DEVELOPMENT OF LABELLED PLATELETS

AS RADIOPHARMACEUTICALS

by

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Summary

The choice of platelet radiolabelling agent and labelling technique has been evaluated since accurate diagnosis is dependent on retaining normal function of the labelled cells. The two labelling agents, indium-111 oxine and indium-111 tropolone were used and indium-111 oxine was found to be the superior platelet labelling agent. Maintenance of viability was demonstrated using *in vitro* aggregation tests.

Having established a successful labelling method, techniques to determine *in vivo* platelet kinetics were evaluated and protocols for splenic uptake and platelet survival were developed. Hepatic uptake was investigated in order to assess *in vivo* platelet function of the manipulated sample since liver time-activity curves are sensitive indicators of platelet activation. A protocol was developed to compare the blood and liver activity curves. Compartmental analysis techniques for determining splenic blood flow and intrasplenic platelet transit time were also used but found to be difficult to adopt as the calculation is dependent upon a subjective estimate. Further work is required to establish a satisfactory protocol.

Radioactively labelled platelets have successfully been used in the diagnosis of idiopathic thrombocytopenia (5 patients) and deep vein thrombosis (7 patients). The results have been clinically useful in the further management of the patient.

Platelet kinetics were also investigated in patients with inflammatory bowel disease (12 patients). *In vitro* aggregation tests on such patients had demonstrated abnormal platelet function. It was thought that this abnormality may contribute to the increase in thrombotic complications reported in inflammatory bowel disease. Therefore, kinetics studies were performed to determine whether abnormalities in distribution and survival could be detected. However, platelet distribution and survival were found to be within the normal ranges.

Key words

indium-111 labelled platelets
platelet kinetic studies
haematological disorders
radiopharmaceuticals
inflammatory bowel disease

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ABBREVIATIONS

ACD	acid-citrate dextrose
ADP	adenosine di-phosphate
ATP	adenosine tri-phosphate
cAMP	cyclic adenosine monophosphate
Ca^{2+}	calcium ion
C	Crohn's disease
^{51}Cr	^{51}chromium
CVT	cortical vein thrombosis
DRH	Dudley Road Hospital
DTPA	diethylenetriaminpenta-acetic acid
DVT	deep vein thrombosis
FDP	fibrin degradation products
^{67}Ga	^{67}gallium
GIH	gastrointestinal haemorrhage
HMPAO	hexamethylpropyleneamine oxime
5-HT	5-hydroxytryptamine
IBD	inflammatory bowel disease
ICSH	International Committee on Standardisation in Haematology
Ig	immunoglobulin
^{111}In	^{111}Indium
^{123}I	^{123}Iodine
^{125}I	^{125}Iodine
ITP	idiopathic thrombocytopenia purpura
LE	labelling efficiency
PE	pulmonary embolism
Pg	prostaglandin

SBF	splenic blood flow
TBV	total blood volume
$^{99}Tc^{m}$	^{99}technetium^{m}
TIA	transient ischaemic attack
tp	intrasplenic platelet transit time
Tx	thromboxane
UC	ulcerative colitis
^{133}Xe	^{133}Xenon

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1. INTRODUCTION

1.1 PLATELETS

1.1.1 Structure and Function

Platelets are formed in the bone marrow by fragmentation of the cytoplasm of megakaryocytes. The time interval between differentiation of the stem cell and production of platelets averages 10 days in man. The circulating platelet count is constant, the normal range in man being 150 - 400 x 10^9L^{-1} (1,2). Any disturbance, such as depletion or infusion of platelets, is rapidly followed by compensatory changes in platelet production. An increase in platelet production is mediated via a humoral factor termed thrombopoetin. On entering the circulation newly formed platelets are sequestered in the spleen for about 3-4 days (3). This platelet pool has been estimated to be as great as one-third of the total platelet mass in man (1). Platelet life span is between 8-10 days (2). Senescent platelets are cleared from the circulation by the reticulo-endothelial system (3).

The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury (4). The term haemostasis is usually taken to apply to the arrest of haemorrhage from damaged blood vessels and involves interaction between platelets and components of the vessel wall. So long as the endothelial lining of the vasculature remains intact, haemorrhage does not occur (1,4). The endothelial cell was formerly thought to be inert with respect to platelets and blood coagulation factors, but it has now been recognised that it has an active role in haemostasis. Endothelial cells are the chief site of prostaglandin I_2 production, an inhibitor of platelet aggregation (1,4). The structure and properties of platelets are such

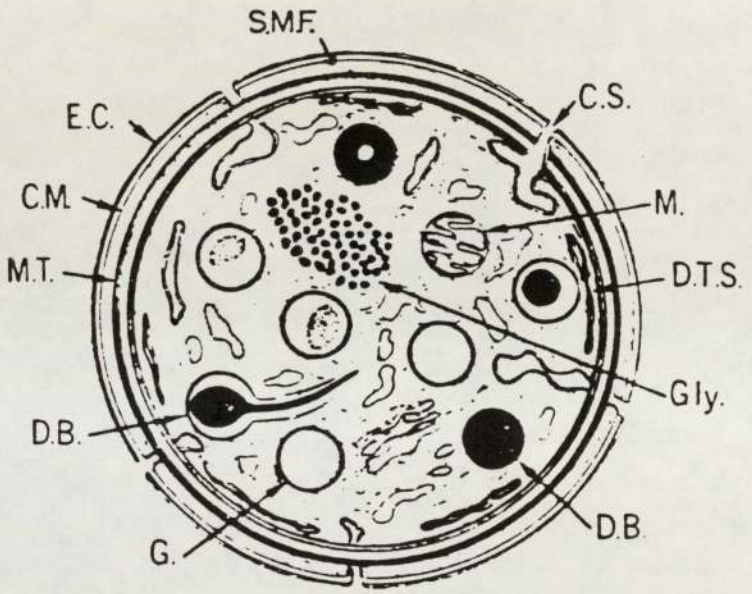
that they play a central role in the sequence of events which follows injury to a blood vessel. Platelets in their resting state have a round/discoid shape with a smooth surface (figure 1.1 (1)).

The surface of the platelet contains glycoproteins which are the site of absorption of various plasma proteins and plays an important part in adhesion and aggregation. Microtubules below the membrane have a skeletal function helping maintain platelet shape. During aggregation they contract towards the centre causing the platelet to change its shape to spheres with psuedopodia of varying lengths from the surface of the cell. Microfilaments are widely distributed throughout the platelet cytoplasm and are involved in the change of shape of platelets in response to stimulation. Platelet granules termed dense bodies and α-granules, contain many substances which are active in platelet aggregation and initiation of coagulation, including fibrinogen, 5-hydroxytryptamine (5-HT), adenosine tri-phosphate (ATP), adenosine di-phosphate (ADP), adrenaline and calcium ions (1,4).

The haemostatic function of platelets depends on their 3 fundamental activities of adhesion, contraction and secretion. When vascular endothelium is damaged and subendothelial tissue, particularly collagen, is exposed, platelets adhere to the site and initiate the formation of an haemostatic plug. This process is continued by aggregation of platelets (1,4).

The platelets then contract and initiate the release reaction. The substances released from the dense bodies and α-granules of platelets will constrict small blood vessels and further the process of aggregation. The whole process of haemostasis is very rapid, adhesion and aggregation taking place within seconds of the injury.

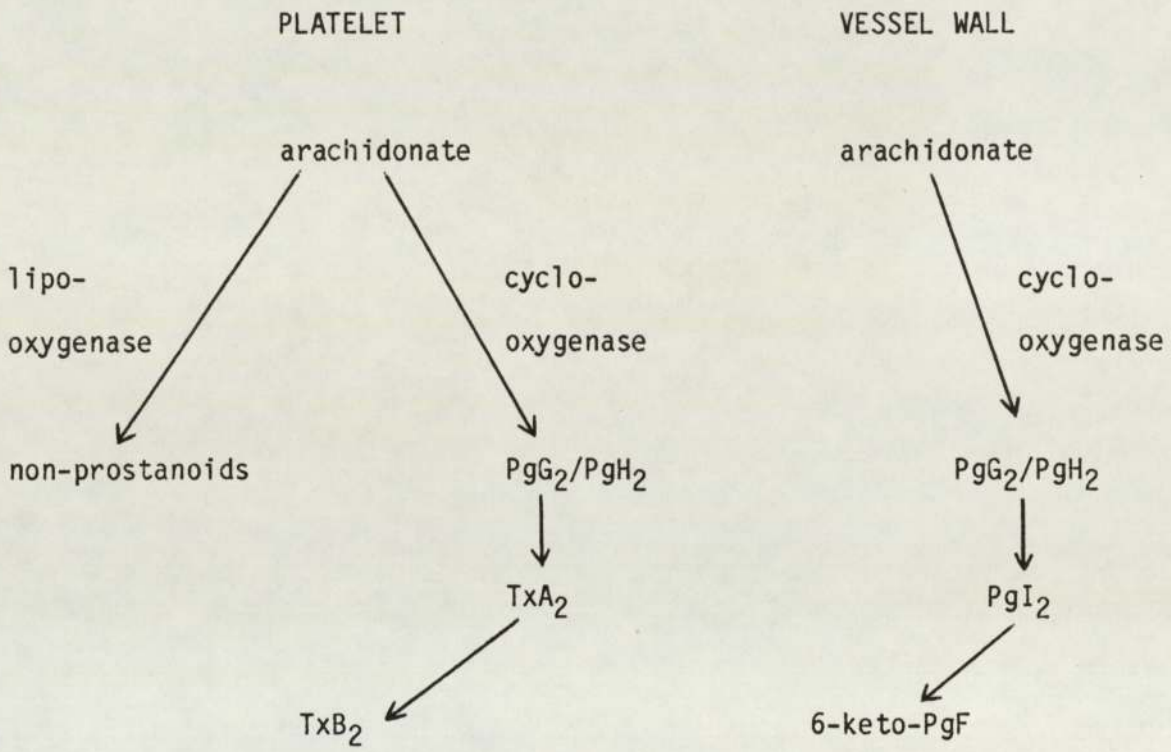
The release reaction triggers the synthesis of thromboxane A_2 from arachidonic acid within platelets. Thromboxane A_2 is a promoter of



E.C.	external coat
C.M.	cell membrane
M.T.	microtubules
D.B.	dense body
G.	alpha granule
Gly.	glycogen granule
D.T.S.	dense tubular system
M.	mitochondrion
C.S.	surface-connected system

Figure 1.1 Diagram of a Platelet in Equatorial Section

aggregation. In contrast, arachidonic acid metabolism in the vessel wall is also stimulated to synthesize prostaglandin I_2, an inhibitor of aggregation. Each pathway is shown below (1).



Pg = prostaglandin

Tx = thromboxane

The product of each pathway interacts competitively to maintain an haemostatic balance. Each pathway requires the presence of Ca^{2+}. The process can therefore be inhibited by agents which increase cyclic adenosine monophosphate (cAMP) concentrations within platelets and thus remove available Ca^{2+}. These include: i) cyclo-oxygenase inhibitors: aspirin, indomethacin, ii) membrane stabilizers: chlorpromazine, propranolol, local anaesthetics, iii) inhibitors of contractile elements: colchcine, vinca alkaloids, iv) components which increase cAMP: PgE_1 , PgD_2, PgI_2, v) phosphodiesterase inhibitors: dipyridamole,

theophylline.

Many attempts have been made to detect abnormalities in arachidonate metabolism. One approach has been to measure the capacity of platelets and vessels to produce thromboxane B_2 and PgI_2. This is determined *in vitro* by measuring the circulating levels of thromboxane B_2 or 6-ketoprostaglandin (a degradation product of PgI_2).

Platelets also appear to have a role in fibrinolysis (1). This is the process by which a fibrin clot is enzymatically degraded into fibrin degradation products (FDP). The dominant mechanism in fibrinolysis is the conversion of plasminogen to plasmin. Plasmin then splits the peptide bond in fibrin. There are a number of plasminogen activators and inhibitors found in body tissues, usually associated with the endothelial layer of vessels (1). A plasminogen activator has been found in trace amounts in platelets, but an inhibitor of plasminogen activation has also been isolated from platelets. The net biological effect of these activities on fibrinolysis is difficult to assess.

All these processes are important in maintaining the haemostatic balance. Each pathway can be investigated using *in vitro* tests which will be discussed in the next section.

1.1.2 Assessment of Platelet Function

Several *in vitro* investigations are available to assess platelet function. These tests include assessment of platelet function in adhesion, aggregation and fibrinolysis.

