

TRANSPORT PHENOMENA AND BLOOD PLATELETS

TRANSPORT PHENOMENA AND BLOOD PLATELETS

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ABSTRACT

This thesis contains four studies of transport phenomena and blood platelets.

The initial stage in thrombus formation is platelet adhesion to a foreign surface: platelets diffuse to the surface and then bind to it. Experiments were performed to examine the influence of haematocrit, platelet concentration, shear rate and drugs upon platelet diffusivity, the platelet wall binding rate constant and the saturation level of platelet adhesion to the surface. The level of platelet adhesion to collagen coated glass tubes were determined by measuring the axial distribution of ^{51}Cr - γ -ray emissions from adherent ^{51}Cr labelled platelets. The results demonstrate that the model of Grabowski et al (1972) adequately describes platelet adhesion to foreign surfaces, that platelet adhesion obeys intermediate kinetics, that the plasma skimming layer can be ignored and that platelet diffusivity is enhanced by the complex motion of red cells. An explanation for contradictions in the literature regarding the effect of drugs upon adhesion is suggested.

A design for data collection and analysis was devised for experiments in which the distribution of tracer in multicompartement systems is followed. The design requires

the provision of multiple responses and analysis with a Bayesian multivariate technique. It deals with the problems of lumping and ill-conditioning inherent in descriptions of tracer distributions. The design proved successful in characterising the exchange of serotonin between the granules and cytoplasm of rabbit platelets and their surrounding medium in in vitro experiments using washed rabbit platelets.

In vitro, serotonin is freely exchangeable between platelets and their surrounding medium. To describe the potential behaviour of platelet serotonin in vivo, models were devised and fitted to the data resulting from dual label, ^{51}Cr and ^{14}C -serotonin, survival curves. The simplest model that adequately described the data was one in which serotonin was assumed to exchange at a finite rate between circulating platelets. The exchange rate constant from platelet to surrounding was similar to that determined in vitro.

The behaviour of adenine nucleotides in vitro and in vivo have been investigated experimentally (Reimers et al, 1975b). The in vitro exchange of adenine nucleotides between a metabolic pool and a granule pool is adequately described by first order kinetics. This information was used in a model that was fitted to data from adenine nucleotide survival and release studies. No significant metabolism or release of platelet adenine nucleotides was found in vivo.

SUMMARY

This thesis describes four studies upon various aspects of platelet behaviour. Part 1 reports upon an investigation into the adhesion of platelets to surfaces and in particular the influence of haematocrit and shear rate upon the process. Part 2 is a study of the use and analysis of multiple responses in multicompartment cellular systems. This is illustrated by a description of the exchange of serotonin between platelets and their surrounding medium. In Part 3 the use of survival curves to determine the in-vivo behaviour of platelet serotonin is described. Part 4 is a study of the release and metabolism of platelet adenine nucleotides.

Part 1 PLATELET ADHESION.

Platelets are cell fragments suspended within the blood. As a perversion of their haemostatic role, platelets are involved in the formation of thrombi. The initial stage of thrombogenesis is platelet adhesion to a foreign surface. Platelet adhesion is considered as a process in which platelets diffuse to a surface and then bind to it, adhesion continuing until saturation of the surface with platelets is achieved. The literature relevant to platelet adhesion is reviewed.

An experimental procedure was designed to measure platelet adhesion to glass, collagen-coated glass or polyurethane-coated glass. Suspensions of ^{51}Cr -labelled washed pig platelets were passed through glass tubes, which were coated if desired. The velocity and duration of the flow could be varied, as could the concentration of platelets and red blood cells within the suspension. After exposing the tubular test surfaces to the platelet suspension and rinsing, the levels of platelet adhesion to the surfaces were determined by counting the ^{51}Cr - γ -ray emissions from the surfaces.

The theoretical model of Grabowski et al. (1972) was adopted to describe platelet adhesion. This model assumes the convective diffusion of platelets to the surface where they bind at a rate first order with respect to the platelet concentration adjacent to the surface and first order with respect to the fraction of the platelet adhesion

sites that are unoccupied. The theory was extended to account for the presence of a red cell depleted 'skimming layer' adjacent to the surface. An expression was derived for the platelet collision frequency with the surface.

Experiments were devised to test the validity of the experimental procedure. It was concluded that the adhesion measured was not influenced by the tube rinsing steps and that platelet adhesion was irreversible over the duration of the experiment. A series of experiments and calculations were conducted to investigate the model of Grabowski et al. (1972), which hitherto has been accepted without test. No contradiction between data and model was found.

The effects of shear rate and haematocrit upon the platelet diffusivity, the surface binding rate constant and the saturation level of platelet adhesion to a collagen-coated surface were investigated. Platelet diffusivity is related to shear rate and haematocrit in such a way that it was concluded that the enhancement of platelet diffusivity induced by red cells arises from the complex motion of the red cells. The binding rate constant was found to increase with increasing haematocrit. The saturation level of platelet adhesion was shown to increase with increases in shear rate and haematocrit.

In contrast to collagen, it appears that not all platelets are capable of adhesion to glass or polyurethane-coated glass. The fraction of platelets that is capable of adhesion to these surfaces rises in the presence of

aggregates, as does the saturation level of platelet adhesion.

The model of the adhesion process used was compared to other models that assume diffusion limited kinetics. These models were significantly worse in their ability to fit data. It was proposed that previous conclusions that the adhesion process obeyed diffusion limited kinetics arose because, in devices relying upon the convective diffusion of platelets, the binding step is generally of less importance than the diffusion step in determining the rate limiting stage in platelet adhesion. This may also explain why, in this study, no inhibition of platelet adhesion was found in the presence of aspirin. A Couette flow device was demonstrated to be better able to distinguish inhibition of the binding step. The apparatus used in this study is more useful in determining the diffusivity and the saturation level of platelet adhesion.

Part 2 MULTICOMPARTMENT ANALYSIS WITH MULTIPLE RESPONSES.

Within multicompartment cellular systems in the steady state, the distribution of tracer as a function of time is described by sums of exponentials. A design for data collection and analysis is proposed to deal with the problems of lumping and ill-conditioning inherent in such a mathematical description. This design requires the provision of multiple responses and can be analysed by a Bayesian multivariate technique. Although only one response may be capable of completely characterising the system, there is much to be gained from additional responses in terms of precision in parameter estimation. A time series is used to account for autocorrelated error in the responses. The design is compared to other least squares approaches and is demonstrated in an investigation of the transport and storage of serotonin in blood platelets. The kinetic parameters describing serotonin movement are estimated and their joint confidence regions plotted. By these means the effects upon the transport and storage of serotonin induced by the treatment of platelets with thrombin, reserpine and imipramine have been determined. Platelet serotonin was found to be distributed between cytoplasmic and granule pools in the platelet. Thrombin causes degranulation of the platelets resulting in fewer serotonin granule binding sites. Reserpine inhibits the transfer of serotonin from the cytoplasm to the granule pool. Imipramine inhibits the transport of serotonin

across the platelet plasma membrane. The exchange studies supported the description of the movement of serotonin between platelets and their surroundings as a 'pump-leak' system.

Part 3 SEROTONIN BEHAVIOUR IN VIVO

Serotonin has been shown to be freely exchangeable between platelets and their surrounding medium in vitro. To investigate the behaviour of serotonin in vivo a platelet survival experiment was devised whereby rabbit platelets, radioactively labelled with ^{51}Cr and ^{14}C -serotonin, were reinjected into rabbits. The levels of ^{51}Cr - and ^{14}C -serotonin within circulating platelets were measured at times up to 180 hours after reinjection of the platelets. At all times the fraction of initial ^{14}C -serotonin within the platelets was observed to be greater than the fraction of initial ^{51}Cr . The ^{51}Cr platelet survival curve, corrected for the elution of ^{51}Cr , is an indicator of platelet survival. Various models were proposed to describe the ^{14}C -serotonin survival curve. The models that gave the best fit to the data assumed a finite rate of serotonin exchange between platelets through the plasma. However, no significant improvement of the fit of the models to the data was obtained by considering an exchange of serotonin between platelets and tissue. The fractional turnover rate from the platelets into the surrounding medium was identical to that calculated from in vitro exchange studies. This indicates that serotonin is lost from the platelet by diffusion.

Part 4 ADENINE NUCLEOTIDES IN PLATELETS.

The adenine nucleotides are the principal links coupling the energy yielding reaction sequences and the energy requiring ones. In platelets, adenine nucleotides are distributed between a metabolic pool and a metabolically inactive granule pool. There is a slow exchange of adenine nucleotides between these pools. The contents of the granule are liberated in the platelet release reaction. The platelet adenine nucleotides were labelled in vitro by addition of ^{14}C -adenosine to a suspension of washed rabbit platelets. The metabolism of ^{14}C -adenosine and the incorporation of the ^{14}C -label into the adenine nucleotides were modelled. It was shown that the exchange of adenine nucleotides between the metabolic and granule pools, the exchange between the various adenine nucleotides in the metabolic pool and the catabolism of the adenine nucleotides obey first order kinetics. Furthermore it was shown that the metabolic pool of adenine nucleotides could be considered as a single entity.

The behaviour of adenine nucleotides in vivo was investigated experimentally. A suspension of washed rabbit platelets was labelled in vitro with ^{51}Cr and ^{14}C -adenine and reinjected into the rabbits. The ^{51}Cr label acts as a platelet survival indicator. The ^{14}C -label is incorporated into the adenine nucleotides. Survival curves were obtained for each label and, at each sample point, release studies were made to determine the distribution of label between

the granule and metabolic pools. Models were devised to describe the ^{14}C -adenine survival curve and the fraction of total platelet ^{14}C -adenine nucleotides in the granule pool. The ^{14}C -adenine nucleotide and ^{51}Cr survival curves do not differ significantly. No significant improvement in the fit of the models to the release data was obtained by assuming there to be catabolism or release of the adenine nucleotides. It was concluded that in vivo release and metabolism of the platelet adenine nucleotides is negligible and that the platelet adenine nucleotide content is not age dependent and thus is unrelated to platelet senescence.

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PREFACE

This thesis is concerned with the application of mathematical models to topics in medical science. To devise and apply such models is not sufficient to 'prove' any particular hypothesis. The most that can be said of any model is that it is in accord with the experimental data. However, several benefits result from the use of mathematical models. The creation of models prompts clear thought about the system being modelled; hypotheses are logically developed to determine their consequences; the fit of models to data may be compared statistically and probabilities may be estimated for various hypotheses. The role of models is thus to examine hypotheses by a determination of their consequences and through a comparison of alternate models representing different hypotheses. It is this role that is common to the 4 parts of this thesis.

Part 1: THE ADHESION OF PLATELETS TO SURFACES.

1.1 Introduction.

Blood is a suspension of three types of cells or cell fragments: erythrocytes, or red blood cells; leukocytes or white cells; and platelets. In man, erythrocytes are biconcave discs, 7.2 - 7.9 μ in diameter (Wintrobe, 1960). Leukocytes are spherical, nucleated cells, 10 - 15 μ in diameter. The disc shaped platelets are cell fragments, 2 - 4 μ in diameter. Red cells, with a concentration of 5.10^9 ml^{-1} , occupy about 45% of the blood volume. The remaining 1/2% of the disperse phase in blood is composed of leukocytes, 10^7 ml^{-1} , and platelets, $2.5 - 5.0 \cdot 10^8 \text{ ml}^{-1}$, each occupying 1/4% of the volume of whole blood. With red cells possessing such a predominance, both in numbers and volume, the fluid properties of blood would be expected to be those of a suspension of red cells, platelets and white cells having a negligible effect (Meiselman and Goldsmith, 1973).

Plasma, the suspending fluid in blood, is an aqueous solution of proteins and salts with the following composition:

90% H_2O

7% Proteins (albumin, fibrinogen, globulins and lipoproteins)

1% Organics


& 1% Inorganics (Na^+ , K^+ , Cl^- , Ca^{2+} , HCO_3^- and PO_4^-)

The role of blood is to regulate homeostasis by maintaining constant the 'milieu interieur'. The plasma

constituents maintain osmotic pressure and act as buffers controlling the blood pH.

The best known function of platelets is a haemostatic one: they adhere to and aggregate at the site of vessel injury, thus preventing blood loss. An undesirable expression of this protective function is the ability of platelets to promote thrombosis and to accelerate blood clotting. A thrombus is a mass or deposit formed from constituents of the blood on the surface of a blood vessel.

The mechanisms leading to platelet aggregation in haemostasis and thrombosis are, first, the adherence of platelets to the damaged wall, followed by the release of platelet ADP (adenosine diphosphate) which causes platelet aggregation, and, second, the activation of the blood coagulation system, the platelet acting as a phospholipid surface on which several clotting reactions occur (Zucker, 1974). Thrombin, a platelet aggregating enzyme, is formed and this in turn converts plasma fibrinogen to fibrin, creating a fibrous network that stabilises a haemostatic plug. The activation of the blood coagulation system is of special importance in venous thrombosis, as fibrin deposition appears to require stagnant blood, a characteristic of the venous side of the circulation. In the arteries, such thrombotic accumulations would be scoured away (Spaet et al. 1973). Thus, on the arterial side of the circulation, platelet adhesion and aggregation play the dominant role (Baumgartner,



1973). It is with platelet adhesion in arterial and high flow regions that this thesis will be concerned.

While haemostasis is initiated by the rupture of a vessel, arterial thrombosis commences with platelet adhesion to a damaged endothelium (Johnson, 1970), or to subendothelial structures such as collagen (Hugues, 1960), the basement membrane (Hugues and Mahieu, 1970) or the microfibrils around elastin (Baumgartner et al. 1971). On artificial surfaces, adhesion follows the deposition of a layer of protein (Dutton et al. 1969). Following platelet adhesion, aggregation occurs together with the release of adenine nucleotides from the platelet storage granules. A released adenine nucleotide, adenosine diphosphate, causes further platelet aggregation.

The embolisation of thrombi, formed on atherosclerotic lesions of blood vessels, is one of the principal causes of death in humans. Thrombus formation also occurs commonly in devices performing part of the normal body functions (artificial kidney, heart pump etc.). In such devices, platelet adhesion and aggregation leads to a decrease in platelet concentration within the body (Gans and Krivit, 1962) which may add to or cause the bleeding tendency common after cardiopulmonary bypass (Stibbe et al. 1974).

Thus an understanding of the initiating mechanism of thrombosis, the adhesion of platelets to a surface, could aid the design of blood compatible devices and illuminate

the nature and location of thrombi. This first part of the thesis is thus concerned with the characterisation of platelet adhesion to surfaces under conditions typical of flow through arteries and extracorporeal devices. The effect of drugs upon adhesion will be investigated.

1.2 The Mechanism of Platelet Adhesion.

It is evident that, in any consideration of platelet adhesion, the transport of platelets to the surfaces is an important factor. Within the body, atherosclerotic plaques with their associated mural thrombi occur in zones of high shear stress (Texon, 1972). Such sites are thought to experience enhanced platelet deposition (Mustard et al. 1963). Baumgartner and Haudenschild (1972) observed that platelet adhesion to subendothelium increases with the flow rate of circulating blood and Petschek (1968) concluded that platelet adhesion to glass was a diffusion limited process.

In this section (1.2) the mechanism by which platelets diffuse to, and bind with surfaces will be considered. Attempts that have been made to characterise the adhesion process will be described. It is important to emphasize however, that much of the previous work on this topic has involved the uniform adhesion of platelets to a surface. Such adhesion has an unknown relationship to thrombogenesis in view of the observation that gross thrombi grow from specific sites on biomaterials rather than uniformly over the surface (Friedman and Richardson, 1972).

1.2.1 Platelet Diffusion.

The fluid properties of blood are those of a suspension of red cells. The volume fraction of red cells, the

haematocrit, is the property that influences both the apparent viscosity and the degree of non-Newtonian behaviour in blood (Rand et al. 1964). The deformability of red cells is important for the relative ease of blood flow: the viscosity of human blood, relative to plasma, at a haematocrit of 45% is 3; for rigid discs, occupying the same volume fraction, a relative viscosity greater than 200 would be expected.

Mammalian blood is best described rheologically as a Bingham plastic, though an equation of the power law type is more satisfactory for fully dispersed suspensions of red cells (Whitmore, 1968b). Below a haematocrit of 20% (Rand et al. 1964) and above shear rates of 50 sec^{-1} (Whitmore, 1963) the non-Newtonian characteristics of blood become negligible. The rates of shear exhibited in the circulation are shown in Table 1.1. In dialysers and other extracorporeal devices the wall shear rates for flowing blood are $200\text{--}400 \text{ sec}^{-1}$ (Stewart et al. 1975).

Blood may not strictly be treated as a continuum: red and white blood cells are subject to hydrodynamic interactions only. This notwithstanding, the no slip condition of the continuum approach is realistic in arterial flow; recurrent attempts to explain blood flow phenomena by introducing an arbitrary degree of slip are most misleading (Silberberg, 1971).

Goldsmith (1971) has examined the microscopic flow

Table 1.1

Rates of shear in the circulation*

<u>Vessel</u>	<u>Wall Shear Rate</u> (sec ⁻¹)	<u>Diameter of Vessel</u> (cm.)
Ascending aorta	190	2.0 -3.2
Descending aorta	120	1.6 -2.0
Large arteries	700	0.02 -0.6
Capillaries	800	0.005-0.001
Large veins	200	0.5 -1.0
Venae cave	60	2.0

* Taken from Whitmore (1968a)

behaviour of erythrocyte suspensions. In dilute suspensions (0.5% haematocrit), at a shear rate below 20 sec^{-1} , red cells rotate with a non-uniform angular velocity, this velocity being at a minimum when the cells are aligned to the stream flow. At higher shear rates, erythrocytes spend progressively more time in orientations aligned to the flow. Similar behaviour was observed by Klose et al. (1972b) in blood of 45% haematocrit. The tendency of the red cells to align in the direction of flow at higher shear rates marks a transition from solid disc to fluid drop behaviour. Red cell behaviour intermediate between that of solid discs and liquid drops is also exhibited in cell migration effects in suspensions of very low haematocrit (0.03%): at a low particle slip Reynolds number of 10^{-5} (that in arterial flow is about 10^{-3}), red cells migrate from the wall, an effect due to red cell deformation. At higher values of this Reynolds number (10^{-2}), the tubular pinch effect, with lateral migration from the axis and wall, occurs. This behaviour is that of solid discs. A result of the migration of cells away from the wall due to cell deformability (Goldsmith, 1974) is the creation of a cell depleted 'skimming layer' adjacent to the wall. Bugliarello and Sevilla (1970) characterised the skimming layer thickness as a function of haematocrit (Table 1.2). The thickness of the skimming layer was not significantly influenced by the flow velocity or the tube diameter. As would be expect-

ed, because of crowding, with increasing haematocrit the skimming layer thickness decreases.

Table 1.2

Skimming layer thickness*

<u>Haematocrit (%)</u>	<u>Skimming Layer Thickness (μ)</u>
5	25
10	20
20	12
40	8

* Taken from Bugliarello and Sevilla (1970)

In blood of a haematocrit of greater than 20%, the cells move in a crowded milieu. Cells are squeezed and distorted but undergo little rotation (Meiselman and Goldsmith, 1973). Whilst at haematocrits of less than 1%, two body collisions between red cells are common, at haematocrits of greater than 15% the concept of erythrocytes having spare time separated from their neighbours is untenable. In concentrated suspensions of red cells, Goldsmith (1971) observed that the red cells underwent radial excursions (S.D. $\pm 3.1 \mu$ in the centre of a 76μ tube at a haematocrit of 39% and a mean fluid velocity of $0.0015 \text{ cm sec}^{-1}$), though, as Chen (1974) observed, these cannot be regarded as being truly random movements.

The motions of platelets is strongly influenced by

those of red cells. A value of $10^{-9} \text{ cm}^2 \text{ sec}^{-1}$ would be expected for the Brownian diffusivity of platelets. However Goldsmith (1971) and Goldsmith and Marlow (1973) calculated a diffusivity of 10^{-8} to $10^{-7} \text{ cm}^2 \text{ sec}^{-1}$ for 2μ latex microspheres, representing platelets, in a suspension 40% by volume erythrocyte ghosts. It is thus evident that through the imposition of radial fluctuations erythrocytes enhance platelet diffusivity by several orders of magnitude over the value expected for Brownian motion. The mean distance of the microsphere fluctuations (S.D. $\pm 5.8 \mu$) was greater than that for red cells.

Chen (1974) has considered three models to describe the enhancement of platelet diffusivity in the presence of erythrocytes. These models are developed from the concept of platelets being carried in the vortices associated with red cells, transferring from one vortex to another by Brownian motion (Keller, 1971). Chen's first model was a microvortex one. It assumed that platelets drift from one red cell's microvortex to another by Brownian motion and that red cells possess a sinusoidal motion in the axial direction. This model predicted a platelet diffusivity of $6 \cdot 10^{-8} \text{ cm}^2 \text{ sec}^{-1}$ at a shear rate of 80 sec^{-1} and a power law relationship between platelet diffusivity D and shear rate γ of the form:

$$D \propto \gamma^{1/2}$$

Chen's second model, the meandering stream model,

again assumed a semi-random motion for red cells. It assumed that platelets move with small packets of fluid, squeezed and pushed by jostling red cells. These small packets have a sinusoidal motion in the axial direction. Such a motion is observed under conditions of high shear rate and haematocrit. Platelet movement in and between the small fluid compartments is due to Brownian motion. With this model, the same dependence of diffusivity on shear rate is obtained:

$$D_{\text{p}} \propto \dot{\gamma}^{1/2}$$

and a value of platelet diffusivity of $10^{-7} \text{ cm}^2 \text{ sec}^{-1}$ is calculated for a shear rate of 80 sec^{-1} .

The third model assumed random motion for red cells and for the platelets associated with them. For this

$$D_{\text{p}} \propto \dot{\gamma}$$

and a platelet diffusion coefficient of $10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ was estimated for a 80 sec^{-1} shear rate. This represents an upper bound to platelet diffusivity as red cell motion is not in fact completely random.

Several experimental studies have been performed to determine the diffusivity of platelets in blood or in suspensions of red cells: Turitto et al. (1972) determined platelet diffusivity in flowing blood employing a technique based on the classical dispersion experiment of Taylor (1953); Grabowski et al. (1972) examined platelet adhesion to glass resulting from blood flow through a U-shaped tube;

Turitto and Leonard (1972) measured platelet adhesion to a spinning disc, whilst Feuerstein et al. (1975) estimated the platelet diffusivity from the measured time dependence of platelet adhesion to collagen-coated surfaces in Couette flow. All these studies concluded that the platelet diffusion coefficient in blood is several orders of magnitude greater than that calculated for Brownian motion.

There is less agreement on the dependence of platelet diffusivity on shear rate and haematocrit. Turitto et al. (1972) could detect no significant dependence of diffusivity on haematocrit (haematocrit 30-50%) and shear rate ($40-440 \text{ sec}^{-1}$). With the use of an annular perfusion chamber however, Turitto and Baumgartner (1974) found that platelet diffusivity was related to shear rate by the expression

$$D_{\text{p}}^{0.42 \pm 0.09}$$

Grabowski et al. (1972) discovered no effect of haematocrit (30-50%) on platelet diffusivity but calculated a shear rate dependence of the form

$$D_{\text{p}}^{1/2}$$

Feuerstein et al. (1975), studying a wide range of haematocrits, found the diffusivity to rise from $10^{-8} \text{ cm}^2 \text{ sec}^{-1}$ at a haematocrit of 15% to a peak of $1.5 \cdot 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$ at a haematocrit at 35%. At a haematocrit of 45% the platelet's diffusivity fell slightly below that for the 35% haematocrit. That the platelet diffusivity changes little above a

haematocrit of 30% would explain the inability of Turitto et al. (1972) and Grabowski et al. (1972) to discover any dependence of diffusivity on haematocrit.

The frequency of platelet collisions with the wall is determined largely by the motion of red cells. Platelet diffusion from the bulk of the blood to a region adjacent to the wall is enhanced by a shear induced red cell movement. Chen (1974) suggested that the platelet encounter rate with the wall would be proportional to the rate at which red cells jostle into the region adjacent to the wall. Then

$$\text{Collision frequency} \propto N_{\text{RBC}}^{1/3}$$

where N_{RBC} is the red cell concentration at the edge of the skimming layer. Keller (1971) found the rate of red cell collision with the wall to be proportional to the shear rate and to the haematocrit to the power (2/3). However there is no evidence in studies of platelet adhesion that the continuum approach to platelet diffusion must be abandoned in proximity to the wall or that special attention must be paid to the microscopic motion of red cells and platelets in that region.

A feature of the majority of these studies is that they have avoided pulsatile or non-steady state flow. Whilst this is unlikely to affect the diffusion of platelets in a cylindrical geometry with developed flow (Caro et al. 1971), steady state calculations may not be able to predict flow separation in time and space, and thus platelet motion,

when flow is unsteady as in the arteries (Friedman et al. 1973). An indication of the effects of separation may be gained however from steady state studies: Goldsmith (1974) observed that red cells and platelets within an annular vortex migrated to join the mainstream.

To summarize this discussion on platelet motion, previous studies have shown the diffusivity to be significantly enhanced by red cell motion. The diffusivity rises with haematocrit to a plateau value reached above a haematocrit of 30%. The dependence of diffusivity on shear rate is uncertain.

1.2.2 The Binding of Platelets to Surfaces.

The adhesion of platelets to surfaces is an ill-defined process. It is likely that the situation is of such complexity that attempts to define a surface's propensity for platelet adhesion in terms of a single parameter may be futile. Platelet adhesion will however depend upon the charge distribution and the detailed structure of the surfaces of both cell and wall. As platelets, like all mammalian cells, carry a net negative charge, electrostatic repulsion and the electrodynamic attraction due to Van der Waal's forces will influence adhesion (Weiss, 1971). These forces may in turn be modified by the extensive ordering of water adjacent to interfaces that would alter dielectric constants (Drost-Hansen, 1971).

Platelet adhesion to artificial surfaces is preceded by the adsorption of plasma proteins (Madras et al. 1971; Vroman, 1972). Platelets thus have only minimal contact with the foreign surface; instead they contact and adhere to the layer of deposited plasma proteins (Dutton et al. 1969). The surface may exert its influence through this protein layer. However, there is as yet no consensus as to the surface properties that promote adhesion. Lyman and Kim (1971) stated that low energy surfaces resisted interaction with blood components. Baier et al. (1971) postulated that the thromboresistance of low energy surfaces, compared to high energy surfaces, was due to the maintenance of the adsorbed protein's native configuration upon such a surface. In contrast, Lyman et al. (1968) noted an increase in platelet adhesion upon protein denaturation. Zucker (1972) suggested that the 10-15 second time delay in platelet adhesion to a glass surface, after exposure to blood, was the time required for fibrinogen conversion; a monomolecular layer of fibrinogen being formed in 2 seconds after exposure of the surface of blood (Vroman, 1972). Butruille et al. (1975) proposed that this delay however was the time necessary to remove pre-wetting solution from the surface. Related to the surface energy is the critical surface tension. Lyman et al. (1970) determined that platelet adhesion could be correlated to critical surface tension in a series of

neutral hydrophobic polymers. Heterogeneity of biomaterials will however complicate these results (Neuman et al. 1975).

Alternative factors have been suggested as being important in determining the thrombogenicity of surfaces: Bennett and Zisman (1971) found the positive dipole moment in polymers to play a critical role, whilst on metals, the potential across the blood material interface has been proposed as the deciding factor (Gileadi et al. 1972). Contradicting all these results is the work of Friedman et al. (1970) who stated that the adhesion of platelets does not depend upon the nature of the surface. This conclusion must be qualified however by the observation that the adhesion process followed diffusion limited kinetics.

An additional factor in platelet adhesion is the roughness of the surface. Ward et al. (1973) found that platelets adhere preferentially to undissolved glass pockets formed in surface imperfections. Adhesion to surface flaws also occurs at high shear rates (Madras et al. 1971).

There is little agreement upon the mechanism by which platelets adhere to foreign surfaces. On adhering to injured subendothelium (Sheppard and French, 1971) or protein-coated glass surfaces (Jenkins et al. 1973), platelets alter shape, developing long projections, pseudopods (Fig. 1.1). Adherence may be inhibited by interference with the platelet contractile processes that are responsible for shape change and pseudopod formation. Thus adherence of fibrinogen-coated



Figure 1.1

EM of platelets adherent to glass (5000 x magnification).

surfaces is diminished by chelation of divalent ions and by agents, such as prostaglandin E_1 (PGE_1), that increase platelet cyclic AMP (adenosine monophosphate) levels (Jenkins et al. 1973). PGE_1 and adenosine similarly inhibited platelet adhesion to collagen-coated glass (Cazenave et al. 1974c).

Various requirements must be met for platelet adhesion to certain surfaces. Fibrinogen and calcium are necessary for the adhesion of washed or unwashed platelets to glass (George, 1972; Zucker and Vroman, 1969); platelet adhesion increasing linearly with increasing concentrations of purified fibrinogen (Mason et al. 1971). In contrast, Packham et al. (1969) found that the coating of glass with albumin diminished adhesion. For platelet adhesion to collagen fibres, Lyman et al. (1971) stated that fibrinogen was necessary, though Cazenave et al. (1973a) found high levels of platelet adhesion in the absence of fibrinogen. Cazenave et al. (1973a) also found that the removal of divalent ions (calcium and magnesium) diminished platelet adhesion to collagen, a result paralleled by the finding of Baumgartner and Haudenschild (1972) that platelet adhesion to subendothelium was enhanced by the presence of divalent cations.

The molecular mechanism by which platelets adhere to foreign surfaces is unclear. Jamieson et al. (1971) proposed that platelet adhesion was mediated by an enzyme acceptor complex. Lee and Kim (1974) suggested a mechanism involving the formation of enzyme-substrate complex bridges

between platelet glucosyl transferase and surface adsorbed glycoproteins (γ -globulin and fibrinogen). Cazenave et al. (1974b) however could find no experimental evidence that collagen glucosyl transferase plays a part in adhesion to collagen. This hypothesis is still a matter of controversy. It is unlikely that platelet membrane sialic acid is involved in platelet adhesion to collagen or connective tissue, as pretreatment of the platelets with neuraminidase, an enzyme that removes the terminal sialic acid residues from glycoproteins or glycolipids, does not alter rabbit platelet adhesion to collagen or to subendothelium (Greenberg et al. 1975), nor the ability of human platelets to adhere to rat connective tissue (Spaet and Zucker, 1964).

Butruille et al. (1975) determined patterns of platelet adhesion to foreign surfaces that call for adherent platelets to release an adhesion potentiating substance that affects other platelets approaching the surface. George (1972) found that platelet adhesion on a glass surface increased with the level of ADP. In contrast, Cazenave et al. (1973a) found that the adherence of platelets to collagen was not caused or increased by ADP.

ASA (acetylsalicylic acid - aspirin) is commonly used and may have a potential antithrombotic effect (Harker and Slichter, 1974). Its effect upon platelet adhesion has not been clearly determined. Whilst Cazenave et al. (1974a, 1975) observed that ASA markedly decreased platelet adherence

to collagen and subendothelium under conditions in which aggregation and thrombus formation are prevented, Sheppard (1972) and Baumgartner and Muggli (1974) concluded that platelet adhesion to subendothelium was not altered by ASA. Similarly George (1972) found that ASA did not influence platelet adhesion to glass.

Finally in this review of the platelet binding process the effects of temperature and flow need to be considered. Cazenave et al. (1973a) found the adhesion of rabbit platelets to be temperature dependent with negligible adhesion at 5°C, more at 22 ° C and most at 37 ° C, an expected result if adhesion involves metabolic processes. Feuerstein et al. (1975) however found that the levels of pig platelet adhesion to collagen at 22°C did not significantly differ from those at 37°C. A similar conclusion was made by Jenkins et al. (1973) who examined the adhesion of pig platelets upon a fibrinogen-coated surface. Regarding the effects of flow, previous work is contradictory. Butruille et al. (1975) suggested that many platelets interact with the surface without adhering, losing temporarily their ability to adhere, and Goldsmith (1974) concluded that platelets become refractory to aggregation as a result of flow. However, Klose et al. (1972a) proposed that platelets were activated by shear alone.

In summary, it is evident that the processes by which platelets adhere to surfaces are complex and, as yet, ill-defined.

1.2.3 Platelet Adhesion - the Rate Determining Step.

In the previous sections, the factors that influence the diffusion of platelets and their binding to foreign surfaces have been discussed separately. This section will be concerned with platelet adhesion as a combined process of diffusion and binding.

Much of the literature on platelet adhesion has arisen from application of the glass bead test of Hellem (1960). This does not truly represent platelet adhesion but rather platelet retention through the adhesion of platelets and trapping of aggregates. With this test it was observed that platelet retention increased with increasing haematocrit, being negligible with platelet-rich plasma (Hellem, 1960). As ghost cells enhance adhesion as much as do erythrocytes, the enhancement must arise from an increased platelet diffusivity rather from the introduction of ADP or other species into the system (Zucker et al. 1972). This was confirmed by Turitto and Baumgartner (1975). The enhancement of platelet adhesion in the presence of red cells indicates that, at low haematocrits (less than 10%), platelet adhesion obeys intermediate or diffusion limited kinetics. Such was observed by Robertson and Chang

(1974) who considered the glass bead column to be analogous to an ion-exchange column. At higher haematocrits (30%) they concluded that platelet adhesion was limited by the binding rate.

Several quantitative studies have been performed that involved platelet adhesion alone and not aggregation. Petschek (1968), examining the deposition of platelets from virgin blood striking a glass surface with stagnation flow, concluded that adhesion was diffusion limited. Grabowski et al. (1972), employing a U-shaped tube, concurred in this, as did Turitto and Leonard (1972) observing platelet deposition on a rotating disc. Turitto and Baumgartner (1974) found the axial variation in platelet adhesion to be less than that expected for diffusion limited control in an annular diffusion device. On the basis of the earlier conclusion that platelet adhesion was diffusion controlled, they precluded intermediate kinetics, in which both diffusion and binding processes are important in the determination of the rate of the adhesion, as an explanation for their results, ascribing the cause to the partial depletion of the boundary layer before blood reached the subendothelial test surface or to the detachment and reattachment downstream of platelets.

This preclusion of intermediate kinetics is of dubious validity in view of the results of Robertson and Chang (1974), using a glass bead column, and those of Feuerstein et al. (1975). The latter found that intermediate

kinetics gave a significantly better fit to the data than did diffusion limited kinetics. However, as will be observed in section (1.4), Theory, these contradictory results may be resolved as certain geometries and flow rates create conditions in which kinetics that are essentially diffusion limited must arise.

1.3 Experimental Design and Procedure.

The design of the experimental apparatus and procedure must be such as to facilitate discrimination between possible models of the processes involved and to allow the elimination of experimental artefacts.

1.3.1 Design.

Grabowski et al. (1972) demonstrated that if the adhesion process is described by diffusion limited kinetics, there is a considerable axial variation in platelet adhesion along the length of a cylindrical tube if adhesion levels do not approach those of surface saturation. With fully developed flow, where x is the axial distance of a point from the leading edge, the level of platelet adhesion of a point is proportional to $x^{-1/3}$. For adhesion kinetics in which the binding rate of platelets to the surface is rate limiting, there will be no axial variation for platelet adhesion (Fig. 1.2). Thus the examination of platelet adhesion levels in long narrow cylindrical tubes, following their exposure to a flowing platelet suspension, should allow much information about the kinetics of the adhesion process to be gathered. A drawback of such a tube geometry is that kinetics approaching those of diffusion limitation would be followed at lower values of the binding rate constant than is the case with other geometries (section 1.4).

In these experiments washed platelet

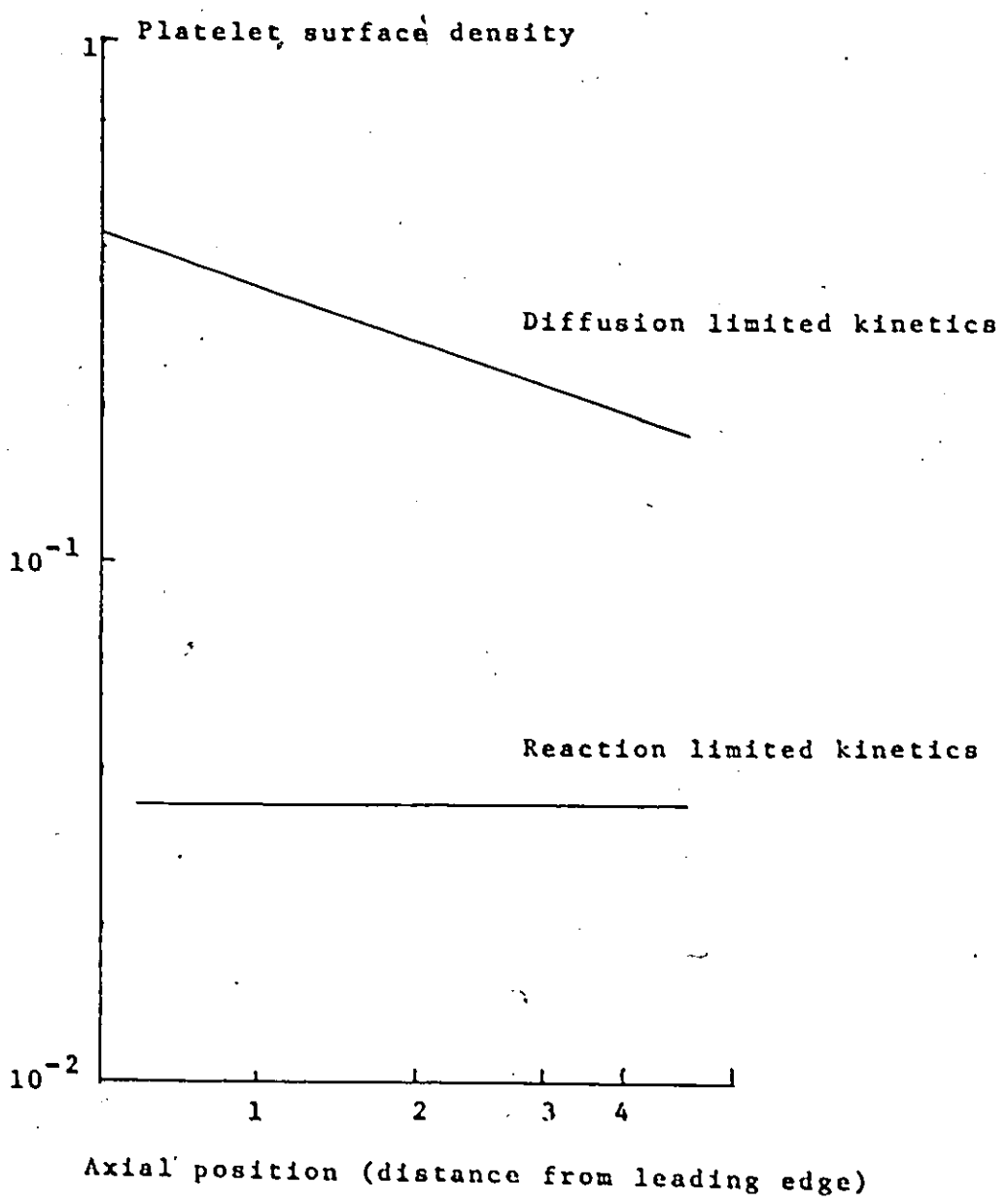


Figure 1.2

Axial variation of platelet adhesion

suspensions were used rather than anticoagulated whole blood or platelet-rich plasma. As a result the effects of anticoagulants are avoided. Using suspensions, experiments may be performed in a controlled manner with predetermined haematocrits and platelet concentrations. This is impossible if the test surface is exposed to blood drawn directly from an animal.

To allow the levels of platelet adhesion on tubes to be compared, experiments should be performed under identical conditions. Eight tubes were thus set in parallel between two manifolds with keys at top and bottom to seal the tubes. The apparatus is shown in Figs. 1.3 and 1.4. The tubes are 0.22 cm internal diameter and 25 cm in length; they are of glass and may be internally coated if desired. To avoid the sedimentation of red cells and platelets upon the test surfaces, the apparatus was mounted vertically.

An infusion-withdrawal pump (Harvard Instrument's 957) provides a steady flow of suspension through the device. The volume flow rate through the apparatus may be varied between 0 and 1 ml sec⁻¹. This range of volume flow rate corresponds to wall shear rates of between 0 and 920 sec⁻¹ depending on the number of tubes open. An air trap is included in the circuit to prevent the passage of air past the cylindrical test surfaces; air bubbles enhance platelet transport, increase platelet reactivity and provide a surface on which platelet aggregates form (Warren et al. 1973). All these

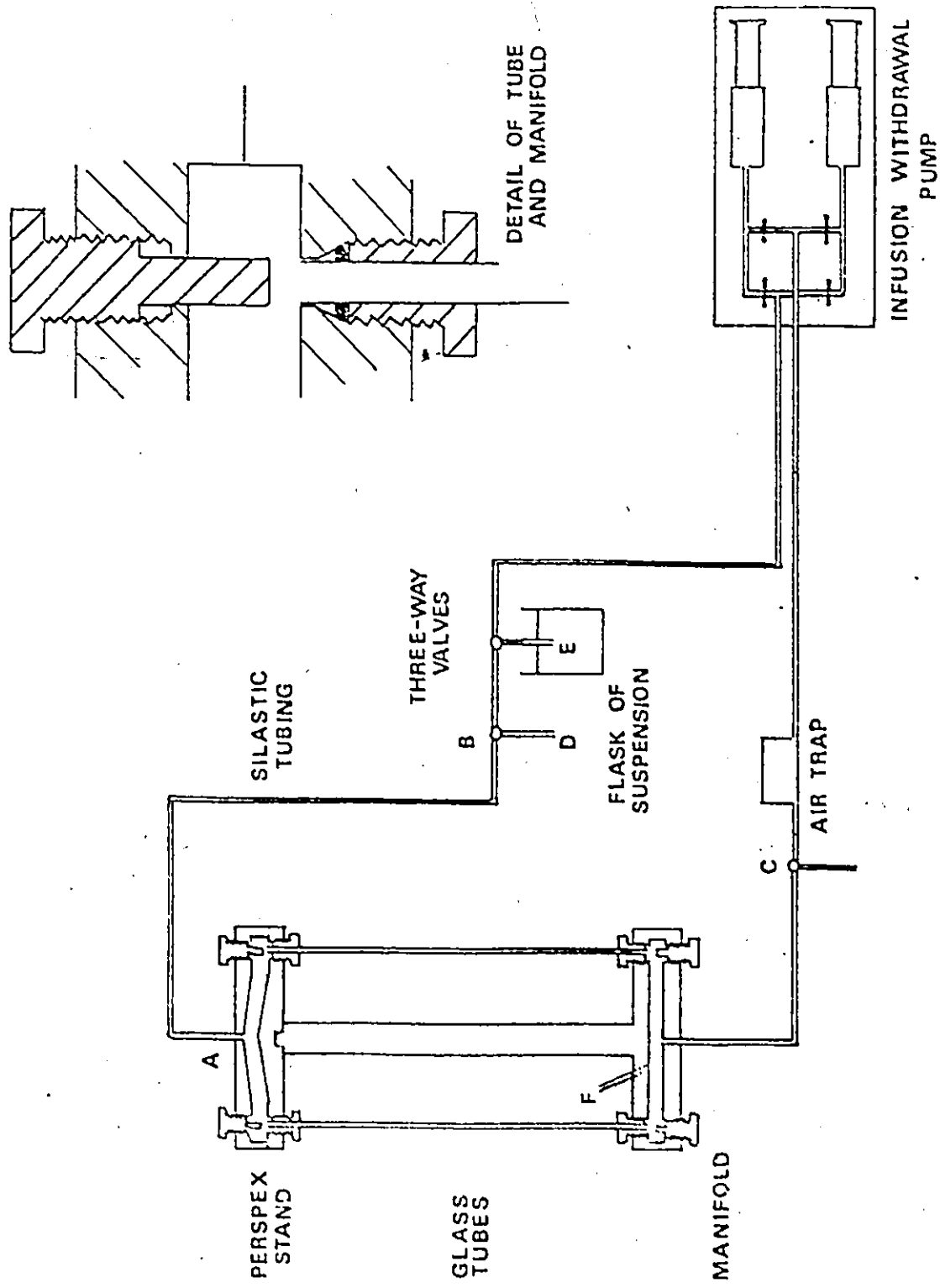


Figure 1.3

Schematic diagram of tube flow device. A-stand and inlet and outlet manifold, B and C - three way valves for prefilling with suspension, D-bleed line, E-beaker containing suspension and F-outlet for Tyrode solution lead fluid.

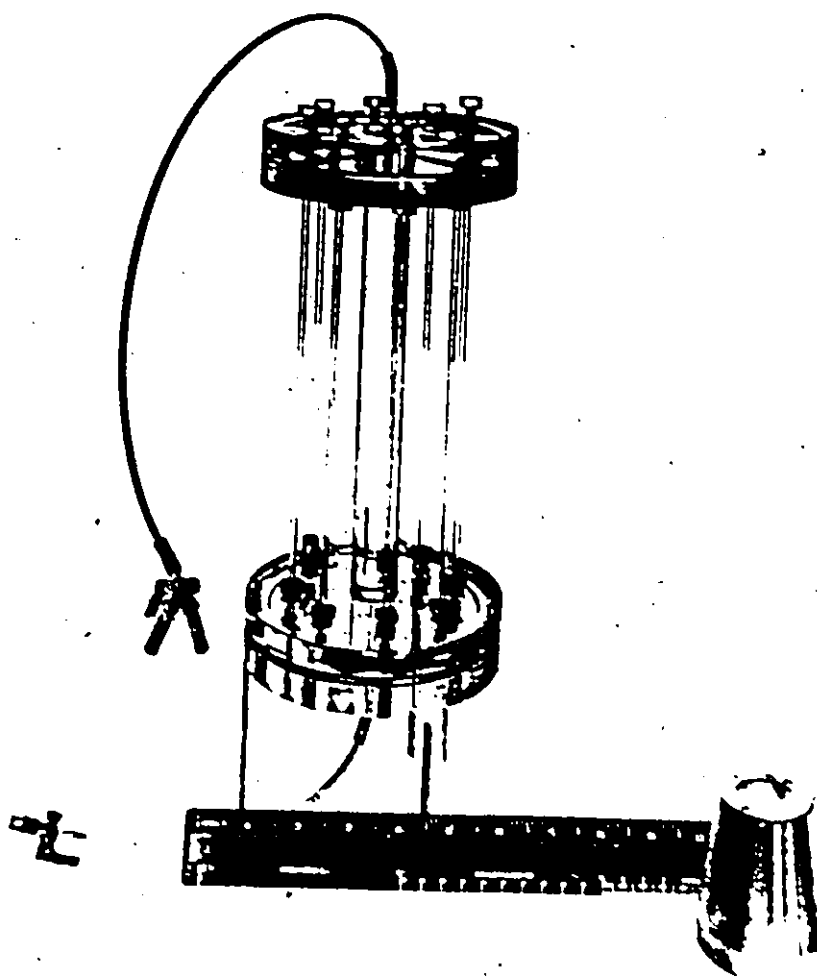


Figure 1.4
Apparatus showing tubes and manifold.

ill-defined effects are undesirable within the test system. The volume of the circuit, including 30 ml syringes, is 80 ml; the tubes and manifolds account for half of the volume.

Visual counting of the levels of platelet adhesion would be both tedious, because of the tube's length, and difficult, because of its curvature. Instead it was decided to give the platelets a radioactive label. Two labels were considered: the γ -ray emitting ^{51}Cr (as sodium chromate) and the β -ray emitting ^{14}C -serotonin. It is unlikely that platelet function is influenced by incubation with ^{51}Cr (Bjornson, 1974) or ^{14}C -serotonin. The use of ^{51}Cr was decided upon for two reasons. First, because of quenching, platelets labelled with ^{14}C must be washed from the surface before counting; however a reproducible washing procedure could not be devised. In contrast, with ^{51}Cr , there is no need to detach the platelets from the glass or polymer surfaces before counting the radiation emanating from the surface. Second, ^{51}Cr is known to label the platelet population uniformly (Busch and Olson, 1973). Thus following the exposure of the surface to a flowing ^{51}Cr labelled platelet suspension, the level of platelet adhesion will be obtained as a radioactive count. The test surfaces for platelet adhesion were glass, polyurethane-coated glass and collagen-coated glass. Collagen is found in the subendothelium of blood vessels. Collagen-coated glass provides an

active surface for platelet adhesion.

1.3.2 Preparations.

Platelet Suspensions.

