasing with increasing haematocrit. That this is not observed is not entirely unexpected, as small variations would be difficult to detect in this region of the curve with the present apparatus. Significantly faster clearance was observed in the tail region of the curves with increasing haematocrit and decreasing flow rate. This appears consistent with the change of velocity profile and increased velocity gradient near to the tube wall. But at slow flows the contribution of diffusion to the dispersion also increases, as shown with ^{131}I HSA in plasma, and the relative influence of diffusion and velocity profile is difficult to determine.

At low haematocrits, and at the flow rates used in the seexperiments, blood behaves like a Newtonian fluid and there will be an essentially parabolic velocity profile. The velocity profile remains the same or altered only slightly in the haematocrit range 0–24 %, yet there was a progressive increase in the rate of clearance of indicator in the tail region of the curve. This finding is consistent with red cells acting to enhance clearance of protein from the wall surface. In the region close to the surface the cells tend to cause mixing and to sweep protein away. Therefore for a given volume flow rate in a vessel of given diameter the presence of cells should reduce the thickness of the 'stagnant' layer at the wall.

Another point of importance is demonstrated in Fig. 5, in respect of the use of multiple indicator injection techniques to study vascular and extravascular compartments. A difference in mean transit times for different indicators is often taken as a measure of extravascular volumes. Striking intravascular separation of indicators of different molecular weights, are possible, for the same flow rate and vessel volume. That all the points in Fig. 5 do not fall on the line $\bar{u}l/\bar{x} = 1.0$, is also at variance with accepted indicator dilution theory. This discrepancy, of the indicator dilution theory in laminar flow, was noted by Bate & Sirs (1971) and Bate *et al.* (1973) and has recently been given a theoretical basis by Lane & Sirs (1974).

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tail pattern taps opened and flow begun by opening the on/off glass tap. Simultaneously, an event marker on the recorder was activated to signal the beginning of the experiment. The solution was collected for the duration of the experiment in a pre-weighted beaker. At the completion of the experiment flow was stopped, an event recorded on the chart paper, the collected liquid was weighed, and the recorded traces were replotted on graph paper in a manner described later, and analysed.

Calculation of flow rate (F), mean transit times ($\bar{t}$, $\bar{t}_n$) and vessel volumes (V, V_n) using the indicator dilution theory

A smooth curve was then drawn by eye through the output dispersion curves on the pen recorder chart. The curves were then replotted as indicator concentration divided by the bolus concentration, c/c_0 , against a dimensionless time parameter, $T = \frac{\bar{u}t}{x}$ (alternatively, $T = \frac{Qt}{V_a}$). x is the midpoint sampling distance. This method of plotting has been used by Sheppard (16) and Bate et al. (43) and facilitates direct comparison of the dispersion of indicators at different flow rates.

The Stewart-Hamilton equation for flow rate is given by $F = \frac{q}{\int \frac{c}{c_0} dt}$, where the symbols are explained in the Introduction. Dividing numerator and denominator on the right-hand side of the equation by c_0 gives $F = \frac{b}{\int \frac{c}{c_0} dt}$, where b is the volume of bolus used. As b is known, all that is required to calculate flow is the area under the c/c_0 against T curve and the appropriate $T \rightarrow t$ conversion factor.

Areas and mean transit times were obtained by planimetry (W.H. Stanley

& Co. Ltd), the mean transit times being obtained and always expressed in a dimensionless form in terms of T . When the dispersion curves with extremely long tails were measured the planimeter did not give reproducible results for mean transit time. An alternative method was to replot the curves as $c/c_0 \times T$ against T and to divide the area so obtained by the area under the c/c_0 against T curves. The newly defined mean transit time was determined by replotting as $c/c_0/T$ against T and dividing the area of the c/c_0 against T curve by the area so obtained.

Solutions, suspensions and indicators

Iodide (^{131}I) in KCl solution

Radioactive sodium iodide, ^{131}I , in weak thiosulphate solution was obtained from the Radiochemical Centre, Amersham, and was used in a 2N solution of potassium chloride which had 1 mg/ml potassium iodide added as a carrier. The KCl solution was made up in distilled water, and to approximately 60 ml (which was to be used as a bolus solution) not more than 0.3 ml of radioactive solution was added, giving a radioactive concentration 1 μ Ci/ml. This was the same solution that was used by Bate (41).

Blood, plasma and red cell suspensions

Whole blood was collected in an abattoir from freshly slaughtered pigs. Usually, approximately 1 litre was collected in a plastic bucket and stored in a plastic bottle. The anti-coagulant used was heparin (6 iu/ml blood) and provided the blood was stored at 4°C. until use (not longer than 30 hours) no precipitation of fibrin was obtained. In the experiments with the model vascular bed the blood was defibrinated

upon collection by stirring with glass rods and discarding the clot obtained.

Plasma was obtained by centrifuging the heparinised whole blood at 1000g for 45 mins and withdrawing the supernatant. Where different haematocrits of whole blood were required, the packed cells so obtained were added in the required quantities back to the plasma. Haematocrits were always determined by measuring the packed cell haematocrit on a Hawksley microhaematocrit centrifuge (12,000g for 5 mins).

For some labelled red cell experiments, suspensions of washed red cells in physiological saline were required. Whole blood was spun for 45 mins at 1000g and the supernatant discarded. The packed cells were then washed 3 times with twice the total red cell volume using saline, spinning each time for 45 mins at 1000g. The cells were then resuspended in the required amount of saline to give the haematocrit desired. Whenever red cell suspensions or whole blood were used, the cells were visually observed with a microscope for signs of crenation before and after each experiment.

Plasma indicators

⁸⁶Rb Cl (Radiochemical Centre) was used as a low molecular weight indicator (m.w. 86) in plasma and whole blood. The bolus solutions were prepared by adding not more than 0.1 ml of the radio-active solution (initial activity 1 m Ci/ml) to approximately 10 ml of solution or suspension.

Before using this with whole blood, consideration was given to

the possibility of red cell Rb uptake. Rubidium is in the same group of the Periodic Table of elements as potassium, which is concentrated intracellularly by the Na/K pump. Love & Burch (47) have shown that the uptake of ^{86}Rb in red cells is the same as potassium. Information on uptake was obtained from preliminary experiments performed with a view to using ^{86}Rb as a red cell label. A modified Krebs Ringer, used by Grahame-Smith & Everest (48) for facilitated ^{86}Rb uptake studies, was prepared as follows: 0.9% NaCl 1 litre; 1.22% CaCl_2 15 ml; 3.92% $\text{Mg SO}_4 \cdot 7 \text{H}_2\text{O}$ 10 ml; 0.1 M phosphate buffer pH 7.4 (17.8g $\text{Na}_2 \text{HPO}_4 \cdot 12\text{H}_2\text{O}$ + 20 ml N HCl, diluted to 1 litre) 210 ml; 100 mg of glucose was added to each 100 ml of this solution before use. Whole blood was then centrifuged at 1000g for 10 mins at 5°C . and plasma and the top layer of cells discarded. Ice cold Krebs Ringer was then used to wash to cells 2 times. The supernatant was discarded at each stage. Approximately an equal volume of Krebs Ringer was added to the packed cells and the suspension incubated for 45 mins at 36°C . The $^{86}\text{Rb Cl}$ solution was then added in the desired concentration (sufficient to produce a final concentration of 10 μ Ci/ml of blood) and the suspension incubated for up to 4 hours.

Samples were removed during incubation and the packed cell haematocrit determined. The % uptake of the cells was calculated by determining the total activity in 3 ml of the suspension and the activity in 1 ml of Plasma using a well counter. Plasma was separated by centrifugation for 10 mins at 1000g. The table below contains typical results of the % uptake of ^{86}Rb into a red cell suspension of 36% haematocrit.

Time after addition of ^{86}Rb (minutes)	30	70	120	180
% uptake by red cells	17	40	55	67

The uptake after 40 mins was therefore 20-25% in Krebs Ringer solution, at the concentration of ^{86}Rb solution used.

In the event, it was decided not to use ^{86}Rb as a red cell label. However, these studies showed that under the most favourable incubation conditions, uptake after 40 mins would not greatly exceed 20%. Tudhope & Wilson (49) have reported an ^{86}Rb uptake of 15% after 1 hour's incubation at 37°C. in whole blood. The indicator dilution experiments in this thesis were performed at 20°C. in whole blood and the ^{86}Rb suspension used immediately after preparation. The bolus concentration was measured upon completion of the experiments. Uptake at this stage was of no consequence as the amount of ^{86}Rb /unit volume in the samples was all that was required. In this way the longest time the red cells were in contact with Rb was 45 minutes and most experiments were completed within 20 minutes. Therefore, it was considered unlikely that ^{86}Rb uptake would greatly affect the results when using whole blood.

Human serum albumin labelled with ^{131}I , HSA - ^{131}I (Radiochemical Centre), was used as a high molecular weight (67,000) indicator in plasma and whole blood. No adverse reaction with pig's plasma or blood could be observed visually when HSA was added. No more than 0.3 ml of the HSA - ^{131}I solution (initial activity usually 1 mCi/ml, in saline) was

added to approximately 60 ml of solution or suspension to prepare the bolus solution. The labelled protein was stored at 4°C. and used within two weeks of delivery.

Sodium iodohippurate (^{131}I) was also used as a plasma label. It is a relatively low molecular weight (m.w. 309) indicator that is known to loosely bind to the plasma proteins, mainly serum albumin. Maher & Tauxe (50), in a study of the renal clearance of various substances in man, have reported around 70% plasma binding. This was determined using dialysis through cellophane bags and ultrafiltration through membranes that pass up to 10,000 m.w. molecules. Uptake by red cells is also a possibility, although because of the time course of the present experiments it is thought that this is unlikely to be a large influence at this time. The dispersion curves of iodohippurate (^{131}I) might therefore be expected to be more like the curves for HSA - ^{131}I in whole blood than ^{131}I in KCl or ^{86}Rb in blood. Sodium iodohippurate (^{131}I) was obtained from the Radiochemical Centre as a sterile aqueous solution containing 1% benzyl alcohol and was stored at 4°C. Final concentrations in the bolus did not exceed 20 $\mu\text{g/ml}$ of blood and had a radioactive concentration of about 1 $\mu\text{Ci/ml}$.

Red cell labelling

It was decided to use ^{51}Cr as a red cell label because this was convenient to use and readily available as $\text{Na}_2^{51}\text{CrO}_4$ solution (5 $\mu\text{Ci/ml}$ of saline) from the Radiochemical Centre. Sodium chromate contains

hexavalent chromium ions which readily enter the red cell (51). The ions are there converted to the trivalent state and bind to the globin part of the haemoglobin molecule. The labelling procedure followed that given by Mollison (52). A citrate phosphate buffer was prepared as follows: 3g trisodium citrate (monohydric), 0.015g sodium dihydrogen phosphate, and 0.2g of dextrose were made up to 100 ml with distilled water. The solution initially has a pH of 7.4, but the chromium uptake at this pH is not very satisfactory (52). The solution was heated for 40 mins at 110°C. in a sealed bottle until caramelisation was apparent. This indicated a pH 6.9 where better uptake is achieved. 100 mls of whole blood were then centrifuged, the white cells discarded and the plasma kept for possible red cell resuspension later. The cells were then washed two times in twice the total red cell volume with the buffer, and resuspended in a few mls of buffer. Up to $\frac{1}{2}$ mC of $\text{Na}_2^{51}\text{CrO}_4$ solution was then added and the red cells incubated for 20 mins at 36°C. in a water bath. The suspension was then centrifuged and the supernatant discarded. The packed red cells were then washed twice with saline as described above, and twice more with either saline or plasma, depending on which was to be the final suspending solution. The red cells were then suspended in saline or plasma and were ready for use. Using this method approximately 95% of the ^{51}Cr was taken up by the cells during the incubation period.

In the interpretation of the experimental results (see Discussion) it is necessary to know whether appreciable quantities of ^{51}Cr were eluted

from the red cells during the course of the experiments. It is well known that ^{51}Cr elutes from the red cells at a rate of approximately 1% per day in vivo, and that the elution rate is affected by the technique of labelling (52). Mollison & Veall (53) have also demonstrated an additional loss of 5% within the first 24 hrs after injection of labelled cells into the circulation which can be accounted for either by a small fraction of the Cr being loosely bound to the cells or by damage to the red cells during labelling (52).

It was considered that not only could Cr elute from the labelled red cells prior to use, but also it could be washed out of the cells during flow down the tube system by the unlabelled suspension. To cover both eventualities the outflow from a series of experiments performed on 7/11/73 (reported in table 4.13a and fig. 4.14) was pooled and tested. A sample from the bolus solution was also tested at completion of the experiments. The % elution of ^{51}Cr from the red cells was determined in a similar manner to the % uptake of ^{86}Rb into the cells. 3 ml of the suspension was taken and 2 ml of plasma removed after centrifugation. Both plasma and cells, and plasma samples were counted in a well counter. Counting was over 3 seconds using a convenient pulse height gate width on the scaler. The following results were obtained for the pooled outflow determination.

	mean	S.D.	No. of 3 sec. observations
Background	5.33	2.38	15
2 ml of plasma	6.09	1.87	11
3 ml of cells & plasma	1027	24.5	8

Similar results were obtained with red cells suspended in saline, labelled red cells left overnight at 4°C. before use, and the bolus solutions left for 4 hrs. It can be seen by inspection, that there is little, if any, ^{51}Cr in the plasma of the sample, almost all of it being attached to the red cell. It was concluded that elution of ^{51}Cr could be ignored in the interpretation of the ^{51}Cr labelled red cell studies in the straight tube system.

Red cell flexibility measurements

It has been demonstrated by Sirs (54) and Rampling & Sirs (55) that the initial rate of packing of red cells in a centrifugal field of 200g provides an unequivocal and quantitative measure of the erythrocyte flexibility. This method of flexibility measurement arose from the consideration that at physiological haematocrits red blood cells are in contact with each other. Rigid red cells at such concentrations are unable to pack into a small enough volume to give a meaningful measure of cell concentration and the rate of packing is extremely slow. With more flexible cells, a smaller packed cell volume and faster rate of packing are obtained. Red blood cell flexibility may be changed by alteration of the red cell membrane or the haemoglobin contained within the membrane. The present study will only be concerned with alterations in membrane flexibility caused by repeated washing and suspension in physiologically normal saline.

The rate of packing was measured in a microhaematocrit centrifuge (Hawksley) using the method of Sirs (54). A microhaematocrit tube was

filled with the blood sample and one end heat sealed. This was spun for 2 minutes on a centrifuge operated at 80 volts, instead of the usual 240 volts. This is equivalent to a centrifugal field of 200g. The apparent cell concentration was determined with a microhaematocrit reader. This process was repeated several times until the rate of change of haematocrit decreased. The operating voltage was then increased to 100 volts and the sample spun for a further two minutes. Finally, spinning at 240 volts for two minutes produced the packed cell haematocrit of the sample. A faster initial packing rate indicated more flexible red cells, if the haematocrits were approximately equal.

C H A P T E R 4

RESULTS - STRAIGHT TUBE SYSTEM

131I in 2N KCl solution

In table 4.1 are the results obtained using the indicator dilution formulae for flow rate, mean transit times and volumes, with iodide in 2N KCl solution. The experiments were performed with mean cross sectional sampling, i.e. without a mixer, and at a temperature of 25°C. The results were obtained over a five month period and illustrate the reproducibility of the method. In the first column is the true flow rate, Q , as directly measured, in units of 10^{-2} ml /sec. Flow rate increases from the bottom to the top of the table. The ratio of true flow rate to calculated flow rate, Q/F (column two) generally increases with increasing flow rate. The calculated flow rate is therefore increasingly overestimating the area under the dispersion curves. Q/F is unity at slow flows and rises to a maximum 1.24.

In the 3rd column the mean transit times derived from the dispersion curves are expressed in terms of T (clearly, $\bar{u}/x = Q/V_a$). V_a/Q is the mean transit time obtained for well mixed flow (see Theory) and $\bar{t}_Q/V_a$ can be thought of as the ratio of observed mean transit time to the 'well mixed mean transit time'. The results show a progressive increase in mean transit time ratio with flow rate. Again, at slow flow rates the value of $\bar{t}_Q/V_a$ is unity, but it rises higher than Q/F to 1.72 at the fastest flow. The newly defined mean transit time, $\bar{t}_n$, is also expressed

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{E}Q}{V_a}$	$\frac{\bar{E}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$	Date
29.0	1.13	1.72	1.18	1.52	1.04	4/1/73
22.7	1.21	1.70	1.26	1.41	1.04	4/1/73
15.8	1.19	1.55	1.17	1.30	0.98	4/1/73
15.8	1.22	1.48	1.22	1.21	1.00	2/10/72
15.5	1.21	1.61	1.26	1.33	1.04	4/1/73
8.30	1.17	1.38	1.17	1.18	1.00	6/9/72
7.99	1.24	1.42	1.26	1.15	1.02	6/9/72
7.27	1.21	1.31	1.13	1.08	0.93	7/9/72
7.24	1.20	1.32	1.15	1.10	0.96	7/9/72
6.60	1.18	1.26	1.12	1.07	0.95	24/8/72
4.89	1.13	1.26	1.17	1.12	1.04	23/8/72
4.46	1.14	1.22	1.14	1.07	1.00	24/8/72
3.24	1.02	1.14	1.08	1.12	1.06	24/8/72
1.90	1.02	1.08	1.04	1.06	1.02	24/8/72
1.07	1.05	1.08	1.05	1.03	1.00	23/8/72
0.54	1.05	1.03	0.99	0.98	0.94	23/8/72
0.54	0.98	1.01	1.01	1.03	1.03	23/8/72
0.28	1.02	1.00	0.98	0.98	0.96	23/8/72

Table 4.1: Results obtained with iodide in 2N KCl without a mixer, at 25°C. For explanation of symbols and results, see text.

in terms of T , and shows the same erratic rise with flow rate as does Q/F .

The ratio of calculated volume, V , to actual (or measured) volume, V_a , is listed in the fifth column. This is calculated from the quotient of columns two and three. There is a rise in V/V_a from unity to 1.52 with increasing flow rate. This corresponds to a maximum overestimate in vessel volume 50%. In contrast to the previous ratios, V_n/V_a is invariant to flow rate and is always close to unity (mean 1.00 ± 0.04 S.D.). It appears that the errors obtained using the dilution formulae for F and $\bar{t}_n$ cancel when V_n , their product, is calculated.