Platelet adhesion can be investigated *in vitro* by determining the extent of platelet retention on glass-bead filters. When blood, anticoagulated with acid-citrate or heparin, is passed through a column of minute glass beads some platelets remain trapped on the column. The percentage of retained platelets can be calculated by performing

platelet counts on the sample before and after passage through the column. Platelet retention is probably due to platelet adhesion to glass and platelet aggregates forming on the column which become trapped by the beads. Normal subjects yield platelet adhesiveness values of 59-99% (5).

The study of platelet aggregation is essential not only in the investigation of a patient with suspected platelet dysfunction but also to assess platelet viability of a manipulated sample. Platelets are very fragile and must be handled with great care. The turbidometric technique for performing *in vitro* platelet aggregation is the most widely used system and consists of a mechanism that monitors light transmission through the sample of platelets in plasma. The addition of an aggregating agent causes increasingly larger platelet aggregates to form with a corresponding increase in amount of light transmitted. Interpretation of these curves is qualitative but in relative terms certain changes can indicate platelet dysfunction, or after *in vitro* manipulation can indicate platelet damage. The aggregation stimulating agents primarily used for these studies are ADP, collagen, arachidonic acid and adrenaline (1).

ADP is normally the first aggregating agent used in the test. A single wave of aggregation (first phase aggregation) is produced by a concentration of 10 $\mu molL^{-1}$ ADP (figure 1.2). Responses are somewhat variable with the weaker ADP concentrations. Commonly, there is a biphasic aggregation (second phase aggregation) at 2.5 $\mu molL^{-1}$ and 5 $\mu molL^{-1}$ concentrations (figure 1.3). When only first phase aggregation is obtained at high ADP concentrations the platelets may deaggregate and the curve returns to base line. This may be observed in damaged cells. Severely damaged cells may not demonstrate any degree of aggregation. During the first phase aggregation, single platelets

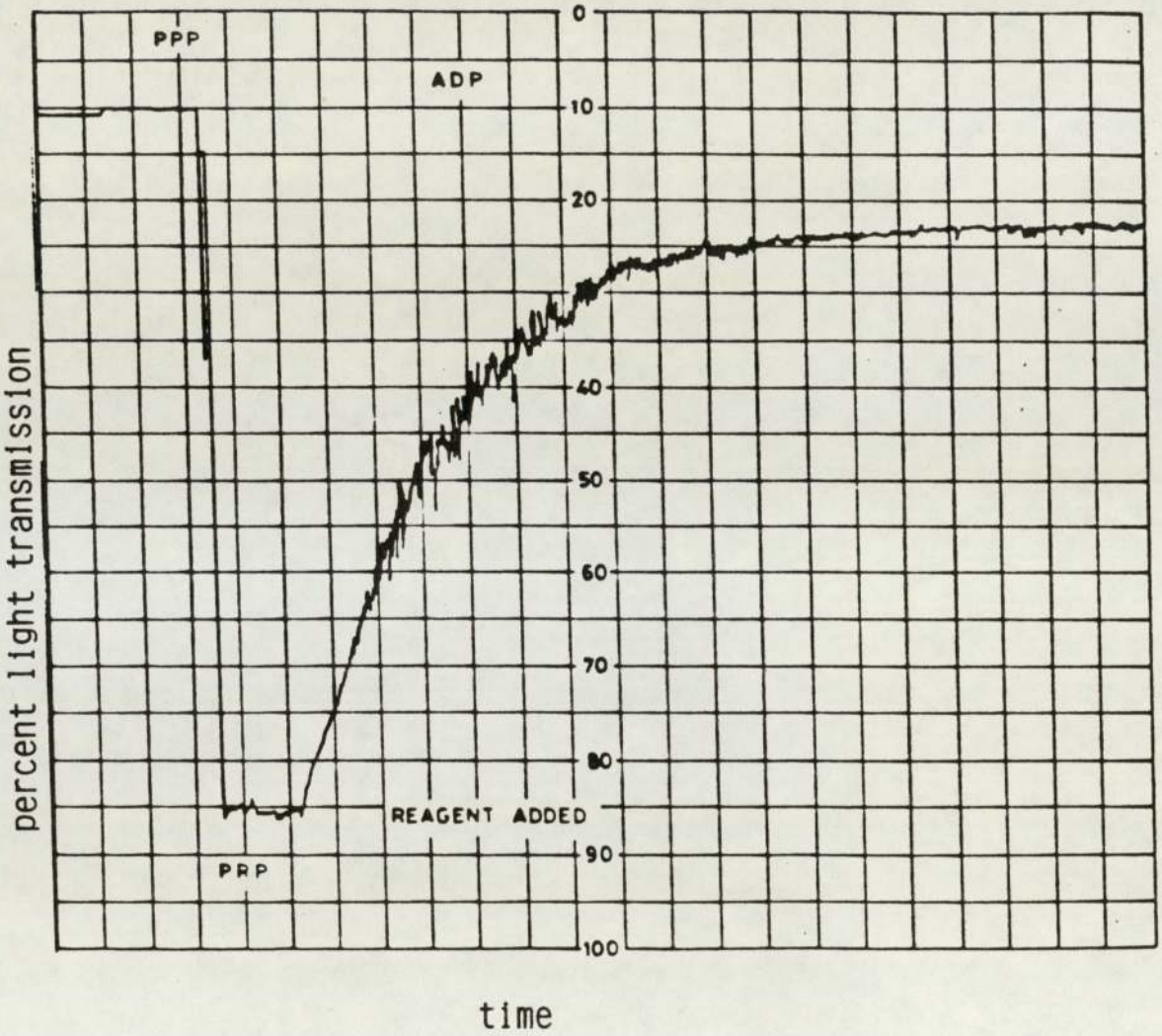


Figure 1.2 Platelet Aggregation in Response to 10 μmol ADP

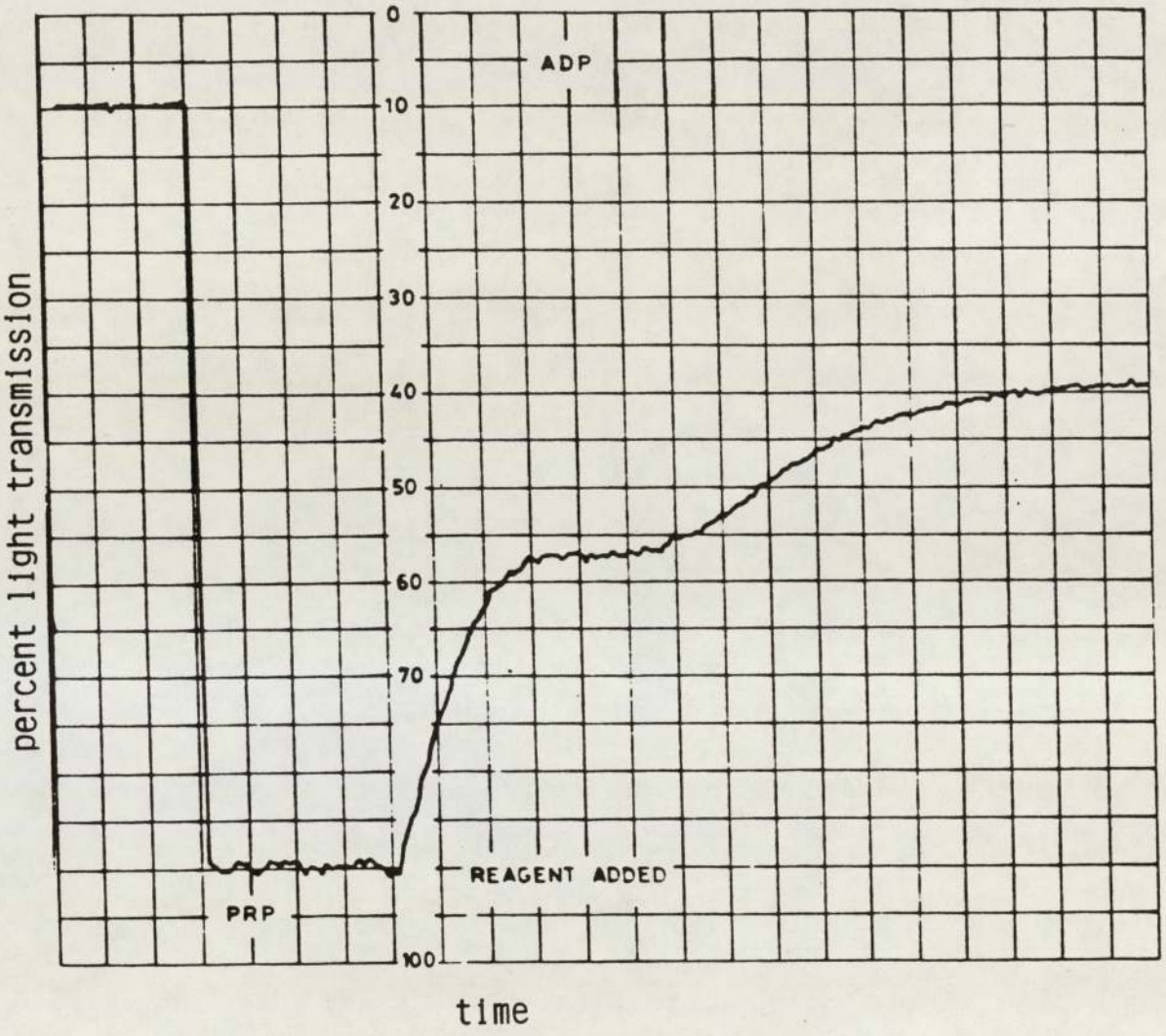


Figure 1.3 Platelet Aggregation in Response to 2.5-5 μmol ADP

adhere to each other to form small aggregates. The second phase aggregation is associated with the release of ADP from the platelets themselves. When assessing platelet function the aim is to add a concentration of ADP that will produce the second phase of aggregation hence demonstrating that the platelet has retained its ability to release ADP after physical handling.

Fibrinolysis can be assessed using the following tests: i) euglobulin clot lysis time, ii) urokinase sensitivity, iii) fibrin degradation products.

Euglobulin clot lysis time is a sensitive test for plasminogen activator which is present together with plasminogen and fibrinogen in the euglobulin fraction of plasma, whereas natural inhibitors of plasminogen conversion are not. The euglobulin fraction of the test plasma is clotted with thrombin and the lysis of the resultant clot is a measure principally of the plasminogen activator. The normal range is 90-240 minutes (2). The urokinase sensitivity test is another method to assess the fibrinolytic pathway. Urokinase converts plasminogen to plasmin which causes clot dissolution. The speed of clot lysis is an indirect measure of the inhibition of plasmin activity by urokinase (2). The measurement of the products of plasmin digestion of fibrinogen or fibrin provides an indirect test for fibrinolysis. FDP can be detected in several ways, the most sensitive method being immunological. They interfere with the thrombin-fibrinogen reaction and cause a prolongation of the thrombin clotting time of plasma (2).

1.2 LABELLED PLATELETS

1.2.1 Labelling Techniques

In nuclear medicine and haematological investigations, blood cells are used to carry gamma-emitting radionuclides around the body. The use of radiolabelled cells allow their biodistribution in the body to be studied. These applications are only of clinical use if the labelled cells behave in the same way as their unlabelled counterparts, i.e. are not modified or damaged as a result of the labelling and if the radionuclide remains firmly associated with the cells throughout the investigation. For non-invasive studies the cells must be labelled with a gamma-emitting radionuclide which can be detected with external monitoring devices such as gamma cameras. The radionuclide must emit gamma rays of a suitable energy and abundance for external detection and have a half life which is sufficiently long to allow investigations to be completed.

Until 1976, blood cells were labelled with one of 3 gamma-emitting radionuclides, ^{51}Cr ^{67}Ga or $^{99}Tc^{m}$. Neither ^{51}Cr nor ^{67}Ga have ideal characteristics for imaging: ^{51}Cr has a long half-life (27.7 days) and the 320 keV gamma-ray used for imaging is produced in only 10% of disintegrations; ^{67}Ga has a shorter half-life (78.3 hours) and emits a mixture of gamma-rays at 93, 184, 209, 300 and 393 keV. $^{99}Tc^{m}$ has a very short half-life of 6 hours but an ideal 89% abundance of the 141 keV gamma-ray. However, ^{51}Cr as sodium chromate, ^{67}Ga as gallium citrate, and $^{99}Tc^{m}$ as reduced pertechnetate do not label platelets satisfactorily although ^{51}Cr has been widely used to label platelets (6).