The preparation of platelet suspensions is that described by Cazenave et al. (1973a). Pig blood, from the carotid artery, was collected in polyethylene bottles. ACDS, the acid-citrate-dextrose solution of Aster and Jandl (1964), (1 part for 6 parts of blood) was used as an anticoagulant. From this blood, suspensions of washed pig platelets were prepared by the method of Mustard et al. (1972) except that all washing and suspension fluids were adjusted to 340 mOsm with 30% NaCl. The working platelet suspension contained approximately 10^9 platelets/cm³ suspended in Tyrode solution (pH 7.35) containing 0.35% bovine albumin. Apyrase ($1 \lambda \text{ ml}^{-1}$) was added to the suspending medium. The activity of this apyrase is defined elsewhere (Mustard et al. 1972). The platelets were labelled with ⁵¹Cr after the first of three washes as described by Cazenave et al. (1973b); 350 μCi of ⁵¹Cr (as sodium chromate, activity 1 mCi ml^{-1}) being added.

Suspensions of washed red cells were prepared from ACDS anticoagulated blood by giving the cells three washes in Tyrode solution containing ACDS.

Suspensions of a given platelet count and haematocrit were prepared by mixing the suspensions of red cells and

platelets in the correct proportions. Further dilution is made through addition of a Tyrode solution containing albumin (0.35%) and apyrase ($1 \lambda \text{ ml}^{-1}$). The suspensions were stored at 37°C.

Acid soluble collagen was prepared as described by Cazenave et al. (1973a). The coating procedure was to fill the tubes with acid soluble collagen at room temperature (22°C). After 5 minutes the tubes were drained and rinsed 4 times with a modified Tyrode solution (pH 7.35) containing no calcium or magnesium. Before insertion in the apparatus, the tubes were allowed to stand and drain for 10 minutes. In this time, collagen fibres are reconstituted on the surface (Cazenave et al. 1973a).

Surfaces.

The basic surface employed was glass tubing. Before use this was soaked in alcoholic KOH (1N) for 24 hours, washed with distilled water, soaked in HCl (3N) for 1 hour, again washed in distilled water and allowed to dry.

Hydrophobic polyurethane was solution coated onto the glass as described by Brash et al. (1972).

1.3.3 Experimental Procedure.

Before the experiment, radioactive counting vials were prepared. 1 ml of modified Tyrode solution was added to those vials that were to receive 1 ml samples of platelet suspension or of its supernatant. To those that were to

receive 2.5 cm lengths of tubing, 2 ml of the Tyrode solution were added. This addition of Tyrode solution ensures that each sample has approximately the same efficiency of counting, the depth of liquid in each vial containing samples being 2.5 cm, a depth identical to the length of glass tubing placed in some of the vials. The background levels of ^{51}Cr γ -radiation were counted in a Beckmann Biogamma counter, the counts accumulated from each vial in two minutes being recorded.

For each experiment 150 μl of platelet suspension was prepared.

Of great importance in the experimental procedure is the avoidance of an air interface with the platelet suspension within the tubular test surfaces. Thus, following the insertion of 8 glass tubes, internally coated if desired, into the apparatus between the two manifolds, the system was primed completely except in the tubing between the points A and B (Fig. 1.3) with a modified Tyrode solution that is isotonic to pig platelets and red cells. The syringes were filled with the platelet suspension, connected to the circuit and manually emptied so as to displace the Tyrode solution between B and C. The syringes were then set into the pump, the glass tubes sealed, tube E is placed in a flask of suspension and the clamp on tube F removed. At a low flow rate, the Tyrode solution was then displaced from the bottom manifold through F, 30 ml of the platelet suspension

being drawn from the flask through E to replace the volume displaced. The flow rate at which the suspension was to circulate was then set and the tubes opened and closed in any desired sequence. For experiments in which the glass tubes were not open concurrently, the tubes were opened in random order. In experiments in which tubes were exposed to the same shear rate for different time periods, the tubes were opened so as to allow their closure at the same time. The first 50 ml of the Tyrode priming solution and platelet suspension was discharged from the circuit through D, this volume being replaced with suspension from the flask. The circuit is then closed. Mixing of the platelet suspension with the priming solution diluted the suspension by 5-10%. The experiment was conducted under conditions of room temperature (22°C).

On completion of the experiment the flow was stopped and the suspension drained from the upper manifold and glass tubes. The tubes were then removed in random order, rinsed by the passage of 20 ml of the modified Tyrode solution through the tube from the constant head device, rinsed again by immersion in Tyrode solution and allowed to drain. The 25 cm long tubes were then cut into 10 equal lengths, each of which was counted separately for 2 minutes in the γ -counter. Four 1 ml samples of the suspension and of the supernatant were also taken for radioactive counting before and after the experiment so as

to determine the ^{51}Cr activity and the extent of lysis. After ensuring that there was no significant change in the levels of background radiation of the vials, the background counts were subtracted from the post experiment radioactive counts so as to determine the counts due to ^{51}Cr attached in the course of the experiment.

The haematocrit was measured. Haemolysis within red cell suspensions could be determined by measuring the level of haemoglobin within a suspension and its supernatant using the cyanmethaemoglobin method (Dacie and Lewis, 1970).

The level of platelet adhesion was determined from the measured level of ^{51}Cr radioactivity upon the surfaces and estimates of the specific activity of the platelets. The platelet concentration within the final suspension is calculated from that measured visually in the platelet suspension before its dilution with the suspension of red cells. The ^{51}Cr radioactivity emanating from samples of the final suspension was counted and thus the radioactivity associated with a single platelet may be estimated. The level of platelet adhesion upon a surface may then be determined by comparison of the radioactivity of the surface with the specific activity of the platelets. Errors in the estimation of the platelet concentration will persist through

the calculations, the calculated level of platelet adhesion to the surface possessing the same percentage error as that made in the visual estimation the platelet concentration.

1.4 Theory.

1.4.1 Introduction.

The adhesion of platelets to a surface follows their diffusion through the suspension. Grabowski et al. (1972) developed a convective diffusion model to describe this adhesion process. It is their model that will be utilized in this study.

1.4.2 Assumptions.

The model was based on a series of assumptions. The validity of these will determine the applicability of the model. The assumptions are as follows (Grabowski, 1972):

1. Blood is an incompressible homogeneous fluid.

It is assumed that blood may be treated as a continuum, the particulate nature of red blood cells and platelets being ignored. This requires that the platelet mean free path be small compared to the platelet concentration boundary layer thickness. It further implies that the effects of red cell sedimentation and finite channel size on the apparent viscosity of blood may be ignored. The cell depleted 'skimming layer' adjacent to the vessel wall is disregarded. However, as will be demonstrated in section 1.4.5, the theory may be extended to account for the skimming layer.

2. Blood is a pseudo-plastic fluid obeying the power law.

Suspensions of washed cells (Meiselman and Merrill, 1967) and whole blood above a shear rate of 50 sec^{-1} (Whitmore, 1963) exhibit Newtonian behaviour.

3. The flow is steady and laminar.

4. A circular cylindrical (tube shaped) geometry will be studied.

5. The flow at the entrance to the tube is fully developed.

6. Platelet transport may be described in terms of convective diffusion, with the effective diffusivity bearing a power law relationship to the fluid shear rate.

This allows for a fluid shear induced red cell augmentation of platelet diffusion.

7. Platelet motion is independent of platelet concentration.

This is realistic given the overwhelming predominance of red cells in blood (Meiselman and Goldsmith, 1973).

8. The inlet bulk platelet concentration is uniform.

9. Transient terms in the convective diffusion equation can be neglected.

This Grabowski (1972) justified.

10. Neither production nor destruction of platelets takes place in bulk flow.

11. All platelets are equally adhesive.

12. At the tube surface the net platelet arrival rate may be equated to a rate of platelet adhesion which is first order with respect to both the platelet concen-

cration adjacent to the surface and the fraction of surface not yet covered by platelets.

This assumption assumes random platelet adhesion. Whilst this is superficially true, Butruille et al. (1975) discovered patterns of platelet adhesion on segmented polyurethane that suggested that adherent platelets release an adhesion potentiating substance that affects other platelets approaching the surface. The patterns suggested that adherent platelets did not exert significant hydrodynamic effects.

13. The effects of diffusion are confined to a narrow boundary layer region adjacent to the wall; the axial Peclet number is large.
14. Both flow and convective diffusion are axially symmetric.

1.4.3 Derivation of Equations.

With the above assumptions, for Fickian diffusion, the convective diffusion results from the local application of the law of the conservation of species. The equation is:

$$\frac{Dc}{Dt} = \nabla \cdot (D\nabla c) \quad (1.1)$$

where c is the local platelet concentration and D is the platelet diffusion coefficient. In cylindrical coordinates, where r is the radial coordinate and x the axial, equation (1.1) may be rewritten as:

$$\frac{\partial c}{\partial t} + u \frac{\partial c}{\partial x} = \frac{1}{r} \frac{\partial}{\partial r} (r D \frac{\partial c}{\partial r}) + \frac{\partial}{\partial x} (D \frac{\partial c}{\partial x}) \quad (1.2)$$

where u is the axial velocity. A power law velocity profile was assumed:

$$u = \bar{u} (1 - (r/a)^{(n+1)/n})^{(3n+1)/(n+1)}$$

where \bar{u} is the mean velocity of flow. A non-Newtonian factor β is defined as

$$\beta = (3n + 1)/4n$$

The diffusion coefficient, which enjoys a power law dependence on shear rate, is expressed as D

$$D = \alpha \left(\frac{du}{dr} \right)^{m/2}$$

$$\text{or } D = \alpha (4\beta \bar{u}/a)^m (r/a)^{m/n}$$

For Newtonian flow, $n = 1$ and $\beta = 1$.

Assumption 12 is equivalent to the boundary condition

$$-D \frac{\partial c}{\partial r} \Big|_{r=a} = kc \bar{s} \Big|_{r=a} \quad (1.3)$$

where k is the adhesion rate constant and \bar{s} is the fraction of surface adhesion sites as yet unoccupied. If ρ_p is the limiting platelet surface density

$$\bar{s} = 1 - \frac{1}{\rho_p} \int_0^t -D \frac{\partial c}{\partial r} \Big|_{r=a} dt \quad (1.4)$$

Assumption 8 may be expressed as

$$c|_{x=0} = c_0, \quad t \geq 0, \quad 0 \leq r \leq a \quad (1.5)$$

Grabowski (1972) then proceeded to transform the above equations (1.2)-(1.5), into non-dimensional forms, eliminating insignificant terms.

With the Peclet number, Pe , defined as ua/D_s , D_s being defined as $\alpha(4\beta\bar{u}/a)^m$, the platelet diffusivity adjacent to the wall, Grabowski (1972) defined

$$\begin{aligned} T &= Pe^{-1/3} a \rho_p / (D_s c_0) \\ \bar{c} &= c/c_0 \\ \bar{x} &= x/a \\ \bar{y} &= (1 - r/a) Pe^{1/3} \\ \bar{t} &= t/T \end{aligned} \quad (1.6)$$

and $K_d = kaPe^{-1/3}/D_s$

he rewrote (1.2) as

$$Pe^{-1/3} (ac_0/\rho_p) \frac{\partial \bar{c}}{\partial \bar{t}} + 4\beta \bar{y} \frac{\partial \bar{c}}{\partial \bar{x}} = \frac{\partial^2 \bar{c}}{\partial \bar{y}^2} \quad (1.7)$$

and (1.3) as

$$\left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=0} = K_d \bar{c} \left|_{\bar{y}=0} \left(1 - \int_0^{\bar{t}} \left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=0} d\bar{t} \right) \quad (1.8)$$

If $Pe^{-1/3} (ac_0/\rho_p) \ll 1$, then the transient term in (1.7) may be neglected. That this reduction of the problem to the quasi-steady state is valid was demonstrated by Grabowski (1972). Thus the convective diffusion model results in a pair of integro-partial differential equations in terms of β , a non-Newtonian factor, and K_d , a measure of the relative

importance of platelet binding and radial diffusion in determining the rate limiting step for the process.

For whole blood, Grabowski (1972) estimated the non-Newtonian factor β to be 1.06. For suspensions of washed cells deviations from Newtonian behaviour ($n = 1$) are smaller (Whitmore, 1968b). Thus the non-Newtonian behaviour of blood only slightly enhances the importance of convection in (1.7).

1.4.4 Solution.

For finite values of the parameter K_d and for finite degrees of platelet coverage the solution must be numerical in form because of the non-linear boundary condition (1.8). Several asymptotic solutions are possible: for infinite values of K_d , the situation is one of diffusion limited kinetics, with the platelet survival rate at the tube surface

$$\left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=0} = \frac{2}{3} \left(\frac{2\beta}{x} \right)^{1/3} \quad (1.9)$$

For small degrees of surface coverage and intermediate kinetics, the platelet arrival rate is (Grabowski, 1972)

$$\left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=0} = \frac{2}{3} \left(\frac{2\beta}{x} \right) / \left(1 + \frac{2}{3} \left(\frac{2\beta}{x} \right)^{1/3} / K_d \right)$$

The numerical solution Grabowski obtained as follows: equation (1.7), without the transient term, may

be integrated with respect to \bar{y} between the limits of 0, the surface, and \bar{d} , the edge of the boundary layer. Certain boundary conditions will be used: it will be assumed that, at the edge of the boundary layer ($\bar{y} = \bar{d}$), the platelet concentration gradient is zero, i.e.

$$\left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=\bar{d}} = 0 \quad (1.10)$$

and that the platelet concentration is at the inlet value, i.e.

$$\bar{c} = 1 \quad (1.11)$$

Integrating, the right hand side of (1.7) becomes

$$\begin{aligned} \int_0^{\bar{d}} \frac{\partial^2 \bar{c}}{\partial \bar{y}^2} d\bar{y} &= \left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=\bar{d}} - \left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=0} \\ &= - \left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=0} \quad \text{from (1.10)} \end{aligned} \quad (1.12)$$

and the left hand side of (1.7)

$$\int_0^{\bar{d}} 4\beta \bar{y} \frac{\partial \bar{c}}{\partial \bar{x}} d\bar{y} = -4\beta \int_0^{\bar{d}} \frac{\partial}{\partial \bar{x}} ((1 - \bar{c})\bar{y}) d\bar{y} \quad (1.13)$$

Now

$$\frac{\partial}{\partial \bar{x}} \int_0^{\bar{d}} (1 - \bar{c})\bar{y} d\bar{y} = \frac{\partial}{\partial \bar{x}} (1 - \bar{c}) \Big|_{\bar{y}=\bar{d}} \bar{d} + \int_0^{\bar{d}} \frac{\partial}{\partial \bar{x}} ((1 - \bar{c})\bar{y}) d\bar{y}$$

But the boundary condition (1.11) states that

$$\bar{c} \Big|_{\bar{y}=\bar{d}} = 1$$

$$\text{Thus } \frac{\partial}{\partial \bar{x}} \int_0^{\bar{d}} (1 - \bar{c}) \bar{y} \cdot d\bar{y} = \int_0^{\bar{d}} \frac{\partial}{\partial \bar{x}} ((1 - \bar{c}) \bar{y}) d\bar{y}$$

Substituting this into (1.13), we have, with (1.12)

$$\left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=0} = 4\beta \frac{\partial}{\partial \bar{x}} \int_0^{\bar{d}} (1 - \bar{c}) \bar{y} \cdot d\bar{y} \quad (1.14)$$

The platelet concentration profile, within the concentration boundary layer, is assumed to be cubic in form. In addition it is assumed that near the tube surface, the diffusive flux is uniform, convection effects being small compared to diffusion effects, and that at the core edge of the boundary layer the platelet concentration is at the value of the inlet concentration, there being no diffusive flux across this edge of the boundary layer.

On these assumptions, defining K_1 as

$$K_1 = K_d \left(1 - \int_0^{\bar{d}} \left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=0} d\bar{y} \right) \quad (1.15)$$

equation (1.8) may be written as $\left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=0} = K_1 \bar{c} \Big|_{\bar{y}=0}$ (1.16)

and the concentration within the boundary layer may be expressed as

$$\bar{c} = 1 - \frac{2}{3} K_1 \bar{d} \left(1 - 3\bar{y}/(2\bar{d}) + (\bar{y}/\bar{d})^3/2 \right) / \left(1 + 2K_1 \bar{d}/3 \right) \quad (1.17)$$

and two simultaneous non-linear partial differential equations may be obtained to describe the convective diffusion of platelets:

$$K_1 = K_d \left(1 - \int_0^{\bar{t}} \left(K_1 / (1 + 2K_1 \bar{d}/3) \right) d\bar{t} \right) \quad (1.18)$$

and

$$\frac{4}{15} \frac{\partial}{\partial \bar{x}} (K_1 \bar{d}^3 / (1 + 2K_1 \bar{d}/3)) = K_1 / (1 + 2K_1 \bar{d}/3) \quad (1.19)$$

with the boundary condition

$$\bar{d} \Big|_{\bar{x}=0} = 0$$

The unknowns in these equations are the boundary layer thickness \bar{d} and \bar{k} ; both are functions of axial position and time.

The platelet flux to the surface is then

$$\left. \frac{\partial \bar{c}}{\partial \bar{y}} \right|_{\bar{y}=0} = K_1 / (1 + 2K_1 \bar{d}/3) \quad (1.20)$$

and its time integral, the dimensionless platelet surface density, is

$$\bar{\rho} = \int_0^{\bar{t}} K_1 / (1 + 2K_1 \bar{d}/3) d\bar{t}$$

The expected platelet surface density is thus

$$\rho(t) = \rho_p \bar{\rho}(\bar{t}) \quad \text{where } \bar{t} = t/T \quad (1.21)$$

A predictor-corrector method may be used to integrate equations (1.18) and (1.19) numerically, given a value

of K_d . With values of \bar{d} and K_1^t calculated, the dimensionless platelet surface density may be determined as a function of \bar{t} and hence the platelet surface density may be found, given ρ_p . Thus in fitting equation (1.21) to experimental data, three parameters need be estimated: T , ρ_p and K_d . From these D_g and K_d may be calculated (1.6). In this study the expanding Simplex method of Nelder and Mead (1964) was employed to obtain those values of the parameters that gave the best non-linear least squares fit of the model to the data.

The solution of equations (1.18) and (1.19) (Fig. 1.5) demonstrates that at values of K_d greater than 4, diffusion limited kinetics are closely approached. This is equivalent to stating that for a value of k such that

$$k > 4D_g Pe^{1/3}/a$$

adhesion will be essentially diffusion limited until the saturation level of adhesion is approached. Thus our ability to determine values of K that significantly differ from ∞ (i.e. from diffusion controlled adhesion) is limited by the tube's dimensions and by the flow rate as well as by the platelet diffusivity and binding rate.

1.4.5 The Skimming Layer.

The theory developed by Grabowski et al. (1972) assumed that the blood was homogeneous. Experimentally a cell depleted 'skimming layer' is observed adjacent to the

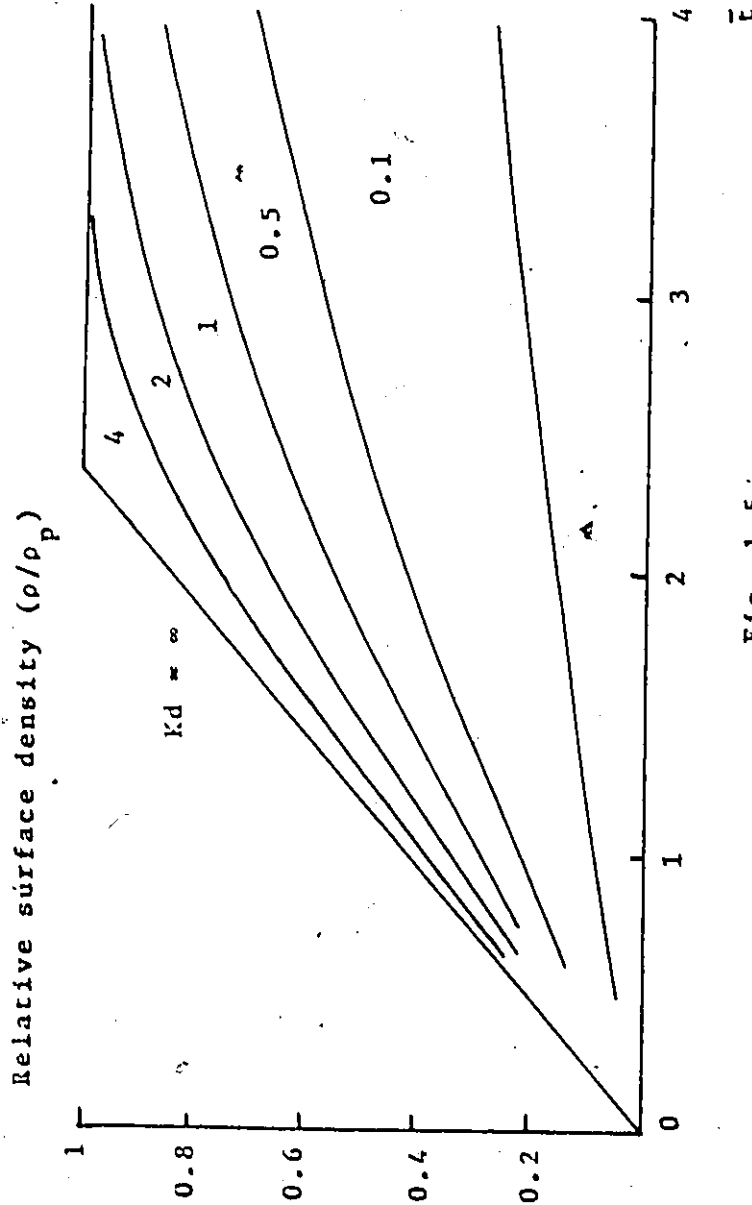


Fig. 1.5

Variation of dimensionless platelet surface density with respect to time at a given axial position ($\bar{x}=8$) for various values of K_d

(After Grabowski et al. 1972)

wall (Bugliarello and Sevilla, 1970). It is thus important to determine if this layer affects the movement of platelets to the surface. An approach similar to that of Grabowski et al. (1972) may be followed.

It is assumed that:

1. Flow is fully developed within a cylindrical geometry. The velocity profile obeys a power law.
2. Between the core containing red blood cells and the wall lies the skimming layer.
3. Within the skimming layer the platelet concentration gradient is linear, the platelet flux uniform.
4. The platelet concentration gradient in the core beyond the skimming layer is described by a cubic equation. It is furthermore assumed that in the core adjacent to the skimming layer diffusive effects are large in comparison to the convective effects.
5. There is no concentration gradient at the edge of the platelet concentration boundary layer, and that, by definition, the concentration there is equal to the bulk platelet concentration.

Following the nomenclature used earlier, if

$$\bar{y} = \bar{d}_e \text{ at the edge of the skimming layer}$$

$$\bar{y}_1 = \bar{y} - \bar{d}_e$$

$$\bar{d}_1 = \bar{d} - \bar{d}_e$$

F is the dimensionless platelet flux to the wall

and $\bar{c} = \bar{c}_w$ at the wall

then, assuming a cubic concentration boundary layer,

$$\bar{c} = a_0 + a_1(\bar{y}_1/\bar{d}_1) + a_2(\bar{y}_1/\bar{d}_1)^2 + a_3(\bar{y}_1/\bar{d}_1)^3$$

$$\bar{d}_1 \geq \bar{y} \geq \bar{d}_e$$

with the boundary conditions

$$\bar{c} \Big|_{\bar{y}_1 = \bar{d}_1} = 1 = a_0 + a_1 + a_2 + a_3 \quad (\text{Assumption 5})$$

$$\frac{\partial^2 \bar{c}}{\partial \bar{y}_1^2} \Big|_{\bar{y}_1 = 0} = 0 = 2a_2/\bar{d}_1 \quad (\text{Assumption 4})$$

$$\frac{\partial \bar{c}}{\partial \bar{y}_1} \Big|_{\bar{y}_1 = \bar{d}_1} = 0 = a_1/\bar{d}_1 + 2a_2/\bar{d}_1 + 3a_3/\bar{d}_1$$

$$\frac{\partial \bar{c}}{\partial \bar{y}_1} \Big|_{\bar{y}_1 = 0} = F = a_1/\bar{d}_1 \quad (\text{Assumption 3})$$

Solving these equations

$$a_0 = 1 - 2a_1/3$$

$$a_1 = F\bar{d}_1$$

$$a_2 = 0$$

$$a_3 = -a_1/3$$

$$\text{and } \bar{c} = 1 - F\bar{d}_1(2/3 - \bar{y}_1/\bar{d}_1 + (\bar{y}_1/\bar{d}_1)^3/3) \quad (1.22)$$

In particular, at $\bar{y}_1 = 0$,

$$\bar{c} = 1 - F\bar{d}_1/3$$

But $\bar{c} = F\bar{d}_e + \bar{c}_w$ at $\bar{y}_1 = 0$ (Assumption 3)

$$\text{thus } F(\bar{d}_e + 2\bar{d}_1/3) = 1 - \bar{c}_w$$

Also $F = K_1\bar{c}_w$ from (1.16)

$$\text{thus } F = K_1/(1 + K_1(\bar{d}_e + 2\bar{d}_1/3)) \quad (1.23)$$

where K_1 is defined as in (1.15).

As before the convective diffusion of platelets may be described by equation (1.7). Integrating this with respect to \bar{y}_1 from the edge of the skimming layer to the edge of the concentration boundary layer we have

$$4\frac{\partial}{\partial \bar{x}} \int_0^{\bar{d}_1} (1-c)\bar{y}_1 \cdot d\bar{y}_1 = \frac{\partial \bar{c}}{\partial \bar{y}_1} \Big|_{\bar{y}_1=0}$$

Substituting (1.22) and (1.23) into this and solving,

$$\frac{4}{15} \beta \frac{\partial}{\partial \bar{x}} (K_1\bar{d}_1/(1 + K_1(\bar{d}_e + 2\bar{d}_1/3))) = K_1/(1 + K_1(\bar{d}_e + 2\bar{d}_1/3)) \quad (1.24)$$

$$\text{and } K_1 = K_d \left(1 - \int_0^{\bar{c}} K_1/(1 + K_1(\bar{d}_e + 2\bar{d}_1/3)) \cdot d\bar{c} \right) \quad (1.25)$$

The dimensionless flux to the surface is F , defined in (1.23). Equations (1.24) and (1.25) may be solved through numerical integration and the platelet flux estimated.

Values of u , a , D_s , k , ρ_p and the skimming layer thickness \bar{d}_c must be known or estimated for this. The estimates of D_s , ρ_p and k chosen are those that give the best non-linear least squares fit of the model to the data.

A comparison of the equations describing platelet flux to the surface with, equation (1.23), and without, equation (1.20), the inclusion of the skimming layer shows that the skimming layer is of importance only when its thickness is greater than or of the same size as that of the platelet concentration boundary layer.

1.4.6 The Collision Frequency of Platelets with the Wall.

Chen (1974) devised an expression for the rate at which platelets encounter the wall. It was based on the frequency at which red cells jostled into the skimming layer adjacent to the wall. An alternative description of the collision of platelets with the wall may be obtained through a continuum approach by analogy to the kinetic theory of gases.

In a suspension of red blood cells and platelets, the principal cause of platelet motion is their interaction with red cells. Platelet motion is assumed to be random with a mean free path independent of platelet concentration. The skimming layer is neglected. Consider an element of surface of area δS on the tube wall. Platelets, with a mean velocity \bar{v} and a mean free path λ , are in collision

with this element. It is assumed that λ and \bar{v} do not vary near the wall and that λ is small in comparison to a , the tube radius. Within the time interval λ/\bar{v} , only platelets inside a hemisphere of radius λ about δS have opportunity to strike δS .

Let $c(y)$ be the platelet concentration at a distance y from the wall. Then, in the time interval λ/\bar{v} , the number of platelet collisions with the element δS is N

$$N = \int_0^\lambda \int_0^{\pi/2} 2\pi r \cos\theta \, dr \cdot r \, d\theta \cdot \delta S \cdot \sin\theta / (4\pi r^2) \cdot c(r \sin\theta) \quad (1.26)$$

where $2\pi r \cos\theta \, dr \cdot r \, d\theta$ is the volume element at a distance between r and $r+dr$ from δS subtending an angle between θ and $\theta + d\theta$ to the normal from S , $\delta S \cdot \sin\theta / (4\pi r^2)$ is the solid angle subtended by δS at a point in the volume element and $c(r \sin\theta)$ (i.e. $c(y)$ at $y = r \sin\theta$) is the platelet concentration within the volume element.

Assuming that the platelet concentration within the concentration boundary layer can be described by the cubic equation (1.17)

$$c(r \sin\theta) = c_0 \left(1 - \frac{2}{3} F \left(1 - \frac{2}{3} r \sin\theta \cdot Pe^{1/3} / (a\bar{v}) + \frac{1}{2} (r \sin\theta \cdot Pe^{1/3} / (a\bar{v}))^3 \right) \right) \quad (1.27)$$

where F is the dimensionless platelet flux to the surface. Equation (1.27) may be written as

$$c(r \sin\theta) = A + B r \sin\theta + C r^3 \sin^3\theta.$$

where $\Lambda = c_0(1-2\bar{d}F/3)$

$$B = c_0 F Pe^{1/3} / a$$

and $C = -Pe \cdot F c_0 / (3a^3 \bar{d}^2)$

Substituting (1.28) into (1.26) and solving

$$N = \delta S (\Lambda \lambda / 2 + B \lambda^2 / 6 + C \lambda^4 / 20) / 2$$

If the collision frequency per unit surface area is f then

$$f = N \bar{v} / (\delta S \Lambda)$$

Now the diffusion coefficient for platelets of mean free path λ mean velocity \bar{v} is, by analogy to ideal gases (Jeans, 1940),

$$D = \lambda \bar{v} / 3$$

Thus $f = ND^3 / (\delta S \cdot \lambda^2)$ or substituting for Λ , B and C

$$f = (3Dc_0 / (4\lambda)) (1 - 2\bar{d}F/3 + FPe^{1/3}\lambda / (3a) - PeF\lambda^3 / (30a^3\bar{d}^2)) \quad (1.29)$$

and this equation applies to platelet collision to the wall irrespective of the rate limiting step in platelet adhesion.

In the situation of diffusion controlled platelet adhesion the net platelet flux to the surface should equal the collision frequency. The net platelet flux

$$\begin{aligned} &= \rho_p F / T \\ &= Pe^{1/3} F D c_0 / a \end{aligned} \quad (1.30)$$

(1.29) and (1.30) may be equated and solved for λ .

1.4.7 Diffusion Limited Kinetics.

Grabowski et al. (1972) often observed a time

dependence for platelet adhesion that indicated intermediate kinetics. Turitto (1975) attempted to explain these experimental results on the basis of diffusion limited kinetics. He assumed that only a fraction of platelet collisions with the wall resulted in adhesion, this fraction being proportional to the fraction of adhesion sites as yet unoccupied. Assuming a constant flux of platelets to the wall, he then devised an expression for the level of platelet adhesion to a tubular surface as a function of time of exposure, platelet flux and the saturation level of adhesion. The flux to the wall at an axial distance x from the entrance of the tube is that calculated for diffusion limited kinetics:

$$F = 2(2\beta u/(xa))^{1/3} c_0 D_s^{2/3} \quad \text{from (1.6) and (1.9)}$$

Of these platelets a fraction $q(\rho_p - \rho)$ adhere on striking the surface. Thus the rate at which platelets adhere to the surface is:

$$\frac{\partial \rho}{\partial t} = Fq(\rho_p - \rho)$$

or

$$\rho = \rho_p (1 - e^{-2(2\beta u/(xa))^{1/3} c_0 (D_s^{2/3} q)t/3}) \quad (1.31)$$

However the assumption of a constant platelet flux, with only a fraction of collisions resulting in adhesion, is tantamount to an assumption that all platelets that make ineffective collisions with the wall are rendered incapable of adhesion. Without excluding the possibility that such platelets could adhere, the platelet concentration profile

could not be maintained constant. Whilst this has been proposed by Butruille et al. (1975), there is no conclusive support for it.

If this assumption cannot be made, then there is an accumulation of reactive platelets in the vicinity of the surface, a state equivalent to the intermediate kinetics that Turitto precluded. It is thus apparent that the validity of Turitto's model is based on an unproven assumption; if that assumption does not hold, intermediate kinetics must result.

1.5 Results and Discussion.

The experimental results will be detailed and discussed in three sections. The first section will demonstrate that the results are valid and not artefacts of the coating, washing or experimental procedures. The second section pertains to the applicability of the model of Grabowski et al. (1972). Finally, in the third section, results based upon the model will be recorded and discussed.

1.5.1 The Validity of the Results.

Experiments were performed to confirm the validity of later results. The following were investigated:

The effect of the order in which the tubular test surfaces are rinsed.

In all experiments, eight tubular test surfaces were set in parallel between the two manifolds. On completion of the experiment, after draining the suspension, the tubes were removed from the apparatus and rinsed. An experiment was performed to determine if the order in which the tubes were rinsed affected the measured levels of platelet adhesion. A suspension of red cells (37% haematocrit) and platelets (4.2×10^8 platelets ml^{-1}) was passed through eight collagen-coated tubes in parallel for 10 minutes at a shear rate of 115 sec^{-1} . The tubes were then removed and rinsed singly, 30 seconds being required for each tube. The average level of adhesion on the tubes was 22.5 ± 2.2 plate-

lets/1000 ².

It was concluded that the order in which the tubes were rinsed did not significantly affect results: the slope of a regression of the level of platelet adhesion upon a tube upon the order of rinsing does not significantly differ from zero, t test, $p > 0.25$ in each of two experiments.

The effect of the direction of rinsing.

In the previous experiment, four tubes were rinsed in the same direction as that of the platelet flow and four in the reverse direction. It was concluded, through a comparison of the mean levels of adhesion resulting from rinsing in each direction, that the direction of rinsing did not influence the level of platelet adhesion measured (t test, $p > 0.25$ in each of two experiments).

Platelet release or lysis.

At the commencement of an experiment, less than 2% of the ⁵¹Cr in the platelet suspension was present as extracellular ⁵¹Cr. This percentage did not change significantly ($p > 0.05$ in 8 experiments) in the course of an experiment. Platelet lysis or release in the course of adhesion is thus negligible, the specific activity of the platelets remaining unchanged.

Haemolysis.

Haemolysis in the course of the experiments was measured as being less than 0.1%: less than 0.1% of the haemoglobin within the suspension was found in the supernatant.

The adhesion of extracellular ⁵¹Cr.

The adhesion of extracellular ⁵¹Cr was investigated.

A suspension of red cells (37% haematocrit) and ^{51}Cr -labelled platelets (4.8×10^8 platelets/ml $^{-1}$) was passed through collagen-coated glass tubes for 10 minutes at a shear rate of 115 sec $^{-1}$. The tubes were then washed, drained and divided into sections for counting. The experiment was repeated with the suspension of red cells (37% haematocrit) and the supernatant of a suspension of ^{51}Cr -labelled platelets. The results are shown below (Table 1.3).

Table 1.3

The adhesion of extracellular ^{51}Cr

	^{51}Cr counts accumulated in 2 minutes	
	Red cells + Platelets	Red cells + Supernatant
In platelets (1 ml suspension)	91354	0
In supernatant (1 ml supernatant)	2175	847
On test surface	30788 ± 2723	140 ± 117

The ratio of extracellular to platelet bound ^{51}Cr adherent to the test surface is 0.004:1. Correcting for the levels of ^{51}Cr in the supernatants of the two suspensions, about 2% of the radioactivity on the test surface appears to originate from extracellular ^{51}Cr . There is thus no apparent preferential adhesion of the extracellular ^{51}Cr .

The level of platelet adhesion as determined by ^{51}Cr levels and electron micrographs.

Brophy (1974) demonstrated that the levels of platelet adhesion determined from measures of ^{51}Cr on a surface did not significantly differ from those estimated by the visual inspection of electron micrographs of the same surface. This was confirmed here (Table 1.4).

Table 1.4

Levels of platelet adhesion as determined by ^{51}Cr radioactivity and EM.

Surface	Level of adhesion (platelets/1000 μ^2)*		
	^{51}Cr	EM	
Glass	11.2	10.0	Fig. 1.6
Collagen	17.8	16.0	Fig. 1.7

*10 minutes exposure to platelet suspension at a shear rate of 115 sec^{-1} .

The removal of collagen from the test surface.

To determine if exposure to a flowing Tyrode solution or erythrocyte suspension removes collagen or in any way modifies a collagen-coated glass surface the following experiments were performed. First, a modified Tyrode solution was circulated through collagen-coated glass tubes for times up to 35 minutes at a shear rate of 115 sec^{-1} . A



Figure 1.6

EM of platelets adherent to glass (2000 x magnification).

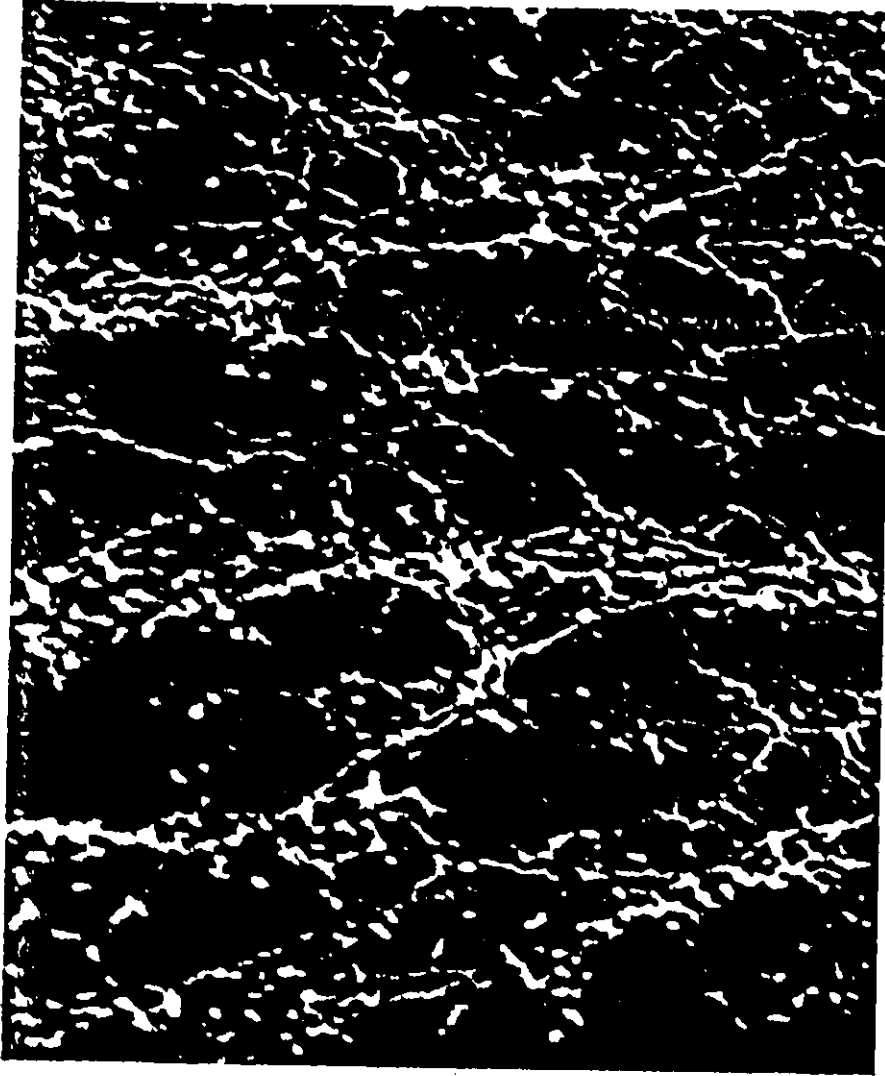


Figure 1.7

EM of platelets adherent to collagen (1000 x magnification).

suspension of platelets (3.2×10^8 platelets/ml⁻¹) and red cells (20% haematocrit) was then passed through the tubes for 10 minutes at a shear rate of 115 sec⁻¹. The resulting levels of platelet adhesion are shown in Table 1.5. The mean level of platelet coverage was 58.38 ± 3.36 platelets/1000 μ^2 . There was no significant change in the level of platelet adhesion with respect to the time of circulation of the Tyrode solution through the tubes (a regression of the level of platelet adhesion with time of circulation is not significant, $p > 0.5$, F test).

The experiment was repeated with an erythrocyte suspension, rather than a Tyrode solution, circulating through the tubes for varying time intervals prior to their exposure to platelets. The results are presented in Table 1.6.

There is no significant change in the level of platelet adhesion with respect to the time of exposure of the collagen to the suspension of red cells prior to its exposure to the platelets by regression (F test, $p > 0.4$ at each shear rate).

A similar experiment was performed (Table 1.7).

This data indicates that there was no significant change in platelet adhesion to the collagen surface (t test, $p > 0.5$). It may thus be concluded that exposure to collagen-coated glass surfaces to a modified Tyrode solution or to suspensions of washed erythrocytes does not significantly modify the surface.

The removal of platelets adherent to the surface.

Two experiments were performed to determine if adher-

Table 1.5

The elution or modification of collagen

Time of exposure to the Tyrode solution (minutes) ^a	0	5	10	15	20	25	30	35
Mean platelet cover- age (platelets/1000 μ^2) on one tube	60.4	60.8	54.9	56.8	54.4	55.8	59.0	64.7

^a Haematocrit 20%, platelet concentration $3.2 \cdot 10^8$ platelets ml^{-1} , shear rate 115 sec^{-1} .

Table 1.6

Red cell induced elution or modification of collagen

Shear rate at which RBC suspension circulated (sec^{-1})	306			30.6		
Time of exposure to the RBC suspension* (minutes)	0	0	15	10	5	5
Mean level of platelet adhesion (platelets/ $1000\mu^2$)**	26.4	28.9	24.8	35.3	33.8	29.0
on one tube				25.6	19.7	

* Haematocrit of suspensions 22%

** Platelet concentration 6.07×10^8 platelets/ ml^{-1}

Shear rate 115 sec^{-1} .

Table 1.7

Red cell induced elution or modification of collagen

Time of pre-exposure to circulating RBC [†] (minutes)	15.	10.	5.	0.
Time of exposure to platelets ^{‡‡} (minutes)	10. 5.	10. 5.	10. 5.	10. 5.
Mean level of platelet adhesion (platelets/1000 μ^2) on one tube	54.1 25.5	49.7 29.5	59.0 31.3	60.4 32.5

[†] Haematocrit 35%

^{‡‡} Haematocrit 38%, platelet concentration 3.77 10⁸ platelets ml⁻¹

Shear rate 115 sec⁻¹.

ent platelets could be removed from collagen-coated surfaces under conditions typical of the experiments to be described later. A platelet suspension containing erythrocytes was passed through collagen-coated glass tubes for 10 minutes at a shear rate of 46 sec^{-1} . The platelet suspension was then drained from the apparatus and replaced by a suspension of washed erythrocytes. This was circulated for varying times at a shear rate of 153 sec^{-1} . The results of this experiment are shown in Table 1.8.

There was no significant removal of platelets with time (regressions are not significant, F test, $p > 0.2$ in each experiment). It is thus unlikely that platelets adherent to collagen may be removed under the flow conditions of these experiments.

A change in platelet adhesiveness with time.

To investigate this possibility, eight collagen-coated glass tubes were inserted in the apparatus. A suspension of ^{51}Cr labelled platelets and red cells was passed through two of the tubes at a shear rate of 184 sec^{-1} . After 10 minutes, two other tubes were opened and the first two closed. This process was continued until all eight tubes had been exposed to the platelet suspension, 40 minutes having elapsed. The results are presented in Table 1.9.

In no case was there a significant change in platelet adhesion in the time period over which adhesion occurred (by regression F test, $p > 0.2$, experiments 1 and 2 and $p > 0.45$ experiment 3). Thus it is unlikely that the adhesivity of circula-

Table 1.8

The removal of platelets adherent to collagen-coated glass

Exposure time of platelet covered surface to RBC* (minutes)	Mean level of platelet adhesion* (platelets/1000 μ^2)			
	0	5	10	15
Experiment 1	29.1	29.7	22.2	25.3
Experiment 2	31.7	31.0	23.3	30.0

* Haematocrit 14%, platelet concentration 3.73 10^8 platelets ml^{-1}

Table 1.9

Platelet adhesivity and the time of flow

Exp. *	Time period when tube exposed to platelets			
	0-10	10-20	20-30	(30-40) (minutes)
1	-	91	94	103
2	137	150	119	124
3	164	174	176	168
Mean level of platelet adhesion to tube ² (platelets/1000μ ²)				
		86	98	112
		114	130	121
		182	195	194

* Experiment 1: haematocrit 36%, platelet concentration $7.50 \times 10^8 \text{ ml}^{-1}$
 2: haematocrit 31%, platelet concentration $4.66 \times 10^8 \text{ ml}^{-1}$
 3: haematocrit 37%, platelet concentration $5.73 \times 10^8 \text{ ml}^{-1}$

In each 10 minute period, two replicates were exposed to the platelets.

ting platelets decreases within a period of 40 minutes. Hence it is improbable that the relative fraction of reactive platelets in the platelet population changes. Brophy (1974) demonstrated that storage of platelet suspensions for up to 3 hours at 37°C did not diminish the adhesive properties of platelets. In these experiments the platelets were always used within 3 hours of their final resuspension.

1.5.2 The Validity of the Model.

Whilst the model of Grabowski et al. (1972) has been employed to determine the parameters that characterize the kinetics of platelet adhesion to a foreign surface, no rigorous examination has been made of the model or of the assumptions upon which it is based. In this section, the results of experiments and calculations designed to test the validity of the model proposed by Grabowski et al. (1972) will be described.

The continuum approach.

For the continuum approach to hold, the platelet mean free path must be much smaller than the concentration boundary layer thickness. The observations of Goldsmith (1971) suggest that, in a suspension containing erythrocytes, the platelet mean free path near the wall is about 2 μ . Fitting the model of Grabowski et al. (1972) to experimental data, the concentration boundary layer thickness may be calculated as a function of axial position and time.

The concentration boundary layer thickness may be expressed as

$$\bar{d} \propto \tau_w^{-1/3}$$

\bar{d} being a function of K_d and \bar{t} . As the Peclet number changes little over the range of shear rates and haematocrits employed in these experiments, the concentration boundary layer thickness at a given value of K_d will be unaffected by changes in shear rate and haematocrit. The results for platelet adhesion to collagen, where K_d took a value of 4.83, are plotted (Fig. 1.8). This is a situation where intermediate kinetics approaching those of diffusion control are obeyed. It will be observed that the boundary layer thickness is, beyond the first 0.1 cm from the entrance, greater than the platelet mean free path. As the binding step becomes more important in limiting the rate of platelet adhesion, the concentration boundary layer will become thinner and the continuum approach of platelet diffusion will be of less validity. However, in these circumstances, the mechanisms by which platelets diffuse through a concentration boundary layer will be of less importance in describing the adhesion process. Thus there is no contradiction revealed in the use of the continuum approach.

Platelet collision frequency.