The behaviour of these parameters is consistent with the theoretical treatment given earlier. At the slowest flow rates diffusion dominates the dispersion and the bolus of indicator is transported along the tube effectively well mixed across the cross section. The theoretical section in chapter 2 predicts that in these circumstances all of the calculated ratios should be unity. With increasing flow rate the area under the concentration against time dispersion curves, Q/F , $\bar{t}^{Q/V_a}$, $\bar{t}_n^{Q/V_a}$ and V/V_a are expected to increase because of the decreasing influence of indicator diffusion. This has been clearly illustrated in table 4.1. The theoretical development also predicts the invariance of V_n/V_a with flow rate, and this has also been demonstrated.

The changes in shape of the dispersion curves, seen in Fig. 4.1, reflect the changing influence of diffusion on the dispersion of the indicator. There are four curves at different flow rates taken from

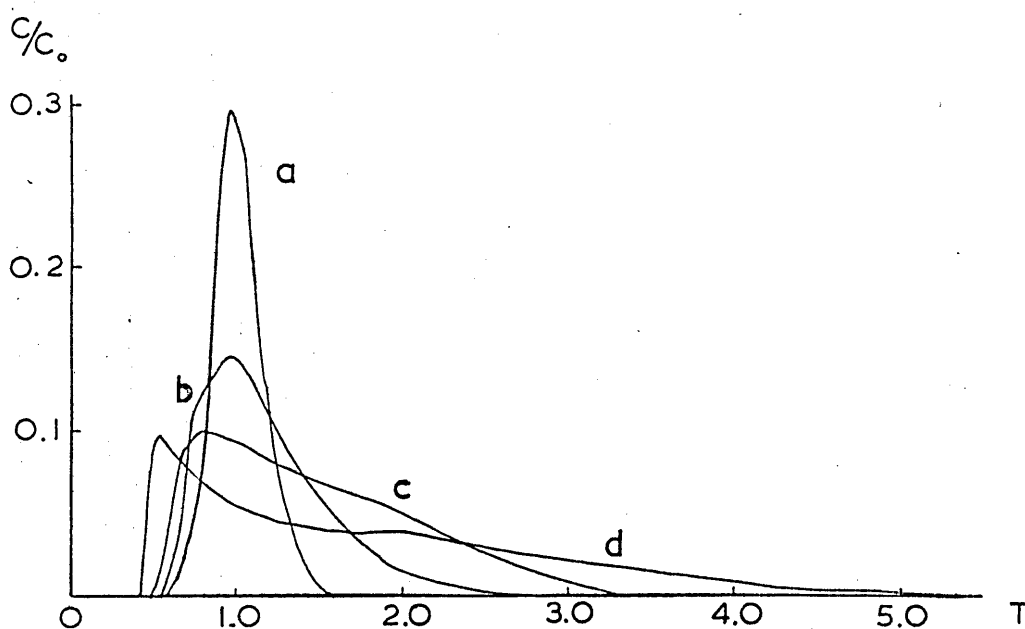


Fig. 4.1: Dispersion curves of ^{131}I in 2N KCl, without a mixer. The observed concentration (c) is divided by the bolus concentration (c_0) and plotted against dimensionless time ($T = \frac{ut}{x}$). These curves are taken from table 4.1. Flow rates (10^{-2} ml/sec). a) 0.54 b) 3.24 c) 8.30 d) 29.0.

table 4.1. The slowest flow, 0.54×10^{-2} ml/sec, where diffusion smooths out the dispersing action of the parabolic profile, the indicator arrives late, rises to a high peak and is cleared relatively early. Use of the indicator dilution equations for F , $\bar{t}$, $\bar{t}_n$, V and V_n gives correct answers. As the flow rate increases (b) c) in fig. 4.1) the curves become more dispersed along the T axis, starting sooner and being cleared relatively later. For the fastest flow rate, 29.0×10^{-2} ml/sec, the velocity profile is dominating the dispersion. Indicator arrives at about $T = 0.5$ and is finally cleared at $T = 5.0$. The increases in Q/F , $\bar{t} Q/V_a$, $\bar{t}_n Q/V_a$ and V/V_a mirror the changing shape of the curves.

Table 4.2 summarises the results obtained with the same solution and indicator, ^{131}I in 2N KCl, but a mixer has been used prior to detection, changing the mean cross-sectional sampling to mean flow proportional. Q/F is now invariant to flow and equal to unity (1.00 ± 0.05 , mean $\pm$ S.D.). The mean transit time and volume ratios $\bar{t} Q/V_a$ and V/V_a again increase from unity with increasing flow rate. The newly defined mean transit time and volume, $\bar{t}_n Q/V_a$ and V_n/V_a are invariant to flow, and are unity (1.03 ± 0.05 , 1.03 ± 0.06 , mean $\pm$ S.D.). Again, these results are consistent with the theoretical development because mean flow proportional sampling should produce dispersion curves with constant (correct) areas and correct values of $\bar{t}_n$ and V_n .

Fig. 4.2 again illustrates the changing shape of the dispersion curves with flow rate. The basic trends are similar to those in fig. 4.1,

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$	Date
25.0	1.11	1.22	1.05	1.10	0.95	1/6/73
23.4	1.10	1.28	1.09	1.16	0.99	1/6/73
23.2	1.02	1.26	0.96	1.24	0.94	4/1/73
17.9	1.00	1.24	1.05	1.24	1.05	27/9/72
16.2	1.09	1.32	1.10	1.21	1.01	1/6/73
14.9	0.99	1.34	1.14	1.35	1.15	1/6/73
12.1	1.02	1.14	1.00	1.12	0.98	4/1/73
10.1	1.06	1.30	1.11	1.23	1.05	1/6/73
10.1	0.99	1.26	1.08	1.27	1.09	1/6/73
8.81	0.98	1.20	1.03	1.22	1.05	27/9/72
8.09	0.98	1.08	0.98	1.10	1.00	4/1/73
8.04	0.99	1.25	1.08	1.26	1.09	1/6/73
5.05	0.98	1.15	1.06	1.17	1.08	27/9/72
3.70	0.93	1.12	1.05	1.20	1.13	27/9/72
3.37	0.94	1.11	1.03	1.18	1.10	27/9/72
2.30	0.97	1.07	1.01	1.10	1.04	2/10/72
1.49	1.03	1.05	1.01	1.02	0.98	2/10/72
1.27	0.94	1.04	1.01	1.11	1.07	2/10/72
0.82	1.01	1.02	0.99	1.01	0.98	2/10/72
0.57	0.94	1.00	0.99	1.06	1.05	26/10/72
0.53	0.94	1.00	0.97	1.06	1.03	26/10/72
0.44	1.02	1.01	0.97	0.99	0.95	2/10/72

Table 4.2: Iodide in 2N KCl with mixer, at 25°C.

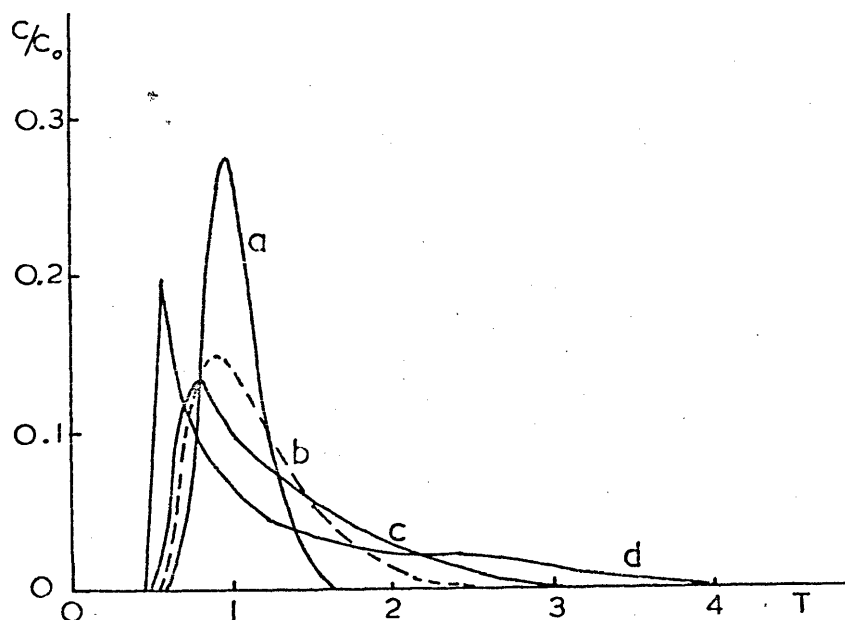


Fig. 4.2: Dispersion curves of Iodide (^{131}I) in 2N KCl, with a mixer, taken from table 4.2. Flow rates (10^{-2} ml/sec).
a) 0.82 b) 3.37 c) 8.81 d) 23.2.

but at fast flow rates, 23.2×10^{-2} ml/sec, the concentration peak is higher and the curves cleared somewhat earlier than without a mixer. This produces the smaller errors associated with the mean transit times and volumes, relative to those without a mixer.

^{86}Rb in plasma and whole blood

Table 4.3 are the results obtained for ^{86}Rb in plasma, without a mixer, at 22°C . The values obtained from the indicator dilution parameters are very similar to those obtained with ^{131}I in 2N KCl, without a mixer. However, a narrower flow range has been considered and the ratios do not approach unity at slow flows (except V_n/V_a). The dispersion curves, fig. 4.3, illustrate the restricted range of flows considered. There is no significant difference in arrival times and the slowest, at 2.39×10^{-2} ml/sec, does not rise to a high peak. Nevertheless, the decreasing influence of diffusion is apparent in the tail region of the curves, the slowest being cleared quicker than the fastest.

The results obtained for the plasma indicator ^{86}Rb in whole blood of haematocrits 40% and 43% are summarised in table 4.4. A mixer was not used. The same pattern emerges as with ^{131}I in 2N KCl and ^{86}Rb in plasma, without a mixer. Q/F , $\bar{t} Q/V_a$ and V/V_a all increase from around unity at very slow flows, 0.33×10^{-2} ml/sec, to a maximum at the fastest flows, although there appears to be a decrease in Q/F after 16.7×10^{-2} ml/sec. V_n/V_a , again shows no systematic change with flow rate. The concentration against time dispersion curves, fig. 4.4, also show a

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{tQ}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
23.0	1.34	1.78	1.36	1.33	1.01
22.8	1.37	1.68	1.32	1.23	0.96
18.6	1.34	1.66	1.27	1.24	0.95
8.79	1.30	1.44	1.27	1.11	0.98
5.22	1.35	1.34	1.26	0.99	0.93
2.39	1.17	1.22	1.16	1.04	0.99

Table 4.3: $^{86}\text{RbCl}$ in plasma without a mixer, 22°C .
All results obtained on 12/6/73.

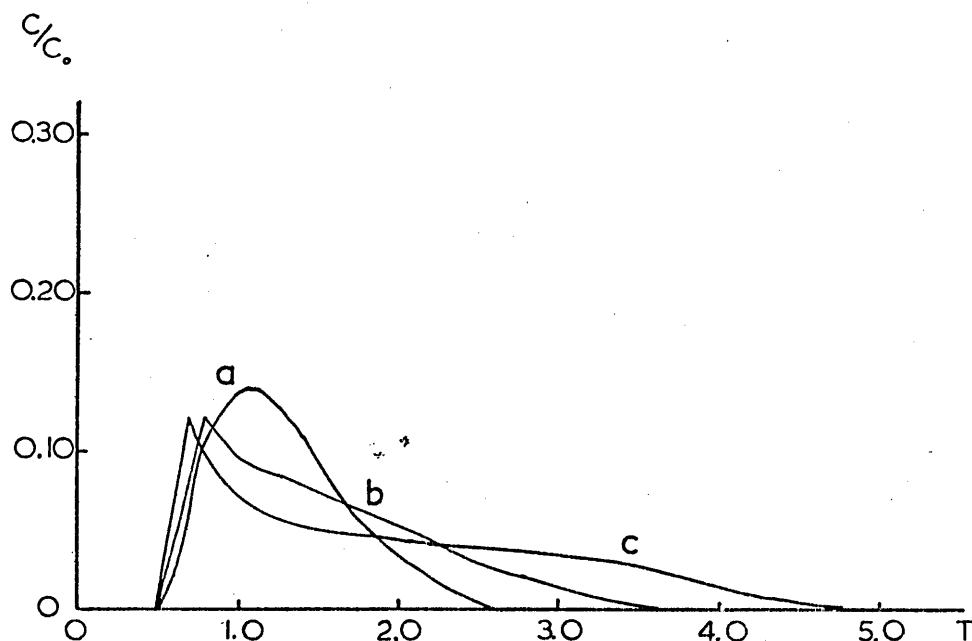


Fig. 4.3: Dispersion curves of ^{86}Rb in plasma without a mixer, taken from table 4.3. Flow rates (10^{-2} ml/sec).
a) 2.39 b) 8.79 c) 23.0.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$	Date
25.2	1.25	1.76	1.36	1.41	1.09	11/4/73
24.8	1.27	1.76	1.31	1.39	1.03	11/4/73
17.4	1.30	1.62	1.31	1.25	1.01	11/4/73
16.7	1.38	1.44	1.38	1.04	1.00	5/4/73
9.08	1.37	1.46	1.26	1.07	0.92	11/4/73
8.65	1.17	1.44	1.16	1.23	0.99	5/4/73
3.60	1.13	1.25	1.12	1.11	0.99	5/4/73
3.48	1.08	1.23	1.10	1.14	1.02	11/4/73
1.30	1.01	1.03	0.98	1.02	0.97	11/4/73
1.15	1.15	1.19	1.15	1.03	1.00	5/4/73
0.33	1.03	1.07	1.06	1.04	1.03	11/4/73

Table: 4.4: ^{86}Rb as a plasma label in whole blood without a mixer.
On 11/4/73 the haematocrit was 43% and temp. 19.5°C .
The haematocrit was 40% and temp. 20°C . on 5/4/73.

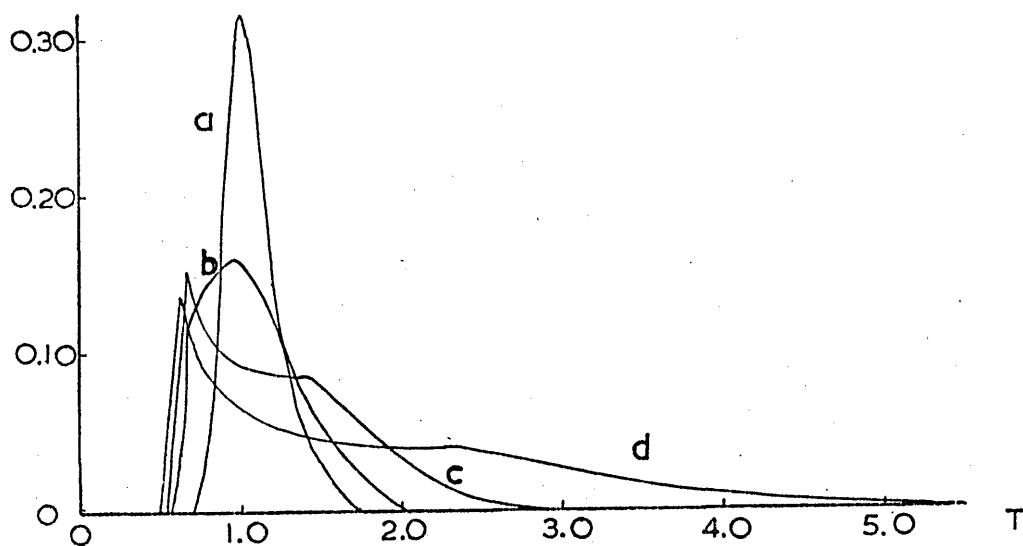


Fig. 4.4: Dispersion curves of ^{86}Rb in whole blood without a mixer, taken from table 4.4. Flow rates (10^{-2} ml/sec). a) 0.33 b) 1.30 c) 3.48 d) 25.2.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
21.2	1.11	1.56	1.31	1.41	1.18
21.2	1.15	1.60	1.18	1.39	1.03
20.1	1.17	1.54	1.19	1.32	1.02
15.6	1.19	1.42	1.20	1.19	1.01
8.51	1.07	1.29	1.06	1.21	0.99
4.02	1.05	1.15	1.02	1.10	0.97
1.41	1.05	1.06	1.01	1.01	0.96
0.80	1.02	1.06	1.03	1.04	1.01

Table 4.5: ^{86}Rb in whole blood with a mixer. All experiments performed 10/4/73, temp. 18°C ., haematocrit 43%.

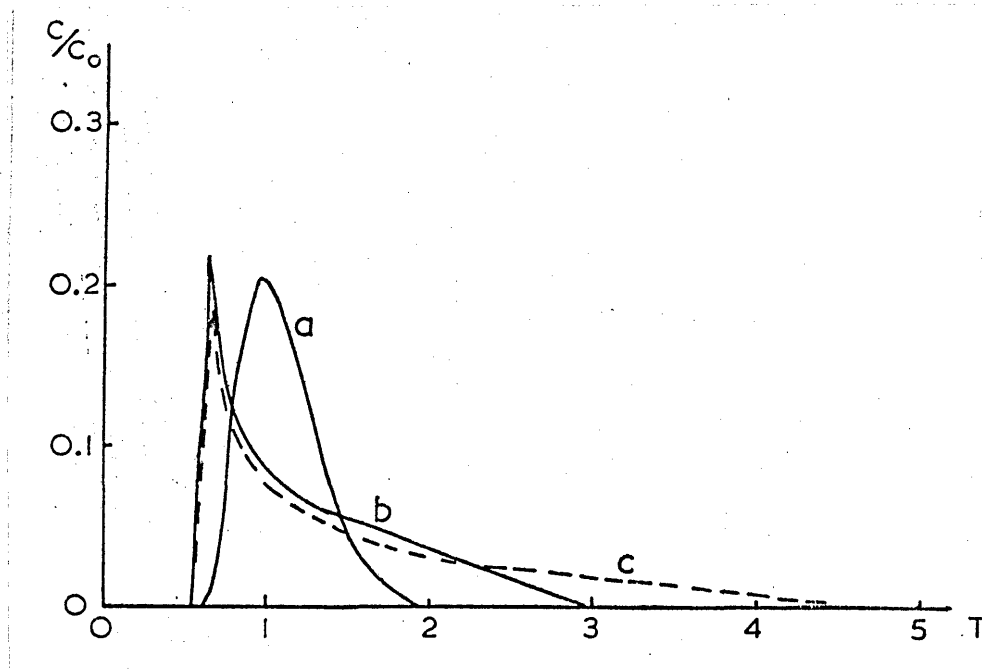


Fig. 4.5: Dispersion curves of ^{86}Rb in whole blood with a mixer, taken from table 4.5. Flow rates (10^{-2} ml/sec).
a) 0.08. b) 4.02 c) 21.2.

qualitative similarity to those of figs 4.1 and 4.3.