In 1976 Indium-111 was introduced as a cell labelling agent (7). It is currently the preferred radionuclide for labelling platelets for several reasons: i) ^{111}In complex remains firmly associated with the

blood cells throughout the investigation ii) ^{111}In emits gamma-rays of suitable energy and abundance for external detection (171 (88%) and 245 (94%) keV) iii) the half-life of ^{111}In is sufficiently long to allow platelet survival investigations to be completed (67 hours).

Ionic indium, as In^{3+}, will not penetrate cells. However, it will form complexes with a wide range of lipophilic bidentate chelating agents (ligand). These include 8-hydroxyquinoline (oxine), acetylacetonate (acac), tropolone, and 2-mercaptopyridine-N-oxide (merc). The mechanism by which ^{111}In complexes label cells is not fully understood, but the proposed scheme is that the ligand transports the ^{111}In through the cell membrane by passive diffusion. Once inside the cell the complex dissociates and the ^{111}In is transferred to some intracellular components which hold it firmly within the cell. The ligand then diffuses out of the cell (8).

Of these ligands, oxine and tropolone are the chelating agents most commonly used. Both of these ^{111}In complexes are not cell specific and cell separation is necessary as part of the labelling procedure. The range of transference mechanisms, centrifugation speeds, anticoagulants and cell suspension media described in the literature is wide. However, the choice is critical for platelets where loss in aggregation ability may be due to physical handling rather than chemical or radiation damage inflicted during labelling (9).

The ability of an ^{111}In complex to label cells is assessed by measuring the labelling efficiency (L.E.). L.E. is affected by many factors so different values can be readily obtained for any ^{111}In complex (6). However, the highest L.E. is not automatically the best. Maintenance of viability and function of the labelled cells is much more important and this may only be achieved with lower labelling efficiences. There are 4 main conditions that can alter L.E.: i) the

proportion of plasma in the medium can effect L.E. as ^{111}In will non selectively bind to cells and plasma proteins. The optimum pH of the medium has been shown to be between 6.5-7.5. At this pH the platelets are less likely to aggregate whilst isolated from plasma (6), ii) the amount of ligand that is mixed with the $^{111}InCl_3$ can alter L.E.. Approximately 10^2 to 10^6 times more ligand than indium is required before cell labelling can occur (6), iii) the cell concentration to give a satisfactory L.E. is routinely 0.5-1.0ml cells at $5 \times 10^8 ml^{-1}$ of platelets (6), iv) incubation time and temperature can effect L.E.. Cell labelling is rapid at 37^oC or at room temperature but much slower at 4^oC (6).

As a platelet labelling agent, ^{111}In oxine suffers from two disadvantages. First, the platelet labelling efficiency in plasma is low when compared with that in plasma free media. This has led many investigators to omit plasma from the incubation medium (10). However, the incubation of platelets in the absence of plasma during the labelling procedure may damage the platelet and alter its behaviour. Secondly, ^{111}In oxine is insoluble in aqueous solution. A small quantity of ethanol was routinely added to dissolve it which was potentially toxic to the platelets. Now a surface active agent, polysorbate 80, is added in the hope of reducing potential toxicity to the platelets (6).

Due to these disadvantages new agents were investigated and tropolone was introduced in 1981 as a new chelating agent (6). Tropolone can label platelets with high efficiency even in the presence of plasma (11). This eliminates the potential of damaging the platelets whilst they are isolated from plasma in the labelling procedure. Another potential advantage of tropolone is that it is soluble in aqueous media. Hence, no solvent that could adversely affect platelet

function is necessary for cell labelling.

However, despite these advantages, tropolone has not replaced oxine as the chelating agent of choice in platelet labelling. The explanation for this is that it is the cell handling during the labelling procedure that is the most important factor for the viability of the platelet population. The practice which is essential for the success of any of the methods has made most nuclear medicine departments hesitant to change to tropolone.

There have been various reports on the comparison of tropolone and oxine in the search for a superior labelling agent (12,13,14). Each study reported a higher labelling efficiency for ^{111}In tropolone compared to ^{111}In oxine. However, no viability loss could be detected using aggregation tests, neither with tropolone nor oxine. The authors all conclude that the *in vivo* kinetics of ^{111}In oxine and ^{111}In tropolone-labelled platelets are similar (12,13,14).

Since the introduction of ^{111}In oxine, many investigators have labelled platelets with ^{111}In oxine using different experimental conditions. Hawker *et al* in 1980 described a method in which they successfully labelled platelets in a plasma free medium using ^{111}In oxine (10). This method is designed to minimise trauma and maintain physiological conditions to retain normal platelet function. The use of acid-citrate as the anticoagulant lowers the pH of platelet suspension to approximately 6.5, making the platelets less likely to aggregate (10). Calcium-free tryrodes solution and prostaglandin E_1 used as the buffer solution are inhibitors of aggregation and assist dispersion of the centrifuged platelets. The platelets are kept in a plasma free state for only 60 seconds at 37^{o}C. These conditions achieve optimum uptake of ^{111}In oxine. The technique describes in detail the careful washing of the platelet button and resuspension in the buffer which

minimises red blood cell, white blood cell contamination and loss of platelets. No special equipment is required but practice is essential for the success of the method. This technique has been used in the clinical investigations performed in this work and is described in section 2.1.

Hawkers technique has been used extensively and has been adopted as the recommended method of platelet labelling for the product leaflet of one commercial supplier of ^{111}In oxine (15). However, this method has not been without its criticism. Mortelmans *et al* reported this procedure to damage platelets when looking at the *in vivo* characteristics of platelets labelled using Hawkers technique (16). A reply to this report stated that Mortelmans did not use the exact method described, highlighting the difficulty in successfully reproducing a technique (17). It appears unlikely that universal agreement on an optimal method will be achieved. Many labelling techniques developed in various departments give the best results in their original environment. However, despite the above report, the method described by Hawker seems to be a notable exception having been used by many authors successfully (18,19,20,21,22).

Tropolone was introduced in 1981 when 2 independent reports appeared in the literature (11,23). Again each department used different experimental procedures. Tropolone has now replaced oxine for clinical use in a few centres (11). The method described by Danpure *et al* includes several important steps which are designed to prevent trauma to the platelets (11). The anticoagulant, acid-citrate dextrose (ACD), is used to maintain the pH in the optimum range of 6.5-7.5. Labelling with tropolone allows the platelets to be labelled in plasma so maintaining a normal physiological environment. The quantity of tropolone added to the indium chloride ($InCl_3$) is critical for

maximal labelling efficiency (11). This method has also been performed and evaluated in this work and is described in section 2.2.

A new agent that has generated a lot of interest in the field of cell labelling is hexamethylpropyleneamine oxime (HMPAO). HMPAO is a lipid soluble complex very similar to oxine and tropolone. The advantage it has is that it is labelled with $^{99}Tc^{m}$. This radionuclide is convenient to use, cheap, widely available and has superior imaging properties when compared with ^{111}In. $^{99}Tc^{m}$ HMPAO is primarily used to label granulocytes because it is most stable on these cells but Becker *et al* have labelled platelets isolated in saline with $^{99}Tc^{m}$ HMPAO successfully (24). The time-activity curves recorded over the cardiac blood pool and spleen were comparable to those of ^{111}In oxine labelled platelets. The maximum activity was in the spleen and a lower activity was seen in the liver. Immediately after the renal perfusion of the labelled platelets a $^{99}Tc^{m}$ complex is excreted by the kidneys and results in kidney and bladder visualisation. At 4 hours post injection intestinal excretion also takes place. This pattern of uptake makes it unsuitable for the biodistribution studies of platelets (24). Also, $^{99}Tc^{m}$ HMPAO complex cannot be used for platelet survival studies. The short physical half-life of $^{99}Tc^{m}$ makes it unsuitable to monitor the survival of a cell that has a normal life span of 9 days. The one area where $^{99}Tc^{m}$ HMPAO labelled platelets may have an advantage over ^{111}In oxine is in the diagnosis of deep vein thrombosis as the use of $^{99}Tc^{m}$ allows earlier imaging (24). The smaller radiation dose to the spleen, compared to ^{111}In-oxine, also makes repeated tests possible.

The main drawback of current techniques for ^{111}In labelled platelets is the technical skill required for cell separation. In a search for new methods of cell labelling using cell specific agents which can label cells in whole blood the potential of monoclonal

antibodies has been considered. Antibodies are produced in response to the introduction of foreign substances (antigens) and these bind to specific sites on the surface of the antigen. Antibodies are therefore exclusively produced in response to a particular antigen (25).

A platelet specific monoclonal antibody has been introduced with the aim of imaging thrombus in man (26). P256 is an IgG mouse monoclonal antibody (26). It is derived from fusion of the P3-NS1/-1Ag4-1 myeloma cell line with spleen cells from BALB/c mice immunised with human peripheral blood mononuclear cells (27). The antibody reacts with the IIb component of the platelet membrane glycoprotein complex IIb/IIIa (27). P256 is specific for the IIb component. The complex is thought to function in platelet-platelet aggregation and to serve on platelets as a receptor for fibrinogen which is instrumental in the participation of platelets in coagulation (28). P256 has been labelled successfully with ^{111}In using diethylenetriaminepenta-acetic acid (DTPA) as the chelating agent (29).

As with ^{111}In oxine labelled platelets, it is important that P256 does not disturb platelet function. This can be studied *in vitro* by measuring the rates of spontaneous aggregation and aggregation in response to proaggregants as described previously (2). It has been shown that platelets carrying P256 on their surface underwent spontaneous aggregation to an extent that was proprotional to the percentage of receptors assumed to be occupied (26). A platelet has been calculated as having 40,000 receptors for P256 (26). At or below an occupancy of 3%, however, no difference in spontaneous aggregation could be detected between platelets with P256 and unlabelled platelets (26). Therefore, one must aim for occupancy of less than 3% if platelet function is not to be disturbed. In these conditions, ^{111}In monoclonal antibodies are a successful and convenient method of labelling

platelets.

1.2.2 Kinetic Studies

Studies of the kinetics of blood platelets were not possible until methods for isotope labelling of a representative population of platelets were developed as described earlier. This technique has made it possible to study various details relating to platelet: i) distribution, ii) survival and iii) sites of destruction.

Distribution

Penny *et al* in 1966 first demonstrated a pool of splenic platelets in dynamic equilibrium with extrasplenic circulating platelets (30). This was confirmed by Aster *et al* on the basis of surface counting over the spleen following injection of ^{51}Cr labelled platelets (31). Since the introduction of ^{111}In there have been many studies on the distribution of platelets within the body (32,33,34,35,36,37). The dynamic equilibrium of platelets within the blood pool and spleen is interpreted on the basis of a two-compartmental model (32,36). The input is taken to be the product of the spleen blood flow and arterial indium concentration and the output proportional to the splenic indium content. i.e.:

$$\text{BLOOD} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} \text{SPLEEN}$$

K_1 is the fraction of the total blood volume supplying the spleen per minute.