The collision frequency of platelets with the wall was calculated, by the method described in Section 1.4.6, for the set of data used above to illustrate the validity

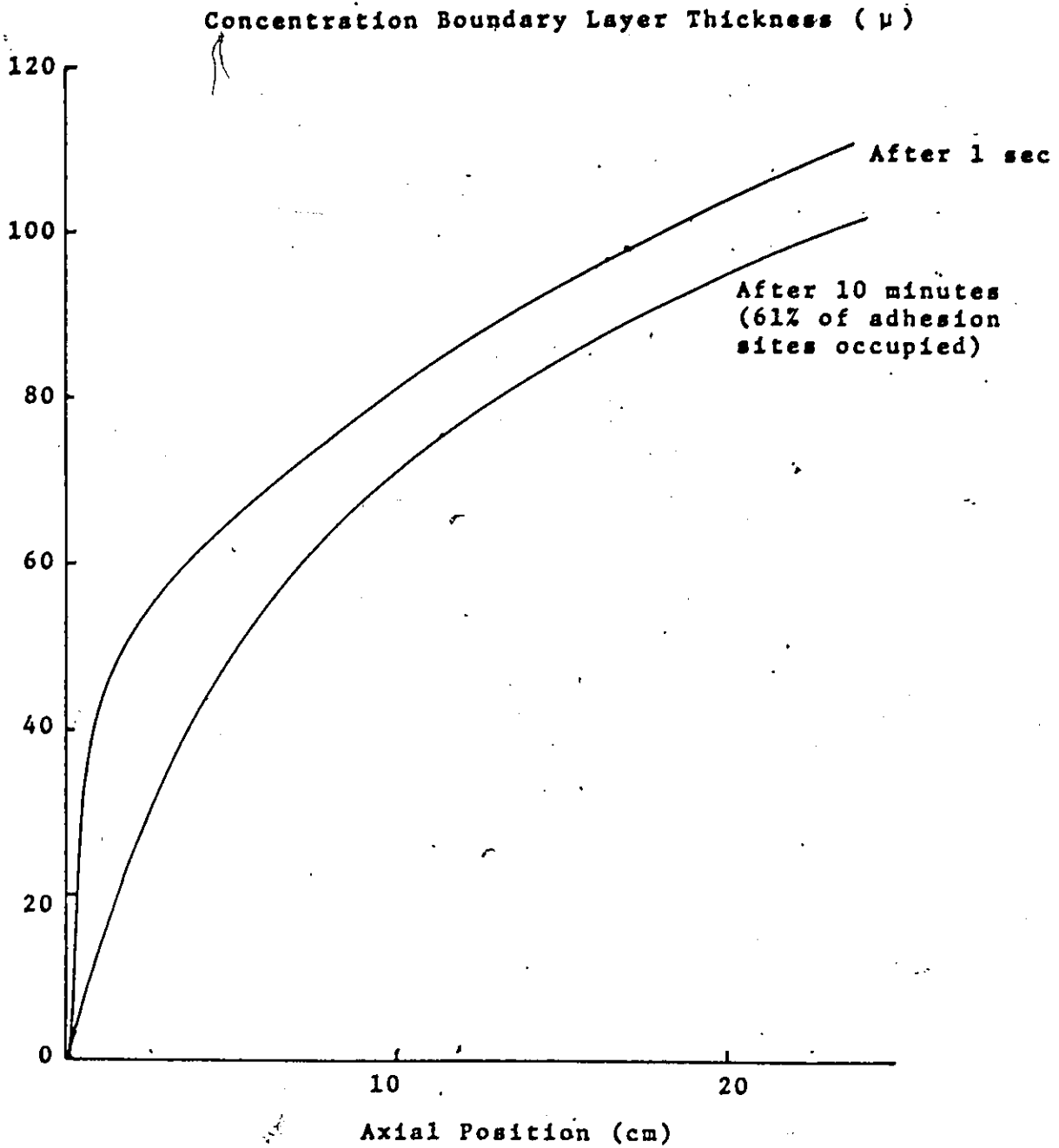


Figure 1.8

Axial variation of concentration boundary layer thickness

of the continuum approach. A platelet mean free path of 2μ was assumed. The axial profiles for the collision frequency and platelet flux are shown in Fig. 1.9. They indicate that initially every collision results in adhesion as would be expected in a diffusion limited process. As the surface becomes covered with adherent platelets, the proportion of collisions that are effective falls whilst the collision frequency increases. This is consistent with a model in which platelet motion is determined by the motion of red cells, the adhesion process obeying intermediate kinetics.

The skimming layer.

The influence of a cell-depleted skimming layer in proximity to the wall is demonstrated by calculation of the diffusivity, the reaction rate constant and the saturation level of adhesion using models that account for, or ignore, the skimming layer. The data, for which the parameter estimates were made, resulted from experiments performed to examine the influence of haematocrit and shear rate upon platelet adhesion to collagen-coated glass. The calculations (Table 1.10) indicate that, if the skimming layer is neglected, the estimates of platelet diffusivity, platelet surface saturation level and binding rate constant differ little from those estimates made when the skimming layer was considered. The inclusion of the skimming layer does not improve the fit of the model to the data (F test, $p > 0.5$, Dell et al, 1973).

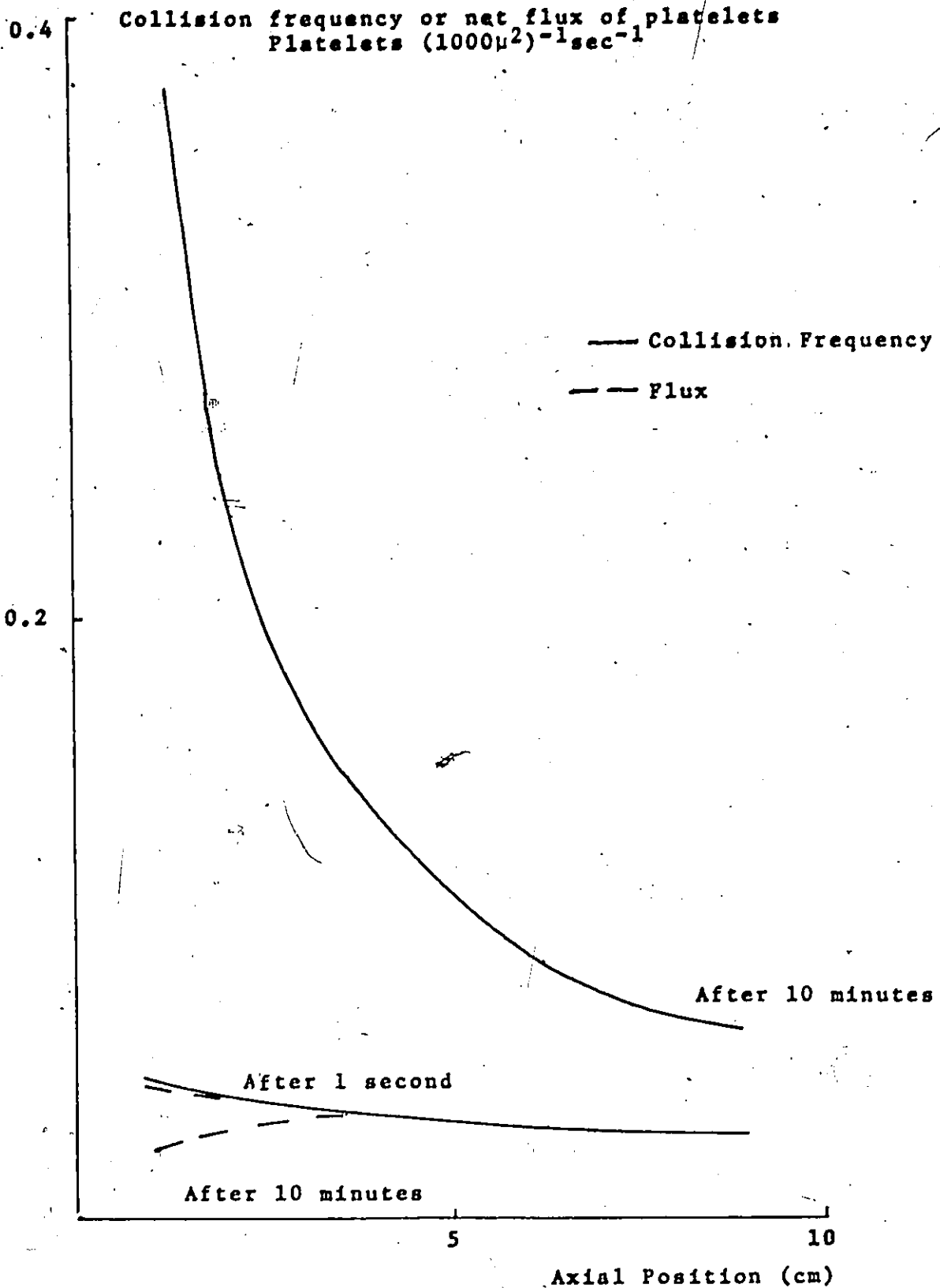


Figure 1.9

Axial variation of the platelet flux and collision frequency
(Kd 4.83, Haematocrit 26%), shear rate 23 sec^{-1} ,
Co $2.53 \cdot 10^8 \text{ platelets ml}^{-1}$)

Table 1.10
Effect of skimming layer

S. of S.	Hct.	Shear Rate (sec ⁻¹)	Skimming Layer Thickness (μ)	D _s cm ² sec ⁻¹	P _p cm ⁻²	k cm sec ⁻¹	K _d
2.09.10 ¹³	34	460	8	2.91.10 ⁻⁷	4.31.10 ⁷	1.87.10 ⁻³	4.20
1.69.10 ¹³			-	2.82.10 ⁻⁷	4.16.10 ⁷	1.87.10 ⁻³	4.31
2.62.10 ¹²		23	8	9.62.10 ⁻⁸	7.45.10 ⁶	3.27.10 ⁻⁴	4.34
2.56.10 ¹²			-	9.64.10 ⁻⁶	7.26.10 ⁶	3.41.10 ⁻⁴	4.34
2.2.10 ¹²	7	460	22	3.57.10 ⁻⁸	5.63.10 ⁶	4.85.10 ⁻⁴	4.39
1.83.10 ¹²			-	2.67.10 ⁻⁸	5.64.10 ⁶	3.77.10 ⁻⁴	4.16
2.45.10 ¹²		23	22	8.05.10 ⁻⁹	7.52.10 ⁵	2.80.10 ⁻⁵	1.86
2.50.10 ¹²			-	8.55.10 ⁻⁹	7.17.10 ⁵	2.63.10 ⁻⁵	1.68

* Values taken from the experimental work of Bugliarello and Sevilla, 1970. At each shear rate, the fit of the models to the data is compared by means of an F test (Dell et al, 1973).

This is expected as the skimming layer is narrower than the concentration boundary layer and thus does not greatly influence the platelet flux (section 1.4.5). The skimming layer thus has a negligible effect upon the diffusion of platelets.

A steady and laminar flow.

The Reynolds number for the flow of suspension in the tube is defined as

$$\frac{(\text{mean flow velocity}) \times (\text{density of the suspension}) \times (\text{tube diameter})}{(\text{suspension viscosity})}$$

For turbulent flow to occur within the flow, the Reynolds number should be greater than 1000. In the experiments conducted with this apparatus the highest Reynolds number to occur was 100. Laminar flow is thus expected and was observed as the suspension of red cells and platelets displaced the solution from the tubes at the start of an experiment.

The infusion withdrawal pump was unable to give a steady flow with the plastic syringes. Analysis of the flow through the apparatus using an electromagnetic blood flow meter (Nycotron 372) however showed the fluctuations from steady flow to be both rapid and small (S.D about 10% of the flow at 56 sec^{-1} , Fig. 1.12).

Fully developed flow.

The length of tube required for flow entering from

a reservoir to become fully developed with a constant velocity profile has been empirically calculated as

$$0.07a.Re$$

where a is the tube's radius and Re the Reynolds number of the flow (Bird et al. 1960). In the apparatus used, the development length would thus vary from 10^{-2} cm at a shear rate of 23 sec^{-1} to about 1 cm at a shear rate of 920 sec^{-1} . The development length is thus at most 5% of the length of the tube employed as a test surface; effectively it will be less: Sparrow et al. (1964) demonstrated that near the tube wall, flow develops earlier than near the tube axis. The flow through the tubes may therefore be considered to be fully developed along the entire length of the tube.

The effect of platelet concentration.

Experiments performed with suspensions of the same haematocrit but containing different platelet concentrations should test the validity of the assumptions that platelet motion is independent of platelet concentration and that the adhesion process is first order with respect to the platelet concentration near the wall and to the fraction of the surface adhesion sites yet unfilled.

The results of two such experiments are shown in Table 1.11. It is evident that the platelet concentration within the suspension does not significantly alter the estimated values for the platelet diffusivity, the binding rate constant or the saturation level of platelet adhesion.

Table 1.11

Effect of platelet concentration

Exp. No. (& Hct)	Platelet concentration (cm ⁻³)	Shear Rate (sec ⁻¹)	D _s (cm ² sec ⁻¹)	ρ _p (pl/1000μ ²)	k* (cm sec ⁻¹)	K _d
1 (39%)	1.69.10 ⁸	460	2.00 ± 3.85.10 ⁻⁷	199. ± 1.84 [†]	1.44.10 ⁻³	4.1
		184	2.20 ± 1.72.10 ⁻⁷	72.9 ± 0	2.68.10 ⁻⁴	0.97
		46	6.5 ± 1.56.10 ⁻⁷	2.40 ± 0	3.55.10 ⁻⁴	4.6
		23	1.1 ± 0.81.10 ⁻⁷	3.9 ± 0	1.95.10 ⁻⁴	2.1
2 (38%)	3.87.10 ⁸	460	2.11 ± 0.34.10 ⁻⁷	177. ± 0	3.46.10 ⁻⁴	4.6
		184	2.10 ± 1.24.10 ⁻⁷	92.1 ± 10.9 [†]	1.51.10 ⁻⁴	0.57
		46	9.83 ± 13.0.10 ⁻⁷	71.0 ± 0	7.23.10 ⁻²	4.5
		23	1.18 ± 1.21.10 ⁻⁷	56.8 ± 0	3.64.10 ⁻⁴	4.0
2 (38%)	1.57.10 ⁸	460	1.88 ± 0.46.10 ⁻⁷	153. ± 0	3.5. 10 ⁻⁴	1.02
		184	1.28 ± 0.79.10 ⁻⁷	107 ± 0	3.06.10 ⁻⁴	1.61
		46	7.79 ± 10.93.10 ⁻⁸	57.4 ± 0	4.42.10 ⁻⁴	5.65
		23	6.53 ± 14.2. 10 ⁻⁸	39.3 ± 0	2.99.10 ⁻⁴	4.95
2 (38%)	3.33.10 ⁸	460	1.83 ± 0.42.10 ⁻⁷	138 ± 0	3.65.10 ⁻⁴	1.12
		184	2.88 ± 2.37.10 ⁻⁷	133 ± 0	5.64.10 ⁻⁴	1.67
		46	7.62 ± 9.84.10 ⁻⁸	60.6 ± 0	3.82.10 ⁻⁴	4.51
		23	8.09 ± 12.85.10 ⁻⁸	60.8 ± 3.73 [†]	3.12.10 ⁻⁴	4.48

*The confidence limits for k, computed by linearising the model in the region of best fit of the model to data, are very large.

†The parameter estimates for each shear rate - haematocrit combination were calculated from the results obtained by exposing one tube to platelets for 5 minutes and a second for 10 minutes except for those marked†. These had a 5 minute exposure only.

There is no contradiction between these results and the model.

The calculations and experiments recorded in this section suggest that, under the conditions of the experiments, the model proposed by Grabowski et al. (1972) is valid. The convective diffusion model, coupled to an adhesion process that is first order with respect to platelet concentration and to the fraction of the adhesion sites that are unfilled, will thus be applied in this study.

1.5.3 Results Based Upon the Model.

Effect of erythrocytes on platelet adhesion.

It was observed that the adhesion of platelets was enhanced by the presence of erythrocytes within the platelet suspension (Table 1.12).

Turitto and Baumgartner (1975) found the enhancement of adhesion to be due solely to the increased diffusion of platelets to the surface. Feuerstein et al. (1975) however, suggested that an increased haematocrit increased the rate constant for the binding of platelets to a collagen-coated surface in addition to enhancing the platelet diffusivity. Neither set of authors could determine if the saturation level of adhesion could be correlated with shear rate or haematocrit. Nor did they or others examine the joint effects of a wide range of shear rates and haematocrits

Table 1.12
Enhancement of platelet adhesion to collagen-coated glass

Exp.	Hct (Z)	Platelet concentration (platelets/ml ⁻¹)	Mean platelet adhesion level (platelets/1000μ ²)
1.	20	4.17 10 ⁸	17.6 ± 3.5
	0	3.85 10 ⁸	2.88 ± 0.8
2.	20	3.15 10 ⁸	21.6 ± 4.0
	0	2.92 10 ⁸	2.5 ± 1.4

* Shear rate 115 sec⁻¹, exposure time 10 minutes

upon platelet adhesion. A series of experiments was thus performed to determine the effect of haematocrit and shear rate on platelet adhesion to collagen-coated glass.

The variation of the diffusivity, the binding rate constant and the saturation level of adhesion of platelets with haematocrit and shear rate.

In random order, collagen-coated tubes were exposed to flowing platelet suspensions of various haematocrits but identical platelet concentrations at one of four shear rates for a period of 5 or 10 minutes. The results are shown in Table 1.13. 95% confidence limits for the diffusivity of the platelets near the wall, D_g , and for the saturation level of platelet adhesion, ρ_p , were calculated assuming linear behaviour for the model in the region of best fit. As in general, the value assigned to the binding rate constant is an approximate one indicating a high rate constant for platelet binding, the kinetics of the adhesion process approaching those of diffusion limited adhesion, confidence limits for the binding rate constants are not given.

The values for the diffusivity and the binding rate constants are in close accord with those given elsewhere (Grabowski et al. 1972; Robertson and Chang, 1974; Feuerstein et al. 1975). The saturation level of platelet adhesion, under conditions of high haematocrit and shear rate, is about $250 \text{ platelets}/1000\mu^2$, a value

Table 1.13

Effect of haematocrit and shear rate upon the platelet diffusivity, binding rate constant and saturation level of platelet adhesion

Exp.	Hct. (%)	Wall Shear Rate (sec ⁻¹)	D _s (cm ² sec ⁻¹)	PP (platelets /1000μ ²)	k (cm.sec ⁻¹)	K _d
1	26	460	2.10.10 ⁻⁷	128.0 ± 1.3	5.66.10 ⁻⁴	1.58
		184	2.05.10 ⁻⁸	120.5 ± 4.7	1.09.10 ⁻³	4.39
		46	1.00.10 ⁻⁷	33.0 ± 1.01	3.68.10 ⁻⁴	4.17
		23	5.72.10 ⁻⁸	28.3 ± 1.1	2.67.10 ⁻⁴	4.83
12		460	8.92.10 ⁻⁸	122.0 ± 2.2	4.29.10 ⁻⁴	2.13
		184	4.90.10 ⁻⁸	98.3 ± 10.7	5.12.10 ⁻⁴	5.11
		46	2.07.10 ⁻⁸	25.7 ± 0.6	1.82.10 ⁻⁴	5.14
		23	7.75.10 ⁻⁸	8.9 ± 0.3	2.90.10 ⁻⁴	1.99
6		460	1.45.10 ⁻⁸	77.9 ± 3.7	3.18.10 ⁻⁴	5.27
		184	1.14.10 ⁻⁸	113.0 ± 19.8	1.62.10 ⁻⁴	4.41
		46	2.14.10 ⁻⁸	6.9 ± 0.2	1.34.10 ⁻⁵	3.73
		23	2.61.10 ⁻⁹	15.6 ± 0.2	2.87.10 ⁻⁵	4.15

2	40	460	2.01.10 ⁻⁷	± 1.21.10 ⁻⁷	175.0 ± 7.8	9.68.10 ⁻⁴	2.78
		184	6.93.10 ⁻⁸	± 2.09.10 ⁻⁷	62.9 ± 11.4	6.08.10 ⁻⁴	4.83
		46	1.55.10 ⁻⁷	± 1.55.10 ⁻⁷	35.5 ± 1.9	2.10.10 ⁻⁴	1.54
		23	1.62.10 ⁻⁷	± 1.37.10 ⁻⁷	24.4 ± 6.5	4.77.10 ⁻⁴	4.28
	20	460	7.04.10 ⁻⁸	± 2.27.10 ⁻⁸	63.4 ± 0.4	8.32.10 ⁻⁵	4.83
		184	3.55.10 ⁻⁸	± 4.15.10 ⁻⁸	68.9 ± 1.1	3.37.10 ⁻⁴	4.20
		46	1.84.10 ⁻⁸	± 5.48.10 ⁻⁸	9.3 ± 0.7	8.18.10 ⁻⁵	4.03
		23	1.43.10 ⁻⁸	± 3.25.10 ⁻⁸	12.0 ± 0.6	8.18.10 ⁻⁵	3.72
	10	460	1.36.10 ⁻⁸	± 7.41.10 ⁻⁸	26.1 ± 5.6	2.51.10 ⁻⁴	4.34
		184	7.50.10 ⁻⁸	± 3.77.10 ⁻⁸	67.9 ± 0.9	4.27.10 ⁻⁴	3.30
		46	7.41.10 ⁻⁹	± 1.25.10 ⁻⁸	9.8 ± 0.6	3.37.10 ⁻⁵	1.87
		23	4.24.10 ⁻⁸	± 5.84.10 ⁻⁸	44.6 ± 3.2	1.88.10 ⁻⁴	4.12
3	34	460	2.82.10 ⁻⁷	± 2.42.10 ⁻⁷	416.6 ± 8.7	1.87.10 ⁻³	4.31
		184	6.96.10 ⁻⁸	± 5.97.10 ⁻⁷	87.6 ± 31.1	4.83.10 ⁻⁴	4.17
		46	1.14.10 ⁻⁷	± 1.09.10 ⁻⁷	102.6 ± 2.5	4.37.10 ⁻⁴	3.93
		23	9.64.10 ⁻⁸	± 1.21.10 ⁻⁷	72.6 ± 3.8	3.41.10 ⁻⁴	4.35
	16	460	9.75.10 ⁻⁸	± 8.41.10 ⁻⁸	199.1 ± 8.2	9.00.10 ⁻⁴	4.19
		184	7.62.10 ⁻⁸	± 1.54.10 ⁻⁷	103.0 ± 10.7	5.60.10 ⁻⁴	4.17
		46	2.85.10 ⁻⁸	± 2.40.10 ⁻⁷	41.2 ± 2.5	2.05.10 ⁻⁴	4.65
		23	3.85.10 ⁻⁸	± 1.97.10 ⁻⁷	37.6 ± 9.5	1.96.10 ⁻⁴	4.61
	7	460	2.67.10 ⁻⁸	± 3.05.10 ⁻⁸	56.4 ± 1.6	1.34.10 ⁻⁴	0.60
		184	5.25.10 ⁻⁸	± 5.63.10 ⁻⁸	42.9 ± 9.2	1.55.10 ⁻⁴	4.51
		46	1.98.10 ⁻⁸	± 2.76.10 ⁻⁸	153.5 ± 7.6	1.44.10 ⁻³	1.69
		23	8.55.10 ⁻⁹	± 1.38.10 ⁻⁸	7.2 ± 0.5	3.46.10 ⁻⁴	4.13

The parameter estimates for each shear rate - haematocrit combination were calculated from the results obtained by exposing one tube to platelets for 5 minutes and a second for 10 minutes.

close to that expected for a complete monolayer of platelets on the surface.

Variation of platelet diffusivity.

The diffusivity may be correlated with both independent variables. A power law relationship of the form

$$D_s \propto H^a \gamma^b$$

was fitted to the results for each experiment. There is a theoretical basis for such a relationship (Chen, 1974). The correlations obtained are listed in Table 1.14.

Table 1.14

Correlations of the diffusivity with the haematocrit (H) and shear rate (γ).

<u>Experiment</u>	<u>Correlation</u>
1	$D_s \propto H^{1.378 \pm 0.124} \gamma^{0.356 \pm 0.061}$
2	$D_s \propto H^{2.118 \pm 0.235} \gamma^{0.314 \pm 0.010}$
3	$D_s \propto H^{2.023 \pm 0.140} \gamma^{0.607 \pm 0.068}$

Taking the mean values of the power law coefficients, the correlation

$$D_s \propto H^{1.839} \gamma^{0.426} \quad (1.32)$$

approximately describes the variation of the diffusion coefficient with haematocrit and shear rate. The shear rate dependence of platelet diffusivity obtained is close to that found by Turitto and Baumgartner (1974). That the power

law coefficient for the dependence of the diffusivity upon haematocrit is greater than 1 suggests that the diffusion of platelets is induced by the interaction of red cells. It is unlikely that the simple method of Keller (1971), in which platelets diffuse in and out of the volume about rotating red cells, holds. For that model, a relation of the form

$$D \propto H\gamma$$

would be expected. The values of the coefficients obtained support the microvortex or meandering stream models of Chen (1974).

The variation in the saturation level of adhesion.

The saturation level of platelet adhesion on a collagen-coated glass surface is seen to increase with both haematocrit and shear rate (Table 1.13). The saturation level, ρ_p , may be correlated with the haematocrit, H , and the shear rate, γ . A power law relationship of the form

$$\rho_p \propto H^c \gamma^d$$

was fitted to the results for each set of experiments. The following correlations were obtained (Table 1.15).

Table 1.15

Correlations of the saturation level of platelet adhesion with haematocrit (H) and shear rate (γ).

<u>Experiment</u>	<u>Correlations</u>
1	$\rho_p \propto H^{1.172 \pm 0.115} \gamma^{0.598 \pm 0.064}$
2	$\rho_p \propto H^{1.072 \pm 0.398} \gamma^{0.666 \pm 0.118}$
3	$\rho_p \propto H^{0.861 \pm 0.295} \gamma^{0.997 \pm 0.151}$

Using the mean values of the coefficients, the correlation

$$\rho_p \propto H^{1.035} \gamma^{0.747} \quad (1.33)$$

describes the variation of the diffusion coefficients with haematocrit and shear rate.

To demonstrate that the above result is not an artefact of the model describing the adhesion process or of the parameter estimation procedure, two further experiments were conducted. In the first, two suspensions were prepared with identical platelet concentrations but with widely differing haematocrits and circulated through collagen-coated glass tubes for times up to 40 minutes. The results are shown in Table 1.16 and Fig. 1.10.

Table 1.16

Effect of haematocrit upon the saturation level of adhesion

Hct %	Time of exposure (minutes)	Mean level of platelet adhesion* (platelets/1000 μ^2)
13	10	12.5
	20	24.4
	30	30.3
42	10	56.9
	20	85.3
	30	107.3

* Shear rate 115 sec^{-1} , platelet concentration 2.31×10^8 platelets ml^{-1} .

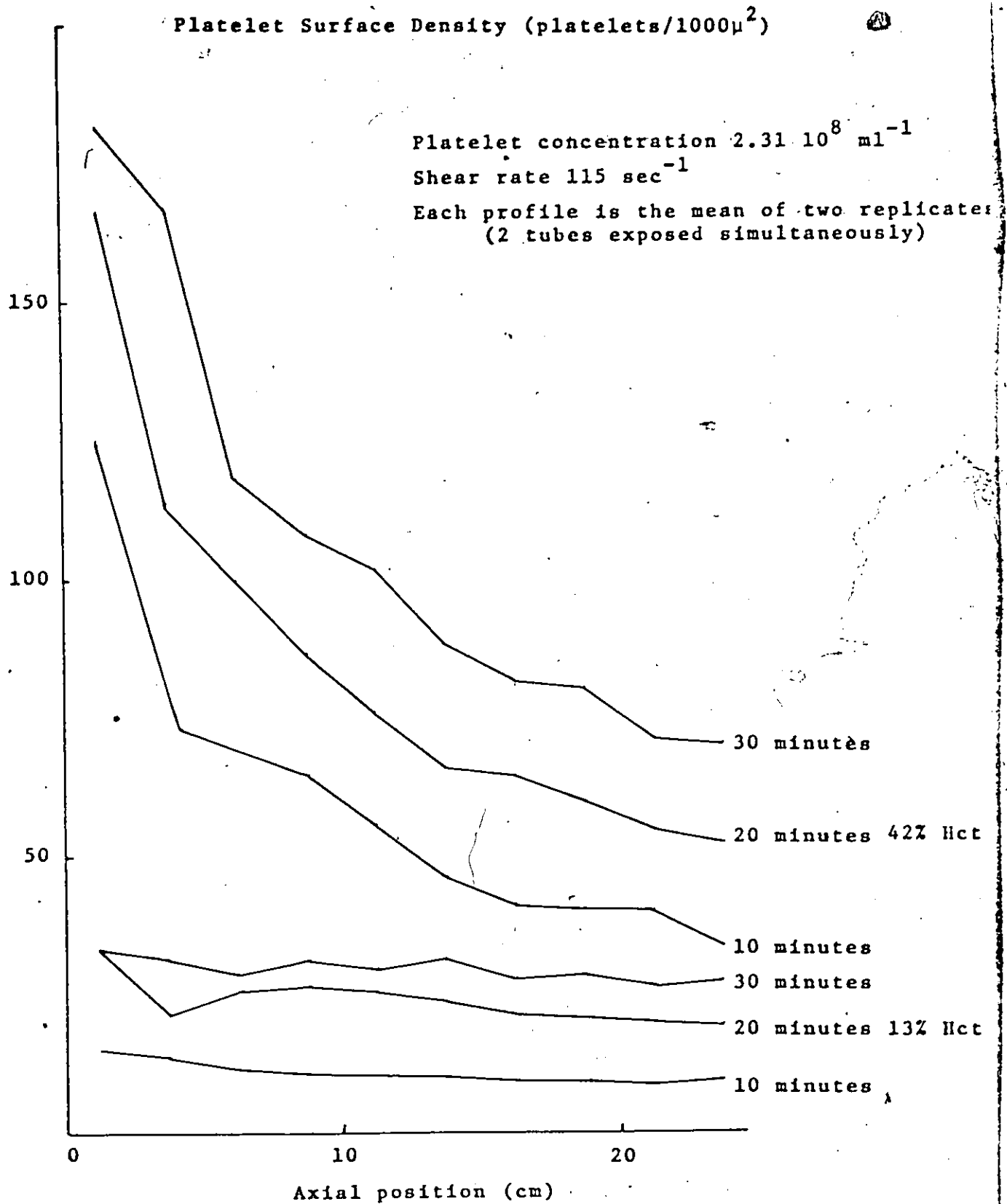


Figure 1.10

Effect of haematocrit upon platelet adhesion

It is apparent that the saturation level of platelet adhesion is dependent on the haematocrit.

In the second experiment, two identical platelet suspensions of the same haematocrit, were circulated through collagen-coated glass tubes for times up to 40 minutes at different shear rates. The results are shown in Table 1.17 and Fig. 1.11.

Table 1.17

Effect of shear rate upon the saturation level of platelet adhesion

Shear rate (sec^{-1})	Time of exposure (minutes)	Mean level of adhesion* (platelets/ $1000\mu^2$)
460	10	214.9
	20	284.8
153	10	94.6
	20	131.5
	40	167.5

* Platelet concentration, 7.7×10^8 platelets ml^{-1} ; haematocrit 15.7.

These results confirm that the saturation level of adhesion increases with increasing haematocrit and shear rate. As it was earlier concluded that the saturation level of platelet adhesion was independent of platelet concentration within the suspension, it is unlikely that the increase in the

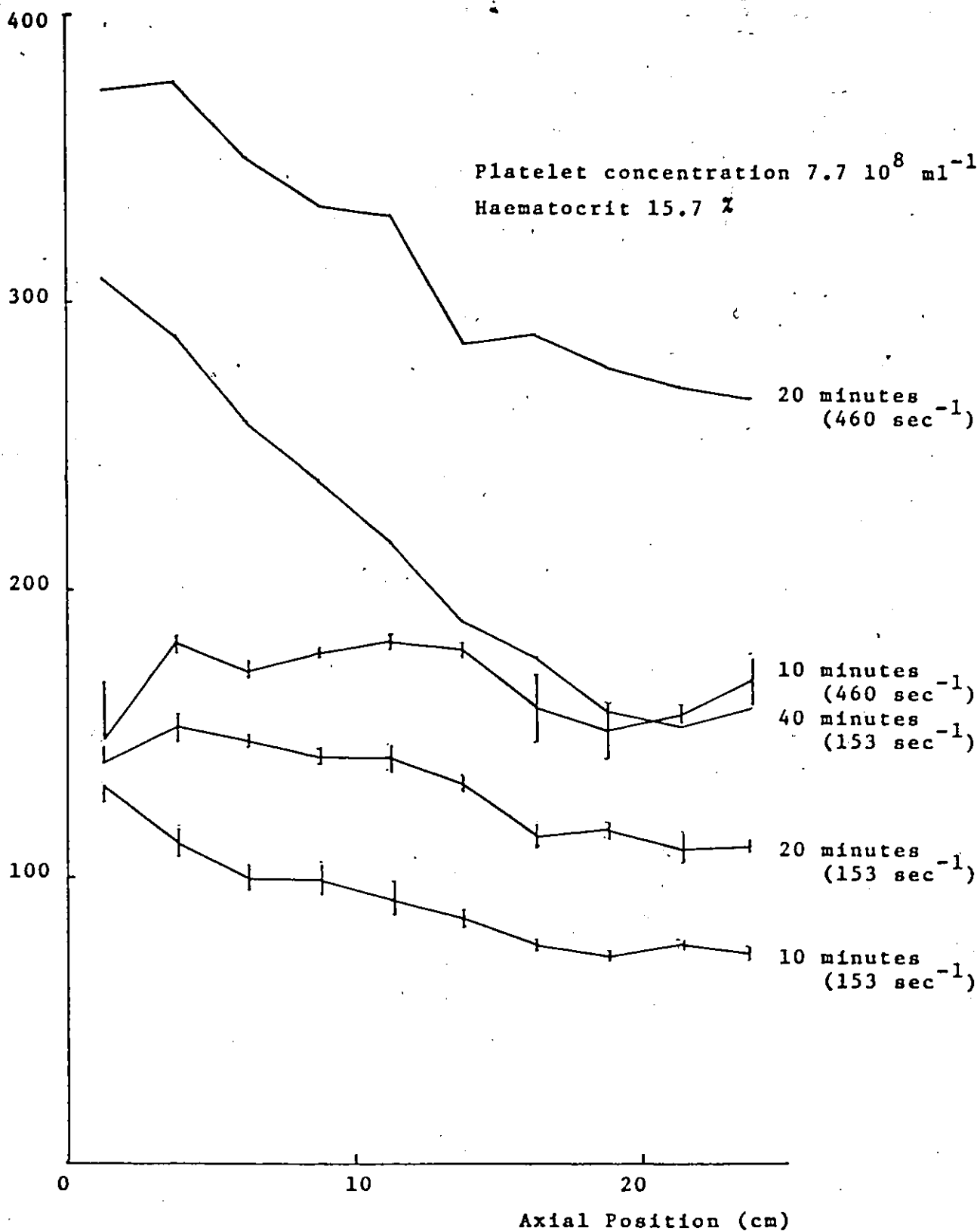
Platelet Surface Density (platelets/ $1000\mu^2$)

Figure 1.11

Effect of shear rate upon platelet adhesion
(showing estimates of standard deviation in data at
each measurement (2 measurements at each point)).

saturation level with increased haematocrit and shear rate arises from an increased platelet collision frequency with the wall or from the release of an adhesion potentiating substance by adherent platelets. It may be speculated that there is a spectrum of adhesiveness within the adhesion sites, some requiring a more violent platelet collision than do others for the platelets to adhere.

Variation of the platelet binding rate constant with shear rate and haematocrit.

In the estimation of the parameters, the highest numerical value that could be taken by K_d was about 4. This value indicates that the overall kinetics of the adhesion process approach diffusion limited kinetics (Fig. 1.5). If K_d takes a value of about 4, then K_d and hence k are not known precisely, but rather have a calculated value that is lower than the actual one. When K_d is about 4, as

$$k = K_d a \cdot Pe^{1/3} / D_s \quad \text{from (1.6)}$$

and as K_d takes a fixed value, then substituting (1.32) for D_s

$$k \propto H^{1.2} \gamma^{0.6}$$

Such a correlation between the binding rate constant, haematocrit and shear rate was found for experiment 3 in Table 1.13 where, at all haematocrits and shear rates, K_d was about 4. For experiments 1 and 2 different correlations were obtained (Table 1.18).

In experiments 1 and 2, a correlation of the form

$$k_{aH}^{1.28} \quad (1.34)$$

holds. This result is in accord with the observation of Feuerstein et al. (1975) that the binding rate constant increases with increasing haematocrit. Such an effect may be related to the skimming layer thickness which decreases monotonically with increasing haematocrit but, like the binding rate constant, is unaffected by the shear rate (Bugliarelló and Sevilla, 1970). It may be postulated that, at higher haematocrits, the force with which red cells and platelets strike the surface is high. It will be recalled that the red cells do not appear to modify the collagen surface so as to affect platelet adhesion (Section 1.5.2).

Table 1.18

Effect of haematocrit (H) and shear rate (γ)
upon the platelet binding rate constant k

<u>Experiment</u>	<u>Correlation</u>
1	$k_{aH}^{0.94 \pm 0.37}$
2	$k_{aH}^{1.63 \pm 0.63}$
3	$k_{aH}^{1.03 \pm 0.20} \gamma^{0.58 \pm 0.11}$

Effects of changes in haematocrit at high haematocrits.

Platelet diffusion coefficients, binding rate constants and saturation levels of adhesion to collagen have been shown to be dependent upon haematocrit over a wide range of haematocrit above 6%. Feuerstein et al (1975) indicated that, above a haematocrit of 30%, platelet adhesion is not affected by changes in haematocrit. This was confirmed in an experiment in which collagen-coated tubes were exposed to platelet suspensions of two different haematocrits. Over a wide range of shear rates ($23-460 \text{ sec}^{-1}$) there was no significant change in platelet adhesion ($p > 0.4$, paired t-test) (Table 1.19).

The effect of apyrase upon adhesion.

Apyrase is an enzyme that degrades ADP, an adenine nucleotide that promotes aggregation. The suspensions of washed pig platelets prepared for this study of platelet adhesion contain a low concentration of apyrase ($1 \lambda \text{ ml}^{-1}$). To determine if the adhesion process observed is dependent upon ADP, experiments were performed to determine the level of platelet adhesion to collagen-coated glass tubes at various apyrase concentrations within the suspension.

The results (Table 1.20) demonstrate that platelet adhesion is unaffected by the level of apyrase (paired t-test, $p > 0.55$) and hence by the level of ADP. It may thus be concluded that the adhesion process observed is not dependent on ADP.

Table 1.19

Effect of changes in high levels of haematocrit upon platelet adhesion

Time of exposure (mins)	Hct	Shear. rate (sec ⁻¹)										
		10	5	184	10	5	10	46	5	10	23	5
Mean level of platelet adhesion to tube	30	174.6	89.4	71.4	44.7	47.1	24.4	27.5	17.3			
	40	188.3	83.4	68.7	46.8	49.4	25.3	30.8	15.2			

Platelet concentration 5.40 10⁸ platelets ml⁻¹.

Table 1.20

Effect of apyrase

Time of exposure (minutes)	Apyrase Level (λ .ml ⁻¹)	Shear Rate (sec ⁻¹)					
		10	5	10	5	184	46
Mean level of plate- let adhesion to tube (platelets/1000 μ 2)	*	202	117	86	62	23	39
	**	209	107	86	60	30	32
		460		10	5	10	5
						23	23

* Haematocrit 33%, platelet concentration 3.71 10^8 platelets ml⁻¹

** Haematocrit 33%, platelet concentration 3.60 10^8 platelets ml⁻¹

Platelet adhesion to glass, collagen-coated glass and polyurethane-coated glass in the presence or absence of aggregates.

It was found that after internally coating the first 5 cm from the entrance of a glass tube with collagen, air bubbles remained attached to the glass-collagen boundary when the tubes were filled with the modified Tyrode solution. Upon passage of a platelet suspension through the tubes, aggregates formed upon these bubbles.

Experiments were performed to examine the adhesion of platelets to glass, collagen and polyurethane-coated glass tubes in the presence or absence of the aggregates formed on glass tubes partially coated with collagen. At a shear rate of 115 sec^{-1} , tubes were exposed to a platelet suspension for 5 to 10 minutes in the presence or absence of these partially coated tubes. The results are tabulated in Table 1.21. Following staining, the tubes were examined microscopically to determine if aggregates were adherent to the surface. No aggregates were found in any tube except those whose leading edges were collagen-coated.

Several observations may be made about these results. Firstly, the saturation level of platelet adhesion to glass and polyurethane is lower than that to collagen. Secondly, in the presence of aggregates, the saturation level of adhesion rises significantly in the case of the glass and polyurethane-coated glass (paired t-test $p < 0.05$). Thirdly, little may be

Table 1.21

Effect of aggregates upon adhesion

Exp.	Type of surface	Aggregates Present?	$2 D$ $cm \cdot sec^{-1}$	$\frac{PP}{1000\mu^2}$ platelets/	k $cm \cdot sec^{-1}$	K
1	Collagen	Yes	$1.298 \cdot 10^{-7} \pm 1.79 \cdot 10^{-7}$	223 ± 14.8	$6.83 \cdot 10^{-4}$	4.171
		No	$1.139 \cdot 10^{-7} \pm 1.27 \cdot 10^{-7}$	202 ± 5.4	$6.86 \cdot 10^{-4}$	4.57
	Glass	Yes	$7.838 \cdot 10^{-8} \pm 1.23 \cdot 10^{-7}$	70.4 ± 3.6	$5.39 \cdot 10^{-4}$	4.61
		No	$5.980 \cdot 10^{-8} \pm 6.10 \cdot 10^{-8}$	28.3 ± 1.1	$4.32 \cdot 10^{-4}$	4.42
2	Collagen	Yes	$7.16 \cdot 10^{-8} \pm 4.01 \cdot 10^{-8}$	125 ± 2.5	$5.16 \cdot 10^{-4}$	4.68
		No	$5.98 \cdot 10^{-8} \pm 4.53 \cdot 10^{-8}$	114 ± 2.1	$4.20 \cdot 10^{-4}$	4.33
	Glass	Yes	$5.53 \cdot 10^{-8} \pm 4.58 \cdot 10^{-8}$	52.8 ± 1.5	$3.89 \cdot 10^{-4}$	4.20
		No	$5.79 \cdot 10^{-8} \pm 1.43 \cdot 10^{-7}$	23.5 ± 1.3	$3.97 \cdot 10^{-4}$	4.15
	Polyurethane	Yes	$1.64 \cdot 10^{-8} \pm 1.1 \cdot 10^{-7}$	26.4 ± 3.4	$1.89 \cdot 10^{-4}$	4.59
		No	$9.50 \cdot 10^{-7} \pm 3.21 \cdot 10^{-8}$	9.6 ± 1.1	$1.54 \cdot 10^{-4}$	5.38

Shear rate: 115 sec^{-1}

Haematocrits: Expt. 1 37%
Expt. 2 35%

Platelet concentrations: Expt. 1 $2.48 \cdot 10^8$ platelets ml^{-1}
Expt. 2 $3.04 \cdot 10^8$ platelets ml^{-1}

said about the binding rate k , as the values estimated for K_d , from which k is calculated, merely indicate that the kinetics approach those of diffusion limited adhesion. Fourthly, it is evident that the platelet diffusivity calculated from the adhesion to a collagen-coated surface is higher than that calculated from the adhesion to glass or polyurethane-coated glass. As it is unlikely that the diffusion of platelets through a suspension will be influenced by the nature of the bounding surface, these results must thus be due to a miscalculation of the concentration gradient of those platelets that are capable of adhering to the surface. Such would occur if a fraction of the platelet population could not adhere to the surface, the effective bulk concentration of platelets being reduced in so far as adhesion is concerned. It will be recalled that D_s is calculated from the estimated values of T and ρ_p :

$$D_s = u^{1/2} (\rho_p / (c_0 T))^{1.5} / a \quad \text{from (1.6)}$$

or $D_s \propto (\text{active platelet concentration})^{-1.5}$

If fewer platelets are capable of adhesion to glass or polyurethane than to collagen-coated glass, then the presence of aggregates increases the size of this reactive fraction. It seems probable that most platelets are capable of adhesion to collagen: Cazenave et al. (1973a) found no significant change in the level of platelet adhesion to collagen-coated surfaces when a platelet suspension was repeatedly exposed to

collagen surfaces, the final platelet concentration of the suspension being diminished by 30%. It is thus unlikely that the correlations between diffusivity and shear rate and haematocrit given earlier arise from varying degrees of platelet reactivity caused by shear and the presence of erythrocytes.

The effect of apyrase in the presence of aggregates.

It was earlier demonstrated that the adhesion of platelets to collagen was not mediated by ADP. In an attempt to see if ADP plays a role in platelet adhesion in the presence of aggregates an experiment was performed to determine the effect of varying levels of apyrase. The results (Table 1.22) show there to be no significant effect (paired t test, $p > 0.25$): the enhancement of platelet adhesion in the presence of aggregates is unlikely to be an ADP effect.

Table 1.22

Effect of apyrase in the presence of aggregates

Time of exposure (minutes)	Apyrase level (λ .ml ⁻¹)	Surface						
		c.g.	1	5	10	5 8.		
Levels of ⁵¹ Cr counts on surface	1*	8305	7850	5938	5883	9100	5852	5111
	10**	7858	7948	5528	5987	7795	5675	4754

* Haematocrit 33%, platelet concentration 3.81 platelets ml⁻¹

** Haematocrit 33%, platelet concentration 3.73 platelets ml⁻¹

1 c.g. - first 5 cm of glass tube collagen-coated

2 c. - collagen-coated glass tube

3 g. - glass tube.

The Effect of EDTA upon Platelet Adhesion

The effect of EDTA upon platelet adhesion to collagen was investigated. EDTA is an agent that chelates the free divalent cations, Ca^{2+} and Mg^{2+} , in solution. An EDTA concentration of $3 \cdot 10^{-3}$ M is necessary to chelate all the free Ca^{2+} and Mg^{2+} within the platelet suspension. A 10^{-3} M EDTA concentration did not significantly inhibit platelet adhesion to collagen over a wide range of shear rates ($23\text{-}460 \text{ sec}^{-1}$) (Table 1.23). A 10^{-2} M EDTA concentration however significantly inhibited platelet adhesion (t test, $p < 0.05$) through the diminution of the saturation level of platelet adhesion and of the platelet-surface binding rate constant (Table 1.24). In the absence of EDTA, the adhesion process obeyed kinetics approaching those of diffusion control. In the presence of EDTA the adhesion process obeyed intermediate kinetics far removed from those of a diffusion controlled process. Thus in the presence of EDTA (10^{-2} M), the binding rate constant could be accurately estimated; in the absence of EDTA this was not so. For platelet adhesion in the presence of EDTA, the platelet surface binding rate constant, k , was unaffected by shear rate tested by the regression of k upon shear rate (t test, $p < 0.1$).

These results suggest that platelet adhesion to collagen may occur in the absence of Ca^{2+} and Mg^{2+} . Perhaps two mechanisms exist for platelet adhesion to collagen, one

Table 1.23

Effect of EDTA (10^{-3} M) on platelet adhesion

Time of Exposure (mins)	Exp.	EDTA Conc. (M)	Shear Rate						
			10	5	10	5	10		
1	0	460	208.5	136.2	73.0	58.1	73.4	44.4	38.9
			170.2	95.0	83.5	47.0	45.7	33.8	29.3
2	0	460	110.1	86.3	42.0	24.7	29.3	17.6	13.5
			107.7	70.2	30.5	23.0	23.9	17.6	12.4
Exp. 1 Hct	33%	Platelet concentration	2.24.10 ⁸		platelets.ml ⁻¹				
Exp. 2 Hct	24%	Platelet concentration	2.94.10 ⁸		platelets.ml ⁻¹				

Table 1.24
Effect of EDTA (10^{-2} M) on platelet adhesion

Shear Rate (sec^{-1})	EDTA conc. (M)	D^* ($\text{cm}^2 \text{sec}^{-1}$)	ρ^* (platelets/ $1000 \mu^2$)	k^\dagger ($\text{cm} \cdot \text{sec}^{-1}$)	K_d
460	10^{-2}	$2.11 \cdot 10^{-7}$	152.7 ± 42.3	$1.84(1.65-3.86) \cdot 10^{-4}$	0.51
	0	$2.60 \cdot 10^{-7}$	280.7 ± 6.7	$1.76(0.49-\infty) \cdot 10^{-3}$	4.24
184	10^{-2}	$2.41 \cdot 10^{-7}$	97.1 ± 14.2	$2.25(2.02-4.72) \cdot 10^{-4}$	0.78
	0	$1.94 \cdot 10^{-7}$	134.2 ± 6.7	$1.09(0.27-\infty) \cdot 10^{-3}$	4.35
46	10^{-2}	$1.54 \cdot 10^{-7}$	81.7 ± 14.7	$1.05(0.74-1.78) \cdot 10^{-4}$	1.66
	0	$7.14 \cdot 10^{-8}$	71.7 ± 2.2	$3.29(0.42-\infty) \cdot 10^{-4}$	4.06
23	10^{-2}	$1.44 \cdot 10^{-7}$	65.9 ± 0	$2.05(0.72-2.46) \cdot 10^{-4}$	0.82
	0	$1.93 \cdot 10^{-7}$	143.9 ± 4.8	$5.22(1.41-\infty) \cdot 10^{-4}$	4.18

* Best fit value \pm 95% confidence limits calculated assuming linear behaviour for model in region of best fit.

† Parentheses contain upper and lower 95% confidence values obtained from plots of joint 95% confidence region of ρ_p and k .

Platelet concentration: $1.77 \cdot 10^8$ platelets ml^{-1}

Haematocrit: 32%

dependent upon divalent cations, the other independent.

Effect of ASA upon Platelet Adhesion

Acetylsalicylic acid (ASA, aspirin) is a widely used anti-inflammatory drug that has been found to inhibit release. Its effect upon platelet adhesion is uncertain: Baumgartner and Muggli (1974) found that ASA did not affect the adhesion of platelets to subendothelium. Cazenave et al. (1975) found the adhesion of platelets to be diminished in the presence of ASA. The effect of ASA upon platelet adhesion to collagen in the present system was thus investigated. A 10^{-4} M ASA concentration was found not to significantly diminish adhesion levels over a wide range of shear rates (Table 1.25) (paired t test, $p < 0.5$).