The effects of changing the mode of sampling on ^{86}Rb in whole blood curves by using a mixer can be seen in table 4.5. The results for Q/F , $\bar{t}_n Q/V_a$ and V_n/V_a were expected to equal unity and be flow invariant, as were those of ^{131}I in KCl (table 4.2). However, Q/F and $\bar{t}_n Q/V_a$, especially, show definite increases with flow rate. Fig. 4.5 illustrates the dispersion curves. The changes in curve shape with flow rate and the increased peak height at fast flows, seen in fig. 4.2, are clearly visible.

HSA - ^{131}I in blood and plasma

Tables 4.6a, b & c, are the results obtained, without a mixer, with the high molecular weight indicator, HSA - ^{131}I , in whole blood. The theoretical prediction of larger values of Q/F , $\bar{t}_n Q/V_a$ and V/V_a than with the lower molecular weight indicators is borne out, although the extension of the flow range will also increase these terms. Table 4.6a illustrates the reproducibility of the results. The six flow rates in units of 10^{-2} ml/sec, are approximately the same (51.0 ± 1.44 , mean $\pm$ S.D.). The means and S.D.'s of Q/F , $\bar{t}_n Q/V_a$, $\bar{t}_n Q/V_a$ and V/V_a are 1.86 ± 0.17 , 3.85 ± 0.18 , 1.84 ± 0.07 , 2.09 ± 0.16 . V_n/V_a is again invariant to flow rate and equal to unity (1.00 ± 0.08 , mean $\pm$ S.D.). Table 4.6b and c show the variation of these terms over a wide range of flow rates. The shape of the dispersion curves, fig. 4.6, illustrates the reason the indicator dilution parameters are larger than with the previous cases. They all correspond to the region of little mixing in the

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
52.6	1.86	4.09	1.85	2.20	0.99
52.4	2.17	3.93	1.95	1.81	0.90
51.3	1.89	3.93	1.76	2.08	0.93
50.6	1.68	3.83	1.85	2.28	1.10
50.5	1.78	3.81	1.77	2.14	0.99
48.7	1.74	3.54	1.86	2.03	1.07

Table 4.6a: HSA - I^{131} as a plasma label in whole blood without a mixer. All results from 24/10/73, haematocrit 45%, temperature 21°C.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
38.4	1.69	3.33	1.82	1.97	1.08
32.1	1.57	3.31	1.50	2.11	0.96
31.8	1.75	3.30	1.76	1.89	1.00
23.5	1.42	2.82	1.53	1.99	1.08
18.2	1.55	2.77	1.62	1.79	1.05
14.0	1.47	2.75	1.57	1.87	1.07
10.5	1.48	2.62	1.55	1.77	1.05
5.50	1.36	2.27	1.42	1.67	1.04
2.54	1.05	1.64	1.05	1.56	1.00

Table 4.6b: HSA - I^{131} as a plasma label in whole blood without a mixer. Results from 26/10/73, haematocrit 48%, temperature 19°C.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
27.8	1.35	3.02	1.43	2.24	1.06
18.2	1.53	2.55	1.44	1.67	0.94
14.1	1.60	2.40	1.49	1.50	0.93
12.2	1.47	2.42	1.47	1.65	1.00
3.50	1.63	2.01	1.51	1.23	0.93
3.50	1.13	1.72	1.10	1.52	0.97

Table 4.6c: HSA - ^{131}I as a plasma label in whole blood without a mixer. Results from 26/1/73, haematocrit 42%, temperature 19°C.

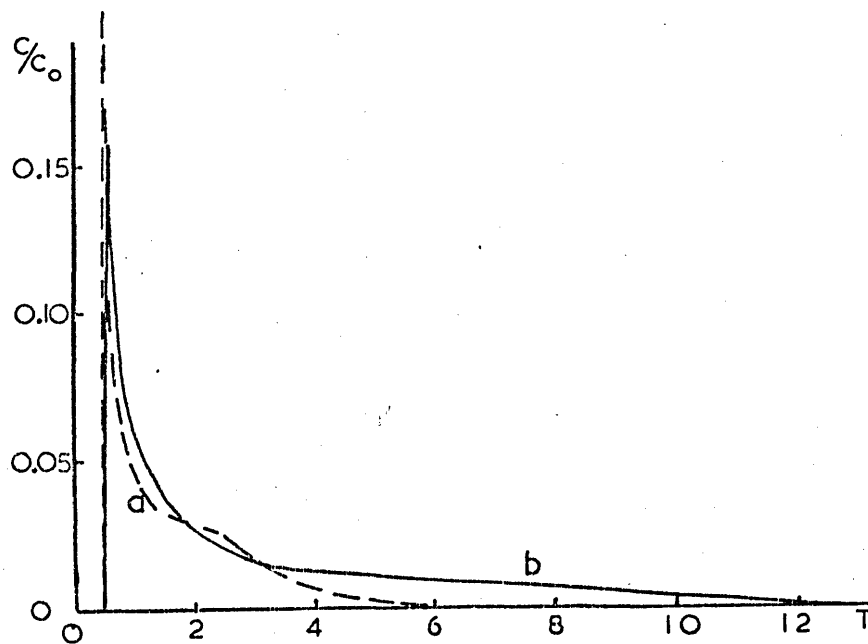


Fig. 4.6: Dispersion curves of HSA - ^{131}I in whole blood without a mixer, taken from table 4.6b. Flow rates a) 2.54 b) 32.1. (10^{-2} ml/sec).

previous diagrams. The velocity profile is dominating the dispersion, resulting in a very late clearance of indicator at most flow rates. With the slowest flow rate illustrated, 2.54×10^{-2} ml/sec, the velocity profile is still the dominant dispersive mechanism, though the change in curve height and earlier clearance of indicator demonstrates the presence of other factors.

The influence of a mixer before the detector on the HSA - ^{131}I in blood curves is shown in tables 4.7a and b and fig. 4.7. In table 4.7a it can be seen that the flow rate calculation, F , is approximately correct because Q/F is invariant to flow rate. V_n/V_a does not give a very satisfactory estimate of volume (it reflects the errors in $\bar{t}_n$) but it is always better than V/V_a . Table 4.7b contains results where a good estimate of Q/F was not obtained, but V_n/V_a was only $\sim 7\%$ in error.

Table 4.8 summarises the results with HSA - ^{131}I in plasma, without a mixer. Because of the smaller volumes of plasma used the results are from several different plasma samples. The trends are very similar to those of HSA - ^{131}I in whole blood. Fig. 4.8 illustrates the changing dispersion curve shape with flow rate. At fast flows, 32.3×10^{-2} ml/sec, the curve is dispersed along the T axis, but as flow is reduced the indicator is cleared progressively earlier. These changes are again reflected in the values of the dilution parameters. Large errors in F , $\bar{t}_n$ and V are obtained at fast flow rates and the errors reduce with decreasing flows. The mixer results are again somewhat equivocal

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{E}Q}{V_a}$	$\frac{\bar{E}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
26.9	0.96	2.42	1.23	2.52	1.28
25.3	1.06	2.37	1.46	2.24	1.38
19.9	1.05	2.18	1.20	2.08	1.14
10.2	1.06	1.88	1.15	1.77	1.08
5.33	1.05	1.75	1.18	1.67	1.12
2.21	1.03	1.52	1.21	1.48	1.17

Table 4.7a: HSA - I^{131} as a plasma label in whole blood with a mixer. Results from 13/3/73, haematocrit 42.5% temperature 18°C.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{E}Q}{V_a}$	$\frac{\bar{E}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
10.8	1.07	1.65	0.99	1.54	0.93
10.2	1.07	1.37	0.99	1.28	0.93
10.1	1.14	1.68	1.06	1.47	0.93
7.16	1.21	1.47	1.10	1.21	0.91

Table 4.7b: HSA - I^{131} as a plasma label in whole blood with a mixer. Results from 23/1/73, haematocrit 46.5% temperature 19°C.

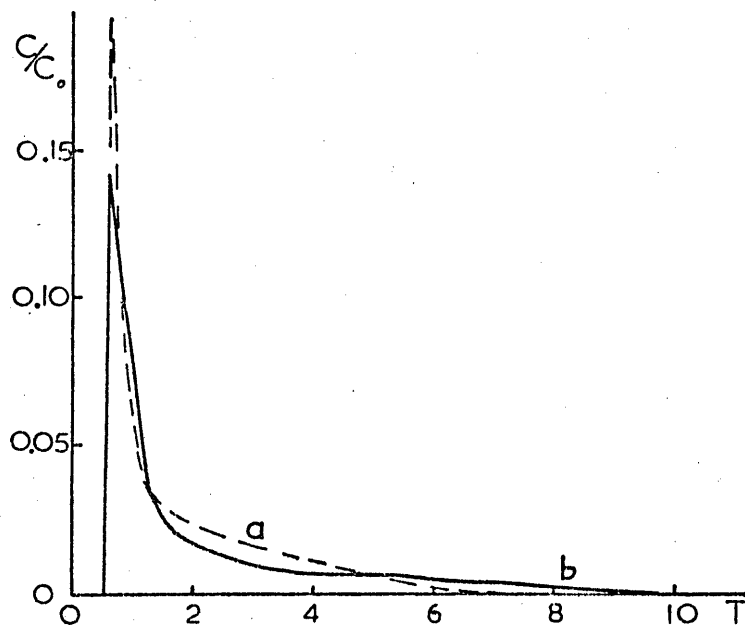


Fig. 4.7: Dispersion curves of HSA - ^{131}I in whole blood with a mixer, haematocrit 42.5%, taken from table 4.7a. Flow rates (10^{-2} ml/sec). a) 10.2 b) 26.9.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{E}Q}{V_a}$	$\frac{\bar{E}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$	Date
42.2	1.68	3.86	1.56	2.30	0.93	15/1/73
32.3	2.07	4.17	1.84	2.01	0.89	9/1/73
25.0	1.92	3.51	1.46	1.83	0.82	7/5/73
21.2	1.78	3.30	1.63	1.85	0.92	9/1/73
15.3	1.94	3.13	1.58	1.61	0.82	9/1/73
9.60	1.82	3.26	1.69	1.79	0.93	9/1/73
8.26	1.64	3.15	1.63	1.92	0.99	16/5/73
4.70	1.61	2.47	1.50	1.53	0.93	16/5/73
3.58	1.82	2.66	1.59	1.46	0.87	9/1/73
0.74	1.32	1.49	1.25	1.13	0.95	16/5/73

Table 4.8: HSA - ^{131}I in plasma, no mixer, temperature 21°C . for all results.

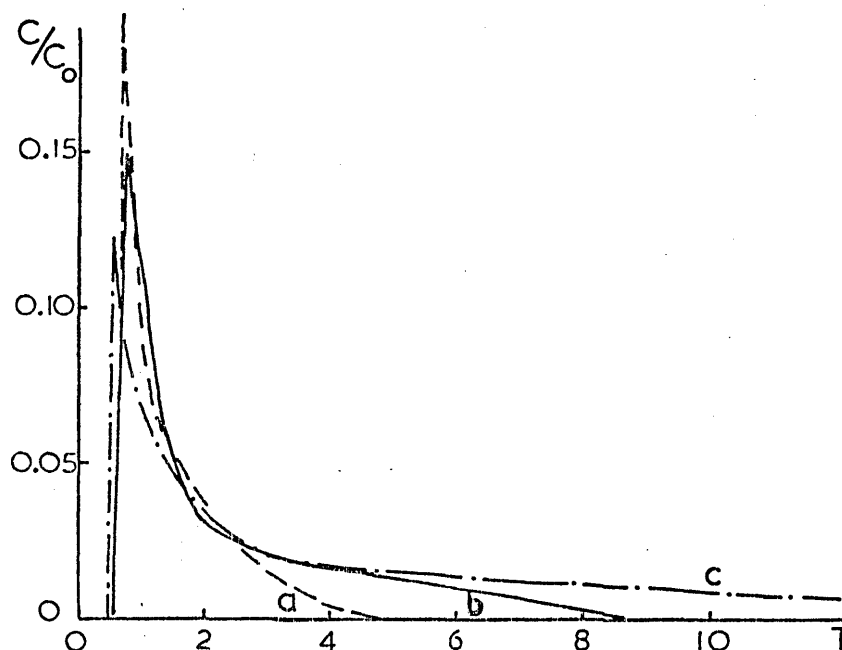


Fig. 4.8: Dispersion curves of HSA - ^{131}I in plasma without a mixer, taken from table 4.8. Flow rates (10^{-2} ml/sec). a) 0.74 b) 4.70 c) 32.3.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{e}Q}{V_a}$	$\frac{\bar{e}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
23.7	1.17	1.72	0.98	1.47	0.84
16.7	1.13	2.00	1.12	1.77	0.99
12.1	1.09	1.94	1.15	1.78	1.06
5.76	1.09	1.63	0.99	1.50	0.91
4.54	1.09	1.66	1.12	1.52	1.03

Table 4.9a: HSA - I^{131} in a plasma with a mixer, temperature 22°C.
All results from 15/1/73.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{e}Q}{V_a}$	$\frac{\bar{e}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
15.0	1.17	2.60	1.30	2.22	1.11
14.1	1.11	2.49	1.25	2.24	1.13
12.8	1.17	2.53	1.22	2.16	1.04
12.1	1.13	2.48	1.24	2.19	1.10

Table 4.9b: HSA - I^{131} in plasma with a mixer, temperature 21°C.
Results from 22/5/73.

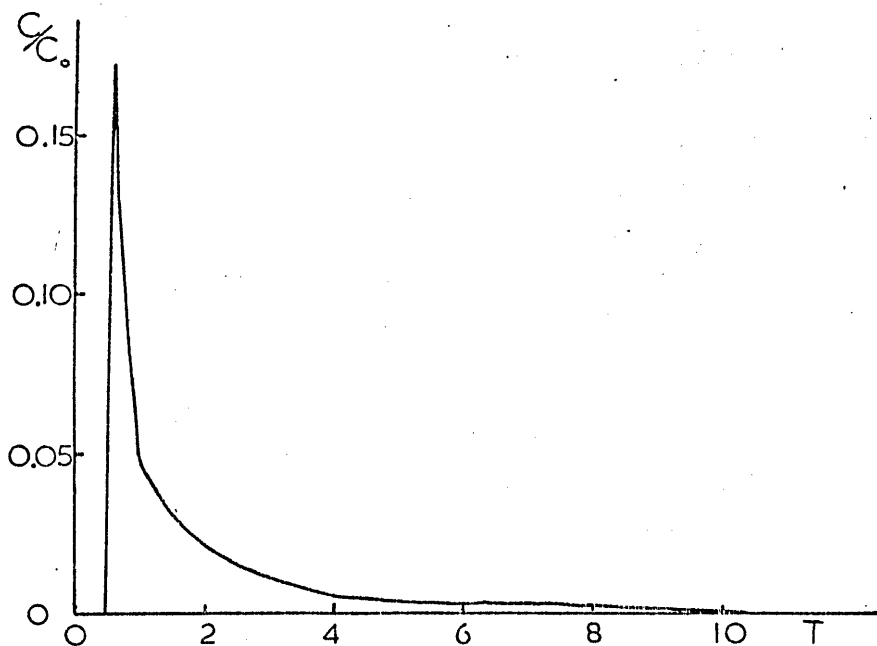


Fig. 4.9: Dispersion curves of HSA - ^{131}I in plasma with a mixer, taken from table 4.9a. Flow rate 23.7×10^{-2} ml/sec.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$	Haemat. %
17.2	1.59	3.53	1.68	2.22	1.06	0
15.2	1.54	3.12	1.52	2.03	0.99	12
16.2	1.47	2.90	1.55	1.97	1.05	23.5
16.6	1.40	2.16	1.27	1.54	0.91	51.5

Table 4.10: Variation of mean transit time with haematocrit of HSA - ^{131}I in whole blood at approximately constant flow. Results from 13/6/73, temperature 21°C .

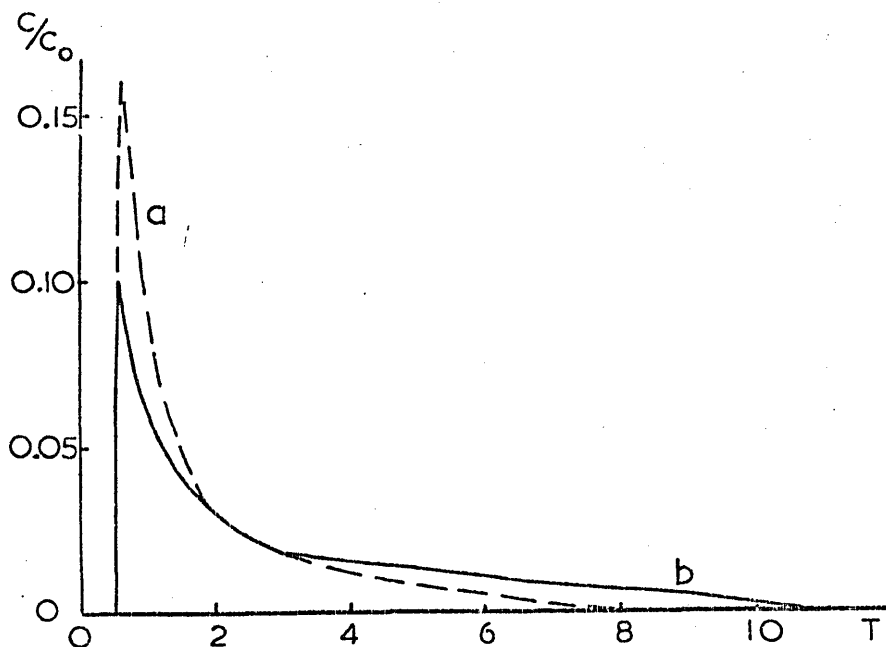


Fig. 4.10: Variation of HSA - ^{131}I dispersion curves with haematocrit taken from table 4.10. a) 16.6×10^{-2} ml/sec, haematocrit 51.5% and b) 15.2×10^{-2} ml/sec, haematocrit 12%.