K_2 is the rate constant of clearance of platelets from the spleen

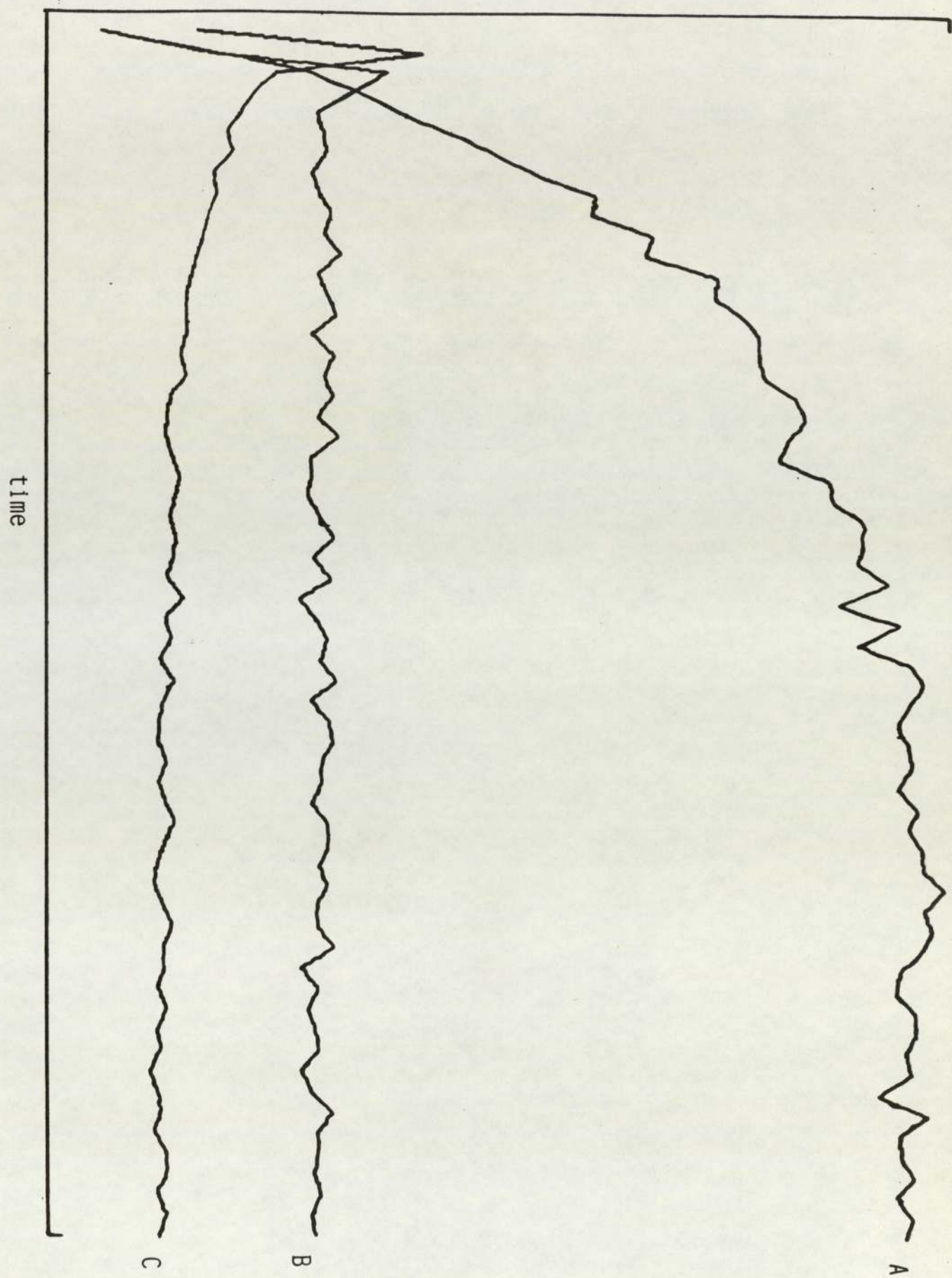
(This two-compartmental model is described in detail in Appendix I).

The platelets have a transit time through the spleen such that at any time, about 30% of the total platelet population is "pooled" in the spleen (32,34,35). Why a platelet spends a third of its life span in the spleen is unknown. The initial *in vivo* distribution has been analysed by monitoring the time-activity curves obtained by a gamma camera over the spleen, liver and heart (figure 1.4). The activity in the spleen increases steadily to reach a plateau at about 15-20 minutes (32,38). The rate of uptake then remains constant.

Quantitative splenic uptake can be calculated using the technique described by Fleming (39). The paper describes a general method for the measurement of activity in an organ using anterior, posterior and lateral views. The method corrects the regional counts obtained on the gamma camera for the varying absorption in body tissue. This is done using a first order geometric mean of the anterior and posterior counts. Expressed in this way, the activity in the organ becomes independent of its depth within the body. Calculation of the splenic uptake using this technique has been used in several clinical situations (12,40). This method for calculation of splenic uptake has been adopted and is described in section 2.4.

From the time-activity curves it can be seen that hepatic uptake is much more rapid than splenic uptake (figure 1.4). Hepatic uptake was reported by Peters *et al* in 1980 and again in 1982 to reach a maximum value within about 6 minutes after injection (32,41,42). The activity then declines at a rate similar to the rate of decline in the blood pool (figure 1.4). These calculations were determined using ^{111}In oxine as the radioisotope. However, in 1985, the same author, using ^{111}In tropolone, reported the liver curve to reach its maximum at 2 minutes and parallel the blood pool curve thereafter (43). Following re-injection of ^{111}In-labelled platelets with oxine or tropolone about 10-

Figure 1.4 Time-activity Curves recorded over Spleen (A), Liver (B) and Cardiac Blood Pool (C)



15% of the dose is rapidly taken up by the liver but because the liver also removes non-viable cells, interpretation of this early activity is uncertain (33,34,36). Platelets undergo prominent but reversible sequestration in the liver immediately after injection, with gradual release over a few hours. This phenomenan has been termed the "collection injury". This is thought to be caused due to exposure of platelets to unphysiological media and the mechanical disturbance of centrifugation. Hence, high initial hepatic activity is a good indication that the platelets have been damaged during labelling (43). Some hepatic activity is expected due to the uptake of severely damaged platelets and fragments which would be expected to be cleared by the liver. In 1985, Peters *et al* assessed the intra-hepatic kinetics of ^{111}In labelled platelets by i) comparing the time courses of ^{111}In activity, recorded by dynamic gamma camera scanning over the cardiac blood pool, spleen and liver and ii) quantifying hepatic sequestration by expressing the count rate over the liver at 10 minutes as a percentage of the 2 minute value (43). For normal functioning platelets hepatic activity should parallel the time course of blood activity indicating that the platelets were reversibly pooling in the liver. This pool is in rapid equilibrium with blood platelets. Accepting that changes in liver activity are the result of bidirectional transport of ^{111}In, hepatic platelet sequestration was expressed at the 10 minute liver activity as a percentage of the 2 minute activity, thereby quantifying the liver time activity curve in terms of its shape (43).

Time-activity curves and a quantification of liver activity were determined for 10 patients taking part in this study and the results are discussed. Hepatic uptake was determined at the 10 minute value as a percentage of the 2 minute value and also at the 10 minute value as a percentage of the 6 minute value since there is discrepancy in the

literature regarding the time at which maximal liver uptake is achieved (32,41,42,43).

Platelet survival

Numerous studies have shown that isotope-labelled platelets disappear from circulating blood within 8 to 10 days (33,34,35,36). Attempts have been made to estimate the mechanism of platelet destruction from the shape of the survival curve. The shape of the survival curve offers some indication of the pattern of platelet destruction. Should the platelets disappear from the circulating blood after a life span of given length, the survival curve on an arithmetical scale will be linear provided the age distribution of the platelet population is homogenous. If destruction occurs randomly there will be an exponential curve on the arithmetical scale and straight line on semi-log scale. After many years of discussion, it is now generally accepted that platelet survival curves of a platelet population consisting of platelets of different random ages are in general neither strictly linear nor exponential (44). The shape of the survival curve is best estimated by the fitting of a mathematical model to the blood radioactivity data. There have been several reports that have assessed and compared the results obtained with several different mathematical models for the analysis of the survival curve (33,44,45). Platelet survival has been calculated from the following 6 mathematical models - i) linear function ii) exponential function iii) weighted mean function iv) multiple-hit function v) Dornhorst function vi) alpha-order function.

On the basis of the results from all 6 methods, Lotter *et al* in 1986 made the following recommendation for estimating mean platelet survival times (44). They stated that the weighted mean, multiple hit, Dorhorst and alpha-order models were all equally satisfactory for the

analysis of platelet survival data. The weighted mean method is a simple method and it is that method which is recommended by the International Committee on Standardisation in Haematology (ICSH), when the main concern is only to obtain an estimate of the mean survival (45). This method is a weighted mean value of the linear estimate and the logarithmic estimate. A recent review has been published by the ICSH which is intended to supplement the previously published recommendations for platelet survival studies (46). It states that the multiple hit method has the advantage of greater coherence and flexibility but if computer facilities required to use this method are not available the weighted mean survival may be calculated. The weighted mean technique has been used in experimental and clinical investigations to determine platelet survival (12,35,37,47). This method was chosen for the estimation of platelet survival in the investigations performed in this work.

The ICSH also makes one other important recommendation relating to the measurement of the radioactive blood samples for determination of platelet survival (45). Measurement of radioactivity in whole blood may be used if red cell contamination can be disregarded. This is determined by comparing the count rates of the whole blood sample to that of standards prepared with ammonium oxalate that will lyse red blood cells. Several authors have advocated that platelet survival data should be based on radioactivity associated with separated platelet samples rather than whole blood samples. However, Hawker *et al* have reported that it is important to use whole blood samples for radioactive counting and the calculation of platelet survival since the freed indium in whole blood is a consequence of *ex vivo* handling eg) centrifugation for separation of platelets from red blood cells (48). They have demonstrated that a consistent proportion is readily lysed by simple

manipulation.

The final problem in the calculation of platelet survival is the determination of total blood volume (TBV). Unfortunately, the normal blood volume for any individual is not simple to determine and although many attempts have been made to derive empirical equations based on the height, weight and sex of the subject, none of these are very successful (49). These estimates all have confidence limits of +/- 15%. Because of these inaccuracies it is important that the same method of calculation is used throughout the investigations. The equations chosen to calculate TBV are those based on the work of Retzlaff *et al* and take into account height and body weight (50).

Sites of destruction

The main sites of destruction are the liver and spleen. Soon after ^{111}In platelet injection the sum of activities in liver, spleen and blood is 100% of the dose (33,34,36). This sum falls progressively throughout the life span of the radiolabelled platelets. This "lost" activity being sequestered by the endothelial component of the bone marrow (36). This is the most likely explanation because there are essentially no losses of ^{111}In via urine or faeces (51) and a bone marrow image becomes increasingly visible (34). Since ^{111}In is relatively stable in the spleen and liver, the increasing bone marrow activity probably represents uptake of platelets rather than that of "free" ^{111}In. Thus, about 40-50% of platelets are destroyed in the bone marrow, approximately as many are destroyed in the spleen.

1.2.3 Splenic Blood Flow

As a progression from the determination of platelet uptake into the spleen, radioisotope techniques have also been used to determine splenic blood flow (SBF) and intrasplenic platelet transit time (tp)

(42,52,53,54,55,56,57). The cause of splenic pooling is unknown and therefore research into factors controlling platelet transit time could be useful.

Techniques for measuring SBF in humans can be divided into two general approaches: i) inert gas clearance techniques and ii) blood cell clearance techniques.

An invasive technique using the inert gas ^{133}Xe involves monitoring its clearance following an injection into the splenic artery (58). An alternative non-invasive inert gas technique supplies gas to the organ by inhalation. The patient rebreathes inert gas until equilibrium between arterial and venous blood is reached, then clearance of gas from the spleen can be monitored following "switch over" of the patient from gas mixture to room air (59). These techniques have the advantage of being independent of total blood volume estimation unlike blood cell clearance techniques.

Erythrocytes labelled with ^{51}Cr or $^{99}Tc^{m}$ have been used to measure SBF. These methods are based on the fact that when appropriately modified, erythrocytes are cleared from the circulation exclusively by the spleen. Therefore, the rate constant of clearance of the erythrocytes is equal to the SBF, expressed as the percentage of total blood volume flowing to the spleen per unit time (53). Based on the same principle as clearance from the spleen antibody coated erythrocytes have been used (53). However, it was shown that the extraction ratio of antibody coated cells varied between individuals depending on the number of immunoglobulin G (IgG) molecules attached and therefore this technique cannot justifiably be used to measure SBF (53).

Determination of SBF using ^{111}In labelled platelets are based on the fact that platelets are in dynamic equilibrium between the circulation and the spleen as previously described. Several authors

have shown that SBF can be calculated by using a compartmental analysis technique as described earlier (32,36,42,57). The technique based on the kinetics of intrasplenic platelet pooling utilizers two parameters i) equilibrium is reached between circulating radiolabelled platelets and platelet pool ii) the fraction remaining in each compartment is constant (see Appendix I).

This analytical technique for the calculation of SBF has been compared with other techniques to validate compartmental analysis as a method of measuring SBF (52). Peters *et al* measured SBF from analysis of the first pass time-activity curve recorded over the spleen by external detection and tp was measured from deconvolution analysis. The results were compared with those obtained from compartmental analysis and were found to correlate, supporting the use of compartmental analysis as a valid technique for simultaneous measurement of SBF and tp. Compartmental analysis has been used for the estimation of SBF in the investigations performed in this work.