Pulsatile and non-pulsatile flow

The previously described experiments were performed with a steady non-pulsatile flow of suspension through the tubes. In the arteries the flow is pulsatile. To determine if the level of adhesion with pulsatile flow significantly differs from that with non-pulsatile flow, an experiment was performed in which identical platelet suspensions were passed through collagen coated glass tubes at the same mean flow rate for 10 minutes (Figs. 1.12-1.13). As expected (Caro et al. 1971) no significant change in platelet adhesion occurred (t test, $p > 0.3$) (Table 1.26).

Table 1.25

Effect of ASA (10^{-4} M) on platelet adhesion

Time of exposure (min)	Exp.	ASA conc. (M)	Shear Rate (sec^{-1})									
			460		184		46		23			
			10	5	10	5	10	5	10	5	10	5
Average platelet surface density	1	0	257.0	145.3	81.5	58.6	79.2	51.5	48.3	25.7		
		10^{-4}	247.2	152.8	79.8	50.3	57.2	34.6	51.0	27.6		
(Platelets/ $1000\mu^2$)	2	0	289.2	158.6	96.6	74.0	90.9	55.5	93.4	60.3		
		10^{-4}	249.3	164.8	90.6	81.6	90.7	70.6	62.6	46.2		

Expt. 1: Platelet concentration $4.47 \cdot 10^8 \text{ ml}^{-1}$.
Haematocrit 33%.

Expt. 2: Platelet concentration $6.14 \cdot 10^8 \text{ ml}^{-1}$.
Haematocrit 38%.

Table 1.26

Effects of pulsatile flow

Frequency of pulsation (Hz)	Mean level of platelet adhesion* (platelets/1000 μ^2)
0	21.2 \pm 2.4
0.67	23.8 \pm 3.1

*Haematocrit 37%, mean shear rate 56 sec⁻¹, platelet concentration 3.90.10⁸ platelets, ml⁻¹.

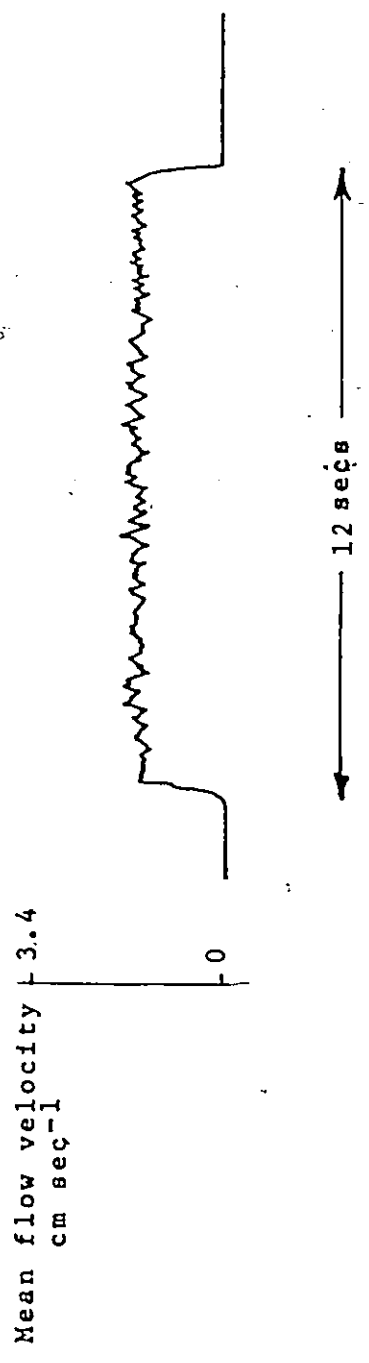


Figure 1.12
Non-pulsatile flow pattern
(wall shear rate 56 sec⁻¹)

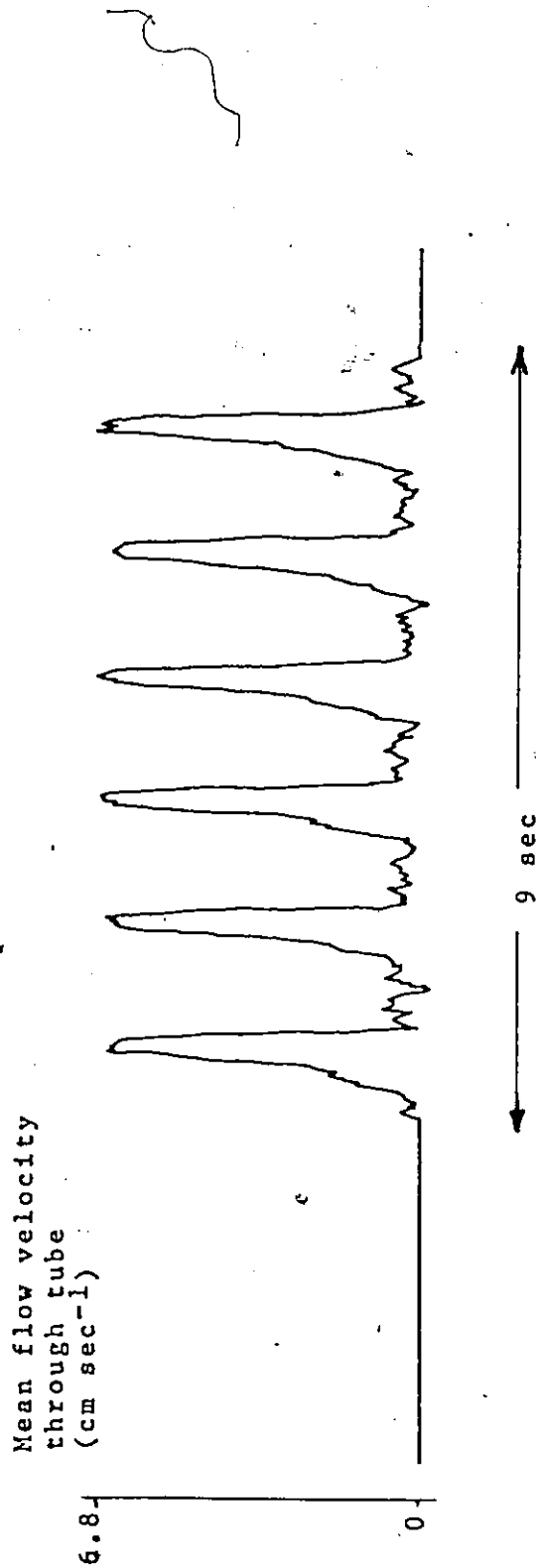


Figure 1.13

Pulsatile flow pattern

(2/3 Hz, wall shear rate 56 sec⁻¹)

It is concluded that the results obtained in steady non-pulsatile flow experiments may be applied to situations in which the flow is pulsatile.

Convective diffusion and Couette flow

Platelet adhesion is a process in which platelets diffuse to and then bind with a surface. If drugs inhibit platelet adhesion, they must do so by inhibition of the binding of platelets to the surface; platelet diffusion is induced by red cell motion and is unlikely to be affected by drugs. Thus if inhibition of platelet adhesion is to be observed experimentally, the adhesion process must obey reaction limited or intermediate kinetics in the presence of the inhibitor. If diffusion limited kinetics are followed nothing may be said about the binding reaction or of its inhibition.

In this study, EDTA (10^{-2} M) was found to inhibit platelet adhesion. However, no significant inhibition of platelet adhesion was obtained in the presence of ASA (10^{-4} M). Similarly Baumgartner and Muggli (1974), also using a device that relied upon the convective diffusion of platelets, found that ASA did not significantly inhibit platelet adhesion to subendothelium. These results are in contrast to those of Cazenave et al. (1975, 1974a) who, using a Couette flow device, concluded that ASA inhibits platelet adhesion. The question thus arises as to whether the different conclusions

drawn about the effect of ASA result from the observation of diffusion limited kinetics in convective diffusion experiments and of intermediate kinetics in Couette flow experiments. The flows in the two systems differ in that, with convective diffusion, platelets are brought by convection into the platelet depleted concentration boundary layer, whilst in Couette flow, where the test surface rotates in the centre of a cylindrical volume of the platelet suspension, no platelets are convected into the concentration boundary layer.

With convective diffusion in a cylindrical geometry, it is possible to estimate values of the binding rate constant above which intermediate kinetics may not be clearly distinguished from diffusion limited kinetics.

Such occurs when

$$k_d \sim 4$$

or

$$ka(ua/D_s)^{-1/3}/D_s \sim 4 \quad \text{from (1.6)}$$

In the experiments analysed, the cube root of the Peclet number, (ua/D_s) , was found to be in the range 100-200. Thus in tubes 0.11 cm in radius, the upper bound upon the value of k that may be clearly distinguished is related to the diffusivity, D_s , so:

$$k = 50000 D_s$$

Similarly, in Couette flow, values of k may be

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estimated above which intermediate kinetics are indistinguishable from diffusion limited kinetics. For Couette flow, the level of platelet adhesion upon a test surface may be approximated by the expression (Feuerstein et al. 1975):

$$\rho = c_0(-D/k + 2(Dt/\pi)^{1/2})$$

The first term in this equation is omitted if diffusion limited kinetics apply. Now the inclusion of statistically insignificant terms in a model leads to imprecision in the estimation of parameters (Julius, 1972). Thus if the test surface is exposed to a platelet suspension for 100 seconds, assuming that this first term, (D/k) may be distinguished if it has 5% of the magnitude of the second term, $2(Dt/\pi)^{1/2}$, then the upper bound upon k for which values of k may be precisely estimated, is

$$k = (D\pi)^{1/2}$$

The bounding values above which k is not clearly discernable are presented in Fig. 1.14. It is evident that at low values of platelet diffusivity ($10^{-8} \text{ cm}^2 \text{ sec}^{-1}$ or below), corresponding to low haematocrits (10% or less), a Couette flow device is able to distinguish higher values of the binding rate constant k than is the convective diffusion device employed in the studies described in this thesis. At higher values of the platelet diffusion coefficient ($10^{-7} \text{ cm}^2 \text{ sec}^{-1}$) that would be found in suspensions of higher haematocrit (30-50%), the convective diffusion and Couette flow devices differ little in their ability to distinguish the higher values of the

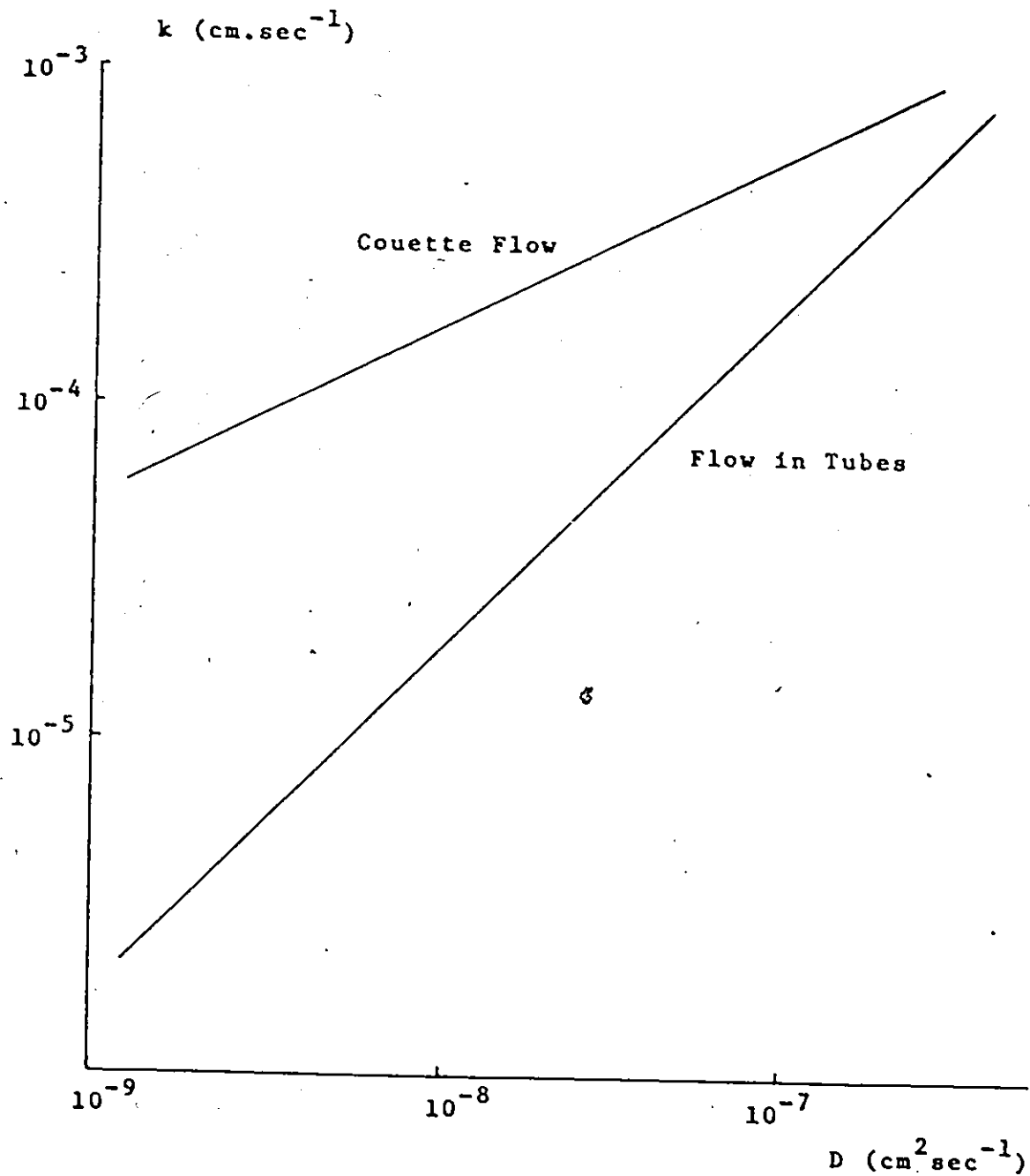


Figure 1.14

Bounding values of k above which adhesion is essentially diffusion controlled (Tubes 0.11 cm radius)

binding rate constant.

As the value of k for platelet adhesion to collagen coated glass varies from $4 \cdot 10^{-4}$ cm.sec⁻¹ at a haematocrit of 30% to 10^{-4} cm.sec⁻¹ at 5% haematocrit and presumably slightly lower values for suspensions free of red cells, it is evident that the inhibitory effects of any drugs are best determined in a Couette flow device by measurement of the level of adhesion from platelet suspensions of low haematocrit. In a tubular flow, convective diffusion device platelet adhesion is more likely to be diffusion controlled and thus not inhibited by drugs. This may explain why Cazenave et al. (1975) found that ASA inhibited platelet adhesion whereas Baumgartner and Muggli (1974) and I did not.

Should there be Taylor vortices within the Couette flow device, the platelet flux to the surface will be increased and thus the effective platelet diffusivity enhanced. Then the adhesion process will be further removed from diffusion limited kinetics, it being unlikely that the binding rate constant is affected by Taylor vortices. In these circumstances any inhibition of the binding reaction is more likely to be detected.

Diffusion limited kinetics or intermediate kinetics
with convective diffusion.

In the last section, it was demonstrated that there are limitations upon our ability to distinguish the intermediate kinetics that describe the process of platelet adhesion. This may explain the conclusion drawn by Friedman and Leonard (1971), Turitto (1975) and Butruille et al. (1975) that platelet adhesion to a foreign surface is a diffusion limited process. Often however, the data is such that, if it were to be described by the model of Grabowski et al. (1972) it would be best fitted by intermediate kinetics. To reconcile this data with diffusion limited kinetics, Turitto (1975) proposed a hypothesis based on the assumption of diffusion limited kinetics, and the assumption that all platelets that fail to adhere on first colliding with the surface lose their ability to adhere (section 1.4.7).

The diffusion limited models of Turitto (1975) and Grabowski et al. (1972) (section 1.4.4 here) were compared using an F test (Dell et al., 1973) to the intermediate kinetics model of Grabowski et al. used in this study. The models were fitted to two sets of experimental data from this study, one set with kinetics approaching diffusion limited adhesion ($K_d = 4.2$); the other with intermediate kinetics ($K_d = 0.6$). The results of the comparison (Table 1.27; Figs. 1.15 and 1.16) show the

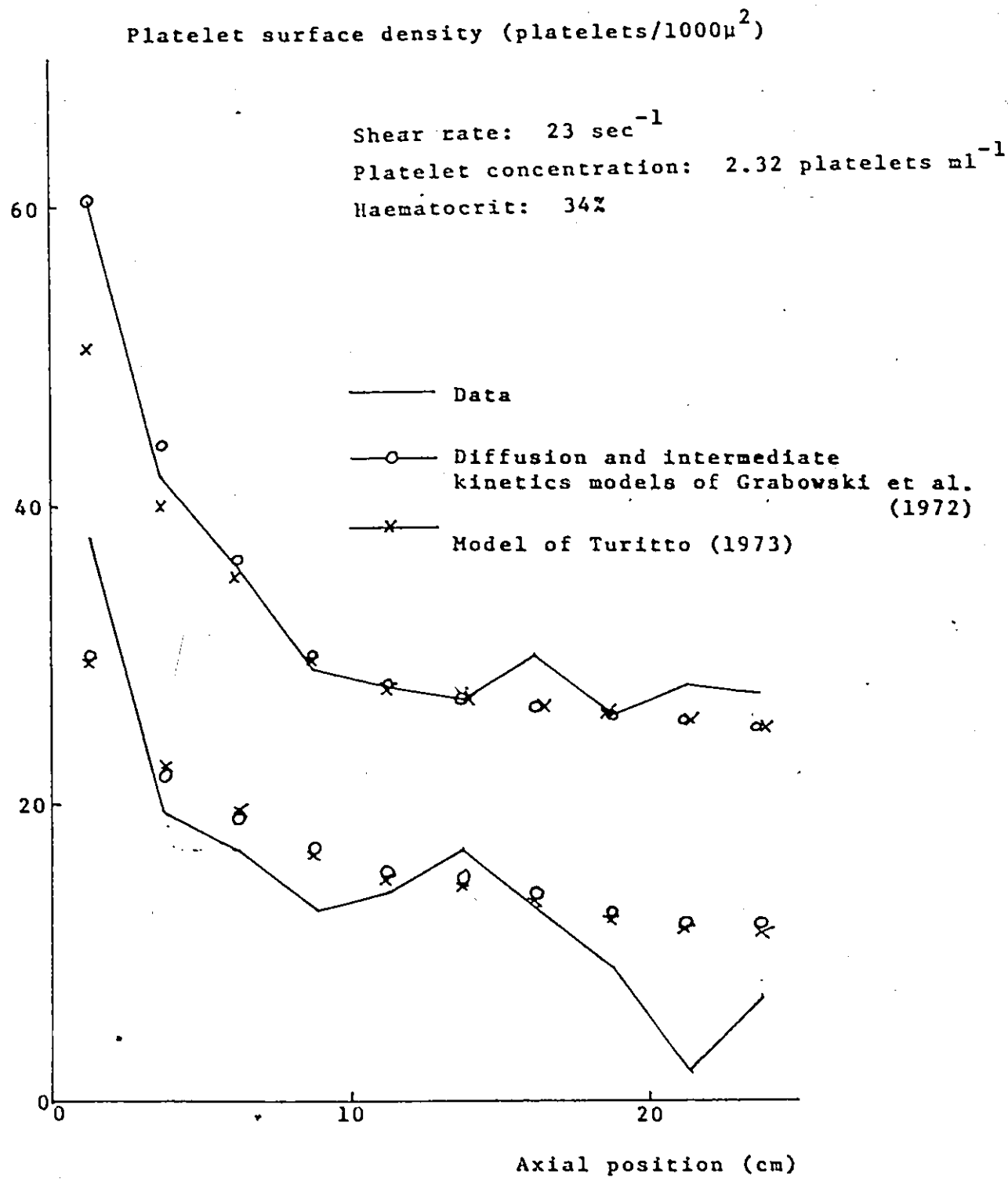


Figure 1.15

Comparison of diffusion limited and intermediate kinetics
(Exp. 1, $K_d=4.2$)

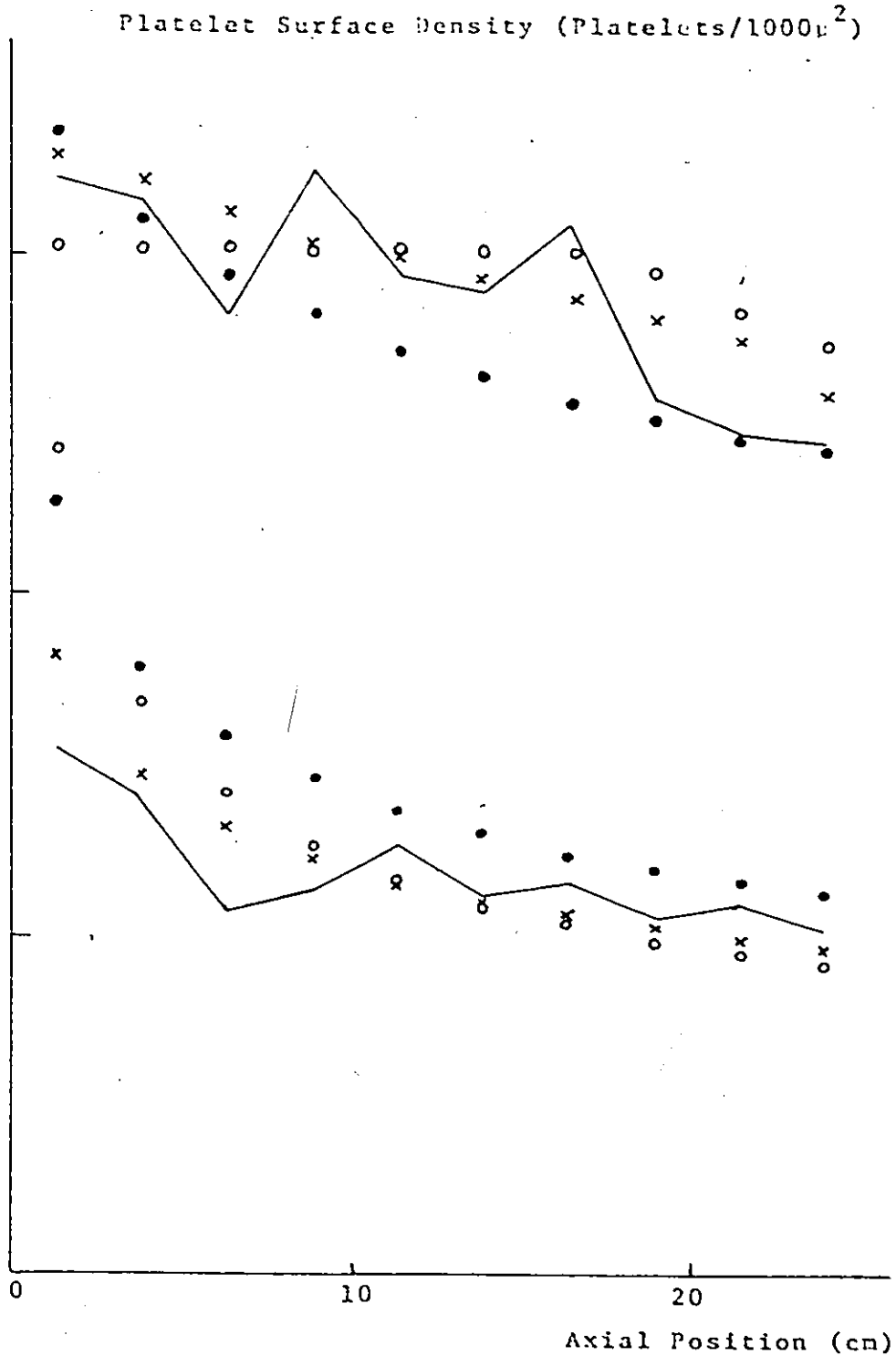


Figure 1.16

A comparison of diffusion limited and intermediate kinetics (Exp. 2, $K_d=0.6$) (— data; o diffusion limited kinetics (Grabowski et al., 1972); • diffusion limited kinetics (Turitto, 1975); x intermediate kinetics (Grabowski et al., 1972)).

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Table 1.27

A comparison of diffusion limited and intermediate kinetics

Model	Exp.	D ($\text{cm}^2\text{sec}^{-1}$)	ρ_p (platelets / $1000\mu^2$)	K_d	S.S.*	Degrees of Freedom
Turitto (1975)**	1	$5.17 \cdot 10^{-8}$	90.7	-	$4.14 \cdot 10^{12}$	18
Diffusion limited kinetics***		$3.86 \cdot 10^{-8}$	74.9	-	$2.36 \cdot 10^{12}$	18
Intermediate kinetics***		$3.55 \cdot 10^{-8}$	68.9	4.2	$2.19 \cdot 10^{12}$	17
Turitto (1975)**	2	$3.68 \cdot 10^{-8}$	35.1	-	$2.75 \cdot 10^{12}$	38
Diffusion limited kinetics***		$2.03 \cdot 10^{-8}$	30.1	-	$3.08 \cdot 10^{12}$	38
Intermediate kinetics***		$2.62 \cdot 10^{-8}$	33.6	0.6	$2.15 \cdot 10^{12}$	37

*Sum of squares of deviations of model from data.

**Section 1.4.7

***Section 1.4.4

Exp. 1: Haematocrit 20%, Shear rate 184 sec^{-1}

Exp. 2: Haematocrit 13%, Shear rate 115 sec^{-1}

model of Turitto (1975) to give significantly worse fits to the data than does the intermediate kinetics model of Grabowski et al. (1972) (F test, $p < 0.01$, experiments 1 and 2). In particular it is evident that in the case where intermediate kinetics approaching diffusion limited kinetics apply, the model of Turitto is unable to account for a continued high rate of adhesion when partial coverage of the surface has occurred. If intermediate kinetics may be assumed, a high rate of adhesion is expected to persist until coverage of the surface with platelets is almost complete, an increased collision frequency (Fig. 1.9) compensating for a decreased probability that collision results in adhesion.

As would be expected, where K_d took the value 4.2, there is no significant difference (F test, $p > 0.1$) in the fits given by models assuming intermediate kinetics and diffusion limited kinetics. At the lower value of K_d ($K_d = 0.6$) however, intermediate kinetics give a significantly better fit to the data than do diffusion limited kinetics (F test, $p < 0.01$).

It is thus evident that the process of platelet adhesion in tube flow is best described by intermediate kinetics. Models that assume diffusion limited kinetics are inferior in their ability to fit the experimental data. There is no support for the model of Turitto (1975) or for its implicit assumption that the platelets that do not adhere on first contacting the wall are rendered incapable of adhesion.

1.6 Conclusions.

It has been established that, following the labelling of platelets with the γ emitting ^{51}Cr , the γ -radiation emanating from a surface provides a good measure of the level of platelet adhesion to the surface. It has been demonstrated on the test surface employed here the platelet adhesion so measured is not influenced by rinsing or by other steps in the preparation of tubes for counting.

The ability of platelets to adhere has not been found to alter significantly over the duration of an experiment. Platelet adhesion to collagen was shown to be irreversible over the duration of the experiment even upon exposure of the surface to flowing suspensions of washed red cells.

The convective diffusion model proposed by Grabowski et al. (1972) was adopted to describe the adhesion process. This model takes platelet adhesion as being a process in which platelets diffuse to a reactive surface where they undergo a binding reaction.

In time the surface becomes saturated with platelets. It is assumed that the motion of platelets may be considered to occur in a continuum. The use of such a model in the current experiments was justified. The presence of a skimming layer did not appreciably alter the calculated values of platelet diffusivity, binding rate constant or saturation level of platelet adhesion. These three parameters were not significantly affected by

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the platelet concentration within the suspension containing erythrocytes.

Subsequently the diffusion of platelets was characterized. The diffusivity was correlated with the haematocrit and shear rate. It was found to be highly dependent upon the haematocrit and dependent upon the shear rate. Such results support the concept of platelets motion being induced by the complex interaction of red cells.

Similarly, the saturation level of adhesion was correlated with haematocrit and shear rate, increases in both these parameters increasing the saturation level of platelet adhesion. This correlation would suggest that the saturation level of platelet adhesion to a foreign surface increases in regions of high shear. High shear would also cause an increased level of platelet retention in extra-corporeal devices.

It proved to be more difficult to characterize the binding of platelets to surfaces. In general the adhesion process obeyed intermediate kinetics approaching diffusion limited adhesion. However, it was found in some instances that the binding rate constant could be correlated to the haematocrit, the binding rate being enhanced by high haematocrits. However, as the value of the binding rate constant is uninfluenced by the wall shear rate it is unlikely that the increase of the binding rate constant with increased

haematocrit results from an increased red cell collision frequency with the wall or from increased jostling of the fluid layers adjacent to the surface. Perhaps the binding rate constant is influenced by the skimming layer whose thickness is determined by the haematocrit alone. The statement that platelet adhesion follows diffusion limited kinetics (Butruille et al. 1975; Turitto, 1975) was found to be unjustified.

Above a haematocrit of 30%, changes in haematocrit do not influence platelet adhesion.

It appears probable that whilst most platelets can adhere to a collagen surface, fewer platelets are capable of adhesion to glass or to polyurethane coated glass unless platelet aggregates are present.

It was demonstrated that EDTA (10^{-2} M) diminished platelet adhesion to collagen through a diminution of the platelet to surface binding rate constant and a reduction of the saturation level of platelet adhesion. With this apparatus however, there was no significant inhibition of platelet adhesion to collagen in the presence of ASA (10^{-4} M), a non-steroidal anti-inflammatory drug. To examine the binding of platelets to surfaces, it would seem that a Couette flow device is better than the devices employing convective diffusion that have been used hitherto. With convective

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diffusion, adhesion occurs under conditions so close to those of diffusion limited adhesion that it is hard to distinguish any alteration in the platelet binding rate with the introduction of drugs.

Thus it would seem that the device employed in this study is limited to the examination of the diffusion of platelets to a surface and to the saturation level of adhesion upon the surface. The binding of the platelets to the surface is best investigated by other means. In conclusion then, it may be stated the adhesion of platelets to foreign surfaces is greatly influenced by the haematocrit and flow conditions under which adhesion occurs. Any examination of the adhesion process must take account of such fluid mechanical factors.

Part 2 THE USE AND ANALYSIS OF MULTIPLE RESPONSES IN MULTI--
COMPARTMENT CELLULAR SYSTEMS: THE TRANSPORT AND
STORAGE OF SEROTONIN IN PLATELETS

2.1 Introduction

For the understanding of biological phenomena, knowledge of the distribution of compounds within and exchange between different compartments is frequently required.

Generally, radioactive tracer experiments provide the data set from which the parameters describing compartmental size and exchange rates may be estimated. This data set may be compatible with different models of the system. The object of data collection and analysis must therefore be to discriminate between possible models. A statistical discrimination technique commonly relies upon information obtained from a least squares fit of model to data. This thesis will demonstrate the advantages of obtaining multiple responses for analysis. It will describe the application of the Bayesian multivariate technique of Box and Draper (1965) to the characterization of compartmental systems and will compare it to other least squares techniques.

2.2 Theory

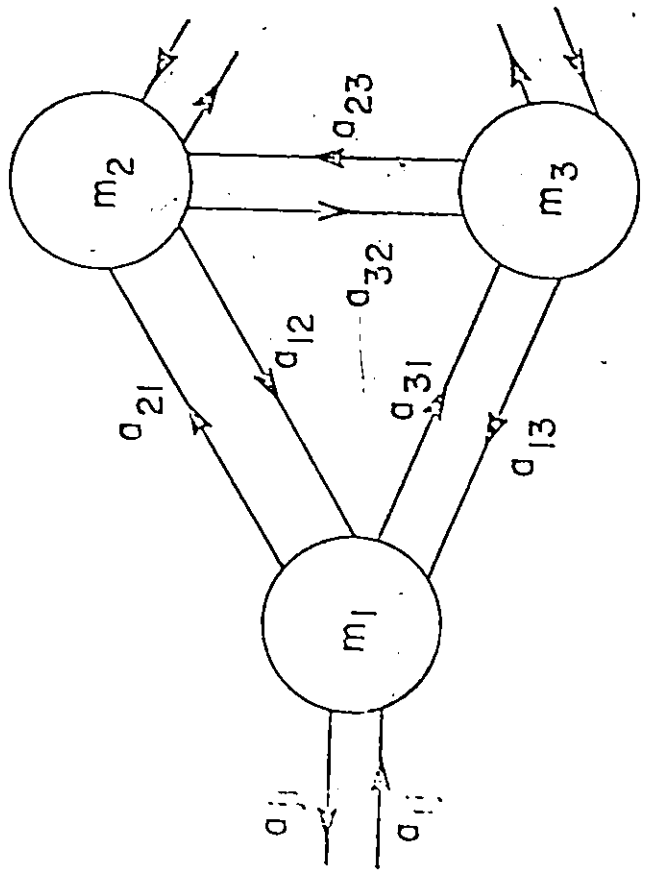
Radioactive tracer experiments have provided much insight into the dynamic behaviour of biological systems.

Generally a system is treated as a series of distinct homogeneous compartments. Within a particular compartment, the tracer molecules have the same probability of change irrespective of their time of entry into that compartment. A stochastic description of the movement of tracer molecules may give a more realistic representation of the system than does the assumption of idealised compartments (Bergner, 1961; Bergner and Lushbough, 1967). However, the use of compartments is justified in small closed systems in which compartments are physiologically evident; it is with these that I will deal.

A Model for Tracer Distribution

Consider a system of distinct, homogeneous compartments. It is assumed that the transport of tracer may be described as a first order process; either the system is in the steady state or the transport process for the naturally occurring isotope has first order kinetics. Let a_{ij} be the fractional amount of tracer or natural isotope in the j th compartment flowing to the i th per unit time (Fig. 2.1). $(-a_{ii})$ is the total turnover from the i th compartment per unit time. Then the mass balance for the tracer in the i th of n compartments, m_i , is:

$$\frac{dm_i}{dt} = \sum_{j=1}^n a_{ij} m_j$$



m_i — Fraction of tracer in i th compartment

a_{ij} — Fractional turnover rate j th to i th compartment

Figure 2.1

A compartmental system

Defining $\underline{\underline{A}} = [a_{ij}]$

in vectorial notation we have

$$\frac{d\underline{\underline{m}}}{dt} = \underline{\underline{A}}\underline{\underline{m}}$$

This may be solved by Laplace transforms (Berman and Schoenfeld, 1956) or matrix diagonalisation (Amundson, 1966). The solution, assuming distinct eigenvalues for $\underline{\underline{A}}$, is:

$$\underline{\underline{m}} = \underline{\underline{G}}[e^{\lambda_i t}] \quad (2.1)$$

and

$$\underline{\underline{A}} = \underline{\underline{G}} \underline{\underline{\Lambda}} \underline{\underline{G}}^{-1}$$

where $\underline{\underline{\Lambda}}$ is the diagonal matrix of the n eigenvalues λ_i of the matrix $\underline{\underline{A}}$ and $\underline{\underline{G}}$ is a matrix function of fractional turnover rates and the initial ^{or} distribution of tracer between the compartments. The equations describing the amount of tracer in each compartment differ only in their pre-exponential constants G_{ij} .

The solution for a closed system of compartments is unconditionally stable, the matrix $\underline{\underline{A}}$ being singular. Furthermore, if detailed balanced kinetics apply (i.e. $a_{ij} > 0$ implies $a_{ji} > 0$; if $\underline{\underline{m}}(\infty)$ is the equilibrium vector, $a_{ji}m_i(\infty) = a_{ij}m_j(\infty)$), the eigenvalues have no imaginary parts and thus no oscillation may occur in the solution (Horn, 1971). In a closed system, one of the eigenvalues of $\underline{\underline{A}}$ is zero, the associated eigenvector being the

equilibrium vector $\underline{m}(\infty)$. For such a system, the dilution method of estimating compartment size is exact. Here the assumption is that the final distribution of tracer isotope is identical to that of the natural isotope (Berman and Schoenfeld, 1956).

In a catenary system, where transfer is to the adjoining compartment in the chain, the sampling of one compartment is capable of providing sufficient information to fully characterise the system, provided the initial tracer distribution is known (Berman and Schoenfeld, 1956). In the analysis, a model of the form

$$\underline{m} = \underline{B}[e^{Ct}]$$

is fitted to the data obtained by the sampling of one compartment and from this the kinetic parameters are estimated.

2.3 Statistics

It is important to precisely characterise the system when assessing the significance of any change caused by a change in experimental conditions. The tracer distribution in a system of n compartments should be represented by equations comprised of a sum of n exponential terms. However, because of the lumping together of exponential terms, the true compartmentation may not be revealed in an adequate description of the experimental data. This lumping effect should not be confused with the lumping that may theoretically

occur (Mann and Gurpide, 1969; Hearon, 1969) with certain linear combinations of compartmental labelling where less than the full set of eigenvalues are excited (Sheppard, 1971). A further complication associated with the lumping with which we are concerned is that where there may be overparameterization in the description of the data, the parametric covariance matrix of a sum of exponentials tends to be ill-conditioned (Julius, 1972).

Additional responses will provide information that will allow a more precise characterization of the model. In compartmental analysis, one measured response is the amount of one tracer in one compartment as a function of time. Further responses are obtained by measurement of the same tracer in different compartments or of different tracers in the same compartment. For the simultaneous analysis of multiple responses, several generalized non-linear least squares techniques have been proposed: by Turner et al. (1963); Beauchamp and Cornell (1966, 1969) and Box and Draper (1965). These multivariate techniques recognize and account for the correlations between responses. They assume that the vectors of errors in the responses, obtained at a given time, are independent random vectors, sampled from a multivariate normal distribution.

These techniques determine the maximum likelihood estimates of the unknown parameters. The method of Turner

et al. (1963) assumes that the covariance matrix of errors within the vectors of observations obtained at a given time is known. In general this is not so. The procedures of Beauchamp and Cornell (1966, 1969) allow for the estimation of the covariance matrix but are difficult to apply.

The Bayesian approach of Box and Draper (1965) provides a simple criterion by which to determine the set of parameters $\underline{\theta}$ with highest posterior probability. The approach begins by assuming a locally uniform prior distribution for the $\underline{\theta}$'s. The Bayesian estimate of parameters is that which minimizes the determinant of the matrix \underline{S} where S_{ij} , the element of that matrix resulting from the i th and j th responses is

$$S_{ij} = \sum_{u=1}^L (m_{iu} - \hat{m}_{iu}) (m_{ju} - \hat{m}_{ju})$$

if L is the number of observations, m_{iu} the u th observation of the i th response and \hat{m}_{iu} the corresponding value of the model's response. Formally the criterion may be stated as

$$\text{Max. } P(\underline{\theta}|\underline{m}) = \text{Max. } |\underline{S}|^{-L/2} \quad (2.2)$$

This criterion provides estimates of the parameters which maximize the posterior density Function (Box & Draper, 1965).

In a model for which the above criterion holds, it is essential that there should be no evidence of lack of fit (non-random deviations between model and data) in each response individually. The lack of fit of one response can affect the weight given to another in the overall criterion

(Box and Draper, 1965).

The determination of the number of statistically significant compartments is an approximate procedure: the model is non-linear in the parameters and particular exponential terms in the model may not be assigned to the presence of a particular compartment. The decision as to whether the addition of a compartment leads to a significant improvement in modelling the data can be made by comparing the criteria of best fit with and without the additional compartment. As there is no appropriate multiresponse test of significance for non-linear models, the responses are compared individually. The ratio of the sums of squared deviations from the data of the models with c and $(c+1)$ compartments acts as an approximate test statistic with an F -distribution (Dell et al., 1973). Hence at a particular confidence level the $(c+1)$ th compartment is significant for the i th response if

$$\frac{(S_{ii})_c}{(S_{ii})_{c+1}} > 1 + \frac{x}{L-x-y} F(x, L-x-y, 1-\alpha)$$

where Y is the number of parameters required to describe a c compartment system, $(Y+X)$ the number required for a $(c+1)$ compartment system and $(S_{ii})_c$ is the i th diagonal element of S for a model with c compartments. The significance of a compartment may be shown in the models for any or all of the responses.

In the modelling of experimental data, Sharney et al. (1963) postulated the following precepts:

1. Approximating equations should be simple, containing only a priori terms which are detectable and statistically significant.
2. The approximating equations should lend themselves to interpretation as solutions for the behaviour of mathematical models which represent an "idealisation" of the processes considered. They should not contradict physiological reality.
3. Such models should elucidate processes beyond the mere numerical representations of the experimentally available data.

Given a model, the confidence regions for the estimated values of parameters are important if differences between the parameters are to be determined. For unbiased parameter estimates, linear dependencies in the data or expected values of the responses should not occur (Box et al. 1973). Individual confidence regions may be obtained by a sensitivity analysis (Kekki et al. 1973) provided by the eigenvalues and vectors of the Hessian matrix of the determinant objective function at the maximum likelihood estimate. This assumes a model linear in the parameters which implies ellipsoidal confidence regions. Alternatively, it is most informative to plot the joint confidence regions of two parameters, the others being held at their maximum likelihood

values. A contour of the confidence region is constructed according to Box and Cox (1964)

$$\log P(\underline{\theta})_{\text{M.L.}} - \log P(\underline{\theta}) = 1/2 X_Y^2$$

or

$$\frac{|S(\underline{\theta})|}{|S|_{\text{M.L.}}} = e^{X_Y^2/2}$$

if γ is the number of parameters in the model and M.L. refers to the maximum likelihood estimate. Joint confidence regions based on the modelling of a single response may be constructed according to Draper and Smith (1966). The confidence contour is then such that, for the i th response,

$$\frac{S_{ii}}{S_{ii\text{M.L.}}} = 1 + \frac{\gamma}{L-\gamma} F(\gamma, L-\gamma, 1-\alpha)$$

The multiresponse techniques described above assume the vector of observations at a given time to be independent of those taken at a different time. Natis and Hartley (1971), in their stochastic compartmental analysis, demonstrated autocorrelation in the responses resulting in biased parameter estimates when an ordinary least squares data fitting technique is applied. An empirical method to account for the autocorrelated error structure is to use a time series (Box and Jenkins, 1970). Correct accounting for the error content of a response allows one to analyse terms in the response model which are significant only over a short initial period and which may be buried in an incorrectly

specified error.

2.4 Design of Experiments so as to Provide Multiple Responses

With a single tracer, an obvious group of responses exists if several compartments may be sampled. Where only one compartment may be sampled, a first response is provided by the initial addition of a tracer to the sampling compartment. Additional responses may be given by other tracers possessing different initial distributions among the compartments. As the initial distributions of these tracers are unknown except in a two compartment system, the responses they provide are incapable of characterizing the system. However, the responses are described by equations with the same exponential constants and, in a closed system, by the same equilibrium value as the first response.

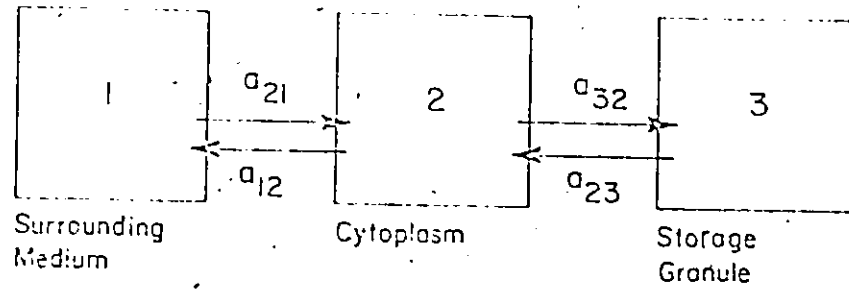
The application of these techniques will be demonstrated in the in vitro investigation of serotonin (5-HT, 5-hydroxytryptamine) exchange between blood platelets and their surrounding medium. Serotonin is a biogenic amine derived from the amino acid tryptophan. It is liberated in the platelet release reaction, together with the other granule bound components of the platelet, inducing aggregation (Mitchell and Sharp, 1964) and causing vascular constriction near the site of aggregation (Garattini and Valzelli, 1965). It is metabolized to form 5HT'ol (5-hydroxytryptaphol) and 5HIAA (5-hydroxyindolacetic acid).

Inside the platelet it is largely stored as a complex with ATP within granules (Born, 1956).

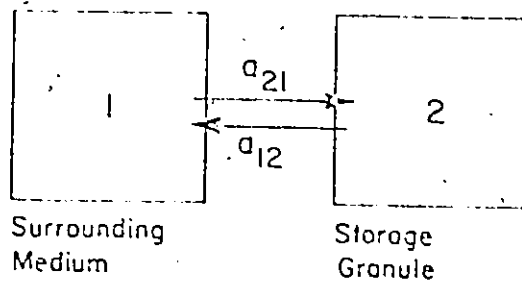
A model of the closed system may consist of two or three catenary compartments: surrounding medium and storage granule or surrounding medium, cytoplasm and storage granule (Fig. 2.2). Experiments were performed under steady state conditions at 37°C, the platelets (10^9 ml^{-1}) being in equilibrium with a high serotonin concentration (123 μM). Sampling is feasible only from the suspending medium as, in intact platelets, internal compartments are not accessible. To provide an additional response, a second tracer, with a different initial distribution between the compartments, was introduced. The first response, to characterize the system was obtained by adding ^{14}C -5HT to the surrounding medium at zero time. Prior labelling of the platelets with ^3H -5HT provided the second response. The distribution of these tracers over time was followed by sampling the surrounding medium at intervals (Fig. 2.3). The detailed experimental procedure is described by Reimers et al. (1975a).

2.5 Verification of the Assumptions

Before giving a detailed description of the results obtained in studies of the exchange of serotonin between rabbit platelets and their surrounding medium, it is necessary to verify the assumptions made about the exchange



3 Compartment System



2 Compartment System

Figure 2.2

Serotonin distribution in a platelet suspension

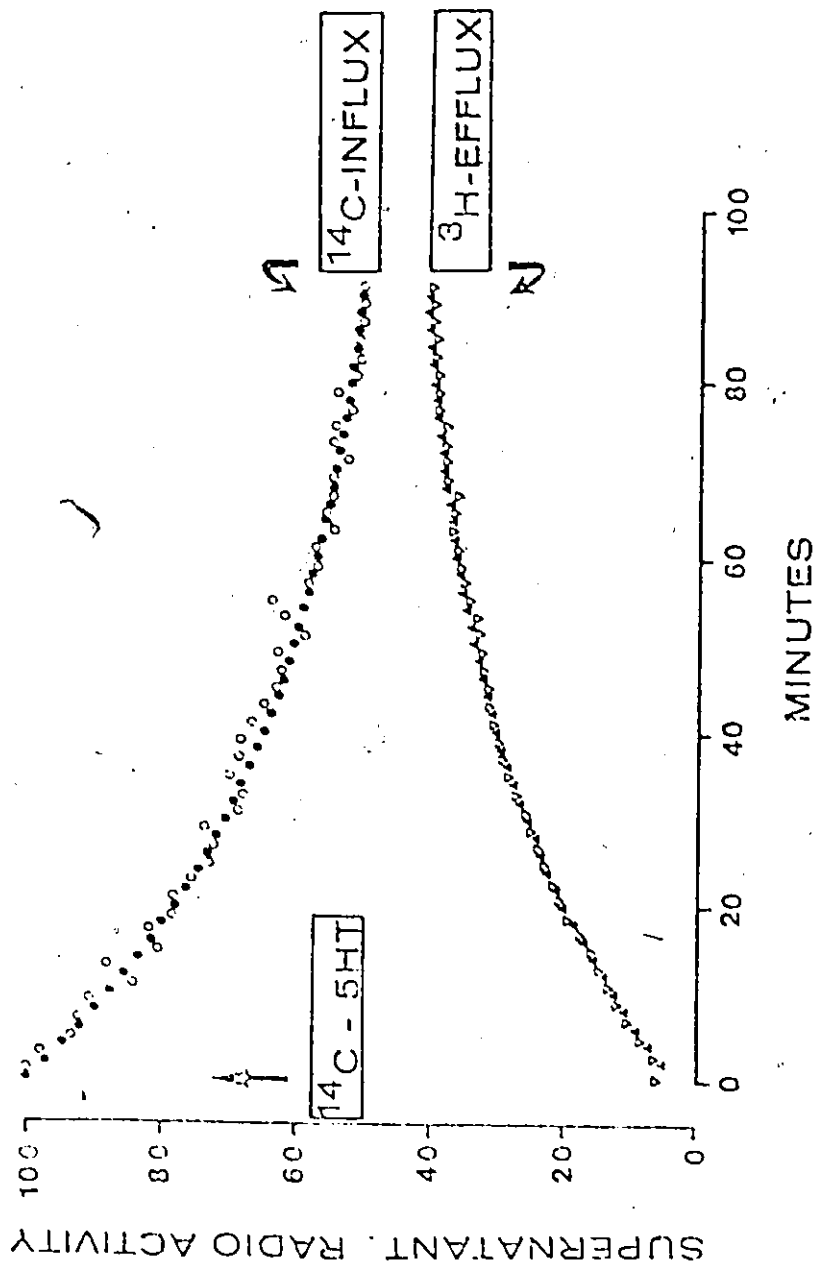


Figure 2.3

Percentage of ^3H -5HT and ^{14}C -5HT in the suspending medium

process. Two assumptions will be investigated: that the serotonin tracer obeys first order kinetics and that the system is closed, the label followed being indeed that of the serotonin tracer.