In tables 4.9a and b the flow calculation, F , is still subject to errors, albeit much less than without the mixer. Similarly, the new volume, V_n , calculations are variable but give a much better result than V .

Iodohippurate (^{131}I) in whole blood

Table 4.11 and fig. 4.11 are the results of iodohippurate as a label in whole blood of haematocrit 41%, without a mixer. The behaviour of F , $\bar{t}$, $\bar{t}_n$, V and V_n , is similar to when using HSA - ^{131}I in whole blood, without a mixer. There is the same increase in Q/F , $\bar{t}^Q/V_n$, $\bar{t}^Q/V_a$ and V/V_a with flow rate, and the constancy of V_n/V_a . The presence of the mixer, table 4.12 and fig. 4.12, reduces the errors in Q/F , $\bar{t}_n^Q/V_a$, while not eliminating them.

Comparison of high and low molecular weight indicators

The results of mean cross sectional sampling (no mixer) are difficult to compare in the tabular form and the lack of identical flow rates prevents close comparison of the dispersion curves. In order to facilitate detailed comparison of the results the mean transit times, expressed in terms of $\bar{t}^Q/V_a$, have been plotted against flow rate, Q , in fig. 4.13. The mean transit time was used because it appeared to be the most sensitive term to the changes in shape of the dispersion curves. Furthermore, it is not dependent on possible errors in bolus concentration determination, as are flow and volume.

In Fig. 4.13 all of the mean transit time curves increase with increasing flow rate. There is a fast rate of increase initially, which becomes more gradual. The mean transit times of the low molecular weight

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{E}Q}{V_a}$	$\frac{\bar{E}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
25.9	1.51	2.54	1.45	1.68	0.96
21.1	1.49	2.61	1.54	1.75	1.03
10.3	1.36	2.13	1.35	1.57	0.99
4.41	1.15	1.72	1.19	1.50	1.03
0.73	1.00	1.27	1.16	1.27	1.16

Table 4.11: Iodohippurate (^{131}I) as a plasma label in whole blood without a mixer. Results from 21/3/73, 21°C., haematocrit 41%.

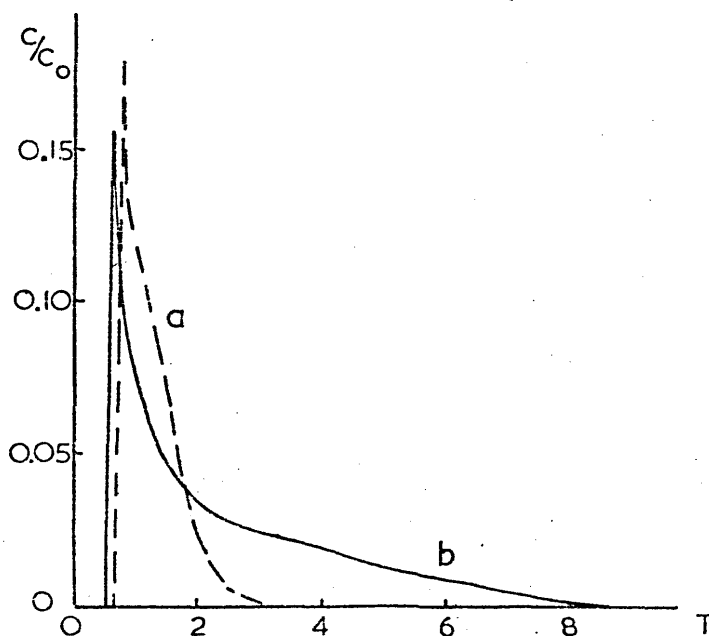


Fig. 4.11: Dispersion curves of Iodohippurate (^{131}I) in whole blood taken from table 4.11. Flow rates (10^{-2} ml/sec). a) 0.73 b) 25.9.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
26.6	1.30	1.88	1.25	1.45	0.96
11.0	1.13	1.90	1.23	1.68	1.09
5.09	1.05	1.51	1.10	1.44	1.05
1.74	0.95	1.27	1.08	1.34	1.14

Table 4.12: Iodohippurate (^{131}I) as a plasma label in whole blood with a mixer. Results from 19/3/73, haematocrit 41.5, temperature 21°C .

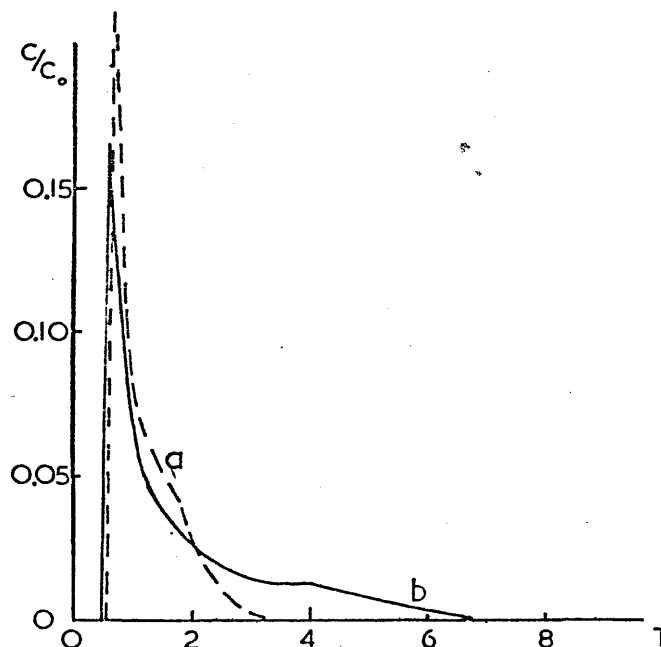


Fig. 4.12: Dispersion curves of Iodohippurate (^{131}I) in whole blood, taken from table 4.12. Flow rates a) 1.74×10^{-2} b) 26.6×10^{-2} (ml/sec).

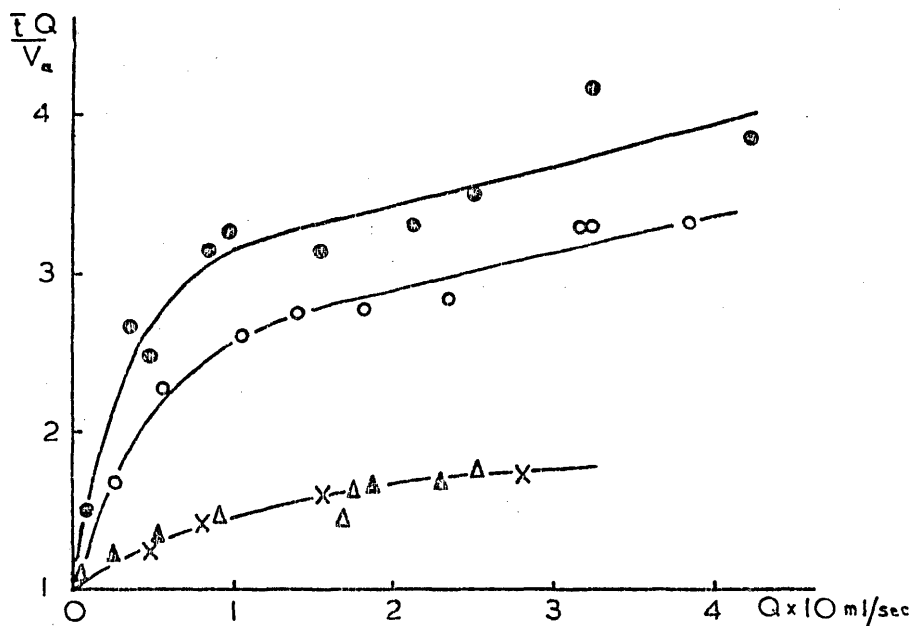


Fig. 4.13: Dimensionless mean transit time against flow rate for different solutes in blood, plasma and 2N KCl (without a mixer). ● HSA - ^{131}I in plasma (table 4.8); ○ HSA - ^{131}I in whole blood, haematocrit 45% (table 4.6a); △ ^{86}Rb in plasma (table 4.3); △ ^{86}Rb in whole blood, haematocrit 40% (table 4.4); × ^{131}I in 2N KCl (table 4.1).

indicator, ^{131}I in 2N KCl , ^{86}Rb in plasma, ^{86}Rb in whole blood, all fall on the same line. There were small differences in the temperature at which the experiments were performed but these do not appear to have influenced the results. The mean transit times of HSA - ^{131}I in plasma are consistently larger than those of HSA - ^{131}I in blood. The presence of the red cells increases the clearance of the high and not the low molecular weight indicators. In order to obtain more information on this increased clearance due to the cells, an investigation was carried out on the effect of red cell concentration on mean transit time. Flows were held approximately constant while the dispersion of HSA - ^{131}I in differing haematocrit red cell suspensions was observed. Table 4.10 shows that adding any concentration of cells decreases the values Q/F , $\bar{t} Q/V_a$, $\bar{t}_n Q/V_a$ and V/V_a . That the cells are producing an increase clearance of indicator is shown in fig. 4.10. In fact, at 0, 12 and 23.5% haematocrits (not all shown) the curve height did not alter significantly, the only changes being in the tail region of the curves. At 51.5% haematocrit there is a change in the height of the curve as well as an increased clearance in the tail.

^{51}Cr labelled red cells in whole blood (without a mixer)

The results of using ^{51}Cr as a red cell label in whole blood are given in table 4.13a, b and c, and fig. 4.14. During some of these labelled cell experiments the relative viscosity, r , was simultaneously measured, as described in the Methods. The results for F , $\bar{t}$, $\bar{t}_n$, V and V_n show the same pattern of behaviour as the other indicators, there

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{v}{V_a}$	$\frac{V_n}{V_a}$	$h \quad r$
87.9	2.44	5.33	2.30	2.18	0.94	5.23
51.5	1.78	4.18	1.87	2.35	1.05	5.82
24.0	1.79	3.51	1.81	1.96	1.01	7.09
9.10	1.48	2.95	1.50	1.99	1.01	10.5
1.98	1.23	1.64	1.34	1.33	1.09	20.7

Table 4.13a: ^{51}Cr labelled red cells in whole blood, haematocrit 43%, temperature 19°C. Results from 7/11/73.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{v}{V_a}$	$\frac{V_n}{V_a}$
29.8	2.00	3.55	1.67	1.78	0.84
26.8	1.75	3.23	1.61	1.85	0.92
22.8	1.59	2.64	1.40	1.66	0.88
20.8	1.74	3.11	1.47	1.79	0.84
15.3	1.66	2.69	1.45	1.62	0.87
10.0	1.71	2.77	1.50	1.62	0.88
5.17	1.53	2.13	1.37	1.39	0.90

Table 4.13b: ^{51}Cr labelled red cells in whole blood, haematocrit 45%, temperature 20°C. Results from 15/9/73.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$	$\eta \tau$
92.2	2.12	4.55	1.87	2.15	0.88	5.28
74.2	2.29	5.46	2.16	2.38	0.94	5.23
43.3	1.84	3.83	1.66	2.08	0.90	5.09
23.4	1.74	3.20	1.64	1.84	0.94	7.38
15.3	1.86	3.60	1.62	1.94	0.87	-

Table 4.13c: ^{51}Cr labelled red cells in whole blood, haematocrit 46%, temperature 19°C. Results from 2/11/73.

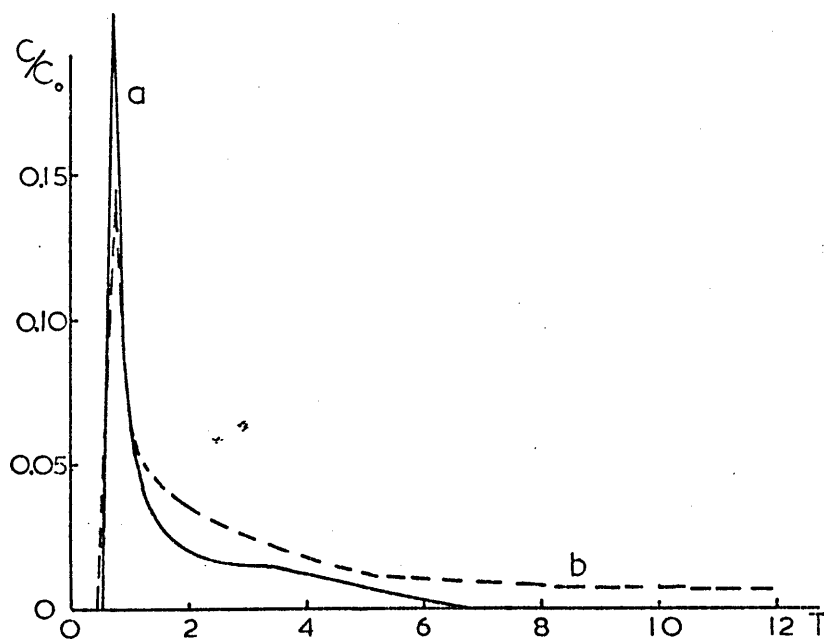


Fig. 4.14: ^{51}Cr labelled red cells in whole blood, haematocrit 43%, taken from table 4.13a. Flow rates (10^{-2} ml/sec).
a) 1.98 b) 87.9.

being the greatest correspondence to the HSA - ^{131}I in whole blood results. Errors in F and $\bar{t}$ of the order of 150% and 430% respectively, are observed at the fastest flow rate. The value of all the terms are lower at slow flow rates, but the decrease is not always progressive. The curves are dispersed widely along the T axis at fast flow rate, arriving at $T \sim 0.5$. At slow flow rates the clearance is faster in the tail region and there is a change in the height of the curves. The relative viscosity, η_r , of the blood decreases with increasing flow rate and approaches a limiting value of $\eta_r \sim 5.3$.

^{51}Cr labelled cells in saline (without a mixer)

In order to be able to assess the influence of red cell - red cell and red cell - protein interactions, the dispersion of labelled red cells in saline at a normal haematocrit, 46%, was studied. The results are presented in tables 4.14a and 4.14b and fig. 4.15. The gross behaviour of F , $\bar{t}$, $\bar{t}_n$, V and V_n , is the same as HSA - ^{131}I in whole blood and ^{51}Cr labelled cells in whole blood. Large errors are apparent in flows, mean transit times and volume. The newly defined volume, V_n , again mostly gives a good estimate of vessel volume, although in table 4.14b the two fastest flow rates have associated errors $\sim 20\%$ in V_n . This is still small compared with the 130% error associated with V . The relative viscosity of the suspensions, while decreasing with increasing flow rate, is lower at all flow rates than that of whole blood. Comparing figs 4.14 and 4.15 it can be seen that the heights of the curves for cells in saline are lower than whole blood curves. The dispersion

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{E}Q}{V_a}$	$\frac{\bar{E}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$	η_r
94.8	2.07	4.86	2.08	2.35	1.00	3.02
77.9	1.86	4.59	1.92	2.47	1.03	3.04
63.7	1.86	5.23	1.82	2.81	0.98	3.49
37.7	1.53	5.14	1.50	3.36	0.98	3.42
14.5	1.47	2.78	1.50	1.89	1.02	4.29
13.7	1.41	2.71	1.52	1.92	1.08	4.96
3.39	1.20	2.03	1.26	1.69	1.05	7.84

Table 4.14a: ^{51}Cr labelled washed red cells in saline. Haematocrit 46%, temperature 21°C. 3/10/73.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{E}Q}{V_a}$	$\frac{\bar{E}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$	η_r
84.7	1.81	4.21	2.13	2.33	1.18	3.36
48.7	1.91	4.52	1.80	2.37	1.24	3.65
31.3	1.91	4.39	1.92	2.30	1.01	4.11
14.7	1.23	2.84	1.36	2.31	1.11	5.60
2.74	1.00	2.13	1.15	2.13	1.15	9.73

Table 4.14b: ^{51}Cr labelled washed red cells in saline. Haematocrit 46%, temperature 21°C. 24/10/73.

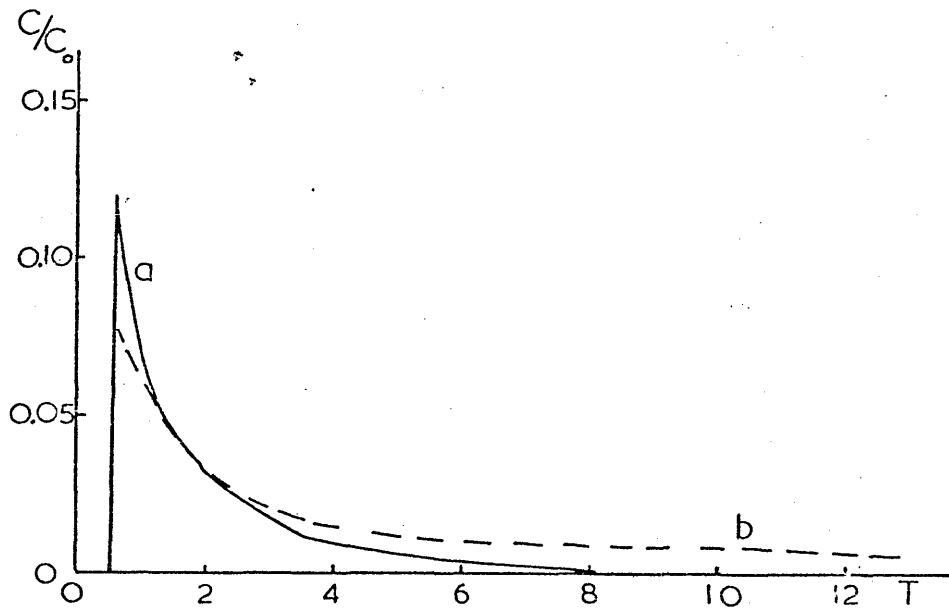


Fig. 4.15: ^{51}Cr labelled washed red cells in saline, haematocrit 46%, from table 4.14a. Flow rates (10^{-2} ml/sec). a) 3.39 b) 94.8.

curve height was, in general, lower for cells in saline than whole blood but the difference was not always so striking as figs 4.14 and 4.15 suggest.

Comparison of ^{51}Cr labelled red blood cell results

The results for ^{51}Cr labelled cells are compared again by plotting dimensionless mean transit time against flow rate. The variations of $\bar{t}^Q/V_a$ are shown in fig. 4.16. The results in tables 4.13b and 4.13c have not been plotted. They can be seen by inspection broadly to follow the graph of ^{51}Cr in whole blood, even though there is a large amount of scatter in the table 4.13b transit times. The pattern of all three variations illustrated is the same. With increasing flow rate there is a large initial rise in $\bar{t}^Q/V_n$ followed by a slower rise and a possible levelling out of the curve at $\bar{t}^Q/V_a$ around $4 \rightarrow 5$. There appears to be no significant difference between the mean transit time variation of ^{51}Cr labelled cells in the cell suspension in saline or in whole blood.