SBF is now measured in relation to TBV ie) $\%TBV min^{-1}$. Values taken from 3 recently published papers are listed below:

Author	Year	SBF (SD) ($\%TBV\ min^{-1}$)	Ref
Peters	1984	3.8 (0.9)	52
Peters	1985	4.8 (0.3)	55
Wadenvik	1987	4.8 (1.9)	56

The validity of the two-compartment closed model for splenic platelet pooling is now established. However, there are a number of potential sources of error. Firstly, other sites of platelet pooling,

in addition to the spleen eg) lung and liver would create an apparent distribution volume in circulating blood greater than the true volume, thereby tending to underestimate SBF expressed as a fraction of TBV. Secondly, the label is not cell specific, and therefore labelling of erythrocytes and leucocytes in a contaminated platelet sample will take place. As whole blood activity is counted this could be a potential source of error.

By using this method, Peters *et al* have accumulated considerable information with respect to SBF and intrasplenic kinetics in a variety of clinical settings (42,43,52,55). They have showed a significant positive correlation between SBF and spleen size, although tp did not correlate with spleen size. They have also reported SBF to be a) raised in splenomegaly and b) often raised (without splenomegaly) in diseases associated with elevated immune complexes. Wadenvik *et al* have confirmed the relationship between spleen volume and SBF and lack of correlation between spleen size and tp (56).

The clinical utility of SBF measurement in man is uncertain, and at present limited. However, this non-invasive, reasonably accurate method of measuring SBF in man provides an experimental means of studying splenic blood cell handling in an area where there is relatively little information. The mechanisms whereby the spleen retards the progress of platelets are entirely unknown. Since the diameter of the inter-endothelial clefts of the splenic sinus wall should be large enough to provide access for platelets and since erythrocytes, which are larger than platelets, transit the spleen much more rapidly than platelets, it would appear that splenic platelet pooling is not simply the result of any mechanical effects (56). SBF is the major determinant of the size of the exchangable splenic platelet pool. Peters *et al* have shown Thromboxane A_2, a promoter of platelet aggregation, has no role in these

mechanisms (56). Wadenvik *et al* has shown that an adrenaline infusion produces a decrease in SBF and splenic pool size demonstrating SBF to be the immediate variable governing the size of the exchangable platelet pool (57). Further work is necessary not only to define the mechanisms of pooling but also to clarify the purpose of it.

1.3 CLINICAL USE OF LABELLED PLATELETS

1.3.1 Deep Vein Thrombosis

The diagnosis of deep vein thrombosis (DVT) is important as the formation of thrombi can be followed by pulmonary embolism (PE) - a potentially fatal condition. Ninety-five percent of emboli arise from thrombi in the leg veins so the prevention, diagnosis and treatment of PE is linked to the management of DVT (60). Once a DVT is diagnosed, treatment with anticoagulants should begin immediately in order to prevent PE. The diagnosis of DVT on the basis of clinical signs is difficult. Such signs are due to changes around the thrombus. Venous obstruction causes swelling of the leg, an increase in skin temperature, pain and tenderness. However, two-thirds of patients have no physical signs, so confirmation of a clinical suspicion is important (61). A large number of techniques have been investigated (61). The ideal diagnostic test must be painless, non-invasive, simple, rapid and accurate in detecting site, size and number of thrombi whether recent or old (62). No method currently satisfies these criteria. Techniques which have been used include Doppler flow detection, impedence plethysmography, thermography, ascending venography (61).

The procedure most commonly used is ascending venography. This involves intra-venous injection of a radio-opaque contrast medium into a foot vein, below a tourniquet placed around the ankle, which forces the contrast media into deep veins. When the tourniquet is released, contrast media floods into the veins of the thigh and pelvis. This technique is not without its problems. The muscle veins of the calf are not filled by the dye making the diagnosis of DVT in the lower leg difficult. This technique is also very painful. Superficial thrombophlebitis at the site of injection is common and retrograde flow

of dye into the sole of the foot may cause a superficial gangrene (61). Thus a painless method, acceptable to the patient, is much sought after.

Many radiopharmaceuticals have also been investigated to find an optimum method. These include fibrinogen labelled with ^{123}I or ^{125}I, ^{123}I labelled plasminogen, ^{111}In labelled fibrinogen, $^{99}Tc^{m}$ macroaggregated albumin and ^{111}In labelled platelets. This large number highlights the difficulty of obtaining a test suitable for diagnosing DVT. The 2 tests that have been used the most are ^{125}I fibrinogen and ^{111}In labelled platelets and are described below.

^{125}I fibrinogen was the first isotope to make substantial contributions to the understanding of DVT. The technique depends upon the incorporation of ^{125}I fibrinogen into an active thrombus. As a result the uptake of radio-iodinated fibrinogen is poor when the thrombus is several days old (62). Detection of increased activity using probe counts is also difficult in regions of large blood pool and therefore relatively insensitive to detection of thrombi above the mid-thigh (63).

Platelets labelled with ^{111}In were introduced in the late 1970's as a method of imaging thrombus. It is claimed to be superior to the ^{125}I fibrinogen uptake technique (63). Platelets have been shown to accumulate on the propagating proximal end of the clot (64). The advantages of this method are firstly, it can detect old thrombi although the older the thrombi, the longer the delay between injection of radiolabelled platelets and first visualisation by scanning (65). Secondly, heparin and warfarin have been reported not to affect platelet deposition on the thrombi. This is an advantage as the patient may be started on anticoagulant therapy (62,65). The major disadvantage of ^{111}In labelled platelets is that 24 hours are required to complete the

study as most lesions are not clearly visible until 20-24 hours after re-injection. However, diagnosis of leg vein thrombosis using labelled platelets has been well documented (21,61,62,64,65,66). The authors suggest that this technique is a valuable addition to the methods for diagnosing leg vein thrombosis.

As discussed earlier, there are 2 new agents which seem to be promising for imaging thrombotic lesions. These are $^{99}Tc^{m}$ HMPAO and ^{111}In labelled platelets with the monoclonal antibody P256. The main advantage of $^{99}Tc^{m}$ HMPAO is that it allows early detection of DVT. Fresh thrombotic lesions can be localised 0.5-1 hour after re-injection of the cells using $^{99}Tc^{m}$ HMPAO (24). The platelet specific monoclonal antibody, P256, has been used to image thrombus in man (26). As with all monclonal antibodies, the main disadvantage of this technique being the development of immunity or sensitivity to P256 (26).

In summary, of the radiopharmaceuticals agents available for detection of DVT, ^{111}In labelled platelets is at present the most widely used technique. This method has been evaluated in this work and is currently available as an alternative diagnositc technique to ascending venography. $^{99}Tc^{m}$ HMPAO and ^{111}In monoclonal antibody have potential in this area but require further investigation before becoming available on a wide scale.

1.3.2 Idiopathic Thrombocytopenia

^{111}In labelled platelet kinetic studies have become an accepted method for the clinical and experimental investigation of thrombocytopenia. The kinetic data obtained has helped in the understanding of the disorder and therefore influenced therapy of the disease.

Causes of thrombocytopenia are numerous and varied. The majority

are secondary to an underlying disease process and are frequently associated with other haematological abnormalities. Isolated thrombocytopenia is less common. In broad terms this can be associated with either reduced platelet production (eg marrow aplaisia, megakaryocyte abnormalities,), or increased destruction or utilization of platelets in circulation (immune related, intravascular coagulation), or pooling of platelets in the spleen (1).

Idiopathic thrombocytopenia purpura (ITP) is a term used to describe thrombocytopenia in which, by process of exclusion, no underlying disorder or drug cause can be detected. Excessive destruction of platelets is due to an underlying immune mechanism. An anti-platelet antibody has been isolated and found to be present in the IgG fraction of plasma (1). In almost all cases of immune thrombocytopenia there is an increased amount of immunoglobulin on the platelet surface and the quantity appears to correlate well with the severity of the disease and resistance to various forms of treatment.

The mainstay of initial therapy of symptomatic ITP is steroids which exert their effects on the immune system. Corticosteroids have no effect on platelet survival or platelet count in normal individuals (1). Reduction of surface-bound and serum antibody is usually achieved by the administration of corticosteroids over a period of days to weeks (1). Complete remissions are achieved in 15-60% of patients treated with corticosteroids, the response taking several days or weeks to become apparent. Unfortunately sustained remissions following discontinuation of therapy are much less common. When steroid therapy has failed, splenectomy is indicated. The spleen is also responsible for production of autoantibody and levels of both platelet-bound and circulating anti-platelet immunoglobulin are usually reduced by splenectomy (1). The overall response to splenectomy varies from 70 to

90% but up to 10% of patients will subsequently relapse. In reaching a decision with respect to splenectomy, platelet kinetic studies may be of value.

Platelet life span in patients with ITP is shortened in almost all cases. The shortest life span is commonly seen in acute ITP and there appears to be a good relationship between platelet survival and platelet count (1). Imaging has demonstrated various patterns of sequestration of the destroyed platelets. Splenic, hepatic and combinations of sequestration site have been reported, the more severe and more resistant to therapy usually being associated with hepatic sequestration and the less severe and more amenable to treatment showing splenic sequestration. Therefore, platelet labelling may be of help in the decision to plan for splenectomy.

Platelet survival studies were difficult in ITP when ^{51}Cr, as sodium chromate, was used as this radiopharmaceutical labels platelets with a very low efficiency. Thus large volumes of blood had to be withdrawn and platelet counts had to be greater than 25-50 x $10^{9}L^{-1}$ for platelet kinetic studies (67). This was a particular problem in infants where only small volumes can be obtained (68). These problems have been overcome by the introduction of ^{111}In oxine as a platelet label. This radiopharmaceutical has high platelet labelling efficiency and studies using autologous platelets may be used despite severe thrombocytopenia.

We have successfully labelled platelets with ^{111}In oxine in 5 patients with ITP. The results of the investigations have helped in the further clinical management and are discussed in section 3.3.

1.3.3 Investigation of Altered Platelet Function

Another area in which ^{111}In labelled platelets are useful as a diagnostic tool is renal transplant rejection. Platelets accumulate in

renal allografts undergoing episodes of acute rejection (20,22). Serial measurements of the ratio of ^{111}In platelet accumulation in the grafted kidney versus uptake in a control area, is found to increase in patients where the transplant is being rejected but to remain constant in non-rejected transplants.

^{111}In labelled platelets have also been used in several experimental situations. A study of platelet accumulation and survival in various disease states can often provide an insight into the mechanisms of the conditions. Three examples are listed below:

i) Localizing the site of gastrointestinal haemorrhage (GIH) may be difficult, particularly when bleeding is intermittent. Because of the central role of blood platelets in haemostasis, labelled platelets might be expected to accumulate at the bleeding site causing an increased area of uptake, persisting even after cessation of active bleeding. Schmidt *et al* have reported 3 cases of intermittent GIH in whom focal tracer accumulations after injection of autologous ^{111}In labelled platelets indicated the presence of transient GIH (63).

ii) Patients receiving even small amounts of whole blood or packed red blood cells (3-5 units) are known to develop thrombocytopenia. Bareford *et al* conducted a study to examine the effect of different transfused blood products as well as the role of splenic sequestration (34). The study, using ^{111}In oxine labelled platelets indicated the fall in platelet count was due to increased splenic sequestration. This was attributed to the filter within the giving set causing platelets to adhere to infused microaggregate debris causing their premature removal from the circulation.

iii) The possible role of abnormal platelet function in the pathogenesis of vascular disease in patients with diabetes mellitus remains controversial. ^{111}In labelled platelet kinetic and

biodistribution studies were performed in 7 patients with poorly controlled diabetes mellitus. No significant differences were observed between the diabetic group and the control group in organ uptake and platelet survival (70).

1.4 PLATELET FUNCTION IN INFLAMMATORY BOWEL DISEASE

1.4.1 History

A clinical association between ulcerative colitis(UC) and thromboembolic disease was first reported in 1936 by Bargen and Barker (71). They reported an incidence of 1.2% of 1500 patients who had undergone one or more previous thromboembolic episodes. They went on further to report an incidence of 31% of thromboembolism in 46 cases of UC at post-mortem examination. The authors were particularly impressed at the relatively young age of the patients in which these complications were occurring (between 19 and 31 years). Since then a number of studies have been performed to demonstrate this association (72,73,74). The autopsy studies invariably demonstrate a considerably higher incidence of thromboembolic complications. Various sites of thromboses have been reported including cerebral veins, thoracoepigastric veins, iliofemoral veins, femoral artery, portal veins and pulmonary veins (75).