Steady State Serotonin Exchange

The theoretical development of the equations describing the distribution of tracer between compartments requires that the movement of tracer has first order kinetics; either the movement of serotonin is a first order process or the system is in the steady state. Now the uptake of serotonin is not a simple first order process at low concentrations of serotonin in the surrounding medium, but occurs via active transport obeying Michaelis-Menten kinetics (Born and Gillson, 1959). At higher serotonin concentrations a first order passive diffusion process is superimposed upon the active transfer (Pletscher, 1968). It is thus essential if the analysis is to hold that the experiments be conducted in the steady state with respect to serotonin distribution. This was ensured by incubation of the washed rabbit platelets with a high ^3H -serotonin concentration (123 μM) for 60 minutes before resuspension with the ^{14}C -serotonin, it having been determined that no further net uptake of serotonin occurred after 20 min.

A Closed System and Serotonin Purity

In the analysis of the exchange experiments it was assumed that the radioactive serotonin is not converted to

other compounds to an appreciable extent in the course of the experiment. Pletscher (1968) showed that endogenous serotonin is metabolized in the presence of platelets agglutinated by thrombin, 5-HT'ol being identified as the major metabolite. However, with the high serotonin concentrations used in these studies, there was no statistically significant breakdown of serotonin into its metabolites (Reimers et al., 1975a).

With paper chromatography it was shown that the ^{14}C -5HT used has radiochemical impurities, principally ^{14}C -5HTAA, comprising up to 6% of total radioactivity. Experimentally the uptake of ^{14}C -5HTAA by platelets was examined. It was found that the non-specific uptake of radioactivity could not exceed 0.6% of the added radioactivity. It is thus confirmed that the label being followed is indeed ^{14}C -5HT. Errors of 5% in the determination of the purity of the ^{14}C -5HT do not appreciably alter the values calculated for serotonin exchange rates (Table 2.1).

2.6 Data Evaluation

The data set from each response is described as a sum of exponentials:

assuming two compartments the equations that describe the data are

$$\begin{aligned}
 m_1(^{14}\text{C}) &= B + B_1 e^{-ct} \\
 m_1(^3\text{H}) &= B + B_2 e^{-ct}
 \end{aligned}
 \tag{2.3}$$

Table 2.1
Effect of varying levels of non-specific ^{14}C -radioactivity

	Fraction non-specific ^{14}C -radioactivity**			
	0	0.1	0.2	0.3
Percent of serotonin in the suspending medium transferred into the cytoplasm per minute (a ₁)	4.4	3.7	4.9	6.1
Percent of serotonin in the cytoplasm transferred into the suspending medium per minute (a ₂)	48.8	46.0	48.0	48.0
Percent of serotonin in the cytoplasm transferred into the amine storage granules per minute (a ₃)	9.0	12.6	9.5	7.7

Percent of serotonin in the amine storage granules into the cytoplasm per minute (a₂₃)

2.6 2.9 2.9 3.0

Serotonin content of cytoplasm* (nmoles/10⁹ pl.) 10.58 9.25 11.92 14.49

Serotonin content of amine storage granule (nmoles/10⁹ pl.) 36.5 39.91 38.61_# 37.00

Calculations were made with a set of data from an exchange study with thrombin stimulated platelets.

* The serotonin concentration within the suspending medium is 21.7 ng/ml. The platelet concentration is 10⁹ pl/ml.

** Non-specific radioactivity is principally ¹⁴C-5HIAA and ¹⁴C-5HT'ol.

and assuming three compartments

$$m_1(^{14}\text{C}) = B_{11} + B_{12}e^{-c_1 t} + B_{13}e^{-c_2 t} \quad (2.4)$$

$$m_1(^3\text{H}) = B_{11} + B_{22}e^{-c_1 t} + B_{23}e^{-c_2 t}$$

These equations may be rewritten in terms of the fractional turnover rates from one compartment to another. The fractional turnover rates are those shown in Fig. 2.2.

Assuming two compartments

$$m_1(t) = (a_{12} + (m_1(0)(a_{12} + a_{21}) - a_{12})e^{-(a_{21} + a_{12})t}) / (a_{12} + a_{21}) \quad (2.5)$$

For the ^{14}C -tracer, $m_1(0) = 1$

For the ^3H -tracer $m_1(0)$ may be measured experimentally

$$0 \leq m_1(0) < 1$$

With two compartments, the fractional turnover rates, a_{21} and a_{12} , may be estimated from either response.

Assuming three compartments

$$m_1(t) = \frac{a_{12}a_{23}}{ab} + \frac{a_{12}}{(a-b)} \left(\frac{(a_{23}-a)}{a} \left(\frac{(a_{21}-b)a_{21}m_1(0)}{a_{12}(a_{23}-a_{21})} + m_2(0) - \frac{a_{23}(a_{23}-b)m_3(0)}{a_{32}(a_{23}-a_{21})} \right) e^{-at} - \frac{(a_{23}-b)}{b} \left(\frac{(a_{21}-a)a_{21}m_1(0)}{a_{12}(a_{23}-a_{21})} - \frac{(a_{23}-b)}{b} \left(\frac{(a_{21}-a)a_{21}m_1(0)}{a_{12}(a_{23}-a_{21})} - m_2(0) + \frac{a_{23}(a_{23}-a)}{a_{32}(a_{23}-a_{21})} \right) e^{-bt} \right) \right) \quad (2.6)$$

where if

$$x = (a_{21} + a_{21} + a_{32} + a_{23})$$

$$y = (x^2 - 4(a_{21}a_{32} + a_{21}a_{23} + a_{12}a_{23}))^{1/2}$$

$$\text{then } a = (x-y)/2, \quad b = (x+y)/2$$

For the ^{14}C -tracer the initial distribution of tracer is known; $m_1(1) = 1$; $m_2(0) = m_3(0) = 0$; and thus the fractional turnover rates may be estimated from the ^{14}C -response.

For the ^3H -tracer; $m_2(0)$ and $m_3(0)$ are unknown. Thus no estimates of the fractional turnover rates may be made from the ^3H -response.

The equations (2.3) and (2.4) are fitted to the data. Additional terms are added to the equations to account for autocorrelation in the responses. A first order autoregressive time series model adequately accounted for the autocorrelation in the responses (Box and Jenkins, 1970). The expanding Simplex method of Nelder and Mead (1964) was used to search for the estimates of parameters B_{ij} and C_i with highest posterior probability; the criterion of maximum likelihood was that of Box and Draper (1965) (equation 2.2). Estimation of the kinetic parameters a_{ij} from the parameters B_{ij} and C_i in the model for the ^{14}C -response, was performed for the two compartment model as in Atkins (1969a) and for the three compartment model as in Robertson et al. (1957). The assumption of a second platelet compartment did not always lead to significant improvement

in the lack of fit with untreated control platelets (Table 2.2).

Experiments were designed to confirm the presence of a cytoplasmic compartment by reducing the size of the platelet's granule compartment through the use of reserpine (Pletscher and Tramer, 1967). The experimental procedure is described later. This design was successful in that two platelet compartments were necessary to describe data from all experiments upon reserpine treated platelets (Table 2.2). The presence of a fourth compartment was not statistically significant ($p < .5$, for 4 experiments in which the presence of a third compartment significantly improved the fit of the model to the data).

Multiresponse analysis provides much smaller confidence regions for the parameter estimates than can analysis of a single response. In Figs. 2.4 and 2.5, the joint 95% confidence regions of a_{21} and a_{12} are plotted for reserpine treated and control platelets, comparing multivariate analysis with the unweighted least squares methods upon single (Fig. 2.4) or multiple (Fig. 2.5) responses. A significant contraction is observed when multivariate techniques are used. Furthermore a joint confidence region for an unweighted simultaneous least sum of squares analysis only slightly overlaps that calculated from multivariate analysis (Fig. 2.5). Because of the absence of weighting and the implied absence of cross-correlation between the responses, analysis

Table 2.2

Significance of a 3rd compartment

Significance of
3rd Compartment

Type of Platelet* No. of Samplings

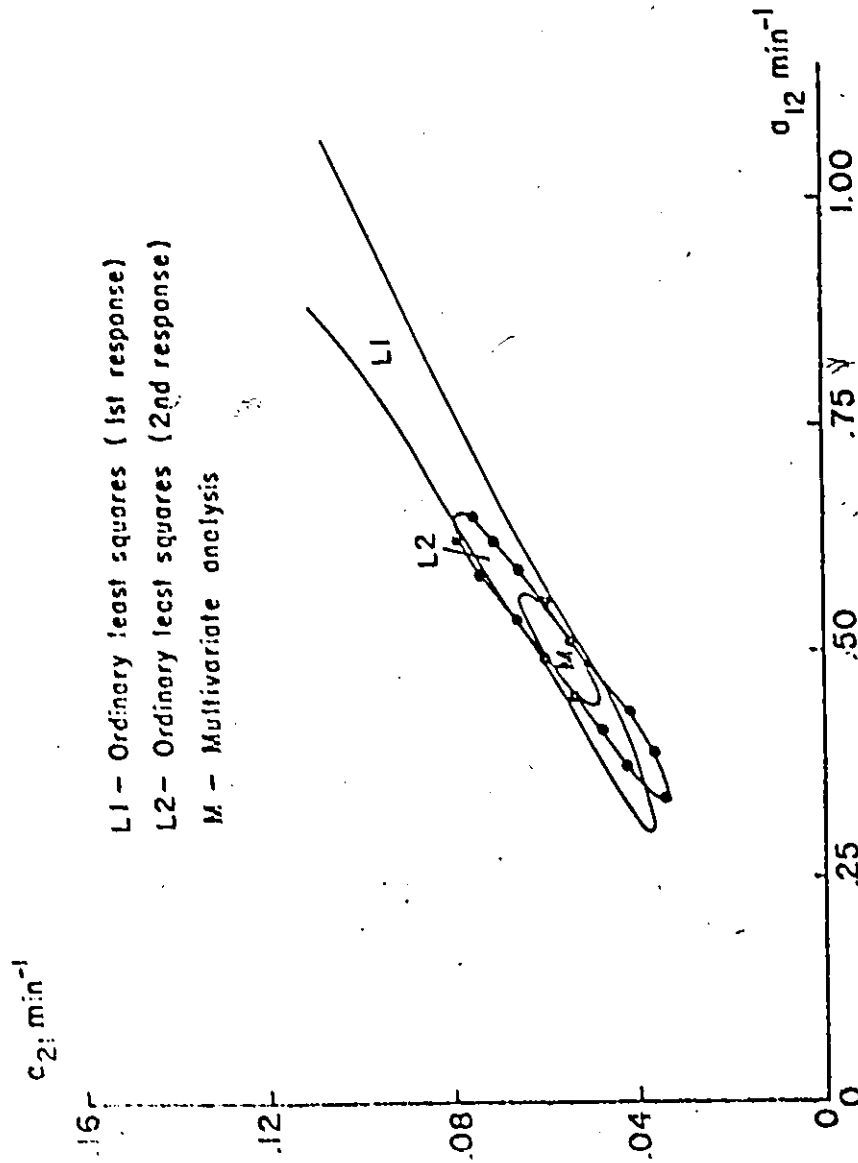
1st 2nd
Response

R	25	✓	✓
C	30	✓	✓
R	30	✓	✓
C	30	X	X
R	30	✓	X
C	30	X	X
R	33	X	✓
C	33	X	X

*R-Reserpine Treated
C-Control

✓ Significant at 95% confidence level

X Not significant at 95% confidence level



L1 - Ordinary least squares (1st response)

L2 - Ordinary least squares (2nd response)

M - Multivariate analysis

Figure 2.4

A comparison of joint 95% confidence regions of a_{12} and a_{21} using the multivariate method and unweighted least squares with a single response.

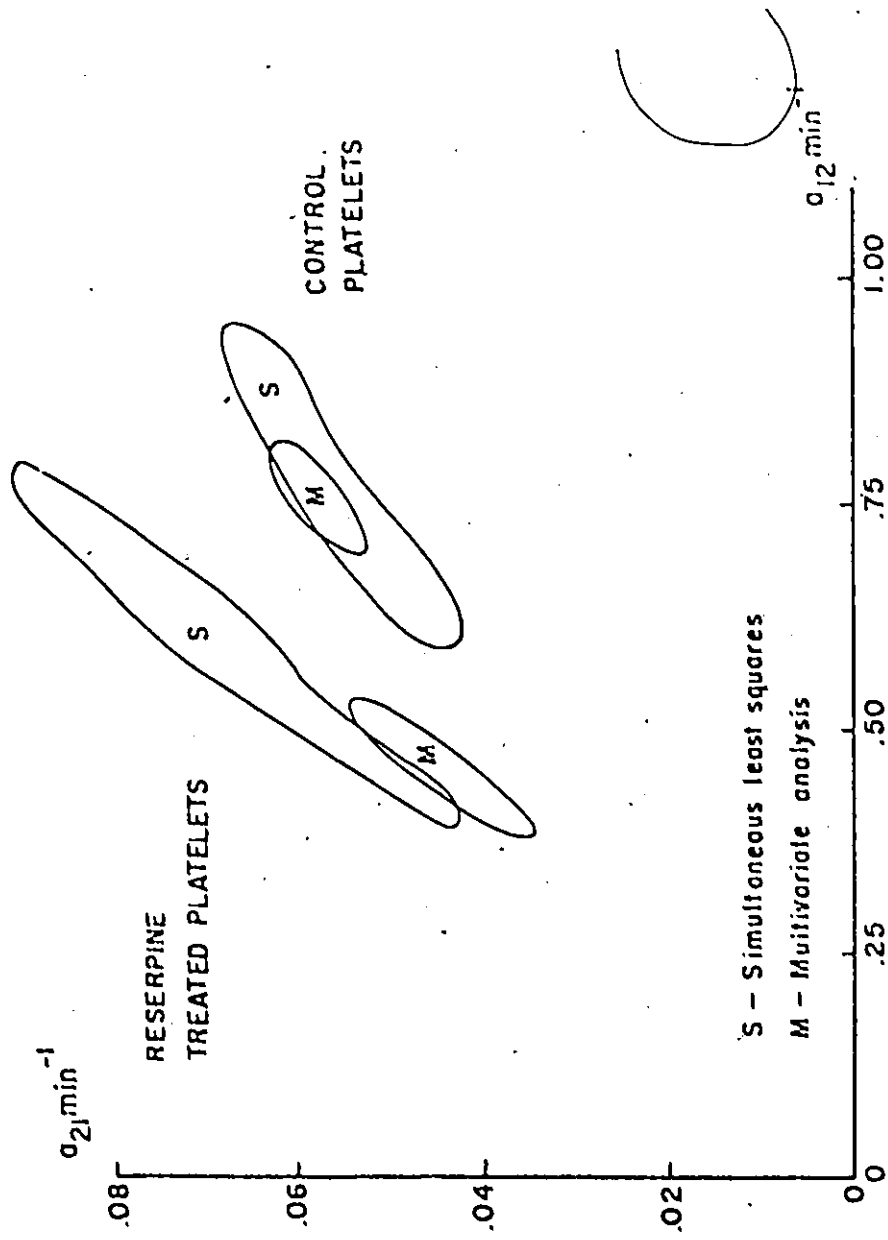


Figure 2.5

A comparison of joint 95% confidence regions of a_{21} and a_{12} using multivariate and unweighted least squares methods with multiple responses.

with an unweighted simultaneous least sum of squares may be assumed to be invalid.

2.7 Serotonin exchange and Storage in Platelets

Three compartments are necessary to give a statistically adequate description of serotonin exchange in platelets (Table 2.2). Earlier the intermediate compartment, between the suspending medium and granules, was referred to as the cytoplasmic compartment. That this is the correct description must now be confirmed.

It may be demonstrated that the intermediate compartment lies within the platelet and is not associated with a serotonin concentration boundary layer in the fluid outside the platelet (Appendix 2.1).

Evidence for the existence of a cytoplasmic compartment may be obtained through release studies. In release, cytoplasmic constituents are not liberated from the platelets (Holmsen et al. 1969b). Markwardt and Barthel (1964) found that 85-95% of the platelet serotonin was in the intermediate compartment (Table 2.6). In reserpine treated platelets, the proportion of total platelet serotonin within the intermediate compartment rises to 25-40%. Upon maximal stimulation with thrombin about 70% of platelet serotonin is released (Reimers et al. 1975b). Thus it is likely that the intermediate compartment represents the pool holding non-releasable serotonin, morphologically corresponding to the cytoplasm.

Further support for the compartmentation defined by exchange studies comes from electron microscopy. The treatment of platelets three times with a low concentration of thrombin caused the loss of 79% of the platelet amine storage granules (Reimers et al. 1975b). Exchange experiments in which control and thrombin treated platelets are compared show a loss of 70% of the amine content upon thrombin stimulation.

It is thus likely that the system described by the exchange experiments corresponds to physiological reality. This is important in view of the suspicion in which compartmental analysis is held (Bergner and Lushbough, 1967), particularly as both compartmental systems and random walk processes, with a continuum of internal states or a large number of discrete states, may describe the same data well (Sheppard, 1971).

That the fractional turnover rates calculated are reasonable is supported by the work of Reimers et al. (1975d) who measured the accumulation of ^{14}C -5HT (10^{-4}M) in platelets (10^7 per ml.) for times between 0.25 to 5 minutes. The fractional turnover rate so calculated for the movement of serotonin from the medium into the platelet was 0.06 min^{-1} , a value close to the value of a_{21} calculated in the exchange experiments (Table 2.3).

Having verified that the model of the platelet system is reasonable, the modification of serotonin exchange

Table 2.3

Effect of thrombin treatment of washed rabbit platelets on serotonin exchange between different platelet compartments and suspending medium

	Control platelets	Thrombin-treated platelets	Significance of difference between means *
Percent of serotonin in the suspending medium transferred into the cytoplasm per minute (a ₂₁)	7.0	6.8	P > 0.05 (not significant)
Percent of serotonin in the cytoplasm transferred into the suspending medium per minute (a ₁₂)	64.8	49.7	P > 0.05 (not significant)
Percent of serotonin in the cytoplasm transferred into the amine storage granules per minute (a ₃₂)	28.0	7.6	P < 0.05 (significant)
Percent of serotonin in the amine storage granules transferred into the cytoplasm per minute (a ₂₃)	2.0	2.6	P > 0.05 (not significant)

Means of 3 experiments.

* In these studies, serotonin transport has been described by non-linear equations containing several parameters. These parameters are highly correlated. Significance of the change in their values upon thrombin-treatment was assessed through the following tests: (1) paired t-test (2) t-test upon the estimates of the values of the constants for each experiment, the covariance matrix for the constants being determined by linearization in the region of the maximum likelihood estimate of the constants (3) direct determination of 95% joint confidence contours for the estimated values in each experiment.

and storage in platelets by treatment with thrombin, reserpine and inipramine was investigated.

The effect of the Thrombin Treatment of Rabbit Platelets

Rabbit platelet suspension was treated three times with thrombin (0.05 u/ml) and deaggregated with PGE₁ (1 μM). A serotonin exchange study was then conducted with the thrombin treated platelet suspension and with a control suspension as described by Reimers et al. (1975a). The results of this experiment are shown in Tables 2.3 and 2.4. Joint confidence regions for the pairs of fractional turnover rates a_{12} and a_{21} , at the platelet-plasma membrane that separates the suspending medium from the cytoplasm, and a_{23} and a_{32} , at the granule membrane that separates the granule from the cytoplasm, are shown in Figs. 2.6 and 2.7.

Analysis of the data shows that thrombin treatment does not alter the transport rate of serotonin across the platelet-plasma membrane and does not significantly alter the fractional turnover rate of serotonin from the granules into the cytoplasm. However, there is a significant diminution in the serotonin content of the granule storage pool ($p < .01$). This is associated with a decreased transfer rate of serotonin into the platelet storage granules. The most probable explanation for this is that after thrombin-induced degranulation there are fewer granules or granule binding sites available for serotonin.

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TABLE 2.4

Effect of thrombin treatment of washed rabbit platelets on serotonin storage capacity and on serotonin exchange rate between different platelet compartments and suspending medium

	Control platelets	Thrombin-treated platelets	Significance of difference between the means
Cytoplasmic compartment (nmoles/ 10^9 plat.)	12 \pm 3	16 \pm 4	p < 0.2
Anine storage granule compartment (nmoles/ 10^9 plat.)	146 \pm 10	40 \pm 6	p < 0.01
Serotonin exchange rate between cytoplasm and anine storage granules (nmoles/min/ 10^9 plat.)	2.86 \pm 0.52	1.06 \pm 0.20	p < 0.05
Serotonin exchange rate between suspending medium and cytoplasm (nmoles/min/ 10^9 plat.)	8.60 \pm 3.23	8.39 \pm 0.52	p < 0.05

Means \pm SE of means of 3 experiments; paired t-test.

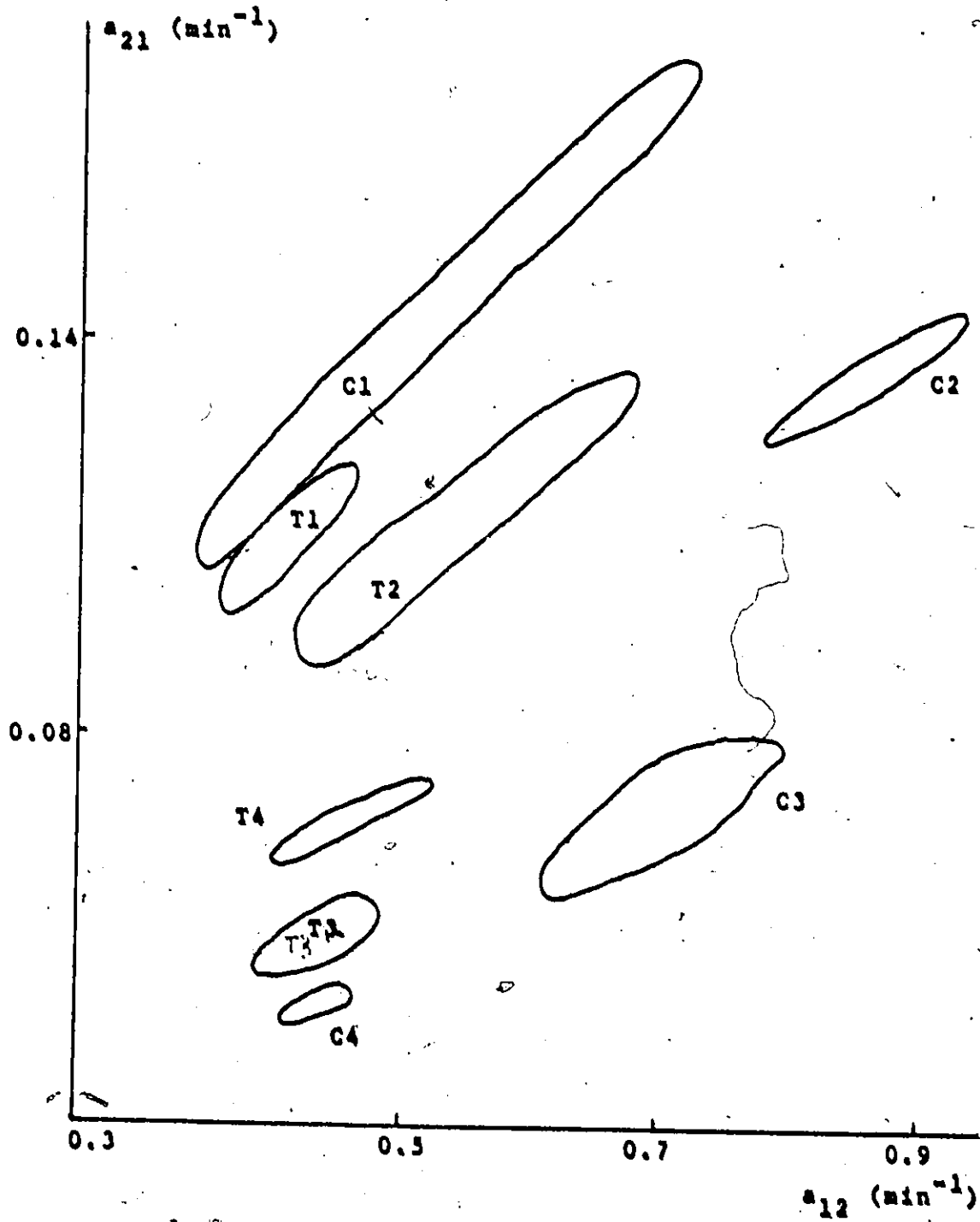


Figure 2.6

95% joint confidence regions of a_{21} and a_{12} -
for thrombin-treated (T) and control (C) platelets

(C1 corresponds to T1 etc.)

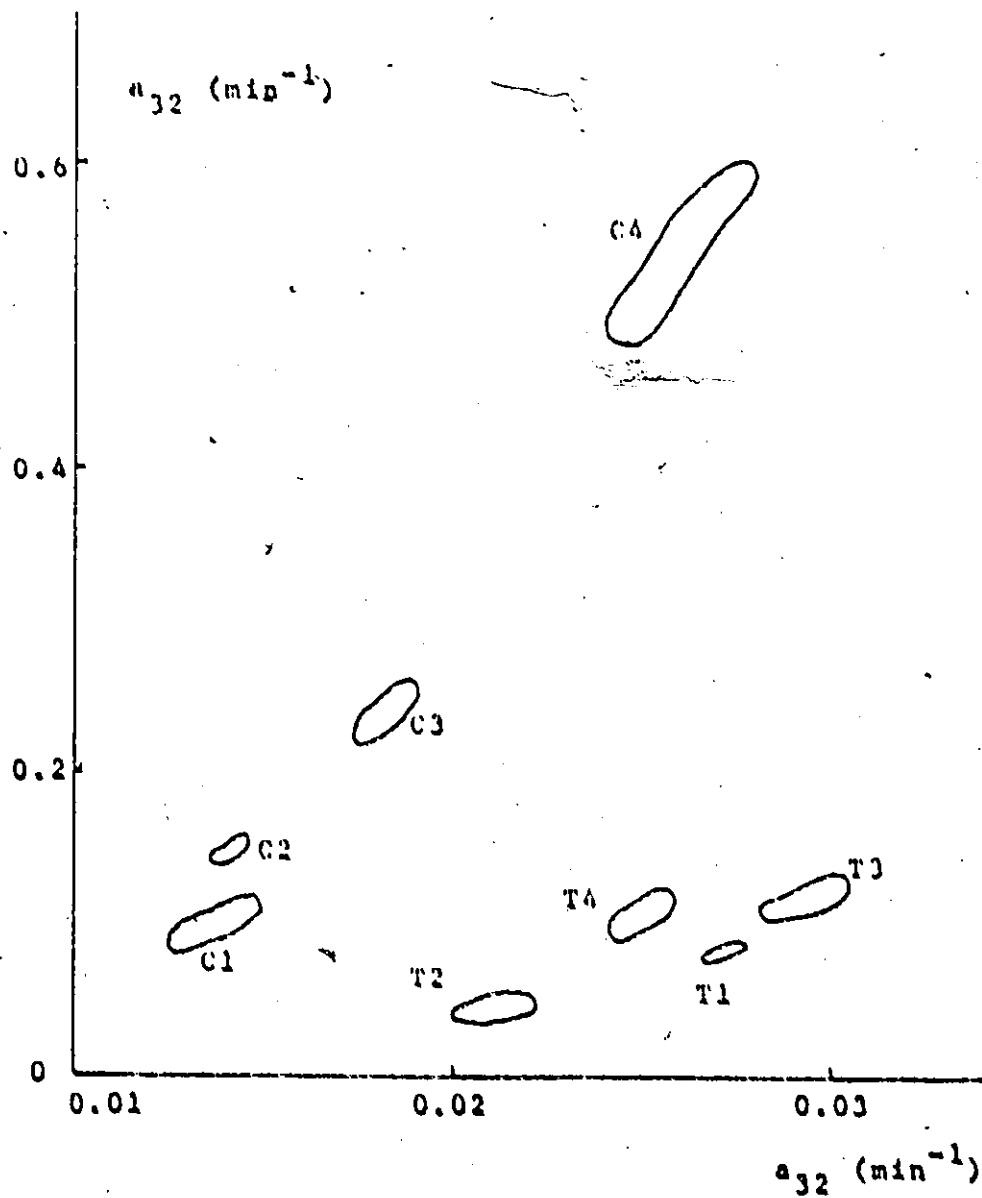


Figure 2.7

95% joint confidence regions of α_{23} and α_{32} for control (C) and thrombin-treated (T) platelets

The Effect of Reserpine and Imipramine on the Transport and Storage of Serotonin in Rabbit Platelets

Studies were conducted into the effect of reserpine (2 μM) or imipramine (20 μM) upon the transport and storage of serotonin in rabbit platelets. The experimental procedure is described in Reimers et al. (1975d). The results are shown in Tables 2.5 - 2.8; joint confidence regions for the pairs of fractional turnover rates a_{21} and a_{12} , describing transport across the platelet membrane, and a_{23} and a_{32} , across the granule membrane, are plotted (Figs. 2.8 - 2.10).

In the presence of reserpine, transfer of serotonin from the cytoplasmic compartment to the granule compartment is greatly inhibited. However, the fractional turnover rate from the granule to the cytoplasmic compartment was not significantly diminished (Table 2.5). Hence there is a diminished serotonin content in the granule pool. Also the fractional turnover rate for serotonin transfer from the cytoplasm to the suspending medium is significantly diminished in the presence of reserpine.

The analysis of data from experiments in which platelets were equilibrated and resuspended in the presence of 20 μM imipramine showed a diminution of serotonin transfer from the suspending medium to the cytoplasmic compartment and from the cytoplasmic compartment to the suspending medium.

Table 2.5

Effect of reserpine on serotonin transfer between suspending medium and different compartments of washed rabbit platelets

Percent transfer between different compartments	Control platelets	Reserpine-treated platelets	Significance of difference between means
Percent of 5HT in the suspending medium transferred into the platelet cytoplasm per minute (a ₁)	7.1 ± 1.2	6.9 ± 1.0	P < 0.05 (not significant)
Percentage of 5HT in the platelet cytoplasm transferred into the suspending medium per minute (a ₁₂)	73.4 ± 8.9	48.5 ± 1.6	P < 0.025 (significant)
Percentage of 5HT in the platelet cytoplasm transferred into the storage granules per minute (a ₃₂)	29.6 ± 6.6	4.5 ± 0.7	P < 0.01 (significant)

Percentage of SHY
in the storage
granules trans-
ferred into the
cytoplasm per
minute (a23)

1.7 ± 0.3

2.4 ± 0.3

P < 0.2
(not significant)

The results are expressed as means ± SEM of 4 experiments. Significance of difference was calculated with the student's t-test.

Table 2.6

Effect of reserpine on SHT content in different compartments of rabbit platelets and its exchange between these compartments

Compartment	Control platelets	Reserpine-treated platelets	Significance of difference between means
Cytoplasm (nmol/10 ⁹ platelets)	10.5 ± 0.8	16.3 ± 3.3	p < 0.2
Storage granules (nmol/10 ⁹ platelets)	177 ± 19.2	28.1 ± 3.3	p < 0.01

Means ± SEM of 5 experiments. The significance of difference by student's t-test.

Table 2.7

Effect of imipramine on 5HT transfer between different compartments of washed rabbit platelets

Percent transfer between different compartments	Control platelets	Imipramine-treated platelets	Significance of difference between means
Percentage of 5HT in the suspending medium transferred into the platelet cytoplasm per minute (a ₁)	8.4 ± 1.6	3.2 ± 1.4	P < 0.01 (significant)
Percentage of 5HT in the platelet cytoplasm transferred into the suspending medium per minute (a ₂)	70.6 ± 2.3	34.7 ± 7.4	P < 0.002 (significant)
Percentage of 5HT in the platelet cytoplasm transferred into the storage granules per minute (a ₃)	23.4 ± 3.0	24.7 ± 7.9	P < 0.1 (not significant)

Percentage of 5HT
in the storage
granules trans-
ferred into the
cytoplasm per
minute (a₂₃)

1.5 ± 0.1

11.2 ± 0.2

P < 0.3
(not significant)

The results are expressed as means and SEM of 3 independent experiments. The significance of difference between the means was calculated as described in Table 2.3. The concentration of serotonin in the surrounding medium was 100 μM.

Table 2.8

Effect of imipramine on 5HI content in different compartments of rabbit platelets and its exchange between these compartments

Compartments	Control platelets	Imipramine-treated platelets	Significance of difference between means
Cytoplasm (nmol/10 ⁹ platelets)	11.3 ± 1.8	8.2 ± 2.1	p < 0.4
Storage granules X (nmol/10 ⁹ platelets)	17.9 ± 2.5	15.5 ± 1.1	p < 0.4

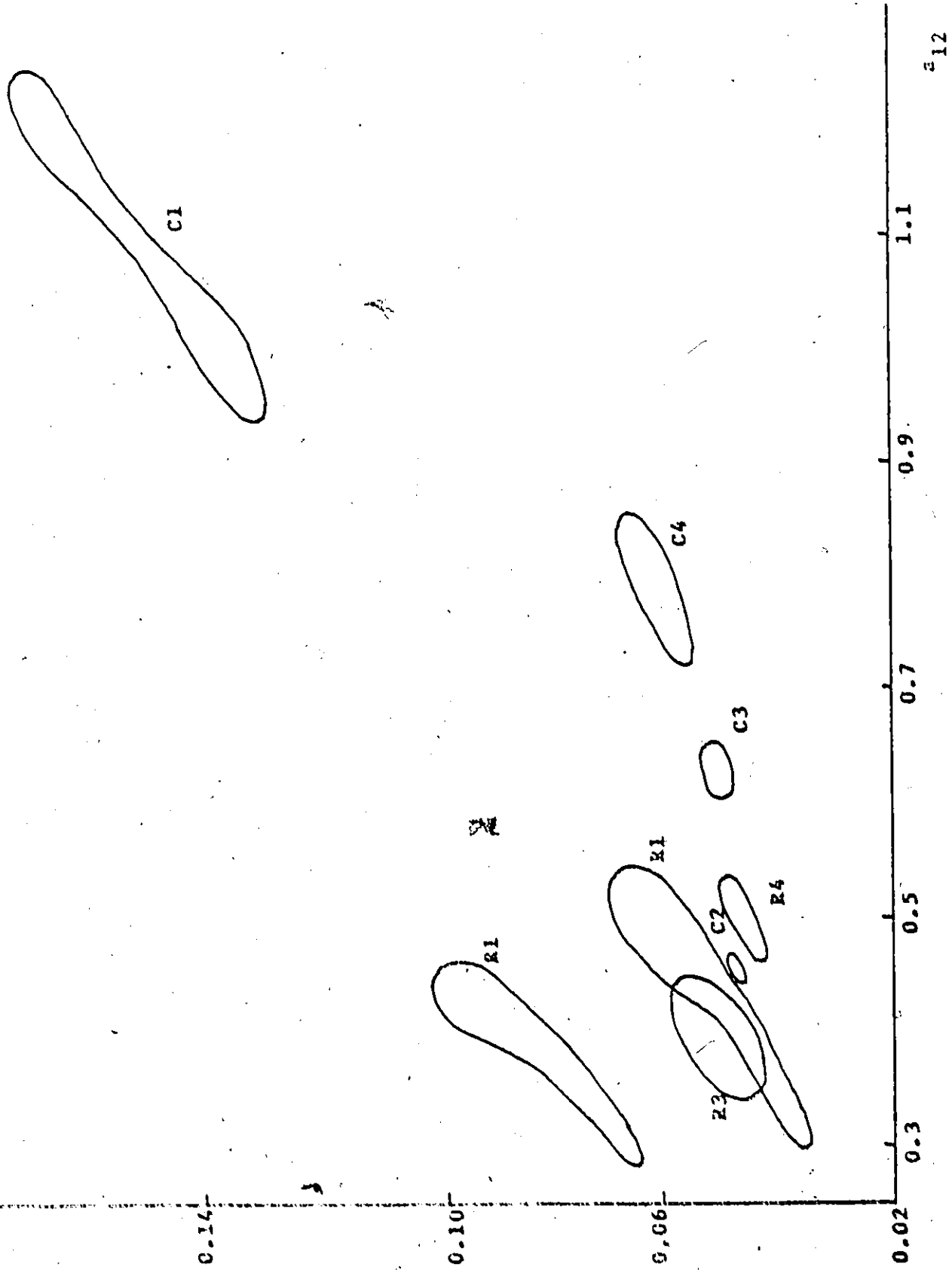


Figure 2.8

95% joint confidence regions of a_{11} and a_{12} for control (C) and reserpine-treated (R) platelets

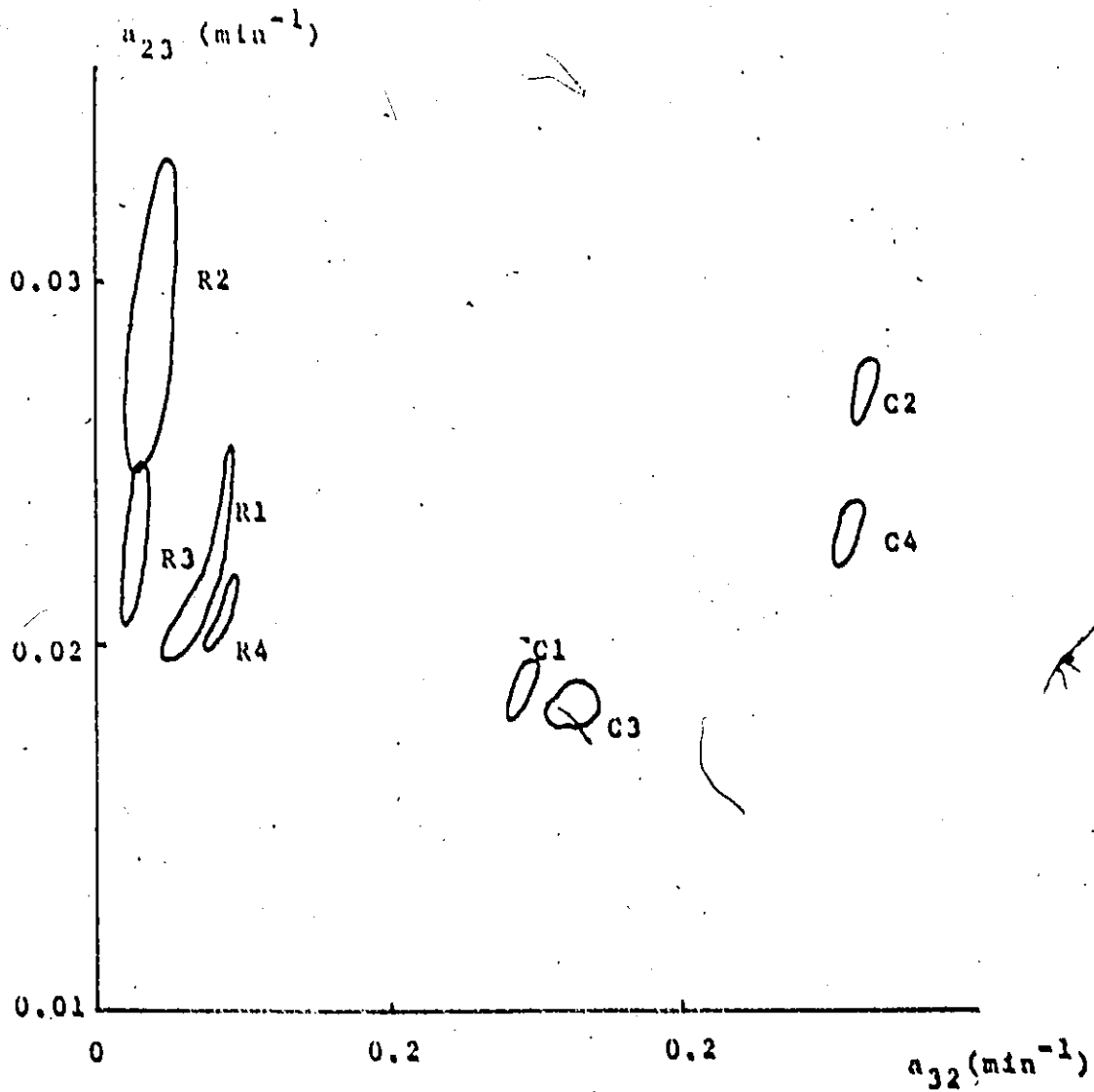


Figure 2.9

Joint confidence regions of a_{23} and a_{32} -
 for control (C) and reserpine-treated (R) platelets

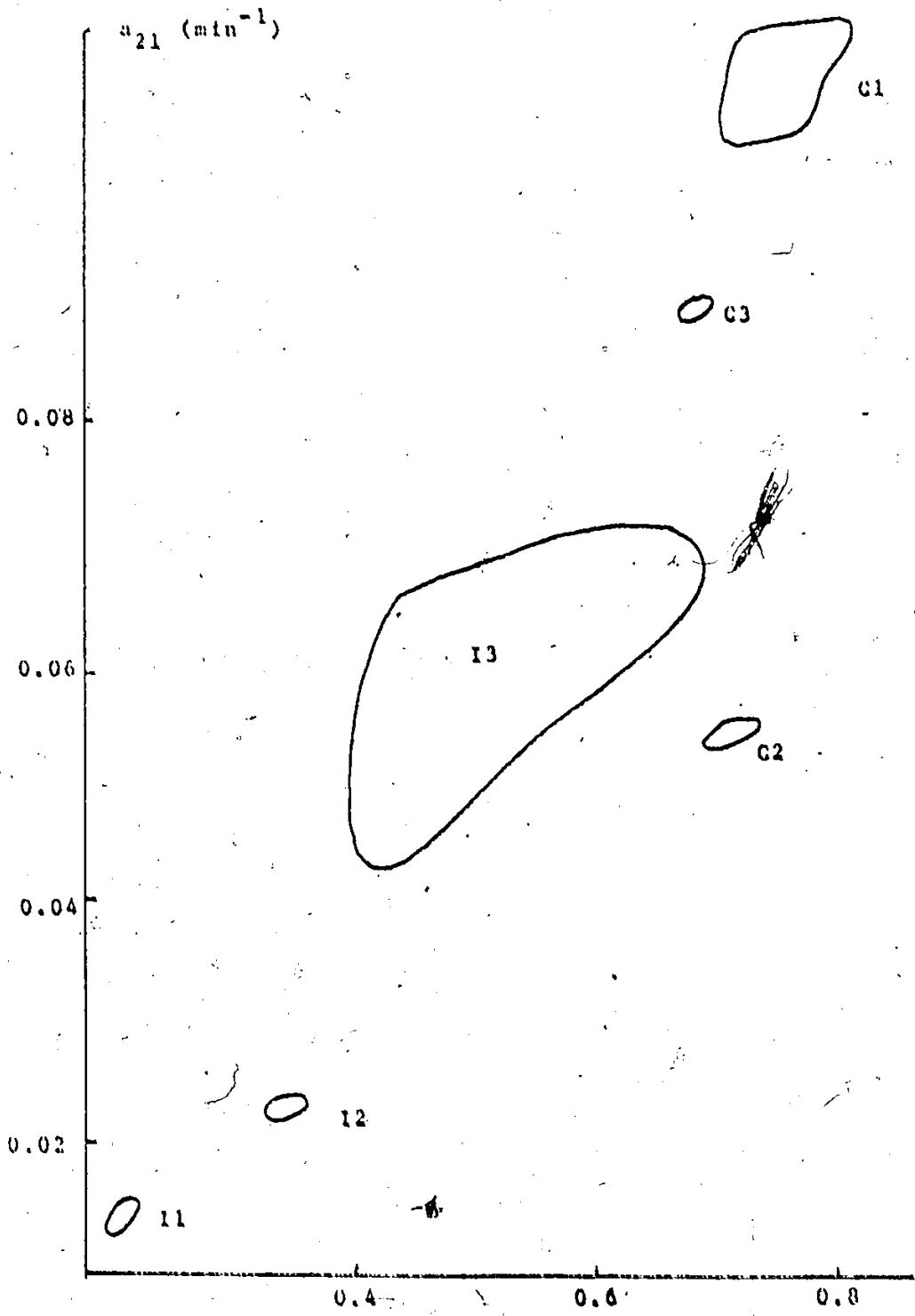


Figure 2.10

Joint confidence regions of a_{21} and a_{12}

The observation that the fractional turnover of serotonin from the amine storage granules to the cytoplasm is unchanged in the presence of reserpine supports the concept that the reserpine acts at the granule membrane in platelets, where it is mainly localized (Da Prada and Pletscher, 1969) rather than interfering with the intragranular complex formation, a reaction observed in vitro (Berneis et al. 1970). Furthermore, the fact that the fractional turnover rate of serotonin from granule to cytoplasm is unchanged is compatible with the assumption of a diffusive mechanism for the transport of serotonin from the amine storage granules into the cytoplasm.

Imipramine has been considered to diminish serotonin uptake at the platelet plasma membrane by competitive inhibition (Pletscher, 1968). This was confirmed in this study where imipramine was found to inhibit serotonin transfer across the plasma membrane but not across the granule membrane.

2.8 Conclusion

Data from tracer experiments performed upon compartmental systems can be described by equations composed of sums of exponential terms. Either non-linear regression or graphical analysis are commonly used to estimate the parameters within these equations.

Dell et al. (1973) have listed the advantages of a

non-linear regression analysis over graphical technique. Chief amongst these is the ability to make statements about the statistical significance of parameters and to provide confidence limits for the parameters describing tracer movement. It may be noted that spline functions have been shown to be excellent empirical functions for the analysis of reserpine curves (Wold, 1974). However, their use does not allow the determination of the properties of single compartments.

With the additional information provided by multiple responses a more precise determination can be made of the number of significant compartments within the system. Multiple responses may be analyzed by the generalized least square techniques of multivariate analysis. A greater precision in parameter estimation results from the recognition of error correlation between the responses. In general the covariance matrix of errors within the vectors of observations will be unknown. This precludes the application of the technique of Turner et al. (1963). The procedures of Beauchamp and Cornell (1966, 1969), whilst allowing for the estimation of the covariance matrix, are, as the authors admitted, difficult to compute. The approach of Box and Draper (1965) allows for the estimation of the covariance matrix and provides an easily computed criterion by which to obtain the maximum likelihood estimates of the parameters.

In an investigation of serotonin kinetics and storage in blood platelets, multiple responses were obtained. Analysis of these by the technique of Box and Draper (1965) gave parameter estimates with smaller confidence regions than those obtained with the more common techniques in which single responses or unweighted multiple responses were analyzed. In this study a time series was employed to account for autocorrelated error within the responses. An expanding Simplex method was used to search parameter space to determine the best estimates.

The technique by which multiple responses are procured to investigate the transport and storage of serotonin in blood platelets has proved capable of illuminating the transfer and more precisely characterising the effect of treatment of platelets by drugs and enzymes.

Thrombin stimulation of platelets are shown to result in a decreased transfer rate of serotonin into the granule storage pool and a diminution of the serotonin content of the granule storage pool. Reserpine is found to inhibit transfer of serotonin from the cytoplasmic compartment to the granule compartment. In the presence of imipramine a diminution of serotonin transfer from the suspending medium to the cytoplasmic compartment and from the cytoplasmic compartment to the suspending medium. The use of this multiple response technique may be extended to an

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examination of platelets from patients with platelet storage pool disease. With these platelets a decreased capacity for the storage of serotonin, similar to that found in the presence of reserpine, is observed (Weiss et al. 1974).

Although this technique of experimental design and analysis has been illustrated by a study in cell physiology, it has application wherever tracers are employed. Through a more precise characterization of the system, the effect of perturbations (drugs etc.) upon the system is more easily ascertained.