Viscometric results

Fig. 4.17 shows the variations of relative viscosity, η_r , with flow rate, Q , for a red cell suspension in saline, whole blood and plasma. The results for cells in saline and whole blood have been taken from the previous tables (4.14a & 4.13a). The curve for whole blood is always above that of the suspended cells. At very slow flows, less than 1.0×10^{-2} ml/sec, the whole blood viscosity increases faster than the cell suspension. The plasma viscosity is constant over most of the flow rate range, but there is a definite small increase below

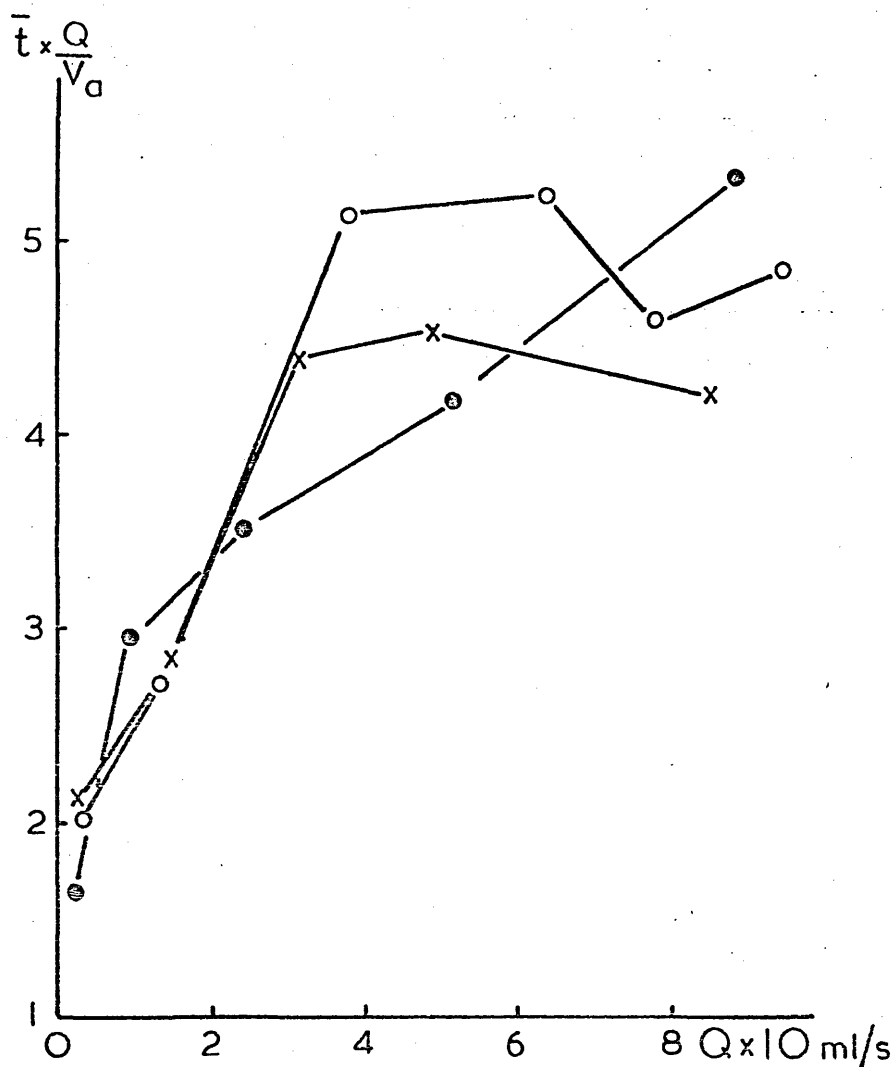


Fig. 4.16: Dimensionless mean transit time against flow rate for ^{51}Cr labelled red cells. O washed cells in saline, haematocrit 46% (table 4.14a); X washed cells in saline, haematocrit 46% (table 4.14b); ● labelled cells in whole blood, haematocrit 43% (table 4.13a).

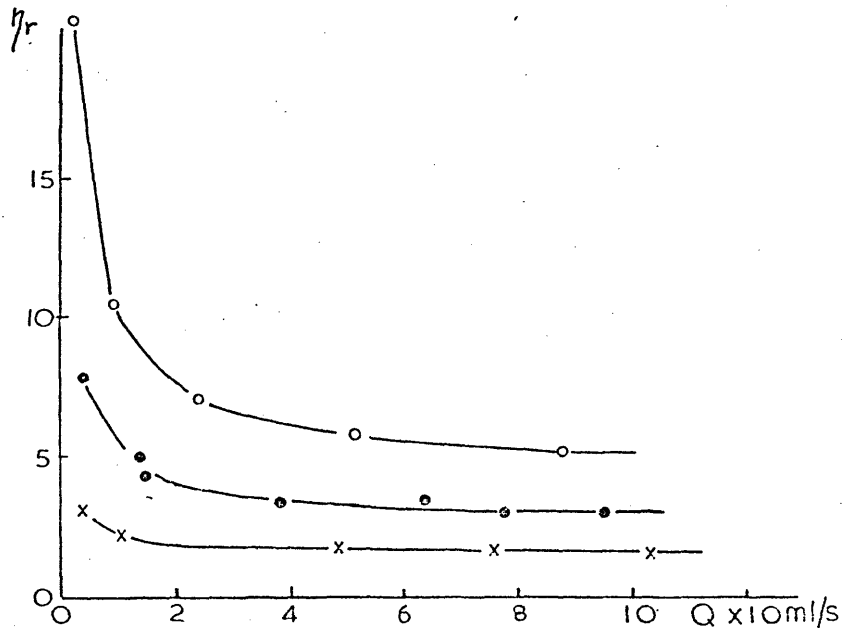


Fig. 4.17: Relative viscosity, η_r , against flow rate, Q . $\circ$ whole blood, haematocrit 43% (table 4.13a); $\bullet$ washed red cells in saline, haematocrit 46% (table 4.14a); $\times$ plasma.

$$Q = 2.0 \times 10^{-2} \text{ ml/sec.}$$

It will be of interest in the Discussion to consider the viscosity in terms of the shear rate at the wall of the straight tube section, D_w . The wall shear rate, D_w , is defined as the velocity gradient $\frac{\partial u}{\partial r}$ at the wall. From Poiseuille's equation $D_w = \frac{4Q}{\pi a^3}$ see for example reference (56). D_w at flow rates of 1.0×10^{-2} and 10.0×10^{-1} ml/sec are 6.52 and 652 sec., respectively.

Flexibility changes in the red blood cells

The rate of packing of red blood cells under a 200g centrifugal force has been shown to be a convenient quantitative measure of red blood cell flexibility, as discussed in the Methods. The rate of packing of red cells in three different situations was measured. The viscosity of the red cell suspensions and the whole blood were measured after the bolus solution had been incubated in the buffer used for red cell labelling. It seemed desirable to examine any effects incubation had on the flexibility of the red cells, and to measure any flexibility changes when the cells are suspended in saline. Fig. 4.18 shows the rate of packing of incubated cells in plasma, whole blood and incubated cells washed and suspended in saline. All three curves were obtained simultaneously. The voltages used on the centrifuge and the spinning times used are marked on the figure. The final point after centrifugation for 2 minutes at 240 volts represent the packed cell haematocrit of the suspension. The rates of packing of whole blood and incubated

Haemat.

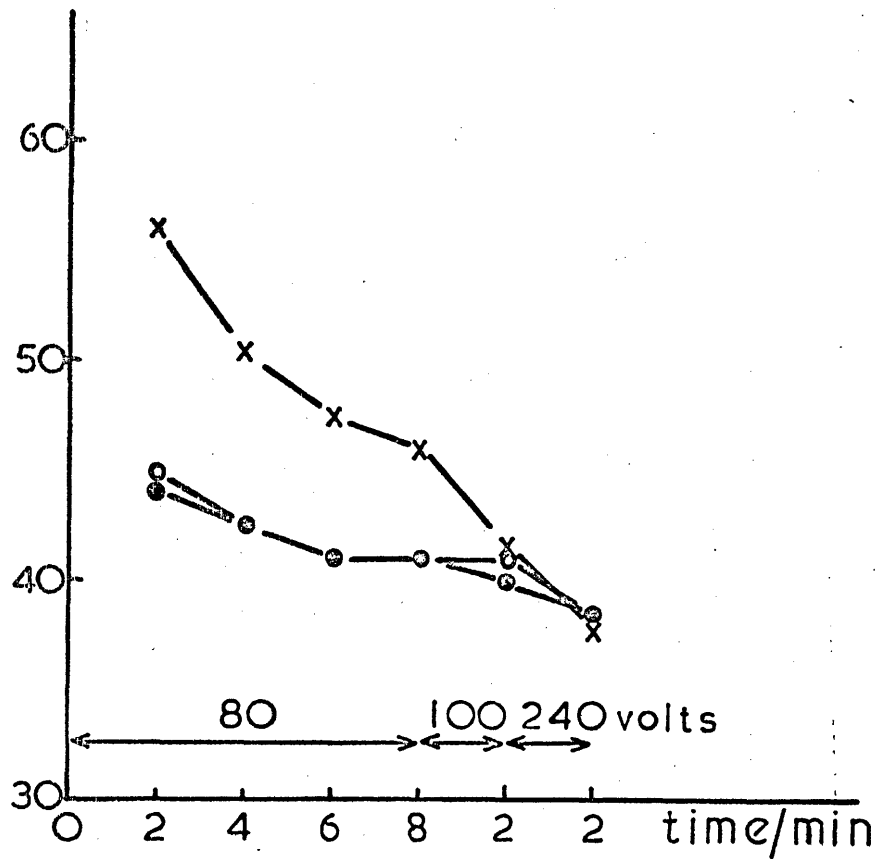


Fig. 4.18: Rate of packing of ○ whole blood, haematocrit 38.5%;
 ● washed and incubated cells resuspended in plasma,
 haematocrit 38.5%; × washed and incubated cells in
 saline, haematocrit 38%.

cells in plasma are identical and significantly faster than the incubated and washed cells suspended in saline. They are therefore more flexible than the washed cells in saline.

CHAPTER 5RESULTS - MODEL VASCULAR BED ^{131}I in 2N KCl solution

All the experiments using the model vascular bed were performed without a mixer before the detector i.e. mean cross sectional concentration sampling was used. The results were expressed in the same form as those of the straight tube system. The actual volume, V_a , of the system was, of course, larger and this led to larger mean transit times. Use of the ratios, however, permits comparison of different indicators and comparison of the two flow systems.

In table 5.1 the indicator dilution results are listed again as a function of flow rate, Q . Q/F increases to a maximum of 1.14 and then decreases to unity at very fast flows, 139×10^{-2} ml/sec. The dimensionless mean transit time, $\bar{t}^Q/V_a$, shows a progressive increase rising to 1.36 and is lower at the same flow rates than in the straight tube system. $\bar{t}_n^Q/V_a$ shows a rise from around 1.0, although there is a lot of scatter. $\bar{t}_n^Q/V_a$ is always less than $\bar{t}^Q/V_a$. Neither gives a good measure of volume at all flow rates. Fig. 5.1 illustrates the dispersion curves, plotted c/c_0 against T . At very slow flows, 1.66×10^{-2} ml/sec, the curve is centred about $T = 1$, is symmetrical and has a high peak. With increasing flow rate the peak height falls and the curves become more dispersed along the T axis. The main changes in the shape of the curve are in the peak height and gradient and position

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
139	1.01	1.36	1.19	1.35	1.18
86.6	1.04	1.32	1.23	1.27	1.19
74.5	1.04	1.24	1.04	1.20	0.95
52.8	1.12	1.28	1.22	1.15	1.09
24.4	1.14	1.20	1.09	1.05	0.96
8.53	1.14	1.10	1.09	0.97	0.96
3.40	1.19	1.03	1.05	0.87	0.88
1.66	1.06	1.00	0.97	0.94	0.92

Table 5.1: Iodide in 2N KCl in model vascular bed. Temperature 18°C. All results 12/12/73.

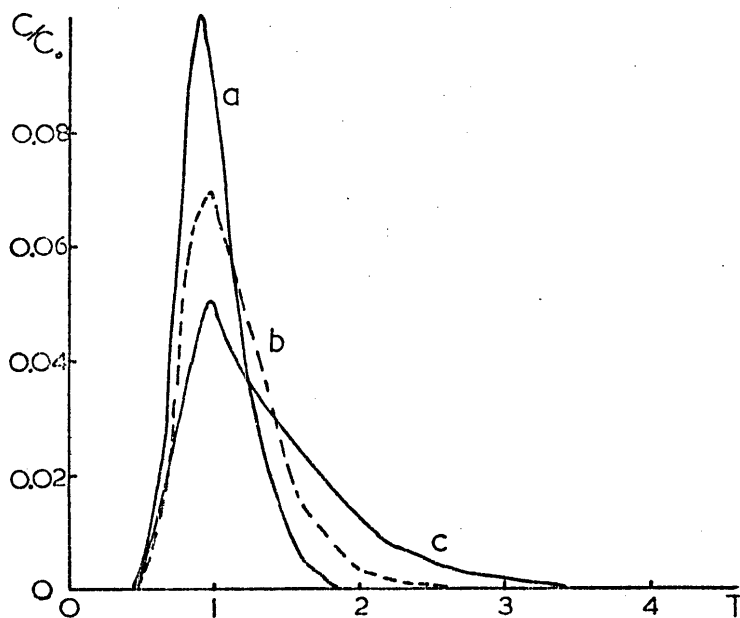


Fig. 5.1: Iodide in 2N KCl in model vascular bed, taken from table 5.1. Flow rates (10^{-2} ml/sec). a) 1.66 b) 8.53 c) 86.6.

of the downslope. The curves are not dispersed along the T axis as much as during flow through the straight tube system.

^{86}Rb in whole blood

Tables 5.2a & b are the results obtained on two occasions for ^{86}Rb in whole blood. Unfortunately, an error was made in the bolus concentration, c_0 , determination in table 5.2a and only the values of dimensionless mean transit time are presented. These show a flow dependence again.

In table 5.2b the results, again, do not progressively increase. $\bar{t}^Q/V_a$ values vary with flow rate, rising from around unity to $1.4 \rightarrow 1.5$. $\bar{t}_n^Q/V_a$ values are lower than $\bar{t}^Q/V_a$ except at the slowest flow rate, 9.04×10^{-2} ml/sec. Neither volume ratios are constant and unity, but V_n gives a better estimate of volume, in general.

Fig. 5.2 shows the dispersion curves of ^{86}Rb in the model bed, in whole blood. They are similar in type to those in fig. 5.1, but flow was not slow enough to obtain a well mixed curve. With increasing flow rate is a lowering of peak height and increased dispersion along the T axis. The dispersion is again less than that in a straight tube.

HSA - ^{131}I in whole blood

Tables 5.3a, b & c are the results obtained with HSA - ^{131}I in whole blood in the model bed. In tables 5.3a & b the values of Q/F tend to be less than unity and shows an increase followed by a decrease with increasing flow rate. The values of $\bar{t}^Q/V_a$ in all three tables

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
84.5	-	1.39	-	-	-
69.8	-	1.36	-	-	-
54.1	-	1.33	-	-	-
27.0	-	1.38	-	-	-
16.1	-	1.34	-	-	-
6.10	-	1.06	-	-	-

Table 5.2a: ⁸⁶Rb in whole blood in model vascular bed.
Results from 15/1/74, haematocrit 39%, temperature 20°C.
Error in c_0 measurement.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
61.8	1.03	1.40	1.24	1.30	1.15
48.5	0.97	1.35	1.21	1.40	1.25
42.8	1.12	1.50	1.33	1.34	1.23
33.7	0.95	1.42	1.32	1.50	1.39
19.7	0.95	1.31	1.30	1.38	1.37
9.04	0.88	1.03	1.10	1.17	1.25

Table 5.2b: ⁸⁶Rb in whole blood in model vascular bed.
Results from 13/1/74, haematocrit 43%, temperature 19°C.

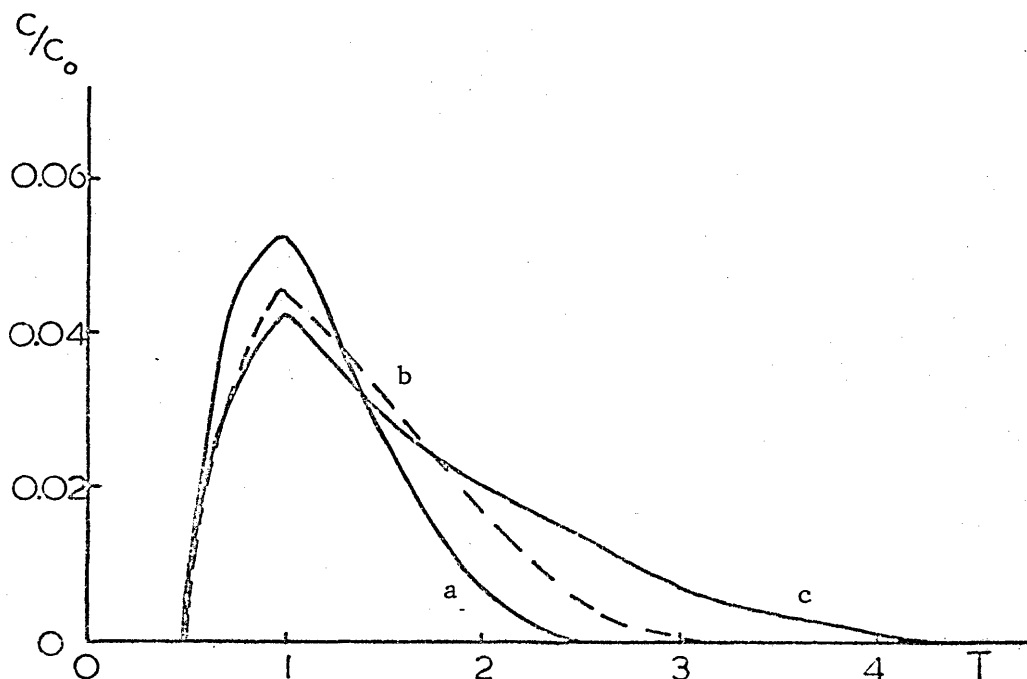


Fig. 5.2: ^{86}Rb in whole blood, haematocrit 43%, in model bed.
 Results taken from table 5.2b. Flow rates (10^{-2} ml/sec).
 a) 9.04 b) 19.7 c) 61.8.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{E}Q}{V_a}$	$\frac{\bar{E}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
86.2	0.79	1.43	1.35	1.81	1.71
64.2	0.84	1.58	1.36	1.88	1.62
45.0	0.95	1.63	1.36	1.72	1.43
27.6	1.04	1.90	1.62	1.83	1.56
17.2	0.93	1.65	1.43	1.77	1.54
2.94	0.84	1.18	1.20	1.40	1.43

Table 5.3a: HSA - I^{131} in whole blood, in the model vascular bed. Results from 22/2/74, haematocrit 41%, temperature 20°C.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{E}Q}{V_a}$	$\frac{\bar{E}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
64.9	0.87	1.50	1.22	1.72	1.40
50.5	1.06	2.00	1.39	1.89	1.31
42.3	0.99	1.84	1.42	1.86	1.43
31.6	0.99	1.75	1.43	1.77	1.44
17.2	0.94	1.51	1.35	1.61	1.44

Table 5.3b: HSA - I^{131} in whole blood, in model vascular bed. Results from 21/2/74, haematocrit 47%, temperature 20°C.

show an increase with flow rate, followed by a decrease. $\bar{t}^Q/V_n$ is larger than in tables 5.2 for all flow rates. $\bar{t}_n^Q/V_n$ also shows an increase followed by a decrease. Large errors in V are obtained and increase to $\sim 80\%$. V_n does not accurately measure the vessel volume, but usually gives a better estimate than V .