In 1968 Lee *et al* investigated the role of platelets in patients with inflammatory bowel disease (IBD). The found, in 16 patients with UC, increased levels of factors II, V, VIII and X and decreased levels of anti-thrombin III. They suggested that a climate of hypercoagulability existed in these patients. These levels, in general, tended to reflect the clinical activity of the disease. In 1966, Morowitz *et al* found significant thrombocytosis in 6 patients with chronic IBD: 5 cases of Crohn's disease and 1 case of UC (77). Other than their bowel disease, no reason was found for these patients elevated platelet counts. A recent study by Talstad *et al* showed that thrombocytosis occurs frequently in both UC and Crohn's disease, and in view of the complication of thrombophlebitis, they suggested that thrombocytosis

should be particularly looked for in these patients and that anticoagulant therapy be evaluated (78).

It has also been the clinical impression of the gastroenterologists at Dudley Road Hospital (DRH) that patients with IBD are prone to thromboembolism. From 200 patients attending the clinic, 8 patients had thrombotic complications and 2 died as a direct result of this complication. The clinical details are listed in table 1.1.

Table 1.1 Clinical Details of Thrombotic Complications in 8 Patients with Inflammatory Bowel Disease

Age (years)	Diagnosis	Event
31	C	PE
32	UC	multiple PE, fatal CVT
36	C	PE
50	UC	multiple PE
55	C	femoral artery embolus
55	C	DVT, multiple TIA, fatal PE
62	UC	DVT, PE
62	C	recurrent DVT

C = Crohn's disease

CVT = cortical vein thrombosis

TIA = transient ischaemic attack

PE = pulmonary embolus

DVT = deep vein thrombosis

It was noted that some of these complications occurred at an early age in agreement with the report by Bargen and Barker (71). In addition to this, it has also been observed at surgery that endarteritis and thrombosis actually occurs in the vessels of surgically removed specimens of the bowel (personal communication, Dr Thompson, Birmingham General Hospital).

1.4.2 *In vitro* Data

As a result of the reports of increased thromboembolic complications, work has been carried out in the gastroenterology department to ascertain whether platelet function is abnormal in patients with UC and C using the *in vitro* tests described in section 1.1.2 (79).

104 patients were randomly selected for this trial; 40 patients with C and 64 patients with UC. Diagnosis of the disease had been previously confirmed by histology and barium studies. The results are shown in table 1.2.

Table 1.2 Platelet Function Tests

Disease	Spontaneous Aggregation	Platelet Hyper-sensitivity	Elevated Euglobulin	Prolonged Urokinase	Elevated FDP's
C	18/40	29/40	7/31	1/24	0/40
UC	17/64	26/64	15/37	1/20	0/64

The results show that urokinase sensitivity and FDP levels were not significantly different from control values. Platelet aggregation and euglobulin clot lysis times were significantly higher in these patients. There was no significant correlation with disease type, disease activity, extent of bowel involvement or treatment.

In summary, *in vitro* tests have shown patients with IBD to have abnormal platelet function. Investigations are currently being performed to examine in more detail various aspects of platelet activation including their secretory role and prostaglandin synthesis.

1.4.3 Hyposplenism

It is of interest to note that studies on splenic function in IBD have previously been reported by one laboratory (80,81). Ryan *et al* reported hyposplenism to be a complication of UC and C but only occurring in those patients with extensive colonic involvement and

suffering either a relapse or a persistently active course. Hyposplenism is associated with impaired immunity and may therefore be associated with the patients inability to control the bowel inflammation. Such a disturbance of immunity may be the crucial factor in the susceptibility to life threatening infections after colectomy. Ryan *et al* determined the variance in spleen function, using the technique of measuring the rate of clearance of ^{51}Cr labelled heat damaged red cells from the circulation (80). The results showed those patients with hyposplenism to have prolonged clearance values indicating a decrease in spleen function. Hyposplenism was found to be associated with thrombocytosis. An association between hyposplenism in UC and septicaemia in the immediate postcolectomy period was demonstrated in this study (80). Four patients with hyposplenism developed life-threatening septicaemia in the early postcolectomy period.

1.5 AIMS OF THE STUDY

For many years numerous studies have tried to develop techniques for labelling platelets. Although there have been many published methods not many Nuclear Medicine Departments offer platelet function tests routinely. These tests can be used in the diagnosis of many disease states. They are also of value in the experimental investigation of platelet function.

Therefore, the aims of the study were:-

i) To evaluate the methods of platelet labelling described in the literature and adopt the method that can be performed most successfully in the facilities available.

ii) To use this technique in the diagnosis of idiopathic thrombocytopenia and deep vein thrombosis.

iii) To investigate platelet function in patients with inflammatory bowel disease using labelled platelets. It was hoped that specific abnormalities in distribution and survival may be discovered.

iv) To investigate uptake of activity into the diseased bowel of patients with inflammatory bowel disease. This was to be achieved by labelling the platelets of patients due for surgery immediately before the operation. Once the bowel had been removed it was to be scanned to see if any abnormal accumulation could be detected in this manner.

2. MATERIALS AND METHODS

2.1 LABELLING WITH OXINE

2.1.1 Materials

All manipulations of blood plasma and platelets were done with sterile plastic materials: 20ml universal containers (cat no. 128C) and 10ml round base, screw cap test-tubes (cat no. 142AS) were obtained from Sterilin, Feltham, England. 3ml sterile plastic pipettes were obtained from Bio-medical Laboratory Supplies, Birmingham, England.

The centrifuge was a MSE Centaur 2 (cat no. 126-010N) from MSE Scientific Instruments, Crawley, England. The brake had been disconnected in order to protect the platelets from damage due to abrupt changes in centrifugal force.

An SE10 waterbath from Grant Instruments, Cambridge, England was used. The temperature was set at 37°C.

Radioactivity was measured using an Amersham Radioisotope Calibrator ARC-120, Capintec Inc, New Jersey, USA.

The aggregometer was a Platelet Aggregation Profiler PAP-4 from Bio Data Corporation, USA.

2.1.2 Reagents

The anticoagulant acid citrate was prepared using the formula: tri-sodium citrate, $2H_2O$ 2.5g, citric acid, $2H_2O$ 1.49g and water to 100ml.

The anticoagulant sodium citrate 3.8%w/v B.P. was obtained from Evans Medical Ltd, Greenford, England.

The buffer solution was prepared using the following procedure. Calcium free tyrodes solution (solution A), obtained from the Sterile Manufacturing Unit, Queen Elizabeth Medical Centre, Birmingham, England

was prepared to the formula of Hawker *et al* (10). Prostaglandin E_1 solution (solution B) was prepared by adding 0.6ml prostaglandin E_1 (Prostin VR 0.5mg/ml, Upjohn, Crawley, England) to 20ml calcium free tyrodes solution. The solution was sterilized by filtration through a 0.22 μm filter (Sabre, Reading, England) and the filtrate was collected in a glass container and stored at -20°C until required. Immediately before use 0.4ml of solution B was added to 20ml of solution A to produce the buffer solution used in the labelling procedure.

The radiopharmaceutical, Indium (^{111}In) Oxine solution (ref. IN.15P) was obtained from Amersham International, Buckinghamshire, England.

The platelet aggregating agent, ADP (cat no. 885-3) from Sigma Diagnostics, St Louis, USA was dissolved in 1ml of distilled water to produce a solution of 100 μ mol L^{-1} ADP. This was further diluted with 0.9% w/v sodium chloride solution to produce concentrations ranging from 2.5 to 10 μ mol^{-1} ADP.

2.1.3 Preparation of ^{111}In Oxine Labelled Platelets

A schematic representation of the method described is shown in figure 2.1. Blood (26ml) was taken by clean venepuncture using a 19 gauge needle. Of this volume 17ml was mixed with 3.0ml of acid citrate in a 20ml container and the remaining 9ml of blood transferred to a sterile round base tube containing 1.0ml of tri-sodium citrate. After mixing by inversion, both tubes were immediately centrifuged at 200g for 10 minutes to generate platelet-rich plasma (PRP). A sample from the citrated PRP (PRP control) was retained for aggregation studies before the remainder was centrifuged at 1000g for 10 minutes to obtain citrated platelet poor plasma (PPP). Five ml of citrated PPP was retained in which to resuspend the labelled platelets before re-injection. The acid-citrated PRP was

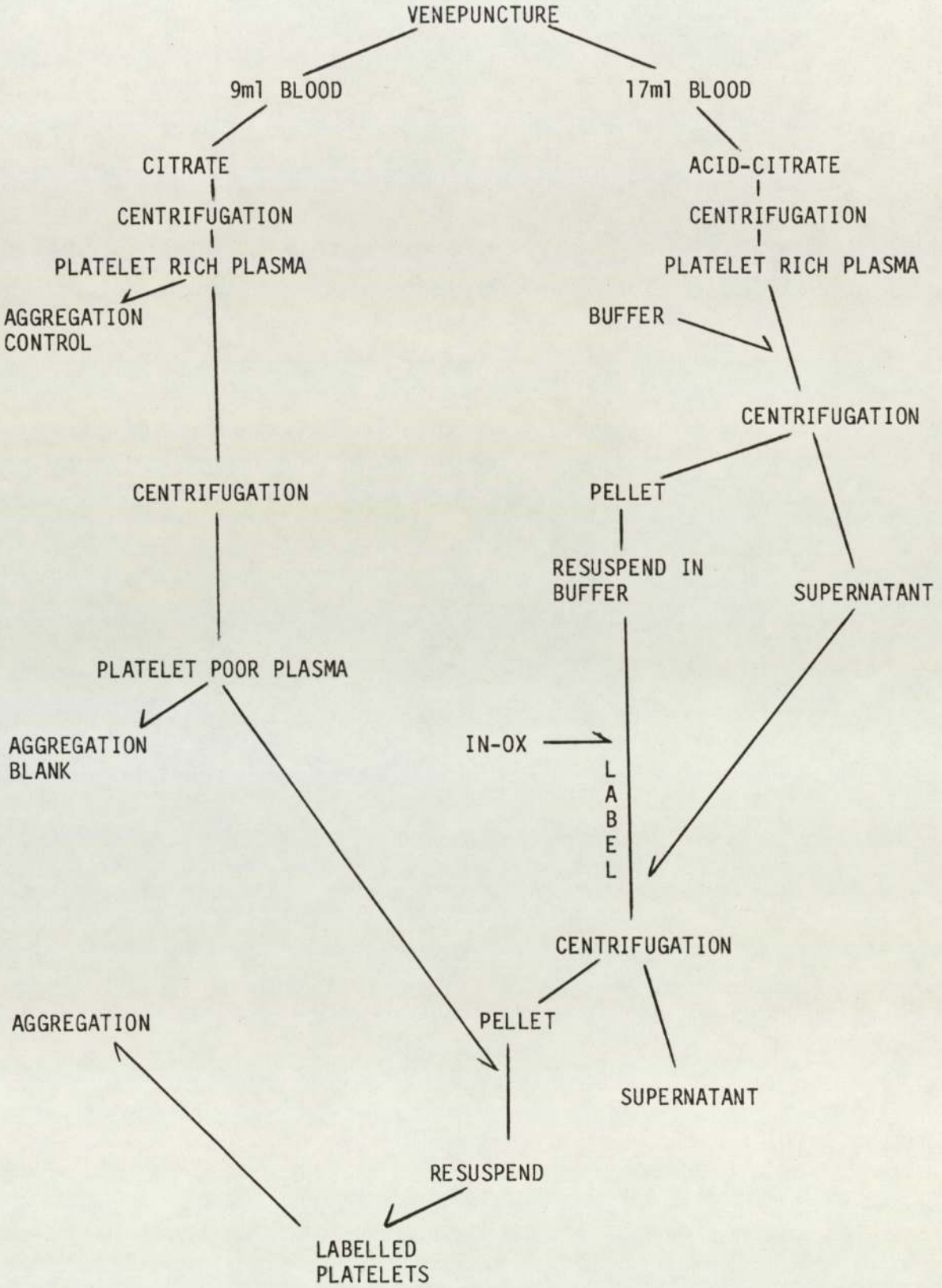


Figure 2.1 Schematic representation of the method described in the text. The left hand column shows the collection of citrated platelets as a control for aggregation respones in vitro, and the collection of citrated plasma in which to finally resuspend the labelled platelets. The right-hand column follows the separation and labelling of acid-citrated platelets.

transferred to a round base tube and made to 10ml with buffer. After mixing by inversion of the tube, the platelets were harvested by centrifugation at 640g for 10 minutes. The diluted plasma was retained aseptically. The platelet button was carefully washed with buffer to remove plasma and then resuspended in 2.5ml of buffer by gentle agitation. 7-8 MBq of ^{111}In oxine was added to the platelet suspension maintained at 37^{o}C. After 60 seconds, 7.5ml of diluted plasma was added, mixed by inversion and the tube centrifuged at 640g for 10 minutes. After removal of the supernatant, the labelled platelets were resuspended in 5ml of citrated PPP. The radioactivity of the ^{111}In labelled platelets after resuspension and of the supernatant, were compared and labelling efficiency calculated. Labelled PRP (PRP test) and unlabelled PRP (PRP control) were assessed for maintenance of platelet function using aggregometry. The samples were incubated at 37^{o}C for 15 minutes to remove the inhibitory effect of prostaglandin in the buffer on the aggregation mechanism. The coulter platelet aggregometer detects changes in optical density of PRP at 37^{o}C as aggregation of platelets occur. ADP (10μ mol L^{-1}) was added to PRP test and PRP control and the responses compared.