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Part 3 THE USE OF SURVIVAL CURVES TO DETERMINE THE IN VIVO
BEHAVIOUR OF SEROTONIN IN PLATELETS

3.1 Introduction

Serotonin, as has been described earlier in Part 2, is a biogenic amine, derived from the amino acid tryptophan. In rabbits, serotonin is primarily located in the gastrointestinal tract (65%), platelets (33.2%) and spleen (1%) (Ernpamer, 1954). The argentaffin cells of the gastrointestinal tract manufacture serotonin. The level of non-platelet bound serotonin within the circulation is negligible. In vitro studies show serotonin to be completely and freely exchangeable between the platelets and their surrounding medium (Born and Gillson, 1959). In part 2 of this thesis the turnover rates that describe this process were determined. In vivo, serotonin exchange between circulating platelets, and between circulating platelets and tissue outside the circulation may occur. If the serotonin within platelets were to be labelled, such an exchange of serotonin would be reflected in the accumulation of label in platelets not present in the circulation at the time of labelling. Another possible source for such an accumulation would be the labelled serotonin of platelets removed from the circulation. The purpose of this study is thus to determine the extent to which serotonin exchange between platelets occurs in vivo. To this end, platelet tracer survival studies were conducted.

Platelets have a finite lifespan; the platelet population is constantly turning over. A platelet tracer survival curve may be obtained by reinjecting platelets, labelled with a tracer, into the circulation and following, over time, the decay in the level of tracer remaining bound to the platelets within the circulation. With the radioactive tracer, ^{51}Cr , platelets may be uniformly labelled, independently of their age (Tsukuda et al. 1971). Furthermore, this label elutes only slowly and at a known rate from the platelets (Aster, 1971). The reinjection of platelets into the circulation, following their labelling with ^{51}Cr , is thus a technique that enables the age distribution of the platelet population to be determined. Various models have been proposed to describe platelet survival. In general these models presuppose a platelet age distribution that is invariant with time. They differ in their assumptions about the removal of platelets from the circulation: Adelson et al. (1957) proposed that platelet death was random; Aster and Jandl (1964) assumed that platelets survived to the same age; whilst Murphy (1971) suggested that platelet loss was best described by a multiple hit model. Without entering into the controversy surrounding the description of the survival process, it is possible to use a ^{51}Cr survival curve as an indicator of platelet survival. Then, following the dual labelling of the platelets

with ^{51}Cr and of the platelet serotonin with ^{14}C -serotonin, ^{51}Cr and ^{14}C -serotonin platelet survival curves should provide information with which the behaviour of serotonin in vivo may be determined. The ^{51}Cr label is attached to the membranes and cytoplasm of the platelet; ^{14}C -serotonin lies chiefly in the platelet granules.

3.2 Models to Describe Serotonin Behaviour

3.2.1 Assumptions

Several common assumptions are shared by models that will be considered here. They are:

1. The platelet age distribution is invariant with respect to time; - the birth and death rates remain equal and constant.
2. There is no loss of platelet serotonin content over time. The effects of release will not be explicitly included in the models. In release the contents of the platelet granules are extruded, if only small quantities of serotonin are released, they will be reaccumulated by the platelets. Larger quantities may diffuse away. Platelets that have undergone release will enjoy a faster turnover of their remaining serotonin content (Part 2) over the lifespan that remains unaltered by release (Reimers et al. 1973). Release will thus be manifested as an enhanced serotonin turnover from the platelets into the surrounding medium.
3. Any serotonin that is transferred into the surrounding medium is immediately reaccumulated by the platelets or tissue. This assumption is justified as the level of non-platelet bound serotonin within the blood is negligible.
4. The labelling of platelets with ^{14}C -serotonin is uniform, being independent of their age and condition. Should the labelling not be uniform but occur preferentially in the younger platelets, then it would be expected that the ^{51}Cr

level would decay faster than ^{14}C -serotonin.

5. A platelet may be considered as comprising of a single homogeneous serotonin compartment. Though it has been demonstrated that platelets that have not undergone release contain both cytoplasmic and granule compartments, the approximation is close as the fractional turnovers of serotonin from the cytoplasmic compartment to the granule compartment are high (Allen et al. 1975).

6. After removal of the platelets from the circulation, any serotonin that is reaccumulated by circulating platelets is taken up immediately.

7. The serotonin accumulated in very young platelets through passage through the gastro-intestinal tract contains no serotonin tracer (Tranzer et al. 1972).

Before presenting hypothetical models with which to describe the behaviour of platelet serotonin in vivo, several terms need be defined:

$\Lambda(t)$ is the fraction of platelets in the circulation at time t after labelling that were present at the time of labelling. $\Lambda(t)$ is determined from the ^{51}Cr survival curve after correcting for the elution of ^{51}Cr from the platelets: if v is the fractional elution rate of ^{51}Cr from the platelets, then $\Lambda(t)e^{vt}$ is equal to the fraction of initial ^{51}Cr present within the platelets at a time t after labelling.

$I(t)$ is the platelet age distribution function. Its integral is equal to $\Lambda(t)$. i.e.

$$\int_t^{\infty} I(x) dx = \Lambda(t)$$

$C(t)$ is the fraction of initial ^{14}C present in the platelets at a time t after labelling.

k is the fractional turnover rate of serotonin from circulating platelets into the plasma.

k_1 is the fraction of serotonin from dead platelets that exchanges into circulating platelets. (Dead platelets are those that have been removed from the circulation).

k_2 is the fractional turnover rate of serotonin from circulating platelets out of the circulation.

3.2.2 Hypotheses

The following hypotheses were considered; the equations associated with each are derived in Appendix 3.1.

- I: There is no exchange of serotonin. The loss of serotonin from the circulation is due to the removal of platelets from the circulation. Then $C(t) = \Lambda(t)$
- II: There is very rapid exchange of serotonin between circulating platelets; no exchange between circulating and dead platelets or other serotonin in tissue. Then the level of ^{14}C serotonin is uniform in all platelets

$$C(t) = e^{-I(0)t}$$

$I(0)$ being the rate of production and removal of platelets from the circulation.

III: No exchange of serotonin between circulating platelets; exchange between circulating platelets, dead platelets and tissue serotonin.

$$\text{Then } C(t) = e^{-k_2 t} (A(t) (1+k_1) \int_0^t I(t-y) C(y) e^{k_2 y} dy)$$

IV: Exchange at a finite rate between circulating platelets alone.

$$\text{Then } C(t) = e^{-kt} (A(t) (1+k) \int_0^t C(x) e^{kx} dx) +$$

$$\int_0^t I(t-y) k \int_y^t C(x) e^{kx} dx dy)$$

V: Exchange at a finite rate between circulating platelets, between dead platelets and circulating platelets and between circulating platelets and tissue serotonin.

$$\text{Then } C(t) = e^{-(k+k_2)t} (A(t) (1+k) \int_0^t C(x) e^{(k+k_2)x} dx) +$$

$$\int_0^t I(t-y) (k \int_y^t C(x) e^{(k+k_2)x} dx +$$

$$k_1 D(y) e^{(k+k_2)y} dy)$$

3.3 Experimental Procedure

3.3.1 Design for Sampling and Radioactive Counting

A design for the sampling of platelets and the counting of their radioactive emissions was devised to minimize the error in determining the levels of ^{51}Cr and ^{14}C within the blood and hence the functions $A(t)$ and $C(t)$. ^{51}Cr emits both γ and β radiation; ^{14}C emits β radiation. Whilst γ emissions may be counted from samples of whole blood, to count β emissions, red cells must be removed from platelet suspensions because of their quenching effect. The experimental results from the survival studies using ^{51}Cr and ^{14}C -serotonin were obtained in the following manner:

1. As a count of the ^{51}Cr γ emissions from a sample of whole blood.

Corrected for the elution of ^{51}Cr from the platelets, the fraction of initial ^{51}Cr , in the blood at a time t after labelling, is $A(t)$.

2. As counts upon separate channels of the β emissions from ^{14}C and ^{51}Cr from a sample of platelet-rich plasma. Corrected for ^{51}Cr elution and expressed as a fraction of the initial ratio, the ratio of these counts is $C(t)/A(t)$.

Obtaining the responses in this manner reduces problems associated with the handling of platelets. Two alternative procedures are to determine either $C(t)$ alone (i.e. the ^{14}C count alone) from platelet-rich plasma, and $A(t)$ from the whole blood sample or to determine both

$C(t)$ and $A(t)$ (i.e. ^{14}C and ^{51}Cr) from platelet-rich plasma, no count of ^{51}Cr radiation emanating from a sample of whole blood being made. These procedures require the number of platelets in the platelet-rich plasma to be counted at all sampling points so that the counts measured may be transformed into counts per circulating platelet. The procedure used did not necessitate this.

3.3.2 Procedure

Suspensions of washed rabbit platelets were prepared in Tyrode solution containing albumin (0.35%) as described by Ardlie et al. (1970). $\text{Na}_2^{51}\text{CrO}_4$ (10 $\mu\text{Ci}/10^9$ platelets) was added to the first washing fluid. Platelets were incubated for 30 minutes at room temperature, resuspended in Tyrode albumin solution and incubated with ^{14}C -serotonin for 60 minutes at 37°C . After incubation, the labelled platelets were resuspended in Tyrode albumin solution. About 10^{10} platelets in 2-3 ml of Tyrode albumin solution were reinjected into each of 3 rabbits. This number of platelets corresponds to approximately 10-15% of the total number of platelets in the recipient rabbit. At intervals up to 180 hours after the injection of the labelled platelets, 6 ml of blood was withdrawn from an ear vein into 1 ml of the acid-citrate dextrose medium of Aster and Jandl (1964). The ^{51}Cr emissions from 1 ml sample of whole blood were counted. From the remainder of the sample, platelet-rich plasma was prepared and the β -ray emissions from ^{51}Cr and ^{14}C from it were recorded.

The platelet preparation and labelling procedure described here is analogous to that described for the dual labelling of platelets with ^{51}Cr and ^{14}C -adenosine by Reimers et al. (1975b). The ^{14}C -serotonin labelling procedure was that of Reimers et al. (1975a).

3.4 Results and Discussion

The ^{14}C -serotonin and ^{51}Cr platelet survival curves are plotted in Fig. 3.1. The equations developed from the models I-V are fitted to the data; the parameters that gave the best non-linear least squares fit of the models to the data being estimated. The parameters estimated or defined for each model are tabulated in Table 3.1. It is evident that the models that assume a non-zero but finite rate of exchange of serotonin between platelets give a significantly better fit to the data than do the other models. However, the inclusion of serotonin exchange between circulating platelets and serotonin pools outside the circulation does not lead to a significant improvement in the fit of the model to the data ($p < 0.5$, F test). Such exchange with non-labelled body deposits of serotonin was postulated by Zucker et al. (1964) to explain the rapid disappearance of ^{14}C -serotonin from platelets in patients with carcinoid syndrome, a disappearance more rapid than that apparent in normal subjects. Without further evidence, the simpler model IV that simply assumes a finite rate of exchange of serotonin between platelets, will be assumed to represent rabbit platelet serotonin behaviour in vivo.

Whilst this analysis is conditional upon the unproven assumption that the uptake and content of serotonin is identical in all platelets, irrespective of their age, it

Fraction of initial ^{51}Cr or ^{14}C -serotonin attached to platelets

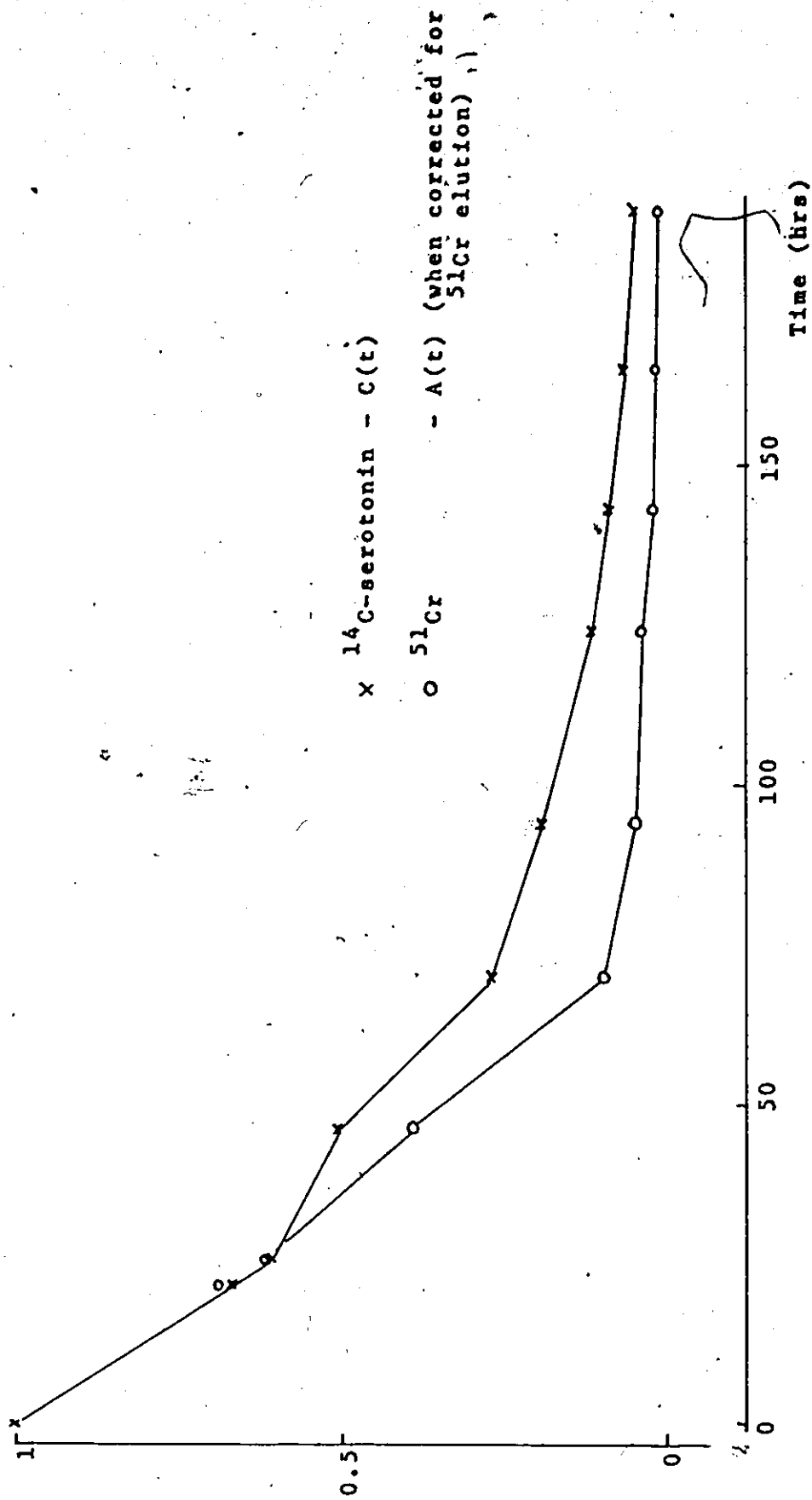


Figure 3.1
 ^{14}C -serotonin and ^{51}Cr survival curves

Table 3.1

Serotonin exchange in vivo

Model	Rabbit	S.S [*]	k (mins ⁻¹)	k ₁	k ₂ (mins ⁻¹)
I	1	7.17 10 ⁻²	0	0	0
	2	1.70 10 ⁻¹	0	0	0
	3	5.99 10 ⁻²	0	0	0
II	1	1.95 10 ⁻²	∞	0	0
	2	1.20 10 ⁻²	∞	0	0
	3	5.44 10 ⁻²	∞	0	0
III	1	4.83 10 ⁻³	0	3.8 10 ⁻¹	9.6 10 ⁻⁵
	2	3.96 10 ⁻²	0	5.2 10 ⁻¹	1.25 10 ⁻⁴
	3	1.00 10 ⁻²	0	8.2 10 ⁻¹	2.80 10 ⁻⁴
IV	1	4.65 10 ⁻⁴	1.18 10 ⁻²	0	0
	2	1.29 10 ⁻³	1.10 10 ⁻²	0	0
	3	5.44 10 ⁻⁴	1.16 10 ⁻²	0	0
V	1	4.30 10 ⁻⁴	8.0 10 ⁻³	1.2 10 ⁻¹	2.3 10 ⁻⁵
	2	8.09 10 ⁻⁴	5.9 10 ⁻³	3.1 10 ⁻¹	3.0 10 ⁻⁵
	3	3.41 10 ⁻⁴	1.31 10 ⁻¹	2.3 10 ⁻¹	2.0 10 ⁻⁵

* Sum of squares of the deviations from the best fit of the model to the data.

The parameter values of 0 and ∞ in this table result from the definition of the models.

has support in a comparison of the fractional turnover rates calculated for the in vivo and in vitro experiments. The mean fractional turnover rate of serotonin from the platelet to the surrounding medium calculated for model IV in these in vivo experiments is 0.0115 mins^{-1} . That calculated in in vitro exchange is 0.011 mins^{-1} (Allen et al. 1975). Hence it may be assumed that the results of in vivo studies upon the effect of drugs upon platelets may also apply to platelets in vivo; the in vivo behaviour of serotonin in platelets being adequately described by a process of slow serotonin exchange between circulating platelets. Furthermore, the use of ^{14}C -serotonin as a platelet survival label is suspect unless agents that prevent ^v serotonin exchange are present.

4. THE EXCHANGE AND METABOLISM OF THE PLATELET ADENINE NUCLEOTIDES.

4.1 Introduction.

Adenosine triphosphate (ATP) and the other adenine nucleotides, adenosine diphosphate (ADP) and adenosine monophosphate (AMP), are the major links in platelets coupling the energy yielding sequences and energy requiring ones. The hydrolysis of ATP to ADP is the primary source of the high energy phosphate group. Further hydrolysis to AMP may also occur. These reactions are reversible: the AMP and ADP may be rephosphorylated.

Within platelets, the adenine nucleotides are distributed between a metabolically active pool, which consists of the platelet cytoplasm, mitochondria and membranes (Holmsen et al. 1969a) and a storage granule pool (Holmsen et al. 1969b). These pools hold 46% and 54% of the platelet adenine nucleotide content respectively (Reimers et al. 1975b). There is a slow exchange of adenine nucleotides between them.

It has been observed that the denser fraction of human platelets contains a higher adenine nucleotide level than does the lighter fraction (Karpatkin, 1969). Older rabbit and human platelets tend to be less dense than younger ones (Amorosi et al. 1971; Charmatz and Karpatkin, 1974). It may thus be inferred that the adenine nucleotide

content of platelets decreases with age. Paulus (1975) however, has disputed the basis for this inference by concluding that platelet size heterogeneity is due to variations in territory growth and demarcation in the megakaryocytes which fragment to form platelets, and not by aging in the circulation. There is thus no consensus as to the extent of the loss of adenine nucleotides from platelets in vivo and hence as to the importance of any such loss in platelet senescence.

If the adenine nucleotide content of platelets were to decrease with age, there are two possible means by which the adenine nucleotides may be lost. The first is through the release of the granule storage pool, an event that will accompany platelet involvement in haemostasis. Associated with release is the irreversible catabolism of 5-25% of the adenine nucleotides in the metabolic pool to hypoxanthine (Holmsen et al. 1969b). The second possible source of loss is the continuous conversion of adenine nucleotides within the metabolic pool to hypoxanthine.

In this study of adenine nucleotide behaviour in platelets an attempt will be made to determine the causes and extent of adenine nucleotide loss from platelets. In doing so the techniques employed may allow the determination of the extent of platelet release within the circulation.

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4.2 The In Vivo Labelling of Platelets by ^{14}C -adenosine.

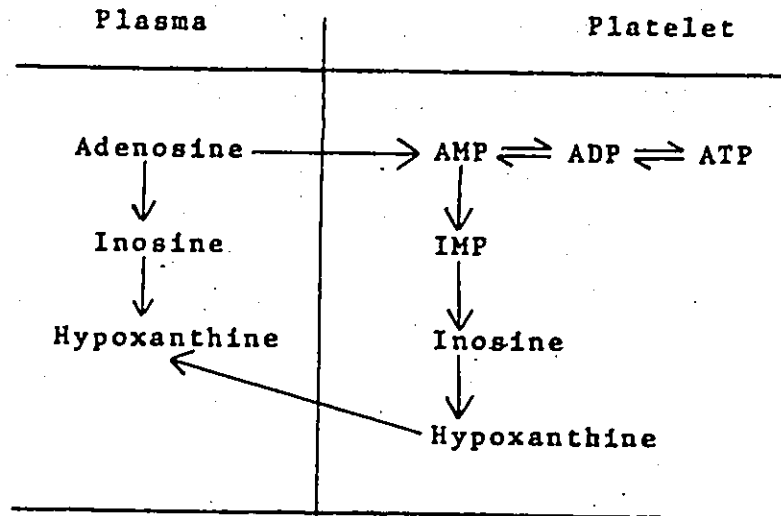
4.2.1 Introduction.

To facilitate the study of adenine nucleotides in platelets, a ^{14}C label was attached to the platelet adenine nucleotides. ^{14}C -adenosine, placed in the suspending medium of a suspension of washed rabbit platelets, is partially incorporated within the platelet adenine nucleotides and partially converted to hypoxanthine. An in vitro study was conducted so as to define the path by which adenosine is incorporated into platelets and to examine the transfer of adenine nucleotides, between ATP, ADP and AMP, and the catabolism of AMP.

4.2.2 Reaction Schemes to Describe ^{14}C -adenosine Labelling.

Holmsen (1971) described the metabolism of ^{14}C -adenosine in platelet rich plasma in a scheme shown in Fig. 4.1. 70% of the ^{14}C -adenosine is converted within the plasma to inosine and thence to hypoxanthine in enzyme catalyzed reactions. The remaining 30% of the ^{14}C -adenosine enters the platelet and is phosphorylated to form ^{14}C -AMP, ^{14}C -ADP and ^{14}C -ATP within the platelet's metabolic pool. A small fraction of the ^{14}C -AMP within the metabolic pool is catabolized to hypoxanthine via IMP and inosine. The enzyme that regulates the conversion of the adenine nucleotides to hypoxanthine is believed to be AMP-deaminase, an

Figure 4.1



Adenosine metabolism in platelet-rich plasma

(after Holmsen, 1971).

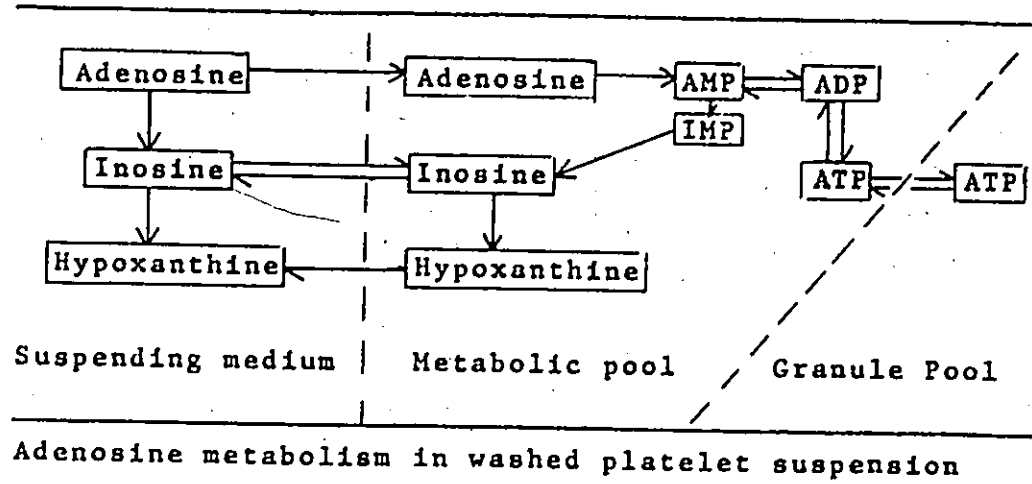
enzyme that is activated by ATP (Holmsen et al. 1974). The uptake of ^{14}C -adenosine by red cell ghosts has been characterized by the Michaelis-Menten kinetics (Berne and Rubio, 1974) that describe enzyme catalyzed reactions.

In light of recent experimental work, Holmsen's model must be extended and modified to describe the metabolism of adenosine in a suspension of washed rabbit platelets. Reimers et al. (1975b) demonstrated a slow but complete exchange of the adenine nucleotides, principally ATP, between the metabolic and granule pools of the platelets. Furthermore Reimers et al. (1975c) showed there to be no significant metabolism of ^{14}C -inosine in the supernatant of a platelet suspension. Within washed platelet suspensions however, inosine metabolism occurred, albeit with platelet inosine levels that did not significantly differ from zero. Adenosine metabolism occurred within the supernatant of the washed platelet suspension. Although reutilisation of hypoxanthine to form adenine nucleotides has been observed (Rivard et al. 1975) it is of negligible importance in the ^{14}C -adenosine labelling of platelets. Thus a modified scheme for the metabolism of ^{14}C -adenosine is presented in Fig. 4.2.

4.2.3 Experimental Procedure.

A suspension of washed rabbit platelets in Tyrode solution containing albumin was prepared by the method of

Figure 4.2

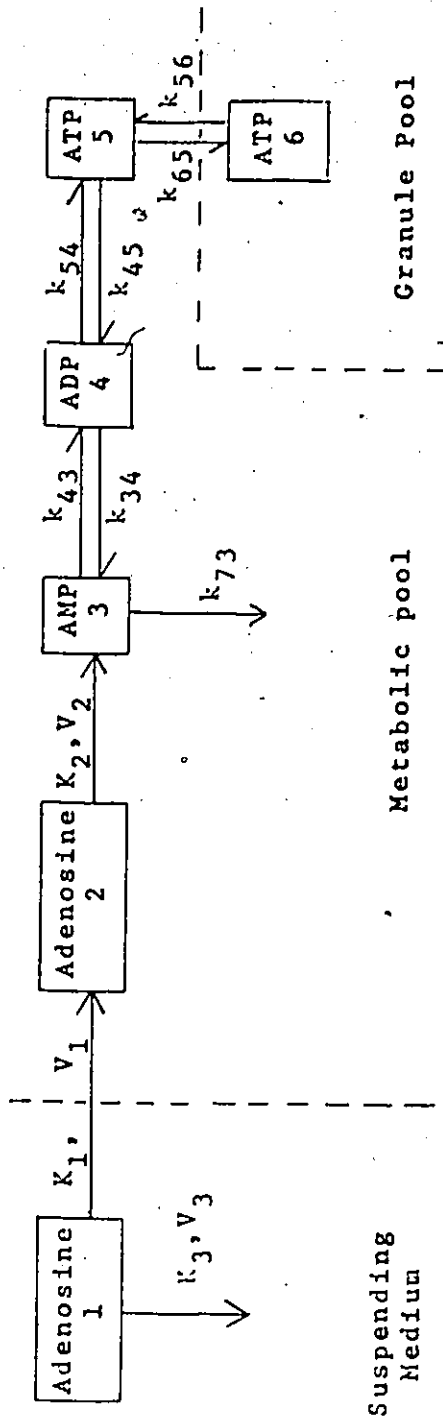


Ardlie et al. (1970). The platelet concentration was 10^9 platelets. ml^{-1} . The platelets were labelled by incubation with ^{14}C -adenosine (8 μM , 10 $\mu\text{Ci}/10^9$ platelets) at 37°C . 5 minutes after the addition of the label, and at 15 minute intervals thereafter, samples of the suspension were taken until 5 hours had elapsed. In addition, samples of the supernatant and of the granule pools were prepared at each sampling time. The latter are obtained by use of a thrombin induced release reaction; this causes the extrusion of the granule contents from the platelet. Using chromatography, the suspending medium (supernatant), platelets and granule pools were analyzed for adenosine, AMP, ADP, ATP, IMP, inosine and hypoxanthine. The detailed experimental technique is that described in Reimers et al. (1975b).

4.2.4 A Model to Describe ^{14}C -adenosine Labelling of the Adenine Nucleotides

The model used is shown in Fig. 4.3. It is a reduced form of that shown in Fig. 4.2 and describes the uptake of ^{14}C -adenosine into platelets, the distribution of the label into the adenine nucleotides and their catabolism. It is assumed that the label is distributed into distinct, homogeneous compartments. In normal platelets the level of adenosine is negligible (Holmsen, 1971). In

Figure 4.3



Model describing ^{14}C -adenosine metabolism and the incorporation of the ^{14}C label into the adenosine nucleotides within a suspension of washed platelets.

the experiment, adenosine equivalent to 11% of the platelet adenine nucleotide content was added to the platelet suspension (8 nmoles adenosine/ 10^9 platelets). Such an amount was found to leave the levels of AMP (0.49 nmoles/ 10^9 platelets), ADP (7 nmoles/ 10^9 platelets) and ATP (55 nmoles/ 10^9 platelets) essentially unchanged. The exchange of nucleotide between ATP, ADP and AMP and the catabolism of AMP may thus be assumed to be quasi-steady state processes, the associated tracer exchange having first order kinetics. The uptake of adenosine into the platelets and its phosphorylation to form AMP and its metabolism in the suspending medium are enzymatic reactions which will be described here by Michaelis-Menton kinetics. For these velocity of reaction = (Maximum reaction velocity) (concentration of substrate)/(Michaelis-Menton constant + substrate concentration).

The compartments and reaction constants are numbered as in Fig. 4.3:

n_i is the tracer content of the i th compartment;

k_{ij} is the first order constant describing transport of tracer from the j th to the i th compartment;

K_i is the Michaelis-Menten constant, being the substrate concentration that gives half the maximal reaction velocity V_i that the enzyme catalyzed reaction may achieve.

The distribution of tracer may be described by the following series of differential equations. These equations result from the mass balance:

Rate of accumulation of tracer in a compartment	=	Rate of influx - Rate of outflux of tracer from the compartment
--	---	--

$$\begin{aligned} \frac{dm_1}{dt} &= -V_1 m_1 / (K_1 + m_1) - V_3 m_1 / (K_3 + m_1) \\ \frac{dm_2}{dt} &= V_1 m_1 / (K_1 + m_1) - V_2 m_2 / (K_2 + m_2) \\ \frac{dm_3}{dt} &= V_2 m_2 / (K_2 + m_2) - k_{34} m_4 - (k_{73} + k_{43}) m_3 \quad (4.1) \\ \frac{dm_4}{dt} &= -(k_{34} + k_{54}) m_4 + k_{43} m_3 + k_{45} m_5 \\ \frac{dm_5}{dt} &= -(k_{45} + k_{65}) m_5 + k_{54} m_4 + k_{56} m_6 \\ \frac{dm_6}{dt} &= -k_{56} m_6 + k_{65} m_5 \end{aligned}$$

with the initial conditions upon the distribution of tracer;

$$\begin{aligned} m_1(0) &= 1. \\ m_i(0) &= 0. \quad , \quad i = 2, 3, \dots, 6 \end{aligned} \quad (4.2)$$

The differential equations may be integrated simultaneously using a 4th order Runge-Kutta technique. Those values of the parameters K_i , V_i and k_{ij} that minimize the

Bayesian criterion of Box and Draper (1965), and thus have the highest posterior probability are adopted. The expanding Simplex method of Nelder and Mead (1964) was used to search for those parameters that gave the best fit of model to data.

4.2.5 Results of the In Vitro Study.

The model proposed in section (4.2.4) was fitted to the data. The experimental data and the model's predictions are shown in Figs. 4.4-4.9. Where more than 2 or 3% of the total ^{14}C is present in a particular form, the fit of the model to the data is good. At lower concentrations of ^{14}C (as in ^{14}C -AMP and ^{14}C -adenosine in the metabolic pool), the errors in chromatography are such that the model does not fit well. The best estimates of the parameters are tabulated below (Table 4.1). As the accumulation of ^{14}C -tracer within the granule was small, the return of ^{14}C from the granule could not be detected. Thus the first order rate constant, k_{56} , that defines the transfer of ATP from the granule storage pool to the metabolic pool was estimated using the equilibrium relationship

$$k_{56}^{m6} = k_{65}^{m5}$$

At equilibrium 54% of the ATP is in the granule pool, the remaining 46% of platelet ATP being within the metabolic

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Table 4.1

Best estimates of the parameter describing the metabolism of ^{14}C -adenosine

Parameter	Estimated Value with 95% confidence limits	Units
V_1	1.27 ± 0.41	$\text{nmoles} \cdot \text{min}^{-1} (10^9 \text{ platelets})^{-1}$
K_1	35.28 ± 10.54	$\text{nmoles} (10^9 \text{ platelets})^{-1}$
V_2	0.59 ± 0.16	$\text{nmoles} (10^9 \text{ platelets})^{-1} \text{min}^{-1}$
K_2	0.27 ± 0.08	$\text{nmoles} (10^9 \text{ platelets})^{-1}$
V_3	1.59 ± 1.02	$\text{nmoles} (10^9 \text{ platelets})^{-1} \text{min}^{-1}$
K_3	32.73 ± 30.	$\text{nmoles} (10^9 \text{ platelets})^{-1}$
k_{73}	0.0097 ± 0.0028	min^{-1}
k_{43}	15.78 ± 3.56	min^{-1}
k_{34}	0.8279 ± 0.299	min^{-1}
k_{54}	3.374 ± 0.99	min^{-1}
k_{45}	0.4454 ± 0.231	min^{-1}
k_{65}	0.00038 ± 0.0001	min^{-1}
k_{56}	0.00032 ± -	min^{-1}