Fig. 5.3 illustrates the changing shape of the dispersion curves with flow rate. At the slowest flow rate, 2.94×10^{-2} ml/sec, there is significant dispersion along the T axis. At a flow rate of 27.6×10^{-2} the curve is maximally dispersed. At a faster flow rate, 86.2×10^{-2} ml/sec, the tail of the curve is cleared somewhat earlier. It is this change that produces the reduction in $\bar{t}^Q/V_n$ at faster flow rates.

Comparison of ^{86}Rb and HSA - ^{131}I dispersion

The dimensionless mean transit time, $\bar{t}^Q/V_n$, is plotted against flow rate, Q , in fig. 5.4 for ^{86}Rb and HSA - ^{131}I in whole blood. It is immediately clear that the HSA - ^{131}I mean transit times are always larger than the ^{86}Rb for a given flow rate. Only at very slow and very fast flow rates do the ^{86}Rb and HSA - ^{131}I lines approach each other. The ^{86}Rb curves show an initial fast rise, followed by a levelling off to a value of $\bar{t}^Q/V_n$ lower than that seen in the straight tube experiments. The HSA - ^{131}I points increase faster than the ^{86}Rb , reach a higher peak and then decrease, approaching the ^{86}Rb lines. The ^{131}I in 2N KCl mean transit times (not plotted) fall on a smooth line just below the ^{86}Rb line, as can be seen by inspection of table 5.1 and fig. 5.4.

$Q \times 10^2$ ml/sec	$\frac{Q}{F}$	$\frac{\bar{t}Q}{V_a}$	$\frac{\bar{t}_n Q}{V_a}$	$\frac{V}{V_a}$	$\frac{V_n}{V_a}$
97.3	1.01	1.38	1.13	1.36	1.12
59.4	1.45	1.63	1.27	1.12	0.88
26.1	1.31	1.61	1.30	1.23	0.99
15.3	1.29	1.68	1.35	1.30	1.05
4.75	1.16	1.32	1.36	1.14	1.17

Table 5.3c: HSA - I^{131} in whole blood in model vascular bed.
From 20/12/73, haematocrit 44%, temperature 21°C.

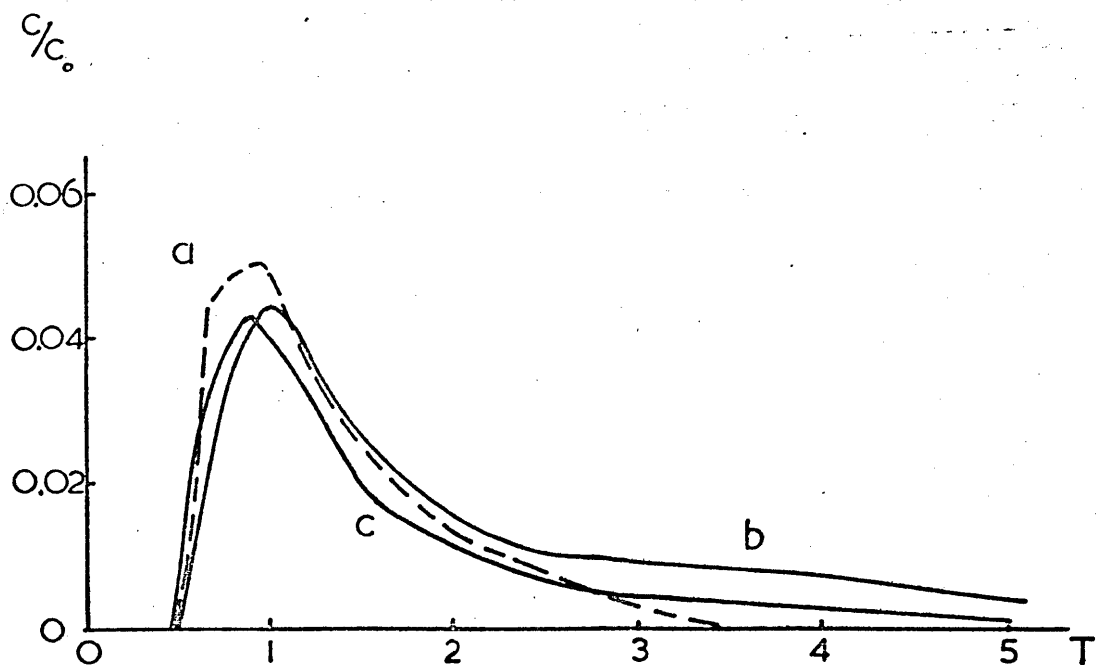


Fig. 5.3: HSA - I^{131} in whole blood, haematocrit 41% in the model vascular bed. Results taken from table 5.3a. Flow rates (10^{-2} ml/sec). a) 2.94 b) 27.6 c) 86.2.

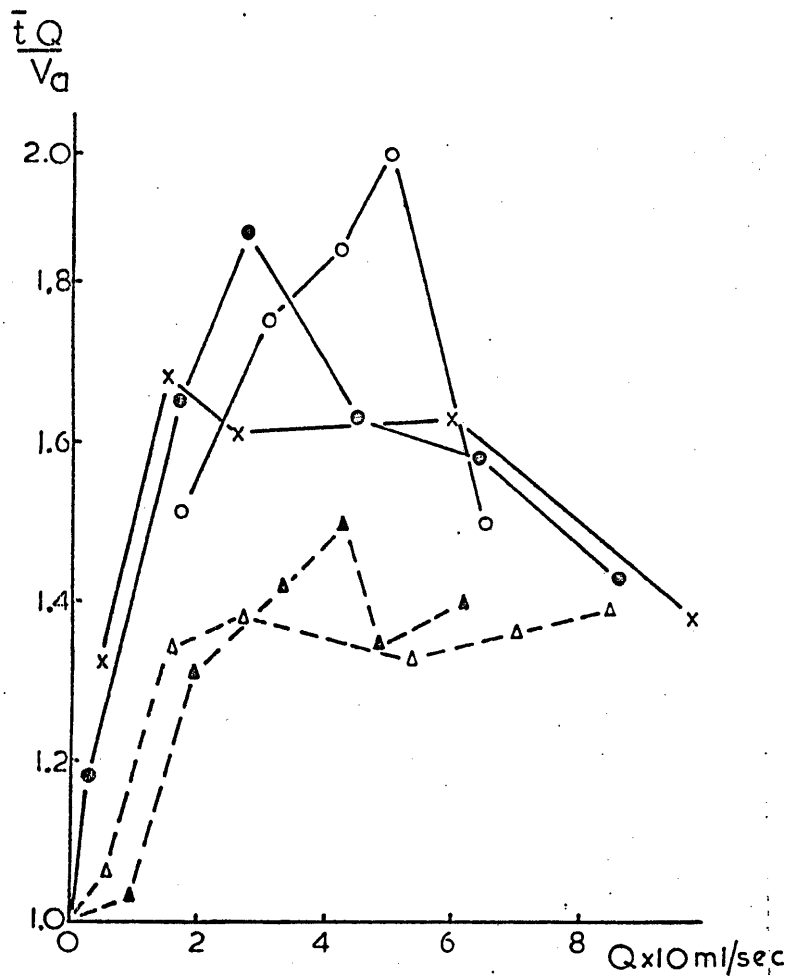


Fig. 5.4: Dimensionless mean transit time against flow rate of solutes in whole blood through the model bed. ▲ ^{86}Rb , haematocrit 43% (from table 5.2b); △ ^{86}Rb , haematocrit 39% (table 5.2a); ● HSA - ^{131}I haematocrit 41% (table 5.3a); ○ HSA - ^{131}I haematocrit 47% (table 5.3b); × HSA - ^{131}I , haematocrit 44% (table 5.3c).

CHAPTER 6DISCUSSIONStraight tube system - no mixer

The results of ^{131}I in 2N KCl, using mean cross sectional sampling, are in general agreement with the work of Bate et al. (43) and confirm the predicted trends derived in the theoretical analysis (chapter 2). The dispersion of indicator is determined by indicator diffusion and the parabolic velocity profile during flow. The shape changes in the dispersion curves, fig. 4.1, and the changes in indicator dilution results, table 4.1, are consistent with the dispersion being controlled by the dimensionless parameter $\frac{\bar{u} a^2}{Dx}$. At flow rates below that for the analytical requirement $\mathcal{L} > 0.8$ of Ananthakrishnan et al. (39) to apply, diffusion dominates the dispersion and all of the indicator dilution parameters give estimates within the experimental errors. The critical flow rate, where $\mathcal{L} = 0.8$, has been estimated to be 0.9×10^{-2} ml/sec, for the present experimental conditions. With increasing flow rate above this there is an increase in the errors associated with the use of F , $\bar{t}$, $\bar{t}_n$ and V . This is predicted from the theoretical equations and agrees with the preliminary results of Bate & Sirs (44).

Accurate fitting of the results to the theoretical curves of Bate et al. was not attempted because it is apparent that both sets of results are in broad agreement and in the ensuing investigation detailed comparison of curve shape was considered impracticable and unnecessary. Results were

generally compared using the dimensionless mean transit times of different solutes. The mean transit times, obtained experimentally for ^{131}I in 2N KCl by Bate & Sirs (44) show good agreement with the present results. However, they only considered a small range of flow rates as they were mainly concerned with changes around the Taylor B condition.

The behaviour of ^{86}Rb and HSA - ^{131}I in plasma are similar to ^{131}I in 2N KCl, as might be expected. The shape changes in the dispersion curves, figs 4.3 and 4.8, indicate that the same mechanisms produce dispersion. Only a narrow range of flow rates was studied using ^{86}Rb and the requirement that $\hat{\tau} > 0.8$ for effective mixing by diffusion was not met. Also, the behaviour of HSA - ^{131}I was necessarily somewhat different because of its much larger molecular weight and, therefore, much smaller diffusion coefficient. The output curves were much more dispersed along the T axis and a much slower flow rate, 0.3×10^{-3} ml/sec at $\hat{\tau} = 0.8$, was calculated for diffusion dominance. Again this requirement was not realised experimentally. Both indicators produced indicator dilution results, tables 4.3 and 4.8, that were consistent with the theoretical development. The ^{86}Rb results showed the same trends as ^{131}I in 2N KCl. The HSA - ^{131}I results were consistent with an indicator having a much higher molecular weight, as the errors were much larger in the appropriate parameters than with the low molecular weight indicators. V_n was also generally invariant to flow rate,

as expected.

The dispersion of ^{86}Rb in whole blood produced similar results to the other low molecular weight indicators. The shape changes in fig. 4.4 and the dilution results in table 4.4 demonstrate again the trends expected using the diffusion and convection model of indicator dispersion. In order to facilitate direct comparison of all the combinations of indicator and solutions the dimensionless mean transit time was used as a measure of indicator dispersion. The results are summarised in fig. 4.13. Using the accepted formulae from indicator dilution theory, all of the points in fig. 4.13 would be expected to fall on the line $\frac{\bar{t}_0}{V_a} = 1.0$. Instead, the mean transit times for all combinations show a progressive increase with flow rate. The initial increase in each curve with flow rate is greater than subsequent increases. The mean transit times of ^{131}I in 2N KCl, ^{86}Rb in plasma and ^{86}Rb in whole blood all fall on the same curve, which is that drawn for ^{131}I in 2N KCl. Apparently, then, the presence of the red cells makes little difference to the dispersion of low molecular weight indicators.

The results for HSA - ^{131}I in whole blood and plasma demonstrate the decreased influence of indicator diffusion on the dispersion at all flow rates. Both sets of results give much higher values of mean transit times than the low molecular weight indicators. Comparing HSA in whole blood and ^{86}Rb in whole blood it is clear that at any given flow rate there is a considerable difference in mean transit times, except at almost zero flow rates where the two curves converge. There

is also a significant difference between HSA in blood and HSA in plasma, the former being cleared earlier at a given flow rate than the latter. There are two possible explanations for the increased clearance of protein by the red cells. The first is due to the red cells having a finite velocity at the tube wall because of the inability of the red cells to approach closer than half the red cell thickness. This produces a reduced red cell concentration near to the wall and has been discussed at length by other workers (57)(58) with reference to the Fahraeus-Lindquist effect. The plasma has a (theoretical) zero forward velocity at the wall. The red cells will therefore sweep the protein from the surface causing an increased clearance relative to plasma solution. This mechanism does not affect the low molecular weight indicators because they are able to diffuse from the wall as shown by the shorter tail regions of the dispersion curves. It is only when indicators have dispersion curves with extremely long tails, indicating little diffusion from the stagnant layer at the wall, that the presence of the red cells alters the clearance characteristics. The second explanation is that the presence of the red cells alters the velocity profile of the plasma, possibly increasing the velocity gradient near the wall. The flow of blood at low shear rates is known to be non-Newtonian and it is therefore expected to have a non-parabolic velocity profile. These two explanations are not necessarily mutually exclusive as an increased velocity gradient near to the wall could cause an increased sweeping by the red cells. Further information on

the increased clearance was obtained by comparing the dispersion curves and mean transit times of various red cell concentrations at the same flow rate. The results summarised in table 4.10 and fig. 4.10 shows that even at haematocrits less than 25% there is an increased clearance relative to plasma. The decreased mean transit times at these haematocrits is mainly caused by changes in the tail region of the outflow dispersion curves (not shown in fig. 4.10). It is well known that at these low haematocrits, where the red cells are not in contact, red cell suspensions behave as a Newtonian fluid at the shear rates used in these experiments (59). It is unlikely, therefore, that the increased clearance here is due to velocity profile changes. At higher haematocrits (51.5%), however, there is a further increase in clearance of the tail region of the curve and, also, the peak height is increased. The change in peak height suggests a change in indicator concentration in the fastest streamlines and is probably due to an altered velocity profile.

The velocity profile induced clearance should be largest at the lowest shear rates where the flow is furthest away from the Newtonian regime. The viscosity changes of plasma and whole blood in the tube system are illustrated in fig. 4.17 and it is clear that over the flow range considered in fig. 4.13 the flow rates fall in the non-Newtonian region. However, at the fastest flow rates of around 4×10^{-1} ml/sec blood flow was almost Newtonian. The expected increase in clearance at low shear rates because of velocity profile changes is difficult to assess because of the concomitant increasing contribution of indicator

diffusion, as seen with plasma. However, at the fastest flow rates the plasma and whole blood mean transit time curves do not appear to converge, which suggests that wall sweeping and not profile changes contributes most to the clearance in these studies. Indeed, unpublished observations following up this work (60) suggest that there is still an increased clearance of HSA in blood relative to plasma at flow rates of 9×10^{-1} ml/sec, a flow rate well within the Newtonian region. To summarise, it is felt that wall sweeping by the red cells and an altered velocity profile can both contribute to the increased clearance of HSA in whole blood relative to plasma. The present evidence suggests that wall sweeping makes the largest contribution especially at high shear rates.

It is also clear from a comparison of (a) the outflow curve shape and dilution parameters of HSA - ^{131}I and (b) the low molecular weight indicators in the Taylor B region, that although the red cells are causing some indicator mixing at the tube wall the behaviour of the protein in blood flow is characterised by a lack of effective mixing in the bulk of the flow. The HSA outflow curves are widely dispersed along the T axis and there are substantial errors in the flow parameters. The red cells, therefore, do not cause substantial mixing of indicator in the laminar flow regime by shear induced motion i.e. rolling and tumbling, as seen in dilute suspensions by light microscopy (61).

The iodohippurate results, fig. 4.11 and table 4.11 demonstrate the effects of protein binding on the dispersion curves of low molecular

weight solutes. The curves and dilution results show more similarity to HSA - ^{131}I than ^{86}Rb in whole blood. Indeed, comparing the mean transit times in table 4.11 with fig. 4.13, it is clear that the dispersion of iodohippurate is almost identical to HSA. Mahe & Tauxe (50) have reported that iodohippurate is around 70% protein bound in man. The results suggest it is likely that large amounts are also bound in the pig's plasma.

With mixer

The preceding discussion was concerned with the dispersion of indicators in inorganic solution, plasma and whole blood when monitored with mean cross sectional concentration sampling. The inclusion of a mixer prior to the detecting crystal made only small differences to the general changes in shape of the dispersion curves. In all cases the influences of diffusion and velocity profile were still clearly discernible. However, with fast flow rates an increase in peak height relative to mean cross sectional concentration sampling was observable, and at most flow rates there was a slightly earlier final clearance of indicator. These changes were due to the re-distribution of indicator across the cross section and subsequent removal at the mean flow rate from the detector. Sheppard et al. (16) were the first to report these effects.