2.2 LABELLING WITH TROPOLONE

2.2.1 Materials

As described in section 2.1.

2.2.2 Reagents

The anticoagulant acid citrate dextrose (ACD) was prepared using the formula: tri-sodium citrate, $2H_2O$ 2.5g, citric acid, H_2O 1.4g, D-glucose anhydrous 2.0g and water to 100ml.

The sedimenting agent Hespan, 6% hydroxyethyl starch, was obtained from Dupont, Stevenage, England.

The chelating agent tropolone from Fluorochem Limited, Glossop, England was dissolved at 4.4×10^{-3}M in Hepes-saline buffer (11).

The radiopharmaceutical Indium ^{111}In chloride in 0.04M HCl (ref. INS.1P) was obtained from Amersham International, Buckinghamshire, England.

2.2.3 Preparation of ^{111}In Tropolone Labelled Platelets

A schematic representation of the method described is shown in figure 2.2.. 7.5ml of ACD was dispensed into a 60ml syringe. 42.5ml of blood was collected in the syringe by good clean venepuncture using a 19 gauge needle and mixed gently. 15ml of this was taken and dispensed into a 20ml sterilin container. The tube was centrifuged at 1500g for 10 minutes to produce PPP. The PPP was removed and 1 part ACD was added to 10 parts of PPP to acidify the solution. This plasma was retained to suspend the cells during labelling with ^{111}In tropolone and for washing and suspending the labelled cells. The remaining 35ml of blood was dispensed into 2 sterilin containers each containing 2ml of Hespan. The samples were mixed and immediately centrifuged at 100g for 15 minutes to give a pellet of

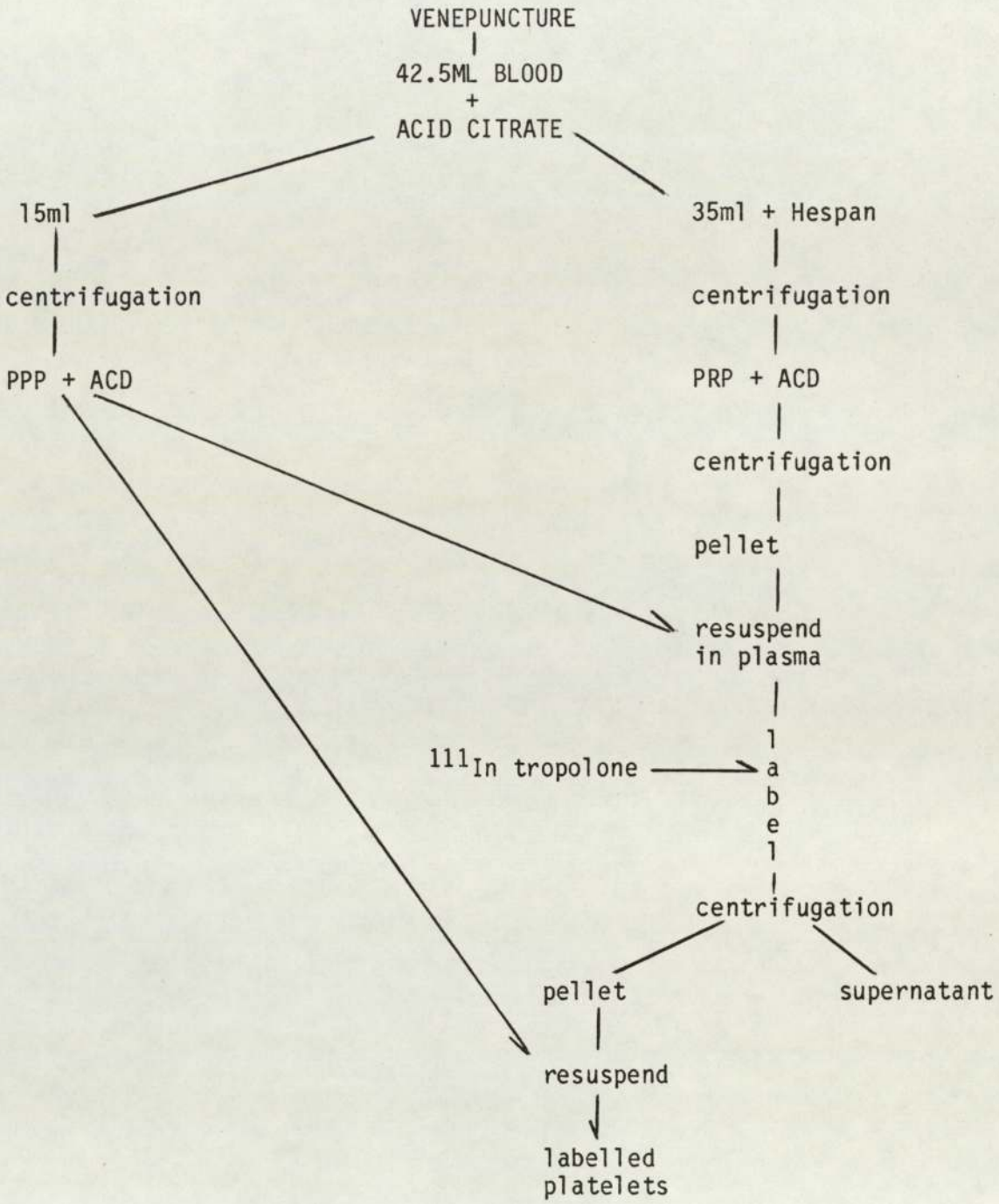


Figure 2.2 Schematic representation of method described in the text. The left hand column shows the collection of citrated plasma in which to finally resuspend the labelled platelets. The right hand column follows the separation and labelling of acid-citrated platelets.

leucocytes and erythrocytes and a supernatant of PRP. The PRP was removed using a sterile plastic pipette and 15ml was dispensed into a sterilin container containing 1.5ml of ACD. The pellet suspension was centifuged at 640g for 5 minutes to produce a platelet plug. The plug was resuspended in 1ml PPP containing ACD for labelling with freshly prepared ^{111}In tropolone, made by adding approximately 11MBq of $^{111}InCl_3$ in 0.04M HCl to tropolone at $4.4 \times 10^{-3}M$ in Hepes-saline buffer. The suspension was incubated for 5 minutes at room temperature. Five ml of acidified PPP was then added to the platelets. The platelets were centrifuged at 640g for 5 minutes. All supernatant was removed and the cells resuspended in 5ml acidified PPP. The radioactivity of the ^{111}In labelled platelets after resuspension and of the supernatant, were compared and labelling efficiency calculated.

2.3 VALIDATION OF OXINE METHOD

2.3.1 Platelet Labelling of Normal Subjects

To assess viability of labelled platelets and labelling efficiency the following parameters were measured:

i) measurement of platelet count - A platelet count was taken of PRP test, PRP control and PPP to calculate the percentage of platelets "lost" during the labelling procedure.

ii) contamination of PRP sample - Contamination of the sample with red blood cell (RBC) and white blood cell (WBC) was monitored at 3 stages during the labelling procedure: a) PRP after first spin at 200g, b) after resuspension of platelet plug, c) PRP test.

iii) microscopic examination - PRP sample was examined under a microscope (magnification X400) to assess the structural integrity of the platelets. "Clumping" of platelets is an indication of aggregation.

iv) effect of temperature at incubation - Two blood samples were simultaneously labelled with ^{111}In oxine. One sample was incubated at $37^{o}C$ for 60 seconds. The remaining sample was maintained at room temperature for 60 seconds. The labelling efficiency of each sample was compared.

2.3.2 Platelet Labelling of Patients with Inflammatory Bowel Disease.

Patients with IBD frequently have an increased platelet count, increased WBC count, and a decrease in haemoglobin. Due to these possible haematological abnormalities the ability to label platelets in these patients was assessed using the following parameters:

i) measurement of platelet count - A count of PRP was taken

ii) contamination of PRP sample - A sample of the final suspension was

counted for RBC and WBC contamination.

iii) aggregation studies - ADP at concentrations of 10 μ mol L^{-1} were added to labelled PRP test and PRP control and the responses were compared.

2.4 SPLENIC UPTAKE

Images were obtained using a Scintronix gamma camera, equipped with a general purpose medium-energy collimator set to acquire both 171 and 245 keV photopeaks of ^{111}In. It was interfaced to a Nodecrest system 3 with a V77 processor. All images were recorded by the computer as 128 x 128 pixel matrices.

2.4.1 Dynamic Study

The camera was positioned anteriorly to visualise the heart, liver and spleen simultaneously. The labelled platelets were injected as a bolus whilst the patient was lying under the camera and data collection begun as the injection was given. Sixty 20 second frames were collected and the time of injection was noted.

2.4.2 Static Study

Immediately after the dynamic study finished, the static study was started. A 10 minute anterior view was acquired followed by a 10 minute posterior view. Finally a left lateral view was acquired and the patients outline was marked using ^{57}Co anatomical markers attached to either side of the abdomen. Further anterior and posterior views were acquired at times decided by the purposes of the study.

2.4.3 Computer Program

A program developed at the Queen Elizabeth Hospital in Birmingham was used to determine splenic uptake. This uses a general method of measurement of activity in an organ using anterior, posterior and left lateral views as described by Fleming (39). This program was modified for use at DRH by N. Tulley (see Appendix II). In order to quantify splenic uptake it was necessary to determine i) attenuation for body tissue, ii)

camera sensitivity, iii) attenuation coefficient for the imaging couch. These were determined using the following procedure: A thin perspex phantom approximately the area of the spleen (12 x 12cm, 0.5cm depth) was filled with a measured activity of ^{111}In. The phantom was placed in a water tank, representing body tissue, next to the camera and imaged at various depths. The variation in count rate with depth was determined. The linear attenuation coefficient for body tissue was obtained from the gradient of the graph of the logarithm of count rate against the depth in the water tank. This was calculated to be 0.114. Camera sensitivity was found by extrapolation of the same graph to find the count rate at zero thickness of the water which was the count rate due to the known activity in the phantom with no attenuation. This was found to be 121453 cts/MBq/10mins. The attenuation factor for the imaging couch was calculated from the variation in count rate when the phantom was placed on the couch and directly on the camera. This was calculated as 1.168.

2.4.4 Data Processing

i) Dynamic study - Sixty 20 second frames were summed to produce a composite image on which three regions of interest (ROI) were drawn over the spleen, liver and heart. Time-activity curves were then generated for each region.

ii) Static study - For each anterior and posterior image an ROI was drawn over the spleen. A background region, below the spleen, was also drawn and used to correct the counts in the spleen ROI. From the left lateral image, the anterior body and posterior body surfaces were determined from the position of the anatomical markers. The anterior spleen and posterior spleen surfaces were also determined visually from the computer image. The computer program (PSPLAT) was run which requires the following information: a) anterior body coordinate, b) posterior body

coordinate, e) injected activity (MBq), f) whether or not correction is needed for attenuation by the couch, g) time(s) after injection (minutes). The splenic activity at each time of imaging was calculated and expressed as a percentage of the injected dose.

2.5 PLATELET SURVIVAL

A 1280 Ultrogamma scintillation counter was used (LKB, Croydon, Surrey, England), to record activity present in each sample.

4 mM EDTA anticoagulant solution was made containing 0.149g EDTA (BDH, Poole, England) in 100ml water. This was used in the preparation of the standard.

Ammonium oxalate 1% solution, as a lysing agent was made containing 1g ammonium oxalate (BDH, Poole, England) in 100ml water. This was used to lyse RBC in the determination of RBC contamination.