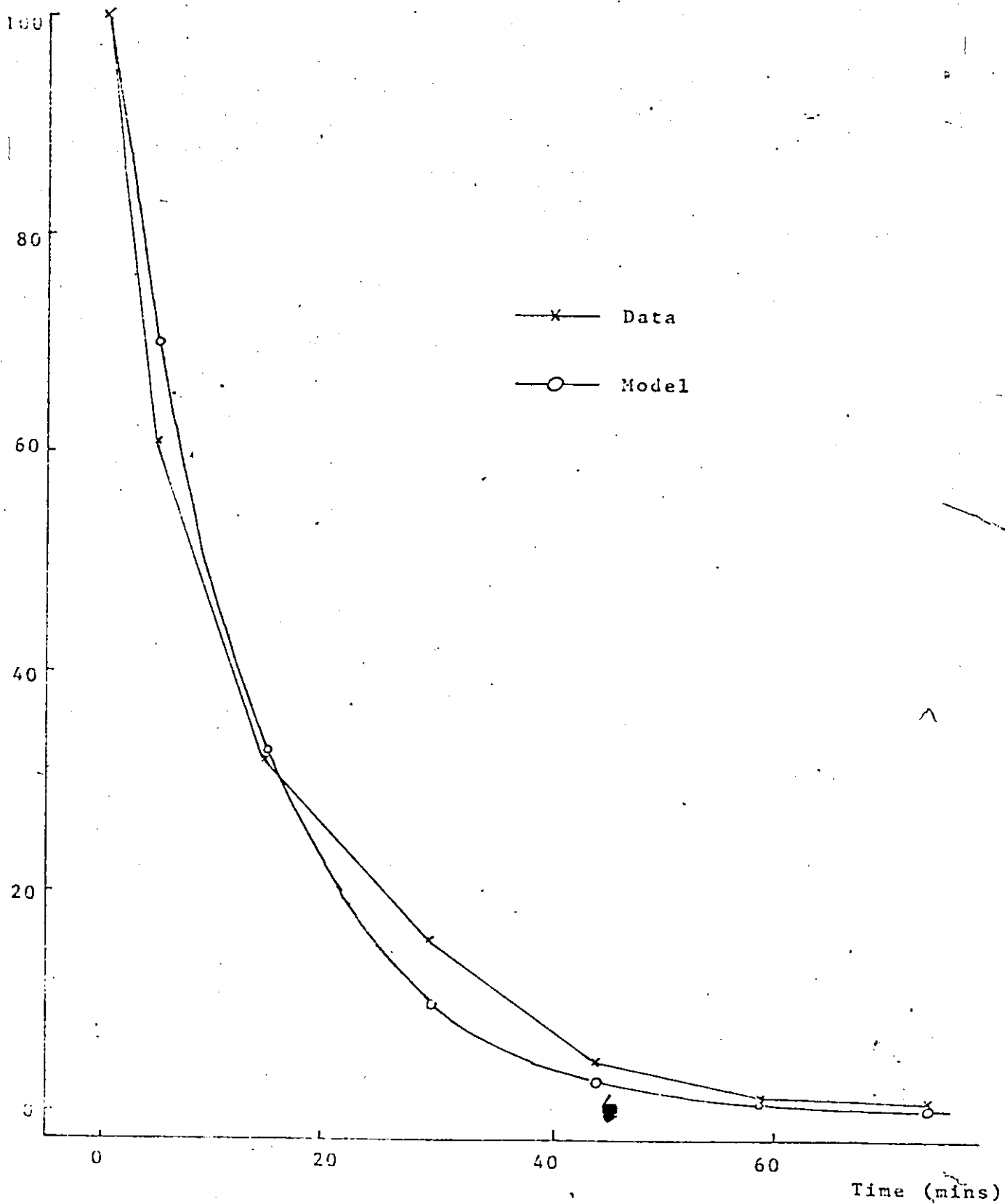


Figure 4.4

Level of ^{14}C -adenosine in suspending medium

% ^{14}C as ^{14}C -adenosine within platelet

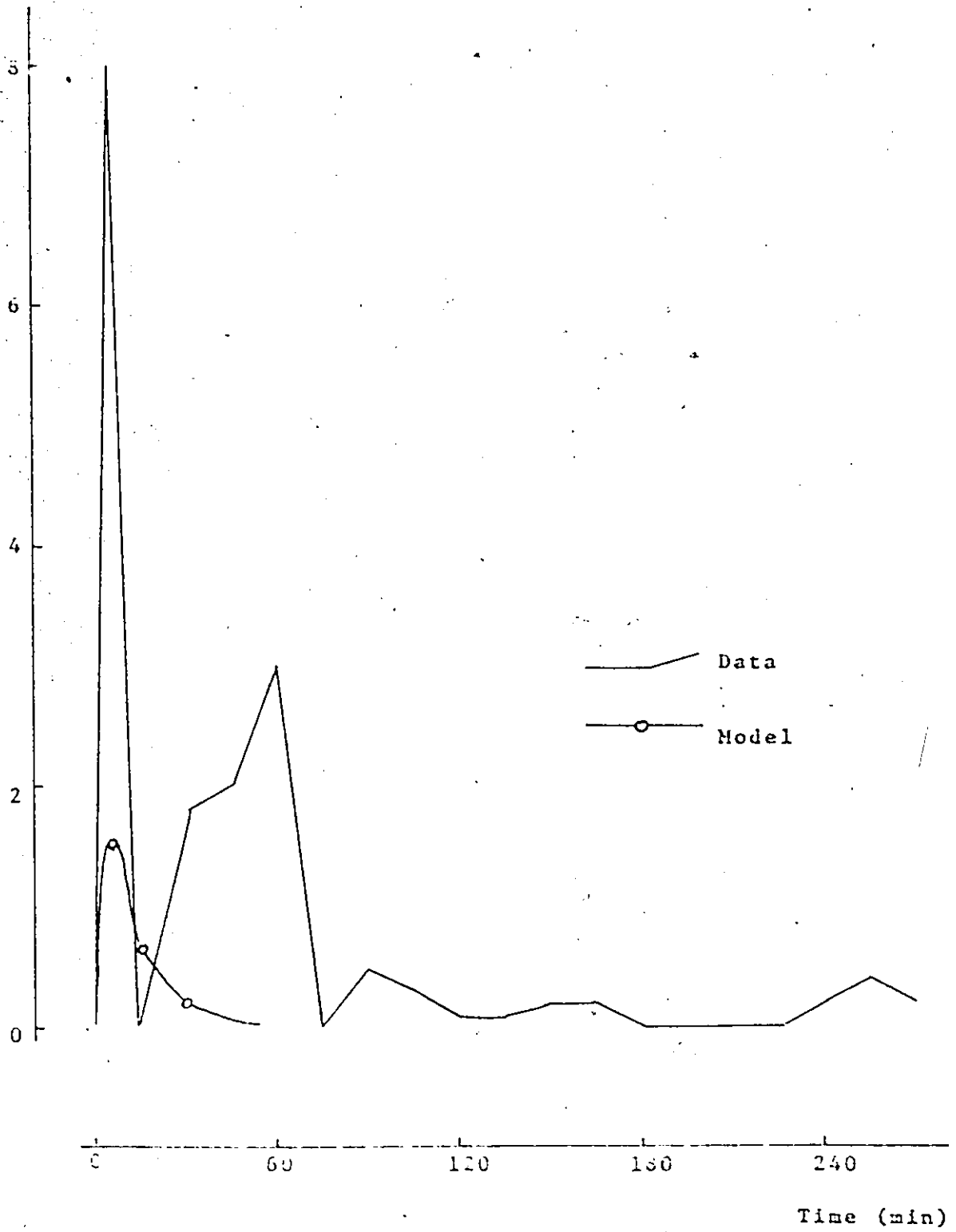


Figure 4.5

Level of ^{14}C -adenosine within platelet

%¹⁴C as ¹⁴C-AMP within platelets

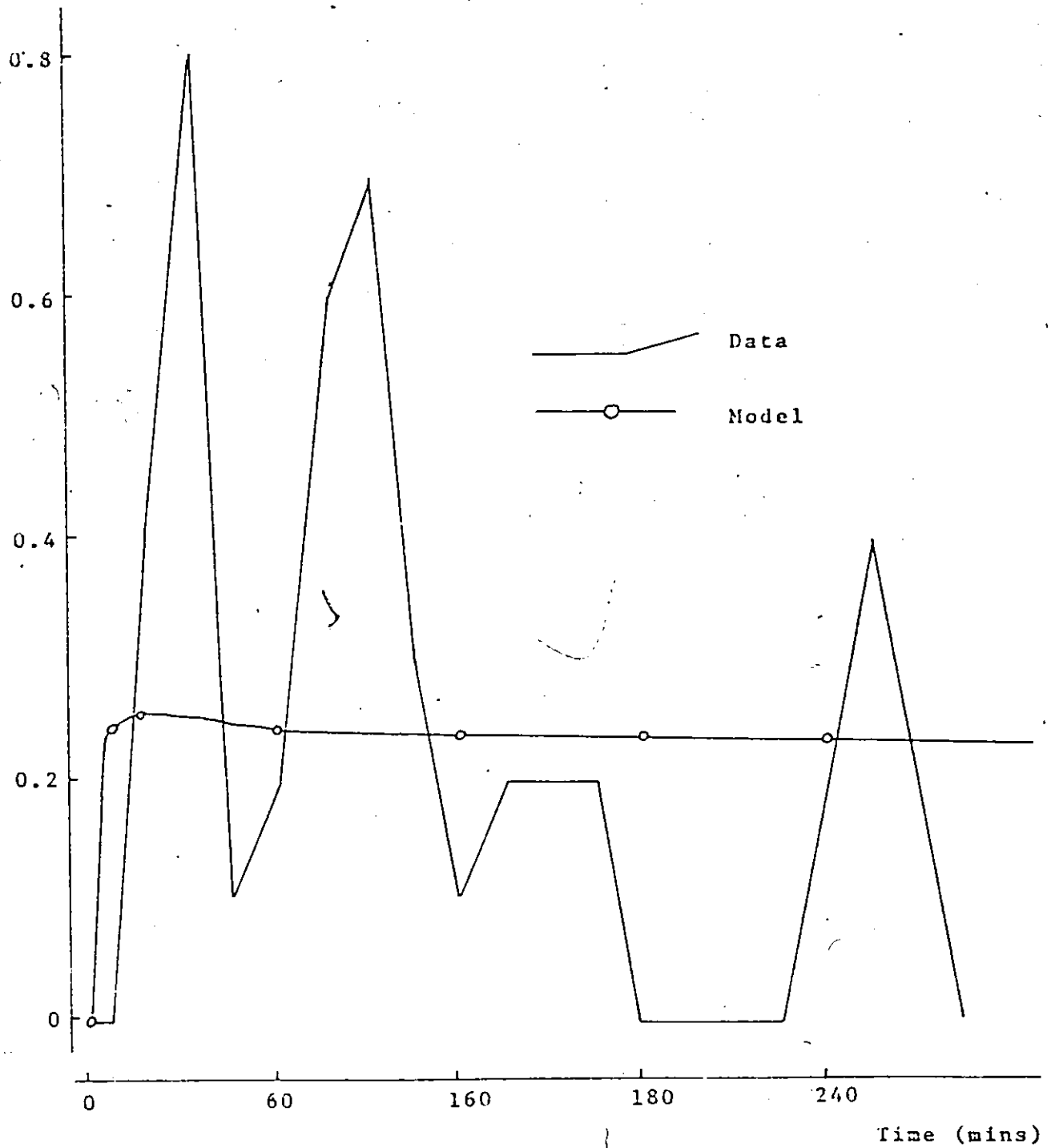


Figure 4.6

Level of ¹⁴C-AMP in platelet

% ^{14}C as ^{14}C -ADP in platelet metabolic pool

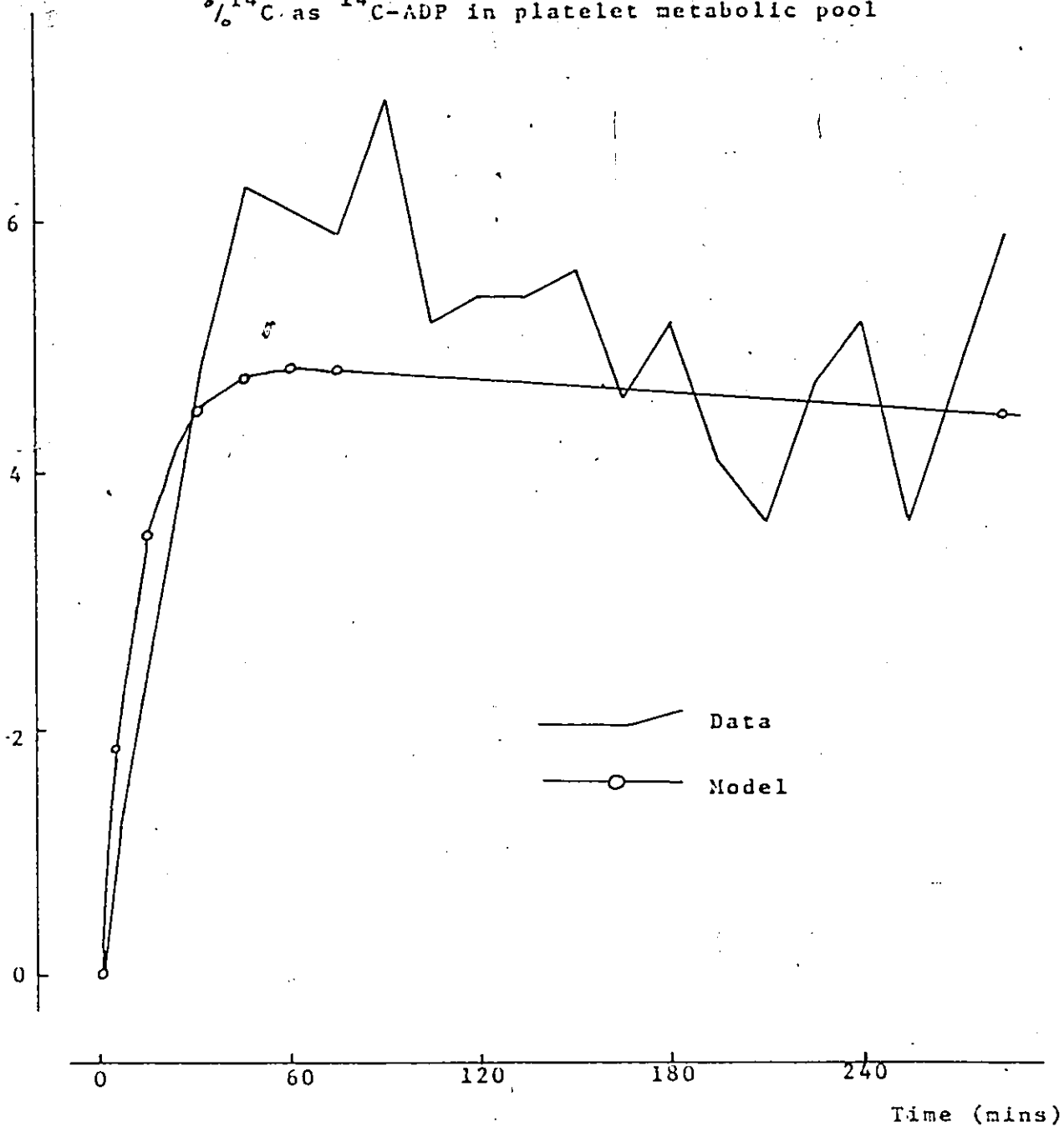


Figure 4.7

Level of ^{14}C -ADP in metabolic pool

$\%^{14}\text{C}$ as ^{14}C -ATP with platelet metabolic pool

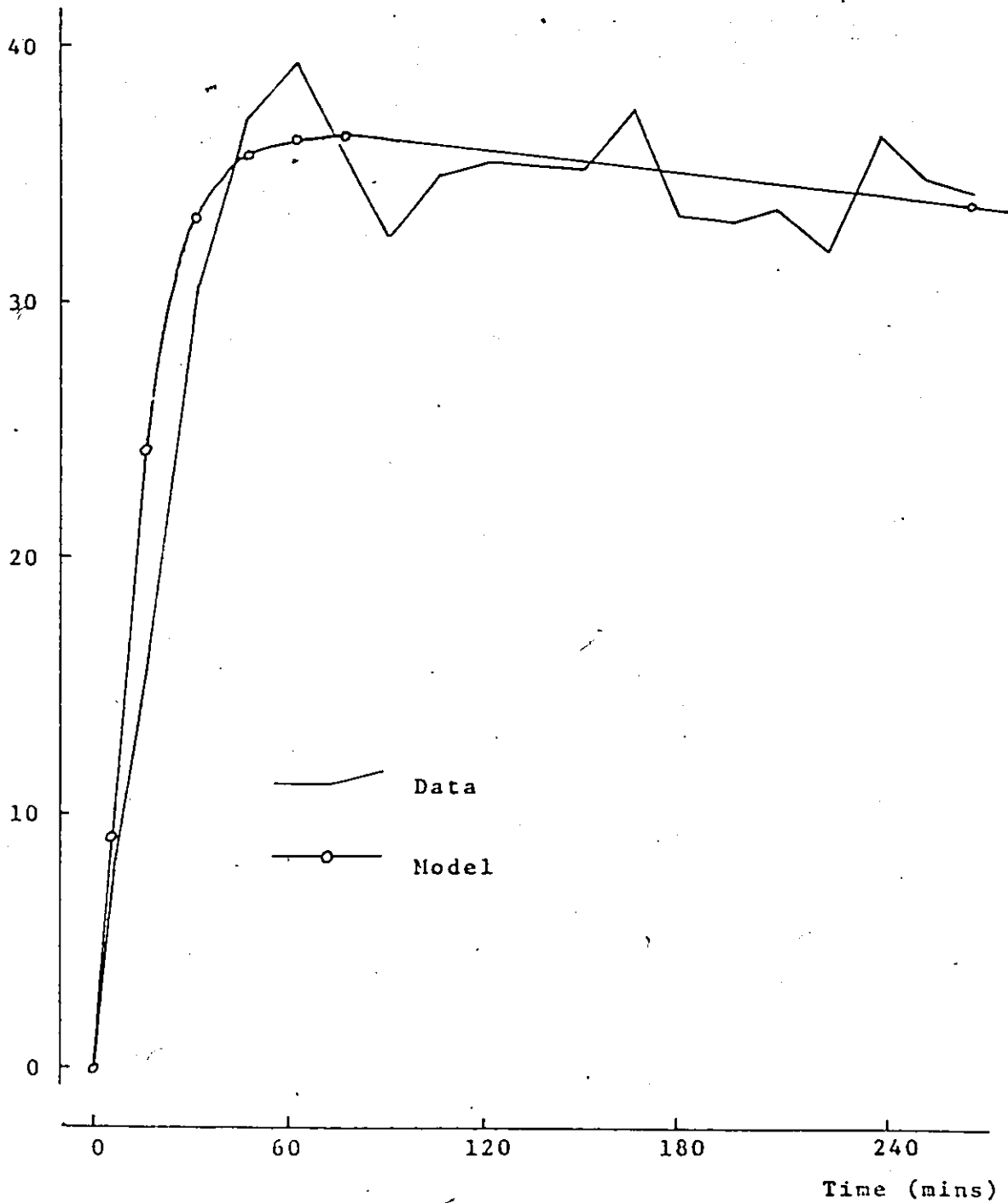


Figure 4.3

Level of ^{14}C -ATP in metabolic pool

%¹⁴C as ¹⁴C-ATP within platelets

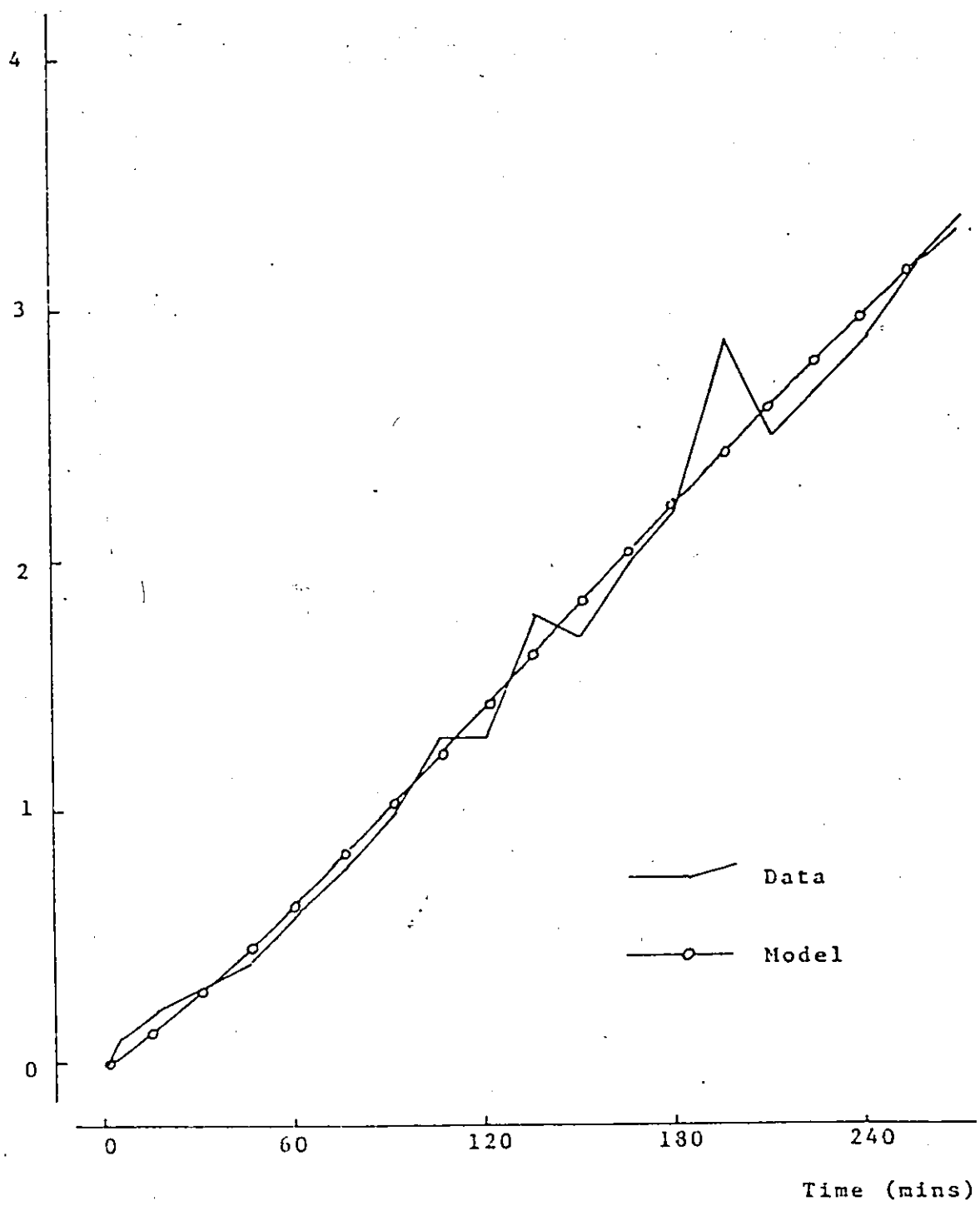


Figure 4.9
Level of ¹⁴C-ATP in platelet granule pool

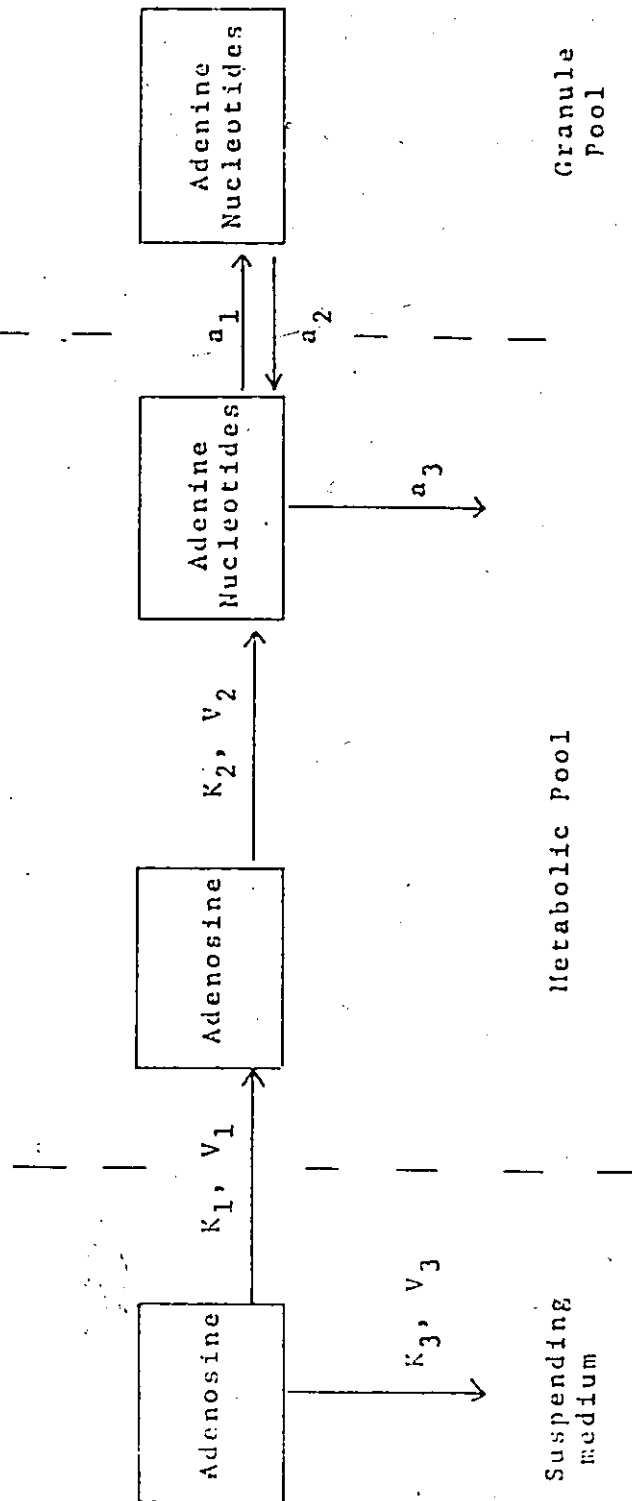


Figure 4.10

Simplified model for adenosine metabolism.

^{14}C as ^{14}C -adenosine in suspending medium

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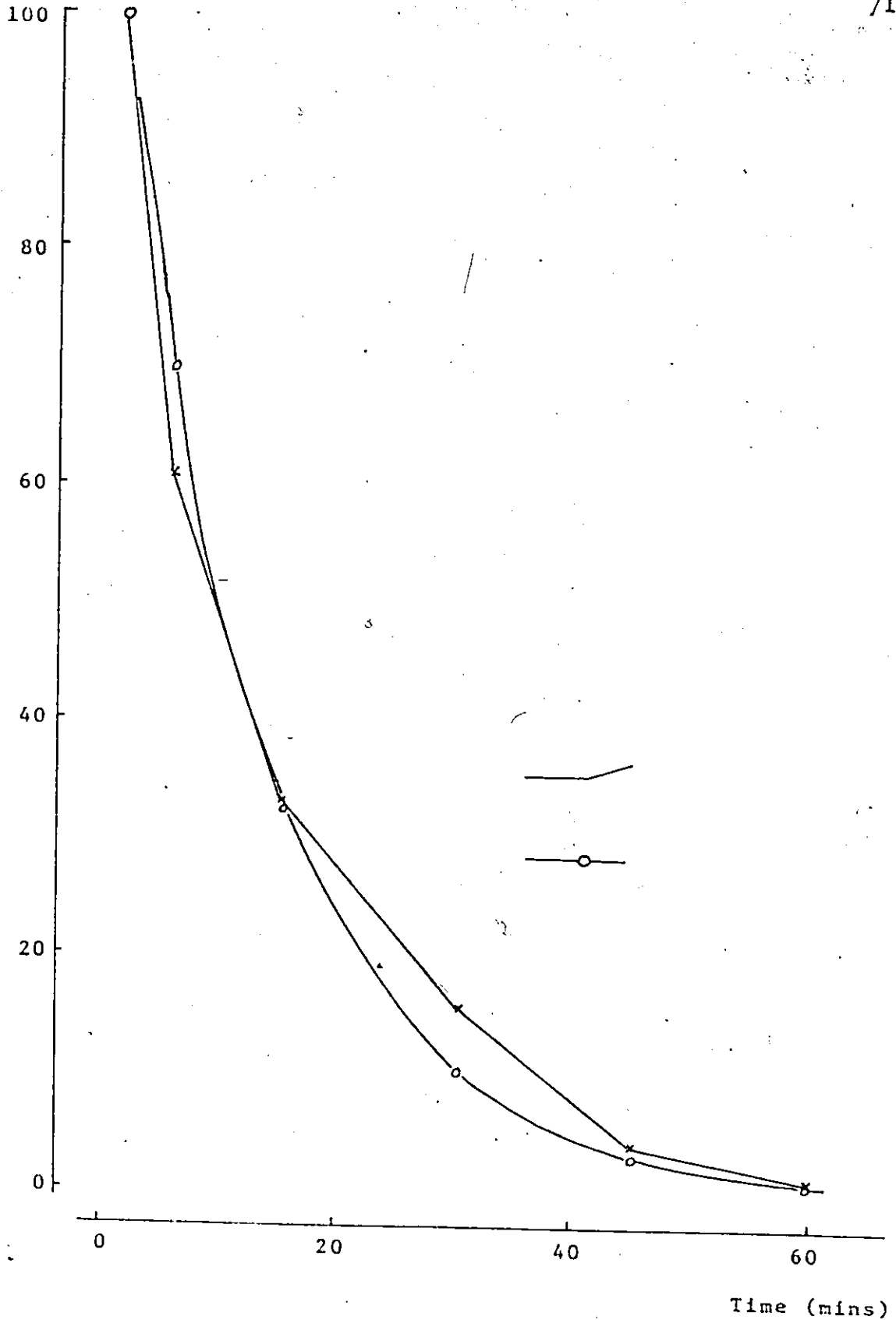


Figure 4.11

Level of ^{14}C -adenosine in the suspending medium

$\%^{14}\text{C}$ as ^{14}C -adenine nucleotides in the metabolic pool

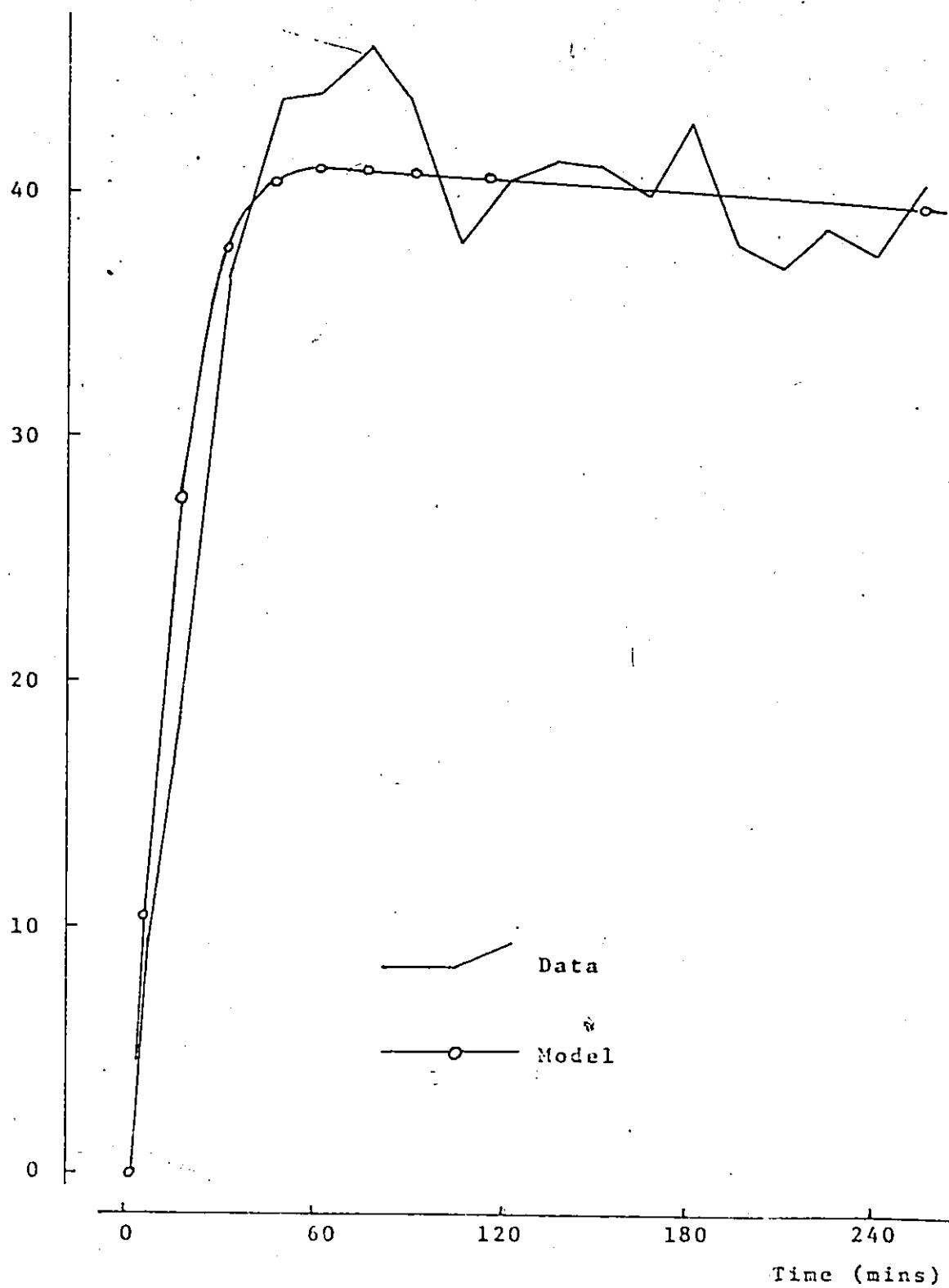


Figure 4.12

Level of ^{14}C -adenine nucleotides in the metabolic pool

pool (Reimers et al. 1975b; Holmsen et al. 1969b); k_{65} is estimated from the experimental data.

Attempts were also made to model the entire metabolic process by which adenosine and the adenine nucleotides are converted to hypoxanthine. Simple Michaelis-Menten kinetics however proved incapable of describing the data, presumably because of the activation and inhibition of the enzymatic reactions (Reich and Sel-kov, 1974).

A simpler model was also considered, in which the three metabolic compartments, of AMP, ADP and ATP are lumped into a single metabolic pool compartment of adenine nucleotides. Such a model is shown in Fig. 4.10. Metabolism of the adenine nucleotides is shown as a first order process as is the exchange between granule and metabolic compartments. This model was fitted to the data; the model's predictions and the experimental data are shown in Figs. 4.11-4.12. The parameter estimates with highest posterior probability are tabulated below (Table 4.2).

It will be noted from the figures that the fit to the data with this model is adequate; there is no significant improvement in the estimated variance of the model's fit to the data with the adoption of a model with separate AMP, ADP and ATP compartments within the metabolic pool ($p < 0.95$). This is expected as the exchange of nucleotide between the compartments of the metabolic pool is rapid (fraction turnover

Table 4.2

Kinetic constants for the metabolism of ¹⁴C-adenosine

Parameter	Estimate with 95% confidence limit	Units
V ₁	1.30 ± 1.51	nmoles(10 ⁹ platelets) ⁻¹ min ⁻¹
K ₁	36.81 ± 78.33	nmoles(10 ⁹ platelets) ⁻¹
V ₂	0.51 ± 0.45	nmoles(10 ⁹ platelets) ⁻¹ min ⁻¹
K ₂	0.32 ± 0.47	nmoles(10 ⁹ platelets) ⁻¹
K ₃	33.1 ± 27.8	nmoles(10 ⁹ platelets) ⁻¹
V ₃	1.56 ± 0.38	nmoles(10 ⁹ platelets) ⁻¹ min ⁻¹
a ₁	0.0002 ± 0.00024	min ⁻¹
a ₂	0.00031 ± -	min ⁻¹
a ₃	0.00026 ± 0.00026	min ⁻¹

*The parameters are those shown in Figure 4.10.

rates greater than 0.44 min^{-1}) in comparison to the transfer of nucleotide from the metabolic to the granule ATP compartments (0.00056 min^{-1}) and the metabolism of AMP (0.0098 min^{-1}).

It is thus apparent that the model of Holmsen (1971), as extended by Reimers et al. (1975b), adequately describes the metabolism of adenosine and its incorporation into the adenine nucleotides of platelets. In particular it has been shown that the metabolism of the platelet's adenine nucleotides and their transport into the granule storage pool may be described as first order processes and that the adenine nucleotides within the metabolic pool may be considered as a single entity rather than as the individual nucleotides AMP, ADP and ATP.

4.3 Adenine Nucleotide Behaviour in Vivo.

4.3.1 Introduction.

The in vitro labelling of rabbit platelets with ^{14}C -adenosine results in the partial incorporation of the ^{14}C -label into the adenine nucleotides of the platelet's metabolic pool. A slow exchange of adenine nucleotides between the metabolic and granule pools is observed. Thus if washed rabbit platelets, labelled in vitro with ^{14}C -adenosine, are reinjected into rabbits, the label should slowly transfer into the granule pool.

In vivo, there are two likely sources of adenine nucleotide loss from the platelets: catabolism or release. In release the granule pool of adenine nucleotides is liberated. In normal rabbits the frequency of release is likely to be small: Reimers et al. (1975b) found there to be no significant change in the levels of ^{14}C -adenine nucleotides within the metabolic and granule pools in a period 18-60 hours after the labelling and reinjection of the platelets.

To investigate the loss of adenine nucleotides in vivo, survival and release studies were performed. The survival studies were made with platelets labelled with ^{51}Cr and ^{14}C -adenine. ^{14}C -adenine is incorporated into the adenine nucleotides of the platelet (Holmsen, 1971). It is used instead of ^{14}C -adenosine as it gives a higher

specific activity of ^{14}C -adenine nucleotides. As in the serotonin studies, the ^{51}Cr was used as a survival indicator. The release studies were conducted so as to periodically determine the ^{14}C -adenine nucleotide levels within the granule and metabolic pools of the platelet population in the survival study. The combination of survival and release studies should allow the source and extent of nucleotide loss from platelets to be determined.

4.3.2 Experimental Procedure.

The preparation of a suspension of washed rabbit platelets and its labelling in vitro with ^{14}C -adenine nucleotides and ^{51}Cr was performed in a manner analogous to that described for the ^{14}C -serotonin studies (sections 3.2 and 4.2). A suspension of the labelled washed platelets (10^{10} platelets in 3 ml) was injected into each of rabbits. At 24 hour intervals commencing three hours after injection, 6 ml blood samples were drawn from the ears of the rabbits. The ^{51}Cr γ radiation emanating from a sample of the whole blood was counted. From the remainder of the whole blood drawn, a suspension of platelet rich plasma was prepared and the ^{51}Cr and ^{14}C β radiation from this suspension was measured. One rabbit was sacrificed and a washed platelet suspension prepared by the method of Ardlie et al. (1970). Release was induced by thrombin in samples of this suspension

so as to obtain samples of the granule pool content of the platelets (Reiners et al., 1975b). The distribution of the ^{14}C -label amongst the adenine nucleotides of the metabolic pool and granule pools was determined by chromatography.

4.3.3 / Models of Adenine Nucleotide Behaviour in Vivo:

Assumptions.

In attempting to model adenine nucleotide behaviour in vivo several hypotheses will be considered. The first assumes that nucleotide loss is due to both metabolism and release the second that the loss is caused by metabolism alone and the third that it is occasioned by release alone. These models are based upon several assumptions. It is assumed that:

1. The platelet age distribution is invariant with respect to time, the platelet birth and death rates being constant and equal. This assumption may be verified by determining the blood platelet concentration at various times.
2. The uptake of ^{14}C -adenine and the incorporation of the ^{14}C -label within the adenine nucleotides is rapid and is independent of platelet age or release prior to labelling. It would seem unlikely that the uptake of ^{14}C -adenine would depend upon whether release had occurred. As an energy dependent process, however, the

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uptake process might depend upon the adenine nucleotide content of the metabolic pool or, more generally, upon the age of the platelet if age were associated with a slowing of the metabolic processes. Experimental studies are required to verify this assumption. Indirect proof would lie in the coincidence of the ^{51}Cr and the ^{14}C -adenine nucleotide survival curves. ^{51}Cr labelling, as performed here, shows no preference for any segment of the platelet population (Tsukuda et al. 1971).

^{14}C -adenine is rapidly incorporated into the adenine nucleotides of the platelet's metabolic pool (Holmsen, 1971).

3. Should release occur, then the fraction of the platelet population that undergoes release in a fixed time interval remains constant. It is further assumed that release occurs independently of platelet age. If a fraction 'a' of those circulating platelets with granules intact releases in unit time, the probability that platelets, of age t, have not undergone release is thus e^{-at} . Platelet survival is independent of release: release, induced by the repeated thrombin stimulation of platelets, has no significant effect upon survival (Reimers et al. 1973).
4. In release, the loss of granule contents is complete. The granules may not be reutilized (Reimers et al. 1975c).

5. The metabolic and granule pools are distinct and homogeneous. In section (4.2) it was found that the adenine nucleotides within the metabolic pool (AMP, ADP and ATP) could be treated together rather than being considered individually.
6. The exchange of adenine nucleotide label between the platelet's metabolic and granule pools and the catabolism of the adenine nucleotides are first order processes. In vitro studies have verified this over a short (5 hours) time period. Justification for this assumption will be provided later.

The model for the platelet is represented in Fig. 4.13.

In this model, let a_1 , a_2 be the first order exchange and catabolic rate constants. a_1 refers to movement of tracer from the metabolic pool to the granule pool, a_2 to tracer transfer from the granule pool to the metabolic pool and a_3 to the catabolism of adenine nucleotides.

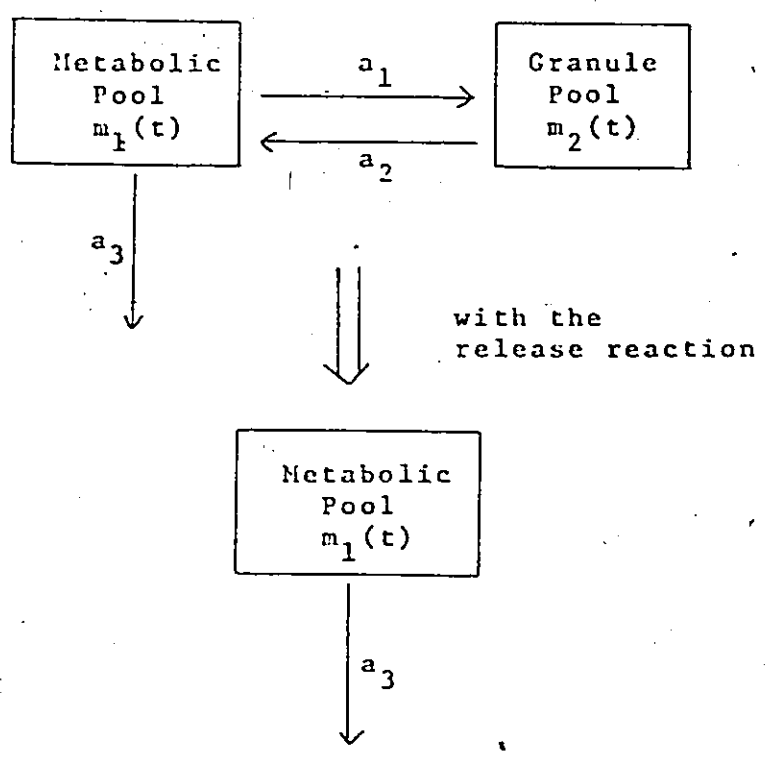
$m_1(t)$ is the fraction of the platelets initial tracer that is present in the metabolic pool at time t , $m_2(t)$ the fraction in the granule pool and $m(t)$ the fraction within the whole platelet.

i.e.
$$m(t) = m_1(t) + m_2(t)$$

The initial conditions are:

$$m_1(0) = 1, \quad m_2(0) = 0, \quad m(0) = 1.$$

Figure 4.13



Model for adenine nucleotide distribution
in the platelet

4.3.4 Nucleotide Loss due to Metabolism and Release.

Survival curves.

As in Part 3, let $A(t)$ be the fraction of those platelets, present in the circulation at the time of labelling, that are still present at a time t after labelling. $A(t)$ may be equated to the fractional level of initial ^{51}Cr present in a sample of blood if the ^{51}Cr level is corrected for a fractional elution rate of 1% per day (Aster, 1971). If $I(t)$ is the age distribution function of platelets within the circulation (i.e. $I(t)dt$ is the fraction of circulating platelets of age between t and $t + dt$), then

$$\begin{aligned} A(t) &= \int_t^{\infty} I(x) dx \\ A(0) &= 1 \end{aligned} \tag{4.3}$$

Let $C(t)$ be the fraction of the initial ^{14}C -adenine nucleotides within the circulating platelets at time t . Then $C(t)$ is made up of contributions from platelets present in the circulation at the time of labelling: from those platelets that had released prior to labelling; from those that had released between labelling and sampling and from those that had not released their granule contents at the time of sampling.

Consider a platelet that had released prior to labelling. At time t it contains a fraction $e^{-\alpha t}$ of its initial label, the remainder having been metabolized. The fraction

of such platelets in the circulation at time zero is

$$\int_0^{\infty} I(x)(1-e^{-ax})dx$$

a being the rate at which intact platelets undergo release.

Thus at time t they contribute to $C(t)$

$$e^{-a_3 t} \int_0^{\infty} I(x+t)(1-e^{-ax})dx \quad (4.4)$$

Those platelets that were present in the circulation at time zero and had not released at time t make up a fraction

$$\int_0^{\infty} I(x+t)e^{-a(x+t)}dx$$

of the platelets in the circulation at time t . Following Atkins (1969b), the level of ^{14}C -tracer within such a platelet at a time t after entry of the tracer into the metabolic pool may be shown to be

$$m(t) = m(0) \left((\lambda_1 - a_1 - a_2) e^{-\lambda_1 t} + (a_1 + a_2 - \lambda_2) e^{-\lambda_2 t} \right) / (\lambda_1 - \lambda_2) \quad (4.5)$$

where λ_1
 $\lambda_2 = ((a_1 + a_2 + a_3) \pm ((a_1 + a_2 + a_3)^2 - 4a_2 a_3)^{1/2}) / 2$

As the result given by Atkins (1969b) is erroneous, the eigenvalues, $-\lambda_1$ and $-\lambda_2$, being incorrectly defined, a correct deviation is given in Appendix 4.1.

Then those platelets, present in the circulation at the time of labelling, that had not released at time t , contribute to $C(t)$

$$m(t) \int_0^{\infty} I(x+t) e^{-a(x+t)} dx \quad (4.6)$$

Finally consider those platelets that release in the time interval y to $y+dy$, $t > y > 0$. Their number, at a time t after labelling,

$$\int_0^{\infty} I(x+t) e^{-a(x+y)} a \cdot dy \cdot dx$$

and they contribute to $C(t)$

$$e^{-ay} a \cdot dy \cdot e^{-a_3(t-y)} m_1(y) \int_0^{\infty} I(x+t) e^{-ax} dx$$

where $m_1(y)$, the level of tracer within the metabolic pool at time y in platelets with granules intact, is

$$m_1(t) = m(0) ((\lambda_1 - a_1) e^{-\lambda_1 t} + (a_2 - \lambda_2) e^{-\lambda_2 t}) / (\lambda_1 - \lambda_2)$$

This expression is derived in Appendix 4.1.

Those platelets that release in the period between labelling and sampling, $0 < y < t$, thus contribute

$$e^{-a_3 t} \int_0^{\infty} I(x+t) e^{-ax} dx \cdot \int_0^t a m_1(y) e^{-ay} e^{a_3 y} dy \quad (4.7)$$

Summing these three sources of contributions (Appendix 4.2)

$$\begin{aligned} C(t) = & (A(t) e^{-at} - a \int_t^{\infty} A(x) e^{-ax} dx) / (\lambda_1 - \lambda_2) \\ & ((\lambda_1 - a_1 - a_2 - a(\lambda_1 - a_2)) / (\lambda_1 + a - a_3)) e^{-\lambda_1 t} + \\ & (a_1 + a_2 - \lambda_2 - a(a_2 - \lambda_2)) / (\lambda_2 + a - a_3) e^{-\lambda_2 t} \\ & + a e^{-(a_3 - a)t} ((\lambda_1 - a_2) / (\lambda_1 + a - a_3) + (a_2 - \lambda_2) / (\lambda_2 + a - a_3)) \\ & + a e^{(a - a_3)t} \int_t^{\infty} A(x) e^{-ax} dx \quad (4.8) \end{aligned}$$

This equation may be fitted to the experimentally determined values of $C(t)$.

Release Studies.

Now an equation for the ratio of granule bound to total platelet ^{14}C -adenine nucleotides will be derived.

This quantity, $R(t)$, may be experimentally determined in release studies.

For platelets that lose nucleotides through metabolism and release, the granule content of platelets is contributed by platelets that had not released at the time of sampling. Thus the fraction of the initial ^{14}C -adenine nucleotides located in the platelet granules at time t is, after (4.6):

$$\begin{aligned}
 G(t) &= m_2(t) \int_0^{\infty} I(x+t) e^{-a(x+t)} dx \\
 &= (A(t) e^{-at} - a \int_t^{\infty} A(x) e^{-ax} dx) a_1 (e^{-\lambda_2 t} - e^{-\lambda_1 t}) / \\
 &\hspace{20em} (\lambda_1 - \lambda_2)
 \end{aligned}
 \tag{4.9}$$

$$\text{and } R(t) = G(t)/C(t) \tag{4.10}$$

4.3.5 Nucleotide Loss due to Metabolism Alone.

In this hypothesis the probability that a platelet undergoes release will be assumed to be negligible, i.e. in the model assuming loss through metabolism and release,

a will be zero. Hence, from (4.8)

$$C(t) = \Lambda(t) \left((\lambda_1 - a_1 - a_2) e^{-\lambda_1 t} + (a_1 + a_2 - \lambda_2) e^{-\lambda_2 t} \right) / (\lambda_1 - \lambda_2) \quad (4.11)$$

or $C(t) = \Lambda(t)m(t)$

Similarly, setting a to zero in (4.9), the fraction of initial ^{14}C within the granules at time t is

$$G(t) = n_2(t) \int_0^{\infty} I(x+t) dx$$

or $G(t) = \Lambda(t) a_1 (e^{-\lambda_2 t} - e^{-\lambda_1 t}) / (\lambda_1 - \lambda_2) \quad (4.12)$

and $R(t) = m_2(t) / n(t) \quad (4.13)$

4.3.6 Nucleotide Loss through Release Alone.

In this hypothesis, metabolism will be assumed to be negligible, nucleotide loss occurring through release alone.

i.e. $a_3 = 0$. It will be assumed that the catabolism of the adenine nucleotides associated with release may be neglected and hence

$$\lambda_1 = a_1 + a_2$$

$$\lambda_2 = 0$$

Substituting these values into (4.8), we have

$$\begin{aligned} C(t) = & \Lambda(t) \left((a_2 + a) / (a_1 + a_2 + a) + a_1 e^{-at} (1 - a e^{-(a_1 + a_2)t}) / \right. \\ & \left. (a_1 + a_2 + a) / (a_1 + a_2) \right) \\ & + (a a_1 \int_t^{\infty} \Lambda(x) e^{-ax} dx) (e^{at} / (a_1 + a_2 + a) \\ & - (1 - a e^{-(a_1 + a_2)t} / (a_1 + a_2 + a)) / (a_1 + a_2) \end{aligned} \quad (4.14)$$

and similarly, substituting them into (4.9),

$$C(t) = (A(t)e^{-at} - a \int_t^{\infty} A(x)e^{-ax} \cdot dx) a_1 (1 - e^{-(a_1+a_2)t}) / (a_1+a_2) \quad (4.15)$$

4.3.7 A simulation of Survival and Release.

To illustrate the value of the survival and release studies in elucidating adenine nucleotide behaviour in vivo, the systems were simulated using the following values for the parameters, estimated from preliminary experiments (Table 4.3). The values of $A(t)$ were taken from a survival experiment. The results of the simulation are shown in Table 4.4.

These simulations show there to be a marked change in the ^{14}C -adenine nucleotide survival curves if loss due to metabolism is assumed to occur. If release alone is assumed to be responsible for the loss of nucleotides, there is little difference between $A(t)$ and $C(t)$. However, if release occurs, with or without metabolism, the values of $R(t)$ are considerably different than if metabolism alone causes nucleotide loss. Thus a combination of release and survival studies should prove effective in determining the means by which adenine nucleotide loss occurs.

4.3.8 Experimental Results and Discussion

The ^{51}Cr and ^{14}C -adenine survival curves from an

Table 4.3

Values for parameters for the simulations

Model	First order rate constants (hrs ⁻¹)			
	a ₁	a ₂	a ₃	a
Nucleotide loss due to				
Release and Metabolism	0.01	0.01	0.01	0.01
Metabolism	0.01	0.01	0.01	0.
Release	0.01	0.01	0.	0.01

Table 4.4

Results of simulations

t (hrs)	Nucleotide loss due to							
	A(t)*	Metabolism		Release		Metabolism & Release		
	C(t)	R(t)	C(t)	R(t)	C(t)	R(t)	C(t)	R(t)
0.	1.	0.	1.	0.	1.	0.	1.	0.
18.	0.67	0.568	0.163	0.661	0.0643	0.555	0.0699	0.0699
42.	0.37	0.262	0.327	0.351	0.0777	0.236	0.0945	0.0945
66.	0.21	0.128	0.438	0.192	0.0656	0.103	0.0897	0.0897
93.	0.09	0.478	0.615	0.081	0.0430	0.081	0.0430	0.0430

*Assuming a ^{51}Cr elution rate of 1% a day (Aster, 1971).

experiment are shown in Fig. 4.14. There is no statistically significant difference between the ^{51}Cr survival curve, corrected for a ^{51}Cr elution rate of 1% a day, (i.e. $A(t)$) and the ^{14}C -adenine survival curve ($C(t)$) (F test, $p > 0.35$, Dell et al, 1973).

The release studies allow the determinations of $R(t)$, the fraction of total platelet ^{14}C -adenine nucleotides present in the granule pool at the time of sampling. The experimentally determined values of $R(t)$ are shown (Fig. 4.15). In modelling the data no improvement in the fit of model to data was gained by assuming there to be metabolism or release of the adenine nucleotides (Table 4.5). A model that considered only the exchange of nucleotides between the metabolic and granule pools was adequate.

It may be concluded that there was negligible metabolism and release of the adenine nucleotides in the platelets, the levels of adenine nucleotides within the metabolic and granule pools of the platelet remaining unchanged over the course of the experiment. If this is so, then the exchange of nucleotides between the pools occurs at a steady rate and thus the exchange of tracer may properly be described by first order kinetics.

The fraction of the adenine nucleotides of the metabolic pool that transfer into the granule pool in unit time (a_1) is 0.0792 hr^{-1} ; the fraction of the granule pool adenine nucleotides that transfer into the metabolic pool (a_2) is 0.511 hr^{-1} . This indicates that 61% of the

Table 4.5

Models for release data

Model: adenine nucleotide loss due to	Fractional turnover rates				S.S.*
	a_1	a_2 (hrs ⁻¹)	a_3	a	
Release and metabolism	0.0792	0.511	0	0	1.797 10 ⁻³
Metabolism	0.0792	0.511	0	-	1.797 10 ⁻³
Release	0.0792	0.511	-	0	1.797 10 ⁻³
No loss	0.0792	0.511	-	-	1.797 10 ⁻³

* S.S - sum of squares of deviations of model from data

Fraction of ^{51}Cr or ^{14}C -adenine attached to platelet

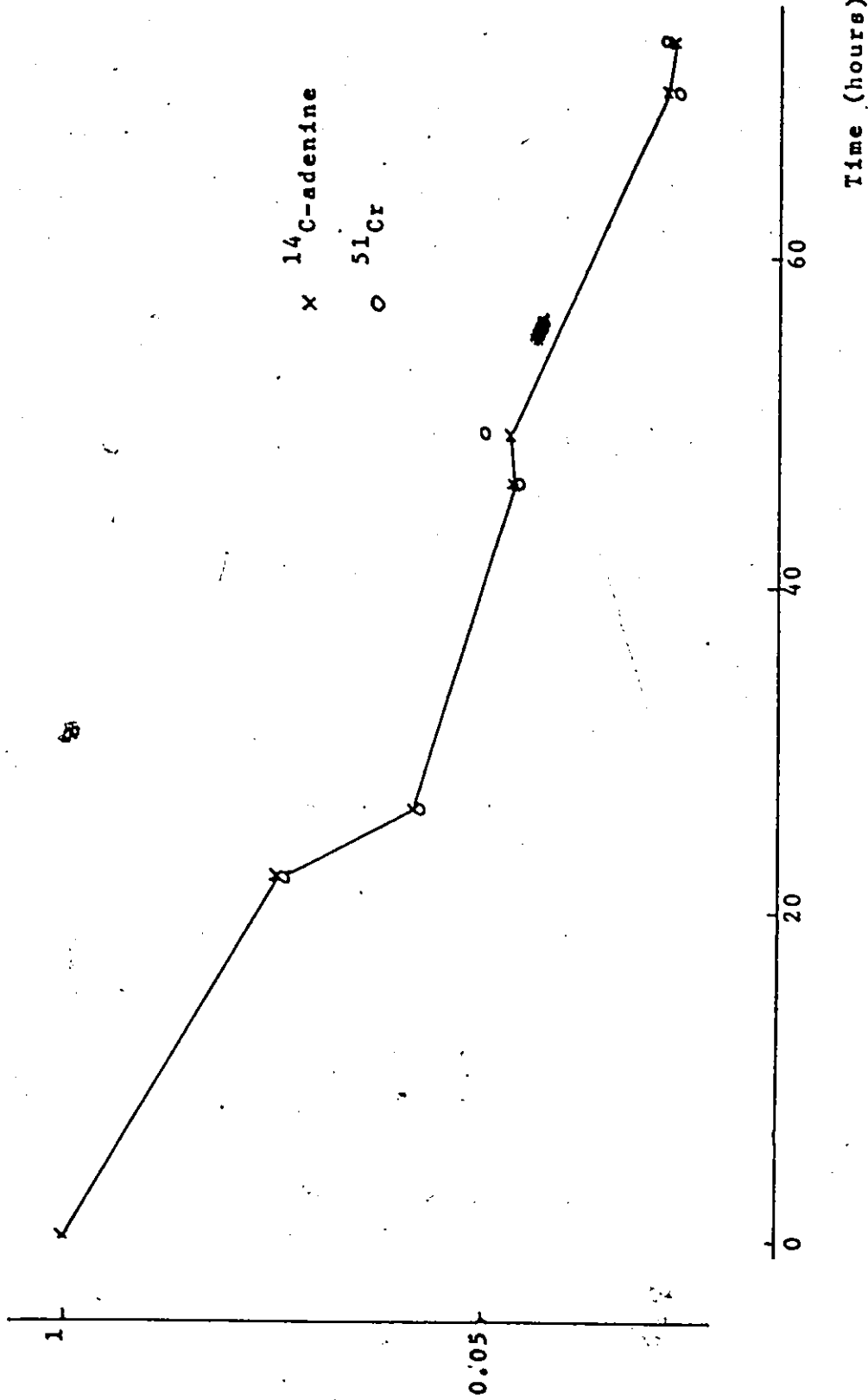


Figure 4.14
 ^{51}Cr and ^{14}C -adenine survival studies

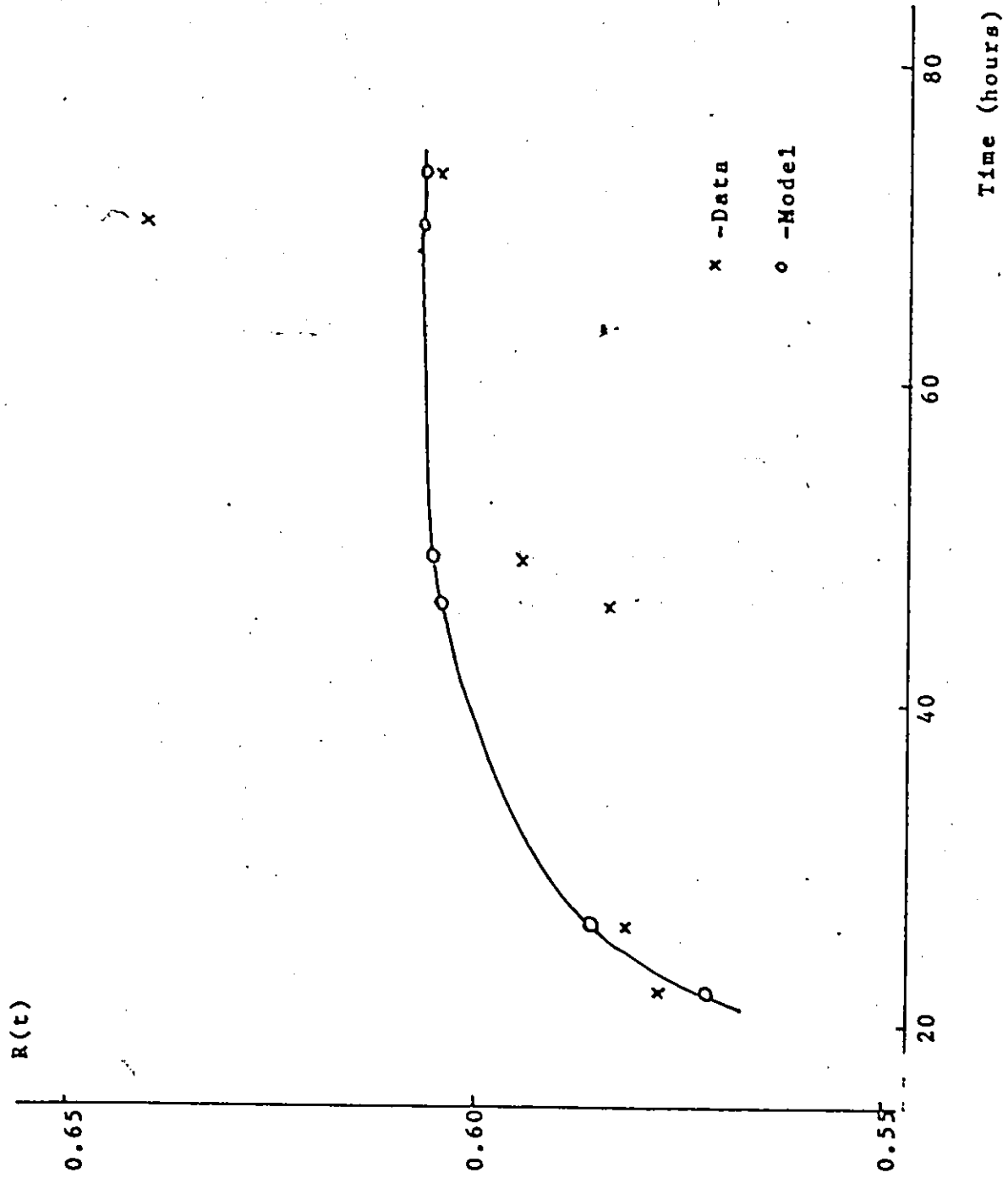


Figure 4.15

Fraction of total platelet ¹⁴C-adenine nucleotides in the granule pool

platelet adenine nucleotides are in the granule pool, 39% in the metabolic pool.

The conclusions that the adenine nucleotide content of platelets is unchanged with age allows three statements to be made: first, senescence of platelets is unrelated to a loss of adenine nucleotides from the platelets; second, if the denser fraction of platelets contains more adenine nucleotides than does the lighter fraction, then heterogeneities in platelet density are unrelated to platelet age: the conclusion of Paulus (1975) is supported; third, platelet release occurs infrequently in the circulation.