The theoretical development predicts that the inclusion of the mixer would effect two main changes in the indicator dilution results. The flow and the newly defined mean transit time ratios, Q/F and $\bar{E}_n Q/V_a$,

would be expected to be unity at all flow rates. Therefore, their product, V_n/V_a , should also be invariant to flow rate equal unity. This constancy in all three ratios is rarely demonstrated. The usual pattern is that either Q/F or $\bar{t}_n Q/V_a$ are close to unity, but rarely both. Sometimes, as in table 4.9b, neither approaches unity. However, the presence of the mixer always reduces the errors in all of the parameters, except V_n , relative to the results without a mixer. V_n was always accurately estimated without a mixer and the unreliability of the mixer can be attributed to the very crude mixer used and the difficulty of mixing the liquids instantaneously across the tube cross-section. Therefore, although the mixer results are not always exactly consistent with the theoretical development, they broadly demonstrate its validity.

Model vascular bed

There are several important objections that can be raised against the application of the straight tube results to the interpretation to in vivo data. The dispersion curves obtained for HSA in whole blood have extremely long tail regions because of limited indicator diffusion, and small changes in this region produce large changes in the magnitude of the mean transit time. However, curves obtained in vivo do not seem to be dispersed to such an extent. It is possible that the tail regions of the curves are influencing the dilution results by an amount that is disproportionate to its influence in vivo. Also, only one type of input

function, the bolus or step input, was used in the theory and experiments. It has been shown that different input functions can give different results for flow (14)(45). Further, in a complex vascular bed there is branching and curving of vessels, changing of haematocrit, loss of fluid through the capillary walls and changing of the nature of the flow regime.

In order to meet some of these arguments, some of the experiments have been repeated in the model vascular bed. This particular model is far from an ideal representation of a vascular system, but it does have some similarities and as such, has often been used before (5)(16). The nature of the indicator input function into the straight tube section after the model bed is clearly not of a simple bolus form. The multiple pathways indicator must traverse imitates, to some degree, the dispersion of indicators through the microcirculation. Because the smallest glass bead diameter was 1.5 mm, the 'capillaries' were not, however, of the same order of magnitude of capillaries in the microcirculation.

With these considerations in mind the dispersion of ^{131}I in 2N KCl was examined at different flow rates. In the interpretation of the results it is not possible to draw only upon indicator diffusion and laminary convection as the dispersive mechanisms. The splitting up of the flow through the multiple pathways will influence in some unknown way the dispersion curves at the outflow. However, because there are still large regions in which fully developed laminar flow may develop, it is likely that the curve shape will change with increasing flow rate because of diffusion and convection effects.

The changing shape of the dispersion with flow rate is seen in fig. 5.1. A much wider range of flow rates compared with fig. 4.1 were studied, but notwithstanding this it is clear that the curves are far less dispersed along the T axis than the corresponding straight tube experiments. At the slowest flow rate, 1.66×10^{-2} ml/sec, the outflow curve is symmetrically distributed about the point $T = 1.0$. Indicator moves along the laminar sections as a bolus, as previously described for the straight tube system, diffusion dominating the dispersion. With increasing flow rates the peak height is reduced and curve becomes more dispersed along the T axis. It is assumed that during the traversal of the model bed section the indicator is always completely mixed at all flow rates, either by diffusion or by the splitting and recombination of the flow stream, and that the changes in the dispersion curves are produced in the straight sections adjoining the model bed. This seems a reasonable assumption to make but it has not been experimentally tested in any way. Zierler (62) has discussed the indicator traversal of the microcirculation in the same terms. The dispersion along the T axis with increasing flow rate is thought to be due to the decreasing influence of indicator diffusion and the increasing influence of laminar convection, in a manner similar to that described in the straight tube system.

The indicator dilution results from the ^{131}I in 2N KCl dispersion curves are presented in table 5.1. The magnitude of the errors incurred in all of the ratios are reduced by the presence of the model bed section,

except those associated with V_n . This can be seen by comparison with the straight tube results in table 4.1. The term $\bar{t}_0/v_a$ shows the progressive increase that might be expected from an examination of fig. 5.1 and this increase is reflected in the values of V/v_a . At fast flow rates errors of up to 30% are obtained in volume calculation using the existing indicator dilution formulae. Even though V_n/v_a does not approach unity, at fast flow rates it always gives as good as, or better estimate of volume than V .

Fig. 5.2 shows the dispersion curves of ^{86}Rb in whole blood. The behaviour of the curves is broadly similar to that shown in fig. 5.1. At slow flow rates a near symmetrical curve is obtained and with increasing flow rates the curves become more dispersed along the T axis. Diffusion is again dominating the dispersion in the long straight section at slow flow rates. With increasing flow rates the influence of laminar convection becomes larger causing increased dispersion in the tail region of the curve.

The dilution results for ^{86}Rb in whole blood, in tables 5.2a and 5.2b, show again the reduced errors associated with the flow parameters relative to the straight tube system experiments, except for V_n/v_a . The shape changes in the curves and the dilution parameters are still largely controlled by the tail region of the curves. The results indicate that the dispersion of ^{86}Rb in whole blood is similar to that of ^{131}I in 2N KCl, in this model bed.

The HSA - ^{131}I whole blood results behave at slow flow rates as

might be predicted from the straight tube system results. Higher errors in mean transit times and volumes are obtained, tables 5.3a, 5.3b and 5.3c, than for ^{86}Rb or ^{131}I . The results for flow in tables 5.3a and 5.3b are similar to the ^{86}Rb results, underestimating slightly the true flow rate. In table 5.3c, however, Q/F is much greater than 1.0, except at the fastest flow rate. V_n/V_a follows the reverse pattern. In Tables 5.3a and 5.3b it is much larger than 1.0 and in table 5.3c it fluctuates around unity. The reasons for these inconsistencies are not clear. Flow and volume measurements were clearly unreliable, there being an unidentified source of error. It is possible that there was some movement of the beads in the bed near to the exit of the bulb, so that the way in which indicator was introduced into the straight tube section altered with time. Alternatively, the haematocrit of the blood could have had a large influence in the dispersion of indicator through the model bed. Because the source of variability was not discovered, these experiments must be regarded, to some extent, as preliminary. However, it should be noted that V_n generally gave a better estimate of volumes than V , as might be expected from the theoretical development.

$\bar{t}_Q/V_a$ was more predictable than these last two terms, as can be seen in fig. 5.4. The range of errors for each indicator was, though, still larger than in the straight tube system. Despite this variability several points may be safely made about the behaviour of the different indicators. a) Both HSA - ^{131}I and ^{86}Rb curves initially increase with flow rate and level out at about 4×10^{-1} ml/sec. At slow flow rates

the influence of diffusion is, therefore, clearly visible. The velocity profile begins to dominate dispersion along the straight sections above 4×10^{-1} ml/sec, and produces maximum errors in $\bar{t}$.

b) The HSA - ^{131}I curve is above that of ^{86}Rb at any given flow rate, demonstrating the greater influence of diffusion on the low molecular weight indicator. c) The HSA - ^{131}I curves all rise to a maximum and then decrease to approach the ^{86}Rb curves. Evidently at flow rates greater than 5×10^{-1} ml/sec there is another mechanism that is causing mixing of the indicator. This is also clearly illustrated in fig. 5.3. This series of dispersion curves is maximally dispersed at $Q = 27.6 \times 10^{-2}$ ml/sec. At faster flow rates the tail of the curve is cleared earlier resulting in a decrease in the dimensionless mean transit time. If it is assumed that the dispersion characteristics of the model bed section do not appreciably alter in this flow region, then the mechanism producing this reduction in transit time must work in the straight tube section. It is possible that eddies are formed at the junction of the glass bulb and straight tube section because of the discontinuity there. These might extend some way down the tube causing lateral mixing of the protein, particularly near to the tube wall. The ^{86}Rb curves would not be affected by this mixing because of the indicator diffusion from the walls. Alternatively, it is possible that with increasing flow rate the volume of distribution of HSA through the model bed section may decrease. At fast flow rates the indicator may preferentially pass down the centre of the bulb.

d) There are small differences between the mean transit times of ^{131}I in 2N KCl and ^{86}Rb in whole blood as can be seen by comparing the mean transit times in table 5.1 with fig. 5.4. The ^{131}I distribution of transit times (not plotted) falls slightly under the ^{86}Rb curve. Further investigation would be required to explain this difference, but it may be related to the way in which the indicators are dispersed through the model bed sections. It would be harder to mix the cell suspensions than the inorganic solution because of their higher viscosity, and the input functions to the straight tube section may therefore differ.

These experiments with the model vascular bed system clearly demonstrate that many of the results obtained from the analysis of indicator dispersion in laminar flow have further applicability than the simple straight tube system. The influences of laminar convection and indicator diffusion are still clearly visible in a system where straight tubing comprises 32% of the total volume. The results from the theoretical analysis of dispersion in a straight tube cannot a priori be applied directly to more complicated systems. However, it is anticipated that situations will occur when conducting in vivo studies, where knowledge of these considerations may be beneficial in the interpretation of results. A discussion of the possible implications of the model of laminar and diffusive dispersion to the use of catheters and to in vivo dispersion will follow.

Implications of dispersion model to catheter sampling & injection systems

The distortion by catheters of in vivo indicator dilution curves has been considered by many authors. Sheppard et al. (16), Rossi & Powers (14) and Gonzalez-Fernandez (45) have approached the problem theoretically by considering the effects of the parabolic profile on concentration against time outflow curves. The dispersive influence of the velocity profile has been examined for different indicator input functions and for different sampling techniques. There have also been many empirical studies (63)(64)(65), because of the problems of applying the theories of indicator dispersion in simple flow systems to blood in complex vascular networks. These empirical studies have been concerned with defining the system of sampling, or injection, that least distorts the shape of the indicator dilution curves. Sherman et al. (63) have assigned a 'figure of merit' to catheters, because of its ease of applicability. They found that in sampling systems the smearing effect of the parabolic profile is least when the volume of the catheter divided by twice the flow rate through it is small compared with changes in indicator introduced at the input of the catheter. Milnor & Jose (65) have produced empirical second-degree polynomial expressions for arrival time, mean transit time, build up time etc. and are in agreement with the work of Sherman et al. (63), in that the ratio V/F should be as small as experimentally feasible for minimal distortion. The general belief in these studies has been that while it is important to obtain an understanding

of the distorting effects of catheters on indicator dilution curve shape, the effect on the dilution parameters is completely predictable. Applying the method of convolution of distribution functions Zierler (3) has predicted that the mean transit times, given by V/F , of catheters and vascular bed are additive. The area under the outflow curve from the catheter should also yield the same area as that under the in vivo curve even though the curve shape will be changed. Yet Rossi & Power (14) and Gonzalez-Fernandez (45) have demonstrated theoretically and experimentally that results for flow and mean transit time, obtained with liquid flow down a straight tube, depend on the nature of the indicator input function and the sampling system used. The present work has confirmed and extended these preliminary findings. When using the theory of indicator dilution as presented by Zierler (3), the mean transit time of a catheter is given by the actual volume (mean cross-sectional area times length) divided by the known flow through it i.e. the sampling or injection rate. In the present experiments $\bar{t}$ is the experimentally determined mean transit time for the first moment of the outflow curve. Therefore, if the theory (as presented by Zierler) is correct, $\frac{\bar{t}Q}{V_a}$ must equal unity at all flow rates. This is obviously not the case. In figs. 4.13 and 5.4 the results of many experiments using bolus injection and mean cross-sectional concentration sampling are presented in a dimensionless form. Not one point lies on the line $\bar{t}Q/V_a = 1$. Discrepancies in $\bar{t}Q/V_a$ of up to 300% for HSA - ^{131}I in plasma and to 200% for HSA - ^{131}I in blood, illustrate the magnitude

of the errors that may be incurred with indiscriminate use of conventional formulae. The effect of indicator diffusion is to reduce these errors, as shown by the ^{86}Rb curve in fig. 4.13. However, the large separation of mean transit times of different indicators with different molecular weights then becomes a possibility. Inspection of the tables in Chapters 4 & 5 show that errors of up to 100% may also arise in the flow measurement during streamlined flow.

It is not only diffusion that can reduce errors in the flow parameters. Any process that induces lateral mixing of indicator will result in more accurate determinations. The same procedures that reduce catheter distortion of curve shape will also reduce the indicator dilution errors because the mechanisms producing distortion and errors are the same. Using small bore catheters and fast sampling is not always possible however, and several workers have questioned all but severe catheter curving as an efficient mixing mechanism (66)(67). It has been suggested that the rolling and tumbling of red cells in velocity gradients (36) may mix indicator. However, the only red cell induced mixing observed in the present study was in the region of the vessel wall and this was only a small part of the total dispersion. It is unlikely, therefore, that this plays a large part in mixing in tubes the size of catheters and therefore reduces these errors.

The present work suggests that for correct use of sampling catheters, mean flow proportional concentration sampling must be used at the catheter outflow. Reliable estimates of flow rate will then be obtained. Use of

mean cross sectional concentration sampling may result in larger curve areas and therefore smaller flow rate estimates than that of the circulatory system. The errors likely to be incurred in the determination of the circulatory mean transit time are larger. By subtracting the 'well mixed mean transit time', $\frac{V_d}{Q}$, from the mean transit time obtained from the first moment of the catheter outflow curve, the dispersion produced by the catheter may be drastically underestimated. An overestimate of the circulatory mean transit time may therefore be made. If mean flow proportional concentration sampling is used the 'well mixed mean transit time' is equivalent to $\bar{t}_n$, as predicted in Chapter 2 and, in general, the error in mean transit time will not be so large as that obtained with mean cross sectional concentration sampling.

It has been demonstrated that there is only one situation where the conventional formulae completely apply, and it can be assumed that the mean transit times are additive. This is when the indicator is completely mixed across the tube cross section at all points along the length of the tube, either by indicator diffusion or by (induced) secondary flow. The applied correction will then accurately correct for the dispersion of the catheter. At all other times, when the indicator is dispersed by a (near) parabolic velocity profile, the mean transit times may not ^{be} additive.

Importance of the diffusion & convection model to the dispersion of indicators in vivo.

The conclusion that must be drawn from the present model studies is that whenever there is streamlined flow the dispersive mechanisms,

with concomitant changes in curve shape and dilution errors, are likely to occur. This must therefore also apply to dispersion of indicators in the mammalian circulation. Bate and co-workers (41)(43), following up the initial work of Taylor (37), first provided a model of indicator dispersion in the circulation based upon indicator diffusion and laminar convection. They have predicted changes in dilution flow parameter determinations and curve shape, from a consideration of dispersion in inorganic solution. The present study provides confirmation that their results are applicable to blood flow in straight tubes and other model systems. Other factors must, of course, be considered when projecting results obtained from relatively simple flow systems to such as the mammalian circulation. The differing results from straight tube and model bed systems illustrate the dangers of premature extrapolation. There are many other variables that have not been considered in the present models, such as pulsatile and retrograde flow, changing haematocrit and extensive branching and curving of large vessels. However, any discussion of the factors that shape in vivo dilution curves must include a consideration of laminar and diffusive dispersion. In the following paragraphs some of the evidence that points to the possible importance of these mechanisms to in vivo studies is reviewed.

Implicit in the diffusion/convection theory is the presence of some form of streamlined flow. The present work has shown that at low shear rates where blood is non-Newtonian (and therefore does not have a parabolic velocity profile) large errors in all parameters are still

possible. A perfectly formed parabolic profile is not, therefore, a pre-requisite for the errors of the type discussed earlier. Investigations of the velocity profile in vivo are difficult and there is therefore not a great deal of quantitative evidence available. However, the weight of evidence points to the presence of some form of streamlining in many regions of the circulation. Streaks of red arterial blood at arteriovenous anastomoses have been reported by Franklin & MacLachlin (68), and Rayston & Taylor (69)(70) have observed streamlining of India ink through a glass section inserted in the arteries of dogs and cats. Helps & McDonald (71) have confirmed the presence of streamline flow in veins varying from the smallest mesenteric of the rabbit to the abdominal vena cava of the dog. They observed the naturally occurring streams of well oxygenated blood, which showed as red streaks against the darker venous blood, and injected Evans blue dye. The computed Reynolds' numbers were found to be well below that necessary for fully developed turbulence but they did find that phasic variations in flow and distortions of the vessels by breathing disrupted the streamlines. However, complete mixing of the streamlines across the vessel lumen was rarely seen, vortices being found mainly in the centre of the vessels. Gachtgens et al. (72) using a photometric double slit technique have demonstrated that the velocity profile is parabolic in the larger vessels of the microcirculation of the cat. Comparing the hepatic distribution of ¹⁹⁸ Au colloid injected into a mesenteric vein and ^{99m} Tc-microaggregated albumin injected into an antecubital vein, Gates & Dore (73) have demonstrated streamline flow

in the human portal vein. They found that the injected gold collected predominantly in the right hepatic lobe because of streamlining. Laminar regions are also implicit in the commonly accepted explanation of axial streaming of red cells (Fahraeus-Lindquist effect). The flow rate in the centre of the blood vessels must be larger than at the vessel walls, as discussed in the Introduction. This phenomenon is well recognised and has been demonstrated repeatedly in many different organs.

Indicator dilution studies have also provided evidence for laminar flow in vivo. Fox et al. (74) have discussed, as have many others, the difficulties of completely mixing the indicator across the cross section at the injection site. Presumably, if the flow system was efficiently mixed, by turbulence or secondary flows, this would present no difficulty. The conclusion that must therefore be drawn is that the flow is at least partly streamlined, even with an inserted catheter. Indeed, Bassingthwaite & Ackerman (75) have discussed the influence of laminar flow at the injection site on mean transit time measurements. Indicator introduced into the slowly moving streamlines near the vessel wall was thought to produce a 30-40% overestimate in this parameter. High pressure injections (74) have been used to induce good lateral mixing of indicator.

In the paper by Bassingthwaite & Ackerman, evidence was also provided against extensive streamlining in the aorta. Injected dye monitored at three positions in the anaesthetised dog (aortic root, lower thoracic aorta and the bifurcation of the aorta) were found to obey the principle of superposition of concentration against time curves. Even cardiac

fluctuations in flow produced relatively little error. From the present work it is considered unlikely that curves from regions in which there is extensive streamlining would obey the principle of superposition. However, because the sampling was performed in the aorta and the superposition did not include curves from injection site to sampling sites, the apparent inconsistency may be resolved. It is possible that flow in the aorta is extensively mixed. Clark & Schultz (76), using a thin film probe, have concluded that inertial factors predominate and viscous forces play little part in aortic flow. However, the time averaging technique they used to calculate the velocity profiles may underestimate the amount of streamlining, and the introduction of the probe through the arterial wall may also encourage instabilities in the flow. This question of the nature of the flow in the aorta has not been conclusively resolved. Frois & Heath (77), in an extensive thermodilution and cineangiographic study, have concluded that flow in the descending aorta and the distal aortic arch is predominantly streamlined, though not entirely laminar, and that flow is disturbed, though not necessarily turbulent, in the ascending aorta and proximal arch. The use of the Reynolds' number to estimate the onset of turbulence has probably added to the uncertainty because its application to the pulsatile flow present in the arteries is questionable (56). Pavek et al. (78) again using thermodilution techniques (interarterial sampling thermistors) have monitored the indicator changes across the aortic arch and have concluded that there is 'grossly incomplete mixing along the ascending

aorta'. This is at variance with the work of Peterson et al. (79) who found that dyes continuously injected into the aortic root were effectively mixed in a short section of the ascending aorta. Pavlek et al. (78) consider this disagreement to be caused by the sampling catheters used by Peterson et al. smoothing out concentration differences.

Several workers have reported differences in results for flow rate when indicator is sampled at different sampling sites. Sleeper et al. (80), comparing curves obtained simultaneously from the right brachial and right radial arteries have concluded that the large discrepancies obtained between the calculated values of cardiac output are caused by the 'non-uniform distribution of dye to the divergent sampling sites'. Also, differences obtained from sampling in different segments of the same arterial stream were attributed to the same cause. In a study of the effects of altering the injecting and sampling sites in dogs, Blackburn and Leigh (81) have concluded that 'differences between transpulmonary and left or right-sided indicator dilution curves were largely due to variations in transit time and washout of the dyed blood during its passage through the lungs'. They were critically examining the influence of coronary and bronchial recirculation on extrapolation procedure and found that these had little effect, other than in profound haemorrhagic shock. The left sided curves gave at least 10% higher estimates in cardiac output than transpulmonary curves.

Zierler (62) has discussed laminar flow through vascular beds and

has concluded that while there is undoubtedly some form of streamline flow, the multiple pathways traversed by indicator is still the dominant dispersive mechanism. The branching and rejoining of the flow system is assumed to make the vasculature an effective well mixed system. The present work provides no direct information on dispersion in the capillaries because capillary flow is likely to be of a different form to the type discussed here (82), although some of the considerations regarding indicator diffusion have already been applied to the micro-circulation (40). It is possible that dispersion through the micro-circulation may be only a small fraction of the total in many studies. Burton (83) has estimated the capillary blood volume to be 10% of the total and Roughton & Forster (84) consider that blood spends only about 0.7 seconds in the capillaries of the lungs which must be compared with a mean transit time from injection to sampling site of about 5 seconds. If the vasculature under consideration has large arteries and veins, laminar effects here may have a substantial influence on the total dispersion. If not, long catheters are required that contain a large volume fraction of the total, and laminar streaming with associated errors may occur here as previously discussed. Milner & Jose (65), for example, have estimated that a one second distortion in mean transit time by catheters may result in a 25% error in studies of the left ventricle because of the relative volumes involved.

Lassen and Crone (85) have provided evidence for the influence of diffusion on the dispersion of indicators contained wholly within the

circulation. Low and high molecular weight indicators simultaneously injected into the cerebral circulation in man produce different curve shapes that can only be interpreted in terms of indicator diffusion (called by Lassen 'Taylor's effect'). The curves are similar to those seen at very slow flow rates in the present work, with diffusion dominating the dispersion. Lassen's low molecular weight indicator curve was initially lower than the reference (protein) indicator, higher at the peak concentration, and subsequently lower again. This produced an initial positive, then a negative, followed by a positive extraction fraction, $E(t)$, (see Introduction) even though there was no indicator passage out of the microcirculation. However, the differences in curve shape are small and detailed examination of the separation does not seem possible. No mention is made by Lassen et al. of differences in mean transit times. This confirmatory evidence is possible only in the cerebral circulation because it is certain that the blood brain barrier prevents the low molecular weight indicator from leaving the circulation through the capillaries in appreciable quantities. In other organs transcapillary exchange can provide severe obstacles to detailed analysis of curve shape (see later).

Norwich & Zelin (86) have tested an indicator diffusion model of dispersion against indicator dilution curves obtained from anaesthetised dogs. Their model draws upon Taylor's theory of indicator dispersion and they produced solutions of the differential dispersion equation similar to Taylor's. They found good agreement between dilution curves from the

cardiopulmonary system and theoretically generated curves. They have also suggested that because indicator dispersion is similar to Brownian motion some modifications of the concept of mean transit time is needed.

Other multiple indicator studies appear to provide evidence against diffusion being an important dispersive mechanism in vivo. It is well known that labelled albumin and ^{24}Na simultaneously injected, produce similar outflow curves from the pulmonary circulation, under physiologically normal conditions (23)(87). Indeed, Chinard & co-workers (21) have collected a mass of data in various organs that show no great differences between some low and high molecular weight indicators in many organs, but they have also stressed the problems of interpretation of dilution curves because of the many shaping influences, some of which are specific for particular solutes in particular organs. However, there is not unequivocal support for the contention that low & high molecular weight indicators produce the same indicator dilution curves. Bauman et al. (88), for example, have presented results that demonstrate a 5-20% loss of small electrolytes in normal subjects during one passage of the lungs. Recirculation of indicators also presents difficulties because of the arbitrary extrapolation procedures often used. While these may not greatly influence the area under the curves, the first moment and therefore mean transit time will depend critically on the extrapolation method. It is this variable that is most sensitive to laminar and diffusive effects and these may therefore be obscured by extrapolation.

In fact, the presence of similar high and low molecular weight curves and indicator dilution variables does not preclude the laminar and diffusive dispersion mechanisms. The results of ^{86}Rb and HSA - ^{131}I during fast flow through the model vascular bed (fig. 5.4) show that similar mean transit times and dispersion along the T axis may be obtained, where the mean transit times still overestimated that directly calculated (from V_a/Q) by 40%.

It is concluded that while there are many contraindications, there is sufficient indirect evidence to support the contention that laminar and diffusive mechanisms are involved in the shaping of indicator dilution curves obtained from many in vivo studies. It will not be possible to assess the importance of these mechanisms until in vivo experiments are designed to look specifically for them. It is unlikely that they will be decisive in many indicator dilution studies but in those where there is extensive laminar flow in the circulating section under examination, they may be dominant dispersive influences. Because these dispersive mechanisms affect both curve shape and dilution flow parameter calculation, these considerations are important. Potentially, they may influence calculations of flow rate, mean transit times, intra- and extra-vascular volumes, capillary filtration and capillary permeability.

The dispersion of ^{51}Cr labelled red blood cells

The dispersion characteristics of labelled red cells in saline and in whole blood are similar to those of HSA - ^{131}I in blood. The same

pattern of increasing errors in the dilution terms, except V_n , are evident in tables 4.13 and 4.14. A larger flow range has been studied and higher errors in F , $\bar{t}$ and V are incurred. The dispersion curves, figs 4.14 and 4.15, are not dissimilar to those of HSA - ^{131}I in whole blood, fig. 4.6.

It is not immediately clear why the dispersion of red cells should be flow dependent. With protein and low molecular weight solutes, indicator diffusion is the main influence that alters the dispersion by the velocity profile. But the red blood cells have an infinitely larger molecular weight than any of the components previously discussed. They are therefore assumed to have a zero molecular diffusion coefficient. What, then, produces this flow dependent dispersion? The amount by which the red cells sediment during the experiments may increase with decreasing flow rate and so increase the red cell clearance in some way. This is considered an unlikely cause for the following reasons. Sedimentation was always minimized by constant mixing of the blood in the reservoir and by keeping the blood flowing whenever possible. A difference in sedimentation, causing a difference in dispersion characteristics, would be expected between washed cells suspended in saline and whole blood. The sedimentation rate for whole blood was of the order of minutes, compared with hours for washed cells. This sedimentation rate difference was readily observed in blood left standing during the experiments and yet the dispersion of washed cells in saline and whole blood were broadly similar, fig. 4.16.

Elution of ^{51}Cr from the red cells is another possibility. The slow flow rate experiments took longer to complete and during this time the label could have diffused out of the cell and into the suspending medium. Ebaugh et al. (89) have shown that eluted trivalent chromium will not re-enter red cells. It would, therefore, behave as a low molecular indicator with a high diffusion coefficient. However, the tests on elution described in the methods conclusively demonstrate that only trace amounts of labelled chromium were free in the suspended medium at completion of the experiments. Elution is therefore not considered to be a significant factor in these experiments.

The explanation most consistent with the available data is that the changes in dispersion curves and the flow parameters are caused by velocity profile changes in the flowing suspensions. Fig. 4.17 demonstrates the viscosity changes with flow rate for both suspensions and plasma. The suspensions are only completely Newtonian for the fastest flow rates, although the viscosity does not abruptly change until flow rates are less than 2×10^{-1} ml/sec. Associated with a progressively increasing viscosity (with reducing flow rate) is an increasing departure from a parabolic velocity profile. Possibly the profile might become more blunt with an increasing velocity gradient, or rate of shear, closer to the wall. Profiles of this sort would result in dispersion curves with later arrival times, higher peak concentrations and earlier final clearance than the curves obtained with a parabolic velocity profile. The experimental curves show no large changes in arrival times,

although differences are evident in fig. 4.14. This was not expected as the present apparatus was not sensitive to change in this region of the curves. Large peak height changes were produced, however, and these were predominantly at the slow flow rates. There were also differences between curves from cells suspended in saline and in whole blood, though not always so large as shown in figs. 4.14 and 4.15. But by far the most significant changes were in the descending portion of the dispersion curves and it was because of these that the dilution parameters changed so much. Fig. 4.16 shows some of the results of the dimensionless mean transit times plotted against flow rate. The results that were not plotted showed the same trends but were subject to more variability, as can be seen by inspection of the tables. Because of this variability any clear distinctions between the two sets of results may not be drawn, although there may indeed be small differences. It is clear, though, that the basic pattern of transit time change is the same for both cell suspensions. With increasing flow rate there is a large initial increase in dimensionless mean transit time followed by a smaller increase and/or a levelling off. It is concluded, therefore, that the present data is consistent with velocity profile changes and that the decrease in mean transit times is, in fact, following the progressive blunting of the velocity profile with decreasing flow rate.

There is one assumption that must be made before this interpretation is accepted. The red cells must not significantly redistribute themselves across the cross section in any other way during flow.

Goldsmith & Marlow (61) have described increased rotation of red cells at low shear rates and small radial movements in suspension containing cell concentrations of less than 5%. However, this is thought to be an unlikely mechanism in the present experiments because of the extensive cell crowding at the haematocrits used.

Washed and resuspended cells differ in several important respects to whole blood in viscometric terms, and these will be discussed prior to the interpretation of the viscosity results. Firstly, the viscometric effects of plasma are removed from the total viscosity by resuspension in saline. This produces a decrease in the relative viscosity, r , of the suspension (90). Secondly, there is no longer a protein-red cell membrane interaction of the type described by Chien et al. (90) and Rampling & Sirs (55). They have concluded that plasma proteins, particularly fibrinogen, interact with the red cell membrane to make it more flexible. Washed cells will, therefore, become relatively inflexible. Chien et al. (91) have demonstrated that red cells made completely rigid by long term suspension in acetaldehyde are almost Newtonian at a cell concentration of 43%, with a relative viscosity of 10-15. It is expected, therefore, that increases in cell rigidity caused by lack of protein-cell interactions will decrease the non-Newtonian behaviour and increase the viscosity of the suspension. Thirdly, washed and resuspended cells (in saline) do not exhibit a red cell-red cell interaction mediated by the plasma proteins, i.e. rouleaux and aggregation. Lack of the interaction would be expected to decrease the suspension viscosity at low shear (or flow) rates (90).

The present results for the viscosity of resuspended cells confirm the importance of cell deformability as a factor in the shear thinning (i.e. decrease in viscosity with increasing shear rate) behaviour of blood. Chien et al. (91) have shown that because there are no protein-red cell and red cell-red cell interactions, it is the deformability of the red cells that enables the fluidity of the suspension to increase with increasing shear. However, the present results also indicate that the plasma protein induced deformability plays little part in the viscometric behaviour of whole blood in these experiments. The centrifugal packing rate experiments, fig. 4.18, demonstrate that there is a significant decrease in red cell flexibility on washing and resuspension. This is shown by the slower rate of packing of cells suspended in saline, as explained in the Methods. There is, however, no appreciable shift to the right of the whole blood viscosity curve on washing and resuspension. The viscosity curves, fig. 4.17, for washed cells is significantly lower than, but parallel to, the whole blood curve. This suggests that over the flow rates considered the main effect of washing and resuspension is the removal of the plasma viscosity from the total viscosity. The mean transit time measurements also suggest that the velocity profiles are equal over the whole flow range, whereas a separation in transit times of the two suspensions would be expected at low shear rates if cell-protein interactions were significant.

It is concluded, therefore, that the main viscometric effect of resuspending the red cells in saline is caused by the removal of the plasma viscosity from the whole blood viscosity and that the red cell-

protein interactions were negligible over the flow range considered. The importance of cell-cell and cell-protein interactions on the viscometric properties of cell suspensions has been elucidated by many workers (90)(92), and the main effects are at the extremely low shear rates of less than 0.1 sec^{-1} . Rotation viscometers with a uniform shear rate and guard rings to prevent surface effects have been used to study this region because of their high sensitivity. However, the physiological importance of low shear viscometry has not been clearly defined, although it has been used as a diagnostic tool (92).

The increase in mean transit times with increasing flow rate provides evidence against the hypothesis of Nubar (93) that increased slip at a vessel wall is responsible for the shear thinning behaviour of blood. Slip is defined by Nubar as 'a finite velocity of fluid at a boundary or in its immediate vicinity', and by postulating that this finite velocity increases with increasing shear stress (i.e. viscosity $\times$ shear rate), a series of theoretical curves describing viscosity changes in blood were produced. These were found to be in good agreement with experimentally obtained curves. An assumption underlying Nubar's hypothesis was that the red cell volume near to the wall region decreases with increasing flow rate. This would be expected to produce faster final clearance of labelled red cells curves, when plotted c/c_0 against T , with increasing flow rate. In fact, the reverse is the case and the curves are cleared faster with decreasing flow rates. Nubar also assumed a flow independent bulk viscosity. However, the changing

shape of the present curves with flow rate demonstrates that changes occur not only at the wall of the tube, but also in the main mass of blood when flowing. This is indicated by the changes in curve peak height and in the downslope of the curves.

Comparison of figs. 4.13 and 4.16 demonstrate that there is no appreciable separation in mean transit times of labelled red cells and albumin over the flow range studied. Such a separation has been predicted from the diffusion/convection theory of indicator dispersion (41) (43) because the red cells have a lower diffusion coefficient than albumin. The outflow concentration against time curves should, therefore, differ significantly. The labelled red cells should arrive earlier, rise to a smaller peak and be cleared later than the albumin producing a longer mean transit time. Groom's (35) mean transit time ratio for cells to albumin $\bar{t}_c/\bar{t}_a$ of greater than unity (see Introduction) could therefore be explained. Bate et al. (43) strongly implied that if adequate correction were made for recirculating indicator, other studies would produce similar mean transit time ratios for cells and albumin. Detailed comparison of the figs. 4.13 & 4.16 is not possible because of the experimental uncertainty, especially in the red cell curves. However, the HSA in whole blood mean transit time curve does appear to be lower than that of ^{51}Cr labelled red cells. This is to be expected from the present experiments because the velocity profile affects the clearance of both cells and plasma and the albumin alone can diffuse across the streamlines. The effect of diffusion is therefore to speed

the clearance of the albumin. However, any difference between the curves is extremely small and the simultaneous determination of albumin and red cell curves would be required to conclusively demonstrate its presence. Because of the magnitude of the difference in the present apparatus and the attendant problems of statistical uncertainty in double tracer experiments, it was decided not to pursue this investigation. It is possible that in vessels the size of the microcirculation the influence of protein diffusion might increase, but here there are other, hydrodynamic, factors that must also be considered. Axial streaming tends to speed the clearance of red cells and it is, in fact, this mechanism that is the dominant factor in most studies, as shown by the usually seen $\frac{\bar{t}_c}{\bar{t}_a} < 1$. The present study, therefore, provides little evidence that the mechanism of red cell and albumin separation as proposed by Bate et al. is an important model for circulatory studies.

Summary

1. A theoretical investigation of the way in which a fluid is dispersed during laminar flow has been carried out from the standpoint of the indicator dilution theory and method. Bolus injection of indicator and two forms of sampling, mean cross sectional concentration and mean flow proportional concentration, have been examined. The results of the analysis have indicated that previous formulations may be substantially in error. It was found that in order to calculate accurately the flow rate using the indicator dilution method, mean flow proportional sampling must be used and a new mean transit time has had to be defined in order to calculate accurately vessel volume.
2. The theoretical predictions have been experimentally tested in a straight tube system and in a model vascular bed using inorganic solution, plasma and whole blood. Broad agreement has been found between the theory and experimental results.
3. In the straight tube system the soluble components of blood have been found to be dispersed by the combined effects of laminar convection and molecular diffusion. With a low molecular weight solute the outflow dispersion curves were qualitatively similar in 2N KCl solution, plasma and whole blood. The dispersion of

3. (cont'd)

- a high molecular weight indicator (HSA) in plasma was dominated by the velocity profile of the flow. The clearance curves in whole blood were similar to those in plasma, except that the red cells were found to speed the clearance of protein in the tail region of the curve. No evidence has been found, however, for appreciable mixing of solutes in the bulk of the flow by shear induced motion of the erythrocytes.
4. The model vascular bed results have demonstrated that the theoretical analysis and the diffusion/convection model have further applicability than to just a straight tube system.
 5. The dispersion of ^{51}Cr labelled red cells during blood and red cell suspension flow down a straight tube has also been examined. Increasing clearance of labelled cells with decreasing flow rates has been attributed to a flow dependent velocity profile. Viscometric measurements have demonstrated the well known non-Newtonian behaviour of blood and red cell suspensions at low shear rates. Increasing the red blood cell rigidity by repeated washing and resuspension in saline was found to have little effect on the velocity profile and dispersion curves.
 6. The implications of this work to the study of the mammalian circulation have been discussed, with particular reference to the use of indicator dilution techniques.

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