Nomenclature - Part 1Latin

- a - tube radius
 c - platelet concentration
 \bar{c} - dimensionless platelet concentration (c/c_0)
 c_0 - platelet concentration at the tube inlet
 D - platelet diffusivity
 D_s - platelet diffusivity adjacent to the wall
 \bar{d} - dimensionless boundary layer thickness (\bar{y})
 \bar{d}_1 - dimensionless distance between the edges of the concentration boundary layer and the skimming layer (\bar{y})
 \bar{d}_e - dimensionless skimming layer thickness (\bar{y})
 F - platelet flux
 f - platelet collision frequency with an element of the wall
 K_d - term indicating the relative importance of the binding reaction and diffusion in determining the rate limiting step in the adhesion process (defined as $ka.Pe^{-1/3}/D_s$)
 K_1 - effective value of K_d accounting for partial platelet coverage of the surface
 k - binding rate constant
 m - power law coefficient
 N - platelet collision frequency with the wall
 n - power law coefficient
 Pe - Peclet number - a number that indicates the relative importance of diffusion and convection (defined as ua/D_s)

- q - fraction of platelets that adhere on collision with the surface
 r - radial coordinate
 \bar{r} - dimensionless radial coordinate (r/a)
 \bar{s} - fraction of surface adhesion sites for platelets that are unoccupied
 T - characteristic adhesion time (defined as $Pe^{-1/3} \cdot a \rho_p / (D_s c_0)$)
 t - time
 \bar{t} - dimensionless time (t/T)
 u - axial velocity
 \bar{u} - mean axial velocity
 \bar{v} - mean velocity of platelets' random movements
 x - axial coordinate
 \bar{x} - dimensionless axial coordinate (x/a)
 \bar{y} - dimensionless distance from the surface (defined as $(1 - \bar{r}) \cdot Pe^{1/3}$)
 \bar{y}_1 - dimensionless distance from the skimming layer boundary ($\bar{y} - \bar{d}_e$)

Greek

- β - non-Newtonian factor
 γ - shear rate
 δS - an element of surface area
 θ - an angle

- λ - mean free path of platelet fluctuations
- ρ - surface density of platelets
- $\bar{\rho}$ - dimensionless surface density of platelets (ρ/ρ_p)
- ρ_p - saturation level of platelet surface density



Nomenclature - Part 2

- a_{ij} - fractional turnover rate from the i th to the j th compartment
 a_{ii} - total fractional turnover rate from the i th compartment
 $\underline{\underline{A}}$ - matrix with elements a_{ij}
 $\underline{\underline{B}}$ - matrix of experimentally determined pre-exponential constants
 C_i - experimentally determined exponential constant
 D - serotonin diffusivity
 $\underline{\underline{G}}$ - matrix of pre-exponential constants
 k - rate constant for uptake of serotonin by platelets
 L - number of samples taken in the course of an experiment
 \underline{m} - vector of responses
 m_{iu} - value of i th response at i th observation
 n - number of compartments in system
 r - a radius
 R - radius of platelet
 S_{ij} - element in matrix S
 X - the increase in the number of parameters associated with the introduction of a compartment
 Y - number of parameters required to describe a system

Greek

- α - confidence level
 θ - parameter

λ_i - eigenvalue of \underline{A}
 \underline{A} - diagonal matrix of λ 's

Nomenclature - Part 3

- A(t) - fraction of platelets in the circulation at a time t after labelling that were present at the time of labelling
- C(t) - fraction of initial ^{14}C present in the platelets at a time t after labelling
- I(t) - platelet age distribution function
- k - fractional turnover rate of serotonin from the platelets into the plasma
- k_1 - fraction of serotonin from dead platelets that exchanges into circulating platelets
- k_2 - fractional turnover rate of serotonin from circulating platelets out of the circulation
- t - time

Nomenclature - Part 4

- $\Lambda(t)$ - fraction of platelets in the circulation at a time t after labelling
- a - fraction of platelets with granules intact that release in unit time φ
- a_1 - fractional turnover rate (metabolic pool to granule pool)
- a_2 - fractional turnover rate (granule pool to metabolic pool)
- a_3 - rate constant for adenine nucleotide catabolism
- $C(t)$ - fraction of initial ^{14}C present in the platelets at a time t after labelling
- $G(t)$ - granule bound ^{14}C -adenine nucleotides
- $I(t)$ - age distribution function of platelets
- K_i - Michaelis-Menten constant
- k_{ij} - fractional turnover rate from the j th to the i th compartment
- m - tracer content of platelet
- m_i - tracer content of i th compartment
- $R(t)$ - ratio of granule bound to total platelet ^{14}C -adenine nucleotides
- t - time
- V_i - maximal reaction velocity

Greek

- λ_i - eigenvalue

REFERENCES

- Adelson, E., Rheingold, J.J. & Crosby, Q.H. (1957) Studies of platelet survival by tagging in vivo with ^{32}P . J. Lab. Clin. Med., 50, 570-576.
- Allen, D.J., Reimers, H.-J., Feuerstein, I.A. & Mustard, J.F. (1975) The use and analysis of multiple responses in multicompartiment cellular systems. Biometrics, 31, 921-929.
- Amorosi, E., Garg, S.K. & Karparkin, S. (1971) Heterogeneity of human platelets. IV. Identification of a young platelet population with (^{75}Se) selenomethionine. Br. J. Haematol., 21, 227-232.
- Amundson, N.R. (1966) Mathematical Methods in Chemical Engineering. Prentice Hall, Inc., Englewood Cliffs, N.J. p. 121.
- Ardlie, N.G., Packham, M.A. & Mustard, J.F. (1970) Adenosine diphosphate induced platelet aggregation in suspensions of washed rabbit platelets. Brit. J. Haematol., 19, 7-17.
- Aster, R.H. (1971) 'Kinetics of isotopically labelled platelets' in Platelet Kinetics, J.M. Faulus (ed.), North Holland Publishing Co., Amsterdam. p. 12.
- Aster, R.H. & Jandl, J.H. (1964) Platelet sequestration in man. I. Methods. J. Clin. Invest., 43, 843-855.
- Atkins, G.L. (1969a) Multicompartiment Models for Biological Systems, Methuen & Co. Ltd., London. p. 118.

- Atkins, G.L. (1969b) Multicompartment Models for Biological Systems, Methuen & Co. Ltd., London. p. 44.
- Baier, R. F., Loeb, G.I. & Wallace, G.T. (1971) Role of an artificial boundary in modifying blood proteins. Fed. Proc. 30, 1523-1538.
- Baumgartner, H.R. (1973) The role of blood flow in platelet adhesion, fibrin deposition and formation of mural thrombi. Microvasc. Res., 5, 167-179.
- Baumgartner, H.R. & Haudenschild, G. (1972) Adhesion of platelets to subendothelium. Ann. N.Y. Acad. Sci. 201, 22-36.
- Baumgartner, H.R. & Muggli, R. (1974) Effect of acetylsalicylic acid on platelet adhesion to subendothelial and on the formation of mural platelet thrombi. Thromb. Diath. Haemorrh. Suppl. 60, 345-354.
- Baumgartner, H.R., Stemeran, M.B. & Spaet, T.H. (1971) Adhesion of blood platelets to subendothelial surface. Experientia 27, 283-284.
- Beauchamp, J.J. & Cornell, R.G. (1966) Simultaneous non-linear estimation. Technometrics 8, 319-326.
- Beauchamp, J.J. & Cornell, R.G. (1969) Spearman simultaneous estimation for a compartmental model. Technometrics 11, 551-560.
- Bergner, P. E. E. (1961) Tracer dynamics: II. The limiting properties of the tracer system. J. Theor. Biol. 1, 359-381.

Light copy

- Bergner, P.E.F. & Lushbough, C.G. (eds) (1967) *Compartments, Pools and Spaces in Medical Physiology*. CONF-661010, Springfield, Va., Clearing House for Federal Scientific and Technical Information.
- Berman, M. & Schoenfeld, R. (1956) Invariants in experimental data on linear kinetics and formulation of models. *J. Appl. Phys.* 27, 1361-1370.
- Berne, R.M. & Rubio, R. (1974) Adenine nucleotide metabolism in the heart. *Circ. Res.* 34-35 Suppl. 3, 109-120.
- Berncis, K.H., Pletscher, A. & DaPrada, M. (1970) Phase separation in solutions of noradrenaline and adenosine triphosphate: influence of divalent cations and drugs. *Br. J. Pharmac.* 39, 382-389.
- Bernett, M.K. & Zisman, M.A. (1971) Surface-chemical aspects of clotting. *Fed. Proc.* 30, 1633-1639.
- Bird, R.B., Stewart, W.L. & Lightfoot, E.N. (1960) *Transport phenomena*, John Wiley & Sons, Inc., New York. p. 47.
- Bjornson, J. (1974) The effect of ⁵¹Cr-labelling procedure on platelet aggregability. *Scand. J. Haematol.* 13, 252-259.
- Born, G.V.R. (1956) The breakdown of adenosine triphosphate in blood platelets during clotting. *J. Physiol.* (London) 113, 61P-62P.

Light copy

- Born, G.V.R. & Gillson, R.E. (1959) Studies on the uptake of 5-hydroxytryptamine by blood platelets. *J. Physiol. (London)* 146, 472-491.
- Box, G.E.P. & Cox, D. R. (1964) An analysis of transformations. *J. R. Statist. Soc. B.* 26, 211-216.
- Box, G.E.P. & Draper, D.R. (1965) The Bayesian estimation of common parameters from several responses. *Biometrika* 52, 355-364.
- Box, G.E.P. & Jenkins, C.M. (1970) *Time Series Analysis, Forecasting and Control*, Holden Day, New York, N.Y.
- Box, G.E.P., Hunter, W.G., MacGregor, J.F. & Irjavec, J. (1973) Some problems associated with the analysis of multiresponse data. *Technometrics* 15, 33-38.
- Brash, J.L., Fritzing, B.K. & Loo, B.H. (1972) Development of materials for heart assist devices. Contract PH 43-64-84. Annual report to National Heart and Lung Institute, April 1972.
- Brophy, J.M. (1974) Mass transport and chemical kinetics of platelet adhesion to biomaterials. M. Eng. Project Report, McMaster University.
- Bugliarello, G. & Sevilla, J. (1970) Velocity distribution and other characteristics of steady and pulsatile blood flow in fine glass tubes. *Biorheology* 7, 85-107.

- Busch, C. & Olson, E.S. (1973) Density distribution of ^{51}Cr -labelled platelets within the circulating dog platelet population. *Thromb. Res.* 3, 1-12.
- Butruille, Y.A., Leonard, E.F. & Litwak, R.S. (1975) Platelet-platelet interactions and non-adhesion encounters on biomaterials. *Trans. Am. Soc. Artif. Intern. Organs* 21, 609- .
- Caro, C.G., Fitzgerald, J.M. & Schroter, R.C. (1971) Atheroma and arterial wall shear. *Proc. Roy. Soc. Lond. (Biol.)* 177, 109-159.
- Carlaw, H.S. & Jaeger, J.C. (1959) *Conduction of Heat in Solids*, Clarendon Press, Oxford. p. 248.
- Cazenave, J.-P., Packham, M.A. & Mustard, J.F. (1973a) Adherence of platelets to a collagen-coated surface: development of a quantitative method. *J. Lab. Clin. Med.* 82, 978-990.
- Cazenave, J.-P., Packham, M.A., Guccione, M.A. & Mustard, J.F. (1973b) Effects of penicillin G on platelet aggregation, release and adherence to collagen. *Proc. Soc. Exp. Biol. Med.* 142, 159-166. *
- Cazenave; J.-P., Packham, M.A., Guccione, M.A. & Mustard, J. F. (1974a) Inhibition of platelet adherence to a collagen-coated surface by non-steroidal anti-inflammatory drugs, pyrimido-pyrimidine and tricyclic compounds and lidocaine. *J. Lab. Clin. Med.* 83, 797-806.

- Cazenave, J.-P., Guccione, M.A., Mustard, J. F. & Packham, M.A. (1974b) Lack of effect of UDI, UDFG and glucosamine on platelet reactions with collagen. *Thromb. Diath. Haemorrh.* 31, 521-524.
- Cazenave, J.-P., Packham, M.A., Guccione, M.A. & Mustard, J.F. (1974c) Inhibition of platelet adherence to a collagen-coated surface by agents that inhibit shape change and clot retraction. *J. Lab. Clin. Med.* 84, 483-493.
- Cazenave, J.-P., Packham, M.A., Guccione, M.A. & Mustard, J.F. (1975) Inhibition of platelet adherence to the damaged surface of rabbit aorta. *J. Lab. Clin. Med.* 86, 551-563.
- Charmatz, A. & Karpatkin, S. (1974) Heterogeneity of rabbit platelets. I. Employment of an albumin density gradient for separation of a young platelet population identified with SE⁷⁵-selenomethionine. *Thromb. Diath. Haemorrh.* 31, 485-491.
- Chen, M.M. (1974) On the continuum formulation of platelet and related transport pertaining to thrombogenesis. A.I.Ch.E. 77th National Meeting (Paper 4a) June 1974, Pittsburgh, Pa.
- Dacie, J.V. & Lewis, S.M. (1970) *Practical Haematology*, J. & A. Churchill, London. p. 37.

- Da Trada, M. & Pletscher, A. (1968) Drugs and their effect upon the transport and storage of serotonin in platelets. *Brit. J. Pharmacol.* 34, 591-597.
- Dell, R.B., Sciacca, R., Lieberman, K., Case, D.B. & Cannon, P.J. (1973) A weighted least squares technique for the analysis of kinetic data and its application to the studies of renal ¹³³Xenon washout in man. *Circ. Res.* 32, 71-84.
- Draper, N.R. & Smith, H. (1966) *Applied Regression Analysis*, John Wiley & Sons, Inc., New York, N.Y. p. 299.
- Drost-Hansen, W. (1971) Role of water structure in cell-wall interactions. *Fed. Proc.* 30, 1539-1548.
- Dutton, R.C., Webber, A.J., Johnson, S.A. & Baier, R.E. (1969) Microstructure of initial thrombus formation on foreign materials. *J. Biomed. Mater. Res.* 3, 13-24.
- Erspaner, V. (1954) Pharmacology of Indole-alkylamines. *Pharmacol. Rev.* 6, 425-487.
- Feuerstein, I.A., Brophy, J.M. & Brash, J.L. (1975) Platelet transport and adhesion to reconstituted collagen and artificial surfaces. *Trans Am. Soc. Artif. Intern. Organs* 21, 427-434.
- Friedman, L.I. & Leonard, I.F. (1971) Platelet adhesion to artificial surfaces. *Fed. Proc.* 30, 1641-1646.

- Friedman, L.I. & Richardson, P.D. (1972) Control of flow in evaluation of blood compatibility of biomaterials. Bull. N.Y. Acad. Med. 48, 429-447.
- Friedman, L.I., Liem, M., Grabowski, E.F., Leonard, L.F., & McCord, C.W. (1970) Inconsequentiality of surface properties for initial platelet adhesion. Trans. Amer. Soc. Artif. Intern. Organs 16, 63-73.
- Friedman, M.H., O'Brien, V. & Ehrlich, L.W. (1973) Wall shear and separation in pulsatile flow through a branch. 26th ACMB 15, 305.
- Gans, H. & Krivit, W. (1962) Problems in hemostasis during open heart surgery. IV. On the changes in the blood clotting mechanism during cardiopulmonary bypass procedures. Ann. Surg. 155, 353-359.
- Garattini, S. & Valzelli, L. (1965) Serotonin; Elsevier Publishing Co., New York, N.Y. p. 169.
- George, J.N. (1972) Direct assessment of platelet adhesion to glass: a study of the forces of interaction and the effects of plasma and serum factors, platelet function and the modification of the glass surface. Blood 40, 862-874.
- Gileadi, E., Stagowski, B., Parmeggiani, A., Lucas, T., Ranganathan, N., Srinivasan, S. & Sawyer, P.N. (1972) Antithrombotic characteristics of cathodically polarized copper prostheses. J. Biomed. Mat. Res. 6, 489-497.

- Goldsmith, H.L. (1971) Red cell motions and wall interactions in tube flow. Fed. Proc. 30, 1578-1583.
- Goldsmith, H.L. (1974) The effect of flow and fluid mechanical stress on red cells and platelets. Trans. Amer. Soc. Artif. Intern. Organs 20, 21-26.
- Goldsmith, H.L. & Marlow, J.C. (1973) The effect of red cells on platelet collisions in blood flow. Fed. Proc. 32, 414.
- Grabowski, E.F. (1972) Momentum and mass transfer in blood with application to thrombus. Ph.D. Thesis. Columbia University.
- Grabowski, E.F., Friedman, L.I. & Leonard, F.F. (1972) The effects of shear rate on the diffusion and adhesion of blood platelets to a foreign surface. Ind. Eng. Chem. Fundam. 11, 224-232.
- Greenberg, J., Packham, M.A., Cazenave, J.-F., Reimers, H.-J. & Mustard, J.F. (1975) Effects on platelet function of removal of platelet sialic acid by neuraminidase. Lab. Invest. 32, 476-484.
- Harker, L.A. & Slichter, S.J. (1974) Arterial and venous thromboembolism: kinetic characterization and evaluation of therapy. Thromb. Diath. Haemorrh. 31, 188-203.
- Hearon, J.Z. (1969) Interpretation of tracer data. Biophys. J. 9, 1363-1370.

- Hellen, A.J. (1960) The adhesiveness of human blood platelets in vitro. Scand. J. Clin. Lab. Invest. 12, Suppl. 51.
- Holmsen, H. (1971) 'Platelet adenine nucleotide metabolism and platelet malfunction' in Platelet Aggregation, J. Caen (ed.), Masson & Cie, Paris. p. 109.
- Holmsen, H. & Rozenberg, M. C. (1968) Adenine nucleotide metabolism of blood platelets. I. Adenosine kinase and nucleotide formation from exogenous adenosine and AMP. *Biochim. Biophys. Acta* 155, 326-341.
- Holmsen, H., Day, H.J. & Storm, E. (1969a) Adenine nucleotide metabolism of blood platelets. VI. Subcellular localization of nucleotides with different functions in the platelet release reaction. *Biochim. Biophys. Acta* 186, 254-266.
- Holmsen, H., Day, H.J. & Stormorken, H. (1969b) The blood platelet release reaction. Scand. J. Haematol. Suppl. 8.
- Holmsen, H., Satkowsky, C. A. & Day, H.J. (1974) Effects of antimycin and 2-deoxyglucose on adenine nucleotides in human platelets. Role of metabolic adenosine triphosphates in primary aggregation, secondary aggregation and shape change in platelets. *Biochem. J.* 144, 385-396.

- Horn, F. (1971) General first order kinetics. Ber. Bunsenges. physik. Chem. 75, 1191-1196.
- Hugues, J. (1960) Accolement des plaquettes au collagene. C.R. Soc. Biol. (Paris) 154, 866-892.
- Hugues, J. & Mahieu, P. (1970) Platelet aggregation induced by basement membrane. Thromb. Diath. Haemorrh. 24, 395-408.
- Jamieson, G.A., Urban, C.L. & Barber, A.J. (1971) Enzymatic basis for platelet: collagen adhesion as the primary step in haemostasis. Nature (New Biology) 234, 5-7.
- Jeans, J. (1940) An introduction to the kinetic theory of gases, Cambridge University Press. p. 58.
- Jenkins, C.S.P., Fackham, N.A., Guccione, M.A. & Mustard, J.F. (1973) Modification of platelet adherence to protein coated surfaces. J. Lab. Clin. Med. 81, 280-290.
- Johnson, S.A. (1970) The circulating platelet, Academic Press, New York, N.Y. p. 378.
- Julius, R.S. (1972) The sensitivity of exponentials and other curves to their parameters. Comput. Biomed. Res. 5, 473-478.
- Karpatkin, S. (1969) Heterogeneity of human platelets. I. Metabolic and kinetic evidence suggestive of young and old platelets. J. Clin. Invest. 48, 1073-1082.

- Kekki, M., Niettinen, T. & Wahlstrom, H. (1973) Modelling and stimulation of cholesterol metabolism in man. Regulation and Control in Physiological Systems, A. S. Iberall & Guyton, A. C. (eds.), International Federation of Automatic Control. p. 71-73.
- Keller, K.H. (1971) Effect of fluid shear on mass transport in flowing blood. Fed. Proc. 30, 1591-1599.
- Klose, H.J., Schmid-Schonbein, H. & Brechtelsbauer, H. (1972a) Shear rate dependent blood coagulation: rheology of plasma in the coagulation of FFP. 1^{er} Congres International de Biorheologie, Lyon, France.
- Klose, H.J., Volzger, E., Brechtelsbauer, H., Heinrich, L. & Schmid-Schonbein, H. (1972b) Micro-rheology and light transmission in blood. Pfluegers Arch. 333, 126-139.
- Lee, R.G. & Kim, S.W. (1974) The role of carbohydrate in platelet adhesion to foreign surfaces. J. Biomed. Mater. Res. 8, 393-398.
- Lyman, D.J. & Kim, S.W. (1971) Interactions at the blood polymer interface. Fed. Proc. 30, 1658-1660.
- Lyman, D.J., Brash, J.L., Chaikin, S.W., Klein, K.G. & Carini, M. (1968) Effect of chemical structure and surface properties of synthetic polymers on the coagulation of blood protein and platelet interaction with polymer surfaces. Trans. Am. Soc. Artif. Intern.

- Organs 14, 250-265.
- Lyman, D.J., Klein, K.G., Brash, J.L. & Fritzing, B.K. (1970) The interaction of platelets to polymer surfaces. *Thromb. Diath. Haemorrh.* 23, 120-128.
- Lyman, B., Rosenberg, L. & Karpatkin, S. (1971) Biochemical and biophysical aspects of human platelet adhesion to collagen fibres. *J. Lab.-Clin. Invest.* 50, 1854-1863.
- Nadras, P.N., Morton, S.A. & Petschek, H.E. (1971) Dynamics of thrombus formation. *Fed. Proc.* 30, 1665-1678.
- Mann, J. & Gurpide, I. (1969) Interpretation of tracer data: significance of the number of terms of specific activity functions. *Biophys. J.* 9, 810-821.
- Markwardt, F. & Barthel, W. (1964) Untersuchungen über die Freisetzung von Serotonin aus Blutplättchen durch Thrombin. *Naunyn-Schmiedeberg's Arch. Exp. Path. Pharmac.* 249, 176-190.
- Mason, R.G., Read, M.S. & Brinkhous, K.M. (1971) Effect of fibrinogen concentration on platelet adhesion to glass. *Proc. Soc. Exp. Biol. Med.* 137, 680-682.
- Matis, H.J. & Hartley, H.D. (1971) Stochastic compartmental analysis: model and least squares estimation from time series data. *Biometrics* 27, 77-102.

- Meiselman, H.J. & Goldsmith, H.L. (1973) Blood rheology, blood flow and thrombosis. *Thromb. Diath. Haemorrh. Suppl.* 54, 273-308.
- Meiselman, H.J. & Merrill, H.W. (1967) Hemorheology: the effect of hemodilution and low molecular weight dextran. *Bibl. Anat.* 9, 288-294.
- Mitchell, J.R.A. & Sharp, A.A. (1964) Platelet clumping in vitro. *Brit. J. Haematol.* 10, 78-93.
- Murphy, E.A. (1971) 'Destruction of Blood Platelets' in *Platelet Kinetics*, J.M. Paulus (ed.), North Holland Publishing Co., Amsterdam. p. 83.
- Mustard, J.F., Rowsell, H.C., Murphy, E.A. & Downie, H.G. (1963) Evolution of the Artherosclerotic plaque, R.J. Jones (ed.) Univ. of Chicago, Ill.
- Mustard, J.F., Perry, D.W., Ardlie, N.G. & Fackham, M.A. (1972) Preparations of suspensions of washed platelets from humans. *Brit. J. Haematol.* 22, 193-204.
- Nelder, J.A. & Mead, R. (1964) A simplex method for function minimization. *Compt. J.* 7, 308-312.
- Neumann, A.W., Hope, C.J., Ward, C.A., Herbert, M.A., Dunn, C.W. & Zingg, W. (1975) The role of thermodynamics in the thromboresistance of biomaterials. *J. Biomed. Mater. Res.* 9, 127-142.

- Packham, M.A., Evans, G., Glynn, M.F. & Mustard, J.F. (1969) Effect of plasma proteins on the interaction of platelets with glass surfaces. *J. Lab. Clin. Med.* 73, 686-697.
- Paulus, J.M. (1975) Platelet size in man. *Blood* 46, 321-336.
- Petschek, H.E. (1968) Flow and thrombogenesis. Res. Rpt. 314 Avco Everett Res. Lab.
- Pletscher, A. (1968) Metabolism, transfer and storage of 5-hydroxytryptamine in blood platelets. *Brit. J. Pharmacol. Chemother.* 32, 1-16.
- Pletscher, A. & Tranzer, J.P. (1967) Action of reserpine and imipramine on the intracellular storage of 5-HT in blood platelets. *Experientia* 23, 289-293.
- Rand, P.W., Lacombe, E., Hunt, H.E. & Austin, W.H. (1964) Viscosity of normal human blood under normothermic and hypothermic conditions. *J. Appl. Physiol.* 19, 117-122.
- Reich, J.G. & Sel'kov, E.E. (1974) Mathematical analysis of metabolic networks. *FEBS Letters* 40, S119-S127.
- Reimers, H.-J., Packham, M.A., Kinlough-Rathbone, R.L. & Mustard, J.F. (1963) Effect of repeated treatment of rabbit platelets with low concentrations of thrombin on their function, metabolism and survival. *Brit. J. Haematol.* 25, 675-689.

Reimers, H.-J., Allen, D.J., Feuerstein, I.A. & Mustard, J.F. (1975a) Transport and storage of serotonin by thrombin treated platelets. *J. Cell Biol.* 65, 359-372.

Reimers, H.-J., Mustard, J.F. & Packham, M.A. (1975b) Transfer of adenine nucleotides between the releasable and non-releasable compartments of rabbit blood platelets. *J. Cell Biol.* 67, 61-71.

Reimers, H.-J., Packham, M.A. & Mustard, J.F. (1975c) Personal Communication.

Reimers, H.-J., Allen, D.J., Cazenave, J.-P., Feuerstein, I.A. & Mustard, J.F. (1975d) The effect of reserpine and imipramine on the transport and storage of serotonin by rabbit platelets. Submitted for publication.

Rivard, G. E., McClaren, J. D. & Brunst, R. F. (1975) Incorporation of hydroxanthine into adenine and guanine nucleotides by human platelets. *Biochim. Biophys. Acta* 381, 144-156.

Robertson, C. R. & Chang, H.N. (1974) Platelet retention in columns packed with glass beads. *Ann. Biomed. Eng.* 2, 361-391.

Robertson, J.S., Rostonon, D.C. & Gamble, J.L. (1957) The determination of exchange rates in three compartment steady state closed systems through the use of tracers. *J. Lab. Clin. Med.* 49, 497-503.

- Sharney, L., Wasserman, L.R., Schwartz, L. & Tandler, D.
(1963) Multiple pool analysis as applied to erythrokinetics. Ann. N.Y. Acad. Sci. 108, 230-248.
- Sheppard, B.L. (1972) The effects of acetylsalicylic acid on platelet adhesion in the injured abdominal aorta. Q. J. Exp. Physiol. 57, 319-323.
- Sheppard, B.L. & French, J.E. (1971) Platelet adhesion in the rabbit abdominal aorta following the removal of the endothelium—a scanning and transmission electron microscopy study. Proc. Roy. Soc. Lond. (Biol.) 176, 427-432.
- Sheppard, C.W. (1971) Stochastic models for tracer experiments in the circulation. III. The lumped catenary system. J. Theor. Biol. 33, 491-515.
- Silberberg, A. (1971) General fluid mechanical effects. Fed. Proc. 30, 1559-1563.
- Spaet, T.H. & Zucker, M.B. (1964) Mechanism of platelet plug formation and the role of adenosine diphosphate. Am. J. Physiol. 206, 1267-1274.
- Spaet, T.H., Stemerman, M.B. & Gaynor, E. (1973) Factors influencing the thrombogenicity of blood vessels. Microvasc. Res. 6, 248.
- Sparrow, I.N., Lin, S.H. & Lundgren, T. S. (1964) Flow development in the hydrodynamic entrance region of tubes and ducts. The Physics of Fluids 7, 338-347.

- Stewart, J.H., Farrell, F.C. & Dixon, M. (1975) Reduction of platelet/fibrin deposition in haemodialysers by aspirin administration. *Aust. N.Z. J. Med.* 5, 117-122.
- Stibbe, J., Ong, G.L., Ten Hoor, F., Nauta, J., Van Der Plas, P.M., Vergroesen, A.J., De Jong, D. & Krenning-Douma, E. (1974) Influence of prostaglandin E_1 on platelet decrease in the heart-lung machine. *Haemostasis* 2, 294-303.
- Taylor, G. (1953) Dispersion of soluble matter in a solvent flowing slowly through a tube. *Proc. R. Soc. Ser. A.* 219, 186-203.
- Texon, M. (1972) The haemodynamic basis of atherosclerosis: the ostial lesion. *Bull. N.Y. Acad. Sci.* 48, 733-740.
- Tranzer, J. R., Da Irada, M. & Pletscher, A. (1972) Storage of 5-hydroxytryptamine in megakaryocytes. *J. Cell. Biol.* 52, 191-197.
- Tsukuda, T., Steiner, N. & Baldini, M. (1971) Chromium 51 -uptake as a function of platelet age. *Scand. J. Haematol.* 8, 270-275.
- Turitto, V.T. (1975) An improved model for analysing platelet deposition on glass surfaces. *Ind. Eng. Chem. Fundam.* 14, 275.

- Turitto, V.T. & Baumgartner, H.R. (1974) Personal Communication.
- Turitto, V.T. & Baumgartner, H.R. (1975) Platelet interaction with subendothelium in a perfusion system: physical role of red cells. *Microvasc. Res.* 9, 335-344.
- Turitto, T.V. & Leonard, E.F. (1972) Platelet adhesion to a spinning surface. *Trans. Am. Soc. Artif. Intern. Organs* 18, 348-354.
- Turitto, V.T., Behis, A.M. & Leonard, E.F. (1972) Platelet diffusion in flowing blood. *Ind. Eng. Chem. Fundam.* 11, 216-223.
- Turner, M.E., Monroe, R.J. & Horner, L.D. (1963) Generalized kinetic regression analysis. *Biometrics* 19, 406-428.
- Vroman, L. (1972) What factors determine thrombogenicity? *Bull. N. Y. Acad. Med.* 48, 302-310.
- Ward, C.A., Dunn, G.W., Herbert, M.A. & Zingg, W. (1973) The role of surface imperfections in the adhesion of platelets to biomaterials. *26th ACMB* 15, 369.
- Warren, B.A., Philp, R.B. & Inwood, M.J. (1973) The ultrastructural morphology of an air embolism: platelet adhesion to the interface and endothelial damage. *Brit. J. Exp. Path.* 54, 163-172.
- Weiss, L. (1971) Biophysical aspects of initial cell interactions with solid surfaces. *Fed. Proc.* 30, 1649-1657.

- Weiss, H.J., Tschopp, T.B., Rogers, J. & Brand, H. (1974)
Studies of platelet 5-hydroxytryptamine (serotonin)
in storage pool disease and albinism. J. Clin.
Invest. 54, 421-432.
- Whitmore, R.L. (1963) Hemorheology and hemodynamics.
Biorheology 1, 201-220.
- Whitmore, R.L. (1968a) Rheology of the Circulation, Pergamon
Press, Oxford. p. 96.
- Whitmore, R.L. (1968b) Rheology of the Circulation, Pergamon
Press, Oxford. p. 76.
- Wintrobe, M.N. (1960) Clinical Haematology, Lea & Febiger,
Philadelphia, Pa.
- Wold, S. (1974) Spline functions in data analysis.
Technometrics 16, 1-11.
- Zucker, M.B. (1962) 'Serotonin (5-hydroxytryptamine):
haematological aspects' in Progress in Haematology,
L.M. Tocantins (ed.), Vol. 2, Grune & Stratton, New
York. p. 206.
- Zucker, M.B. (1972) Discussion of paper by R.G. Mason:
some methods for the in vitro estimation of the
blood compatibility of biomaterials. Bull. N.Y.
Acad. Med. 48, 425-428.
- Zucker, M.B. (1974) The value of the blood platelet in
hemostasis and as a scientific tool. Trans. N.Y.
Acad. Sci. 36, 561-568.

- Zucker, M.B. & Vroman, L. (1969) Platelet adhesion induced by fibrinogen adsorbed onto glass. Proc. Soc. Exp. Biol. Med. 131, 318-320.
- Zucker, M.B., Hellman, L. & Zumoff, B. (1964) Rapid disappearance of ^{14}C -labelled serotonin from platelets with carcinoid syndrome. J. Lab. Clin. Med. 63, 137-146.
- Zucker, M.B., Rifkin, P.L., Friedberg, N.M. & Collar, B.S. (1972) Mechanisms of platelet function as revealed by retention of platelets in glass bead columns. Ann. N.Y. Acad. Sci. U.S.A. 201, 138-144.

Appendix 1.1

The Neglect of Washout Time

Prior to the exposure of the tubular test sections to a flowing platelet suspension, the tubes are filled with a solution so as to avoid the formation of an air interface with the platelet and erythrocyte suspension within the tubes. In this appendix the time for which a platelet free layer remains close to the surface will be calculated.

Experimentally it is observed that the washout time is important only at high velocities ($10 \text{ cm}\cdot\text{sec}^{-1}$) of the platelet and erythrocyte suspension through the tubes. At these velocities a parabolic velocity profile is observed as the suspension displaces the primary solution. At lower velocities ($1 \text{ cm}\cdot\text{sec}^{-1}$), the suspension is observed to displace the solution in plug flow.

Consider fully developed Newtonian flow along a cylindrical tube. The transit time for an element of fluid at distance y from the wall is

$$\frac{t_m a}{4y}, \quad y \ll a \quad (\text{A.1.1.1})$$

where t_m is the mean transit time for flow along the tube of radius a .

If the initial conditions (time $t=0$) upon the platelet

concentration $c(t, r, x)$ at an axial position x and a radial position r are

$$c(0, r, x) = 0, \quad x > 0$$

$$c(r, r, 0) = 1, \quad t \geq 0$$

then the velocity average concentration of suspension emerging from the tube at time t is:

$$\bar{c} = 1 - \left(\frac{t_m}{2t}\right)^2, \quad t \geq t_m/2 \quad (\text{A.1.1.2})$$

Consider the tubes used experimentally. They are of length 25 cm and internal diameter 0.11 cm. For a platelet suspension flowing through the tube at 10 cm. sec^{-1}

$$t_m = 2.5 \text{ sec.}$$

and the transit time for an element of fluid 10μ from the wall is (from A.1.1.1) 70 seconds. If the diffusion of platelets were negligible, a period of 70 seconds would be required to introduce platelets into a zone 10μ from the wall along the entire length of the tube. At this time the velocity average concentration would be 0.9997. The same velocity average concentration could however be achieved within $1.5 t_m$ or 4 seconds if a platelet diffusivity of $10^{-7} \text{ cm.}^2 \text{ sec}^{-1}$ were assumed (Turitto et al. 1972).

It is thus apparent that in this apparatus, because of the red cell induced diffusion of platelets, platelets rapidly enter the zone adjacent to the wall. Hence the solution washout time may be disregarded when red cells are present in the suspension.

Appendix 2.1

Serotonin Exchange between the Platelet and its Surrounding Medium: does the Intermediate Compartment lie within the Platelet or within a Boundary Layer about the Platelets.

If the distribution of serotonin between the suspending medium and platelets is at equilibrium, net transfer of serotonin from the suspending medium to the platelet or vice versa occurring, then the uptake of serotonin by the platelet is described by first order kinetics. Consider the platelet as a sphere of radius R . When a serotonin tracer is initially located within the suspending medium, its net movement is initially described by the following equations:

$$\frac{\partial m}{\partial t} = D \left(\frac{\partial^2 m}{\partial r^2} + \frac{2\partial m}{r\partial r} \right)$$

$$D \left. \frac{\partial m}{\partial r} \right|_{r=R} = -km \Big|_{r=R}$$

$$m = m_0, \quad r \geq R, \quad t=0$$

where m is the tracer concentration, D its diffusivity, r a radius and k the rate constant for tracer absorption into the platelet. These equations have the solution (Carslaw and Jaeger, 1959):

$$m = m_0 \left(1 - KR^2 \left(\operatorname{erfc} \left(\frac{r-R}{2(Dt)^{1/2}} \right) - \exp \left(- \left(\frac{k}{D} + \frac{1}{R} \right) (r-R) + \left(\frac{k}{D} + \frac{1}{R} \right)^2 Dt \right) \cdot \left(\operatorname{erfc} \left(\frac{r-R}{2(Dt)^{1/2}} \right) + \left(\frac{k}{D} + \frac{1}{R} \right) (Dt)^{1/2} \right) \right) \right) / (r(Rk/D+1))$$

With large values of $(k/D+1/R)(Dt)^{1/2}$, at $r=R$,

$$m_{r=0} = m_0 (1 + \pi^{-1/2} R / ((D/Rk+1)(Dt)^{1/2})) / (1 + Rk/D)$$

For serotonin $D \sim 10^{-5} \text{ cm}^2 \cdot \text{sec}^{-1}$

and for platelets $R \sim 10^{-4} \text{ cm}$

and $k \sim 10^{-5} \text{ cm} \cdot \text{sec}^{-1}$

(This value of k is estimated from the experimental observation that 10% of the serotonin in the suspending medium, concentration 123 μM , enters 10^9 platelets min^{-1} under equilibrium conditions. The surface area of a platelet is 10^{-7} cm^2 .)

As then $(k/D+1/R)(Dt)^{1/2} > 30$, for $t \geq 1$ sec., the approximation for large values of $(k/D+1/R)(Dt)^{1/2}$ may be employed.

The minimum possible value for $m_{r \geq R}$ is thus

$$m_{r=R} = m_0 (1 / (1 + Rk/D)) > 0.9999 m_0 \text{ and this value is}$$

attained to within $0.00001 m_0$ by $t=1$ sec.

Thus diminution of tracer level about the platelet due to an initial flux into the platelet is negligible. As at no time is there a significant boundary layer, it is likely that the intermediate compartment lies within the platelet,

Appendix 3.1

Equations describing Serotonin Behaviour.

The proposed models are described in section 3.2. The equations associated with models I and II require no proof. Those associated with III and IV may be treated as special cases of the equation for model V. The derivation of the equation for model V follows.

In Model V it is assumed that there is serotonin exchange between circulating platelets; (between circulating platelets and dead platelets (exchange from the latter occurring immediately following their removal from the circulation) and between circulating platelets and serotonin outside the circulation, the latter containing a negligible amount of tracer.

$\Lambda(t)$, $G(t)$, $I(t)$, k , k_1 and k_2 were defined in section (3.2.1). Let platelets entering the circulation at time y , $0 < y < t$, have a specific activity of serotonin tracer $a(y,t)$ at time t . As platelets entering the circulation are assumed to carry no tracer in their serotonin

$$a(y,y) = 0, \quad 0 < y < t \quad (\text{A.3.1.1})$$

Those present in the circulation at the time of labelling have a specific activity $a(0,t)$ where $a(0,0) = 1$. (A.3.1.2)

The total platelet content of serotonin tracer is composed of tracer from platelets present in the circulation at the

time of labelling and of tracer from platelets that entered the circulation later.

$$C(t) = \int_0^{\infty} I(x+t)a(0,t)dx + \int_0^t I(t-y)a(y,t)dy$$

$$= a(0,t)A(t) + \int_0^t I(t-y)a(y,t)dy \quad (\text{A.3.1.3})$$

Let us apply a mass balance to the tracer in a platelet that entered the circulation at time y .

Then

Rate of accumulation of tracer	Rate of loss to other platelets and from the circulation	+ Rate of gain from other circulating platelets + Rate of gain from dead platelets
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or

$$\frac{da(y,t)}{dt} = -(k+k_2)a(y,t) + kC(t)$$

$$-k_1(a(0,t)\frac{d}{dt} \int_0^{\infty} I(x+t).dx + \int_0^t a(y,t)\frac{d}{dt} I(t-y).dy)$$

(A.3.1.4)

Defining

$$f(t) = \int_0^t a(y,t)\frac{d}{dt} I(t-y).dy$$

(A.3.1.4) may be rewritten as

$$\frac{da(y,t)}{dt} = -(k+k_2)a(y,t) + kC(t) + k_1(a(0,t)I(t)-f(t))$$

(A.3.1.5)

Now differentiating (A.3.1.3)

$$\frac{dC(t)}{dt} = \Lambda(t) \frac{da(0,t)}{dt} - I(t)a(0,t) + I(0)a(t,t) + \int_0^t a(y,t) \frac{d}{dt}(I(t-y)) dy$$

$$+ \int_0^t I(t-y) \frac{d}{dt}(a(y,t)) dy$$

Substituting (A.3.1.5) into this, recalling (A.3.1.1)

$$\frac{dC(t)}{dt} = -(k+k_2)\Lambda(t)a(0,t) + k\Lambda(t)C(t) + k_1(a(0,t)I(t) - f(t))\Lambda(t)$$

$$- I(t)a(0,t) + f(t) - (k+k_2) \int_0^t I(t-y)a(y,t) dy$$

$$+ kC(t)(\Lambda(0) - \Lambda(t)) + k_1(a(0,t)I(t) - f(t)(\Lambda(0) - \Lambda(t)))$$

$$= -k_2C(t) - (1-k_1)(a(0,t)I(t) - f(t))$$

$$\text{as } \Lambda(0) = 1$$

Rearranging

$$(a(0,t)I(t) - f(t)) = \left(\frac{dC(t)}{dt} + k_2C(t) \right) / (k_1 - 1)$$

Substituting this into (A.3.1.5)

$$\frac{da(y,t)}{dt} = -(k+k_2)a(y,t) + kC(t) - k_1 \left(\frac{dC(t)}{dt} + k_2C(t) \right) / (1-k_1)$$

or

$$\frac{d}{dt}(a(y,t)e^{(k+k_2)t}) = (kC(t)e^{(k+k_2)t} - k_1 \frac{d}{dt}(C(t)e^{(k+k_2)t})) / (1-k_1)$$

For $y = 0$, integrating from 0 to t , with the initial conditions

$$a(0,0) = 1, C(0) = 1.$$

$$a(0,t) = (e^{-(k+k_2)t} - k_1 C(t) + k_0 e^{-(k+k_2)t} \int_0^t C(x) e^{(k+k_2)x} dx) / (1-k_1)$$

(A.3.1.6)

Similarly, for $0 < y < t$, integrating from y to t with the

initial condition $a(y,y) = 0$,

$$a(y, t) = \frac{(k_1 C(y) e^{-(k+k_2)(t-y)} - k_1 C(t) + k e^{-(k+k_2)t} \int_y^t C(x) e^{(k+k_2)x} dx)}{(1-k_1)} \quad (\text{A.3.1.7})$$

for $0 < y \leq t$

Substituting (A.3.1.6) and (A.3.1.7) into (A.3.1.3) and simplifying

$$C(t) = e^{-(k+k_2)t} \left(\Lambda(t) (1+k) \int_0^t C(x) e^{(k+k_2)x} dx \right. \\ \left. + \int_0^t I(t-y) \left(k \int_y^t C(x) e^{(k+k_2)x} dx \right. \right. \\ \left. \left. + k_1 C(y) e^{(k+k_2)y} dy \right) \right) \quad (\text{A.3.1.8})$$

This is the equation for model V.

In model IV it was assumed that there was serotonin exchange only between circulating platelets. Then $k_1 = k_2 = 0$.

With these values substituted, (A.3.1.8) becomes

$$C(t) = e^{-kt} \left(\Lambda(t) (1+k) \int_0^t C(x) e^{kx} dx \right. \\ \left. + k \int_0^t I(t-y) \int_y^t C(x) e^{kx} dx \right) \quad (\text{A.3.1.9})$$

This is the equation for model IV.

In model III it was assumed that there was no serotonin exchange between circulating platelets. Then $k = 0$.

With this value, (A.3.1.8) reduces to

$$C(t) = e^{-k_2 t} \left(\Lambda(t) + k_1 \int_0^t I(t-y) C(y) e^{k_2 y} dy \right) \quad (\text{A.3.1.10})$$

This is the equation for model III.

Appendix 4.1

Nucleotide Tracer Distribution in Platelets.

The model to be considered is shown in Fig. 4.13.

The movement of tracer between the compartments is described by the following series of differential equations. $m_1(t)$ is the level of tracer at time t in the platelets' metabolic pool; $m_2(t)$ is the level of tracer in the granule pool and $m(t)$ is the sum of the above - the total level of serotonin within the platelet.

i.e. $m(t) = m_1(t) + m_2(t)$

For the metabolic pool

$$\frac{dm_1}{dt}(t) = -(a_1 + a_3)m_1(t) + a_2m_2(t) \quad (\text{A.4.1.1})$$

For the granule pool

$$\frac{dm_2}{dt}(t) = -a_2m_2(t) + a_1m_1(t)$$

with initial conditions

$$m_1(0) = m(0)$$

$$m_2(0) = 0 \quad (\text{A.4.1.2})$$

Taking the Laplace Transform of (A.4.1.1) and substituting in (A.4.1.2)

$$sm_1(s) - m(0) = -(a_1 + a_3)m_1(s) + a_2m_2(s) \quad (\text{A.4.1.3})$$

$$sm_2(s) = -a_2m_2(s) + a_1m_1(s)$$

solving

$$m_1(u) = m(0) \left((a_2 - \lambda_1) / (u + \lambda_1) - (a_2 - \lambda_2) / (u + \lambda_2) \right) / (\lambda_2 - \lambda_1)$$

$$m_2(u) = a_1 m(0) (1 / (u + \lambda_1) - 1 / (u + \lambda_2)) / (\lambda_2 - \lambda_1)$$

where

$$\lambda_1 = \frac{a_1 + a_2 + a_3}{2} + \frac{((a_1 + a_2 + a_3)^2 - 6a_2a_3)^{1/2}}{2}$$

$$\lambda_2 = \frac{a_1 + a_2 + a_3}{2} - \frac{((a_1 + a_2 + a_3)^2 - 6a_2a_3)^{1/2}}{2}$$

Hence

$$m_1(t) = m(0) \left((\lambda_1 - a_2) e^{-\lambda_1 t} + (a_2 - \lambda_2) e^{-\lambda_2 t} \right) / (\lambda_1 - \lambda_2)$$

$$m_2(t) = a_1 m(0) (e^{-\lambda_2 t} - e^{-\lambda_1 t}) / (\lambda_1 - \lambda_2)$$

and

$$m(t) = m(0) \left((\lambda_1 - a_2 - a_1) e^{-\lambda_1 t} + (a_1 + a_2 - \lambda_2) e^{-\lambda_2 t} \right) / (\lambda_1 - \lambda_2)$$

(A.4.1.4)

Atkins (1969b) in his derivation incorrectly defined the eigenvalues λ_1 and λ_2 . The equations may be expressed as a fraction of the initial platelet bound tracer by setting $m(0) = 1$.

Appendix 4.2

inclusion of loss from platelets during of contributions

Summing the contributions to the level of tracer in the platelets defined in equations (4.4), (4.6) and (4.7) we have:

$$\begin{aligned}
 C(t) &= e^{-at} \int_0^{\infty} I(x+t) (1 - e^{-ax}) dx \\
 &+ \mu(t) \int_0^{\infty} I(x+t) e^{-a(x+t)} dx \\
 &+ e^{-at} \int_0^{\infty} I(x+t) e^{-ax} \int_0^t \alpha m_1(y) e^{-ay} e^{ay} dy
 \end{aligned}
 \tag{A.4.2.1}$$

Before rearranging (A.4.2.1) the following derivation will be made:

$$\int_0^{\infty} I(x+t) e^{-ax} dx = e^{at} \int_t^{\infty} I(x) e^{-ax} dx$$

Recalling that

$$\int_t^{\infty} I(x+t) dx = \Lambda(t)
 \tag{A.4.2.2}$$

then, integrating by parts

$$\begin{aligned}
 \int_t^{\infty} I(x) e^{-ax} dx &= [(\Lambda(0) - \Lambda(x)) e^{-ax}]_t^{\infty} \\
 &+ a \int_t^{\infty} e^{-ax} (\Lambda(0) - \Lambda(x)) dx \\
 &= \Lambda(t) e^{-at} + a \int_t^{\infty} \Lambda(x) e^{-ax} dx
 \end{aligned}$$

Hence

$$\int_0^{\infty} I(x+t) e^{-ax} dx = \Lambda(t) + a e^{at} \int_t^{\infty} \Lambda(x) e^{-ax} dx
 \tag{A.4.2.3}$$

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