2.5.1 Sampling Procedure

Labelled red cell contamination may be disregarded if the counting rate of the standards prepared with saline do not differ more than 10% from those of the standards prepared with ammonium oxalate (45). The following experiment was therefore performed to assess whether RBC contamination could be disregarded in this study.

Standards were prepared in duplicate. 0.1ml of the labelled platelet suspension was added to 4 tubes, 2 contained 2ml of ammonium oxalate 1% solution and 2 contained 2ml of saline. The 4 tubes were centrifuged at 200g for 30 minutes. The supernatants were removed without disturbing the platelet plug and discarded. The 4 tubes were counted and the percentage difference in count rates calculated.

The number and timing of the blood samples was decided by the purposes of the study. A 5ml blood sample was taken at each time from the opposite arm of the injection site and collected in an EDTA tube. Each sample was stored until all the samples had been collected at the end of the investigation. Four ml of each blood sample was accurately pipetted into an EDTA tube for counting. When RBC contamination had been calculated and found to be <10%, a standard for counting was prepared of

similar activity to the blood samples. To achieve this approximately 4-5 MBq of ^{111}In oxine was accurately measured into a 100ml volumetric flask and made up to volume with 4 mM EDTA solution (dilution 1). Two ml of dilution 1 was accurately pipetted into a 200ml volumetric flask and made up to volume with 4 mM EDTA solution (dilution 2).

2.5.2 Counting

Each sample was rolled to ensure constant geometry and then counted on the scintillation counter. The time was set to obtain a minimum of 20,000 counts to reduce statistical error. Three readings were taken of the standard, each blood sample and background counts.

2.5.3 Determination of Blood Volume

Total blood volume was calculated from the sum of the red cell volume and plasma volume (50).

male red cell volume (ml)

820 x height (m) + 17.3 x weight (kg) - 693 (+/-252)

male plasma volume (ml)

2370 x height (m) + 9.0 x weight (kg) - 1709 (+/-358)

female red cell volume (ml)

1640 x height (m) + 5.7 x weight (kg) - 1649 (+/-129)

female plasma volume (ml)

4050 x height (m) + 8.4 x weight (kg) - 4811 (+/-196)

2.5.4 Determination of Activity in Blood

The average count of each sample and standard was calculated and background corrected. The activity injected was decay corrected to the time at which the samples were counted. The activity in MBq, present in the standard was calculated. Knowing the number of counts obtained from

the standard and blood samples, the percentage of the dose in each blood sample was determined.

$$\frac{\text{activity in 4ml sample}}{\text{activity injected (decay corrected)}} \times 100$$

Total activity in blood volume was calculated:

$$\frac{\text{activity in 4ml sample}}{4\ (\text{ml})} \times \text{total blood volume}$$

2.5.5 Calculating Weighted Mean Survival

Platelet survival can be determined from the counts obtained at time, ti, after re-injection for each blood sample using a weighted mean value of the linear and logarithmic estimate (45) (see Appendix III). A computer program was written to calculate platelet survival using the weighted mean method (see Appendix IV).

2.6 HEPATIC ACTIVITY

2.6.1 Time Activity Curves

From the dynamic gamma camera imaging, time activity curves were constructed from ROI's drawn over the cardiac blood pool, spleen and liver using a Nodecrest system 3 computer with a V77 processor and Micas 3000 software. Three 20 second frames were summed to produce 20 successive 1 minute values for each ROI. A curve was constructed for each ROI expressing the count at each minute as a percentage of the 20 minute value. Time activity curves were also constructed for each ROI expressing counts in each 1 minute frame as a percentage of the counts in the 2nd frame.

2.6.2 Hepatic Sequestration

Hepatic sequestration was quantified by expressing the count rate over the liver ROI at 10 minutes as a percentage of the 2 minute value and at 10 minutes as a percentage of the 6 minute value.

2.7 SPLENIC BLOOD FLOW

Compartmental analysis was used to determine splenic blood flow (SBF) and intrasplenic platelet transit time (tp) (52, 56, 57) (see Appendix I). In order to use this method, the following values were calculated:

i) Activity in the spleen at 20 minutes post injection (Qs).

ii) Activity in the blood at 20 minutes post injection (Qb).

iii) Rate constant of equilibrium of ^{111}In labelled platelets between the splenic platelet pool and circulating blood (Ks).

2.7.1 Calculating Qs

The percentage uptake in the spleen at 20 minutes of the total injected dose has been previously calculated. This value was converted to the quantity of ^{111}In present in the spleen in MBq.

2.7.2 Calculating Qb

The activity present in the blood sample at 20 minutes was counted. Knowing TBV, this was expressed in MBq as the activity in the circulating blood at 20 minutes.

2.7.3 Calculating Ks

This was calculated by a computerised maximum likelihood estimate from the dynamic curve recorded over the spleen from 0 to 20 minutes. The protocol was written for a Nodecrest system 3 computer using Micas 3000 software.

The asymptote value taken to calculate Ks is subject to operator error. To estimate this error, the value for Ks was determined by 3 independent observers and the difference in SBF calculated.

SBF and tp were calculated using the following equations (see Appendix I):

$$SBF = Ks \times \frac{TBV}{(1 + Qs/Qb)}$$

$$SBF = y \text{ ml } min^{-1}$$

expressed as a percentage of TBV:

$$SBF = \frac{y}{TBV}$$

$$tp = \frac{1}{(Ks - SBF/TBV)}$$

$$tp = z \text{ min}$$

There have been 2 reports of a positive correlation between SBF and spleen size (55,56). Spleen size was calculated from the anterior images taken immediately after dynamic imaging, expressed as the number of pixels present in the ROI over the spleen.

2.8 PROTOCOLS

2.8.1 Protocol for Assessment of Platelet Function in Patients with Inflammatory Bowel Disease

Ethical committee and ARSAC (Administration of Radioactive Substances Advisory Committee) approval was obtained for the protocol described.

Patient Assessment

Case notes of patient were studied prior to investigation to assess:

i) previous history of thromboembolism

ii) disease state and degree of bowel involvement

iii) previous *in vitro* platelet function

iv) medication which may affect platelet function eg) aspirin.

The patients were sent an information sheet before their appointment (see Appendix V).

Day 1

Patient attended the Medical Physics Department at 9am. A 30ml blood sample was taken and the platelets labelled with 3-4 MBq of ^{111}In. These were injected as a bolus into the arm whilst the patient was lying under the gamma camera and ^{111}In uptake from the time of injection and up to 20 minutes was recorded. Immediately after the dynamic image, a 10 minute anterior and 10 minute posterior image were acquired. Finally a left lateral image with anatomical markers to mark the anterior and posterior surfaces of the body was acquired. A 5ml blood sample, taken from the opposite arm of the injection site, was collected in an EDTA tube. Further anterior and posterior images were acquired at approximately 4 hours after injection and a 5ml blood sample was taken. The times of injection, images and blood samples were noted. The patients height and weight was taken to calculate total blood volume.

Day 2

The patient attended for 10 minute anterior and posterior images taken approximately 24 hours after injection. A further 10 minute anterior image was taken of the lower abdomen. A 5ml blood sample was taken. The time of imaging and sampling was noted.

Day 3-5

The patient attended on days 3,4 and 5 for a 5ml blood sample only. On each visit the time was noted.

The following parameters were determined: i) splenic uptake ii) dynamic hepatic uptake iii) platelet survival iv) splenic blood flow v) intrasplenic platelet transit time.

2.8.2 Protocol for Assessment of Platelet Function in Patients with Idiopathic Thrombocytopenia

Patient Assessment

Case notes of the patient were studied prior to investigation to assess:

i) platelet count

ii) effect of treatment with steroids

iii) medication which may affect platelet function eg) aspirin

Day 1

The same protocol was followed as for IBD

Day 2

The patient attended for 10 minute anterior and posterior images taken at approximately 24 hours after injection. A 5ml blood sample was taken. The time of imaging and sampling was noted.

Day 3-5

The patient attended on days 3,4 and 5 for 5ml blood sample only. On each visit the time was noted.

The same parameters were determined as for IBD.

2.8.3 Protocol for Diagnosing Deep Vein Thrombosis

Patient Assessment

Case notes of the patient were studied prior to investigation to assess:

i) previous history of DVT

ii) results of venogram (if performed)

iii) time of initiation and dose of heparin/warfarin therapy.

Day 1

Time between clinical suspicion of DVT and investigation was kept to a minimum. A 30ml blood sample was taken and platelets labelled with up to 8 MBq of ^{111}In. The injected dose was increased as a greater amount of activity is required to demonstrate accumulation of platelets in the propagating thrombus. This is because of background activity in the blood pool. The dose was injected into the arm of the patient as a bolus dose. Four hours after injection, a 10 minute anterior image of the thighs and a 10 minute posterior image of the calves were acquired.

Day 2

The patient attended for 10 minute anterior image of the thighs and a 10 minute posterior image of the calves taken approximately 24 hours after injection.

Probe Counts

If an increased area of activity was not detected on the images, probe counts were taken. This was achieved using a sodium iodide scintillation detector (NaI(Tl), diameter 2", thickness 2", connected to a J and P Engineering NIM unit, Nuclear Enterprises, Reading, England). Counting points were marked 4" apart up the posterior of the calves from the ankle to the back of the knee and up the anterior of the thighs from the knee to the groin. The activity over each mark was counted for 30

seconds and repeated after repositioning. The counts were noted and a graph of counts against distance from the ankle was plotted.

3. RESULTS AND DISCUSSION

3.1 VALIDATION OF OXINE METHOD

3.1.1 Assessment of Techniques

Labelling with ^{111}In oxine was a simple procedure. As only 26ml of blood was required it was easily acquired by good clean venepuncture causing minimum disturbance to the blood sample. The average time required for the labelling procedure was 60 minutes, thus, keeping the time between venepuncture and re-injection to a minimum. The sample was easy to manipulate - the platelet plug quickly resuspended and RBC and WBC contamination was minimal. The mean L.E. for ^{111}In oxine labelled platelets was 40% (n = 6; range 36-45). Labelling with ^{111}In tropolone required 42.5ml of blood and took 75 minutes to complete. The sample was difficult to manipulate as there was RBC contamination making resuspension of the platelet plug difficult. The mean L.E. for ^{111}In tropolone labelled platelets was 39% (n = 2; 36,41).

3.1.2 Platelet Labelling of Normal Subjects

Practice is essential for the success of any platelet labelling method. Thus, blood from healthy volunteers was labelled with ^{111}In oxine in order to become familiar with the labelling procedure and improve L.E..

Careful washing of the platelet plug was important to remove most of the plasma but retain the majority of platelets. The average percentage of "lost" platelets was 17%, demonstrating that disturbance of the platelet plug is small, hence facilitating platelet labelling.

Blood from one patient was tested for RBC and WBC contamination at 3 stages during the labelling procedure and the results are shown in table 3.1.

Table 3.1. RBC/WBC Contamination of the Platelet Sample in Normal Subjects

Stage in procedure	RBC ($10^{12}L^{-1}$)	WBC ($10^{6}L^{-1}$)	Platelets ($10^{9}L^{-1}$)
PRP after first spin	0.00	0.01	541
PRP after resuspension	0.00	0.06	493
PRP test	0.00	0.10	431

Reference Ranges

RBC (m) 4.7 - 6.1 x $10^{12}L^{-1}$

RBC (f) 4.2 - 5.4 x $10^{12}L^{-1}$

WBC 4.8 - 10.8 x $10^{6}L^{-1}$

Platelets 150 - 400 x $10^{9}L^{-1}$

At each stage there was no RBC contamination and the percentage of WBC present compared to platelets was minimal.

The degree of aggregation in a sample can also be assessed by microscopic examination. PRP test did not show clumping and platelets were visualised as individual cells.

The effect of incubation temperature was found to affect L.E. significantly. From one blood sample L.E. at 23°C was 50% but at 37°C was 65%.

3.1.3 Platelet Labelling of Patients with Inflammatory Bowel Disease

In order to become familiar with manipulating blood samples from these patients, their platelets were labelled with ^{111}In oxine.

Due to the possible abnormal haematological results, contamination of the platelet suspension was thought to be a potential problem. The results are shown in table 3.2.

Table 3.2 RBC/WBC Contamination of the Platelet Sample in Patients with Inflammatory Bowel Disease

Stage in procedure	RBC ($10^{12}L^{-1}$)	WBC ($10^{6}L^{-1}$)	Platelets ($10^{9}L^{-1}$)
PRP test	0.00	0.34	456

Contamination with WBC was slightly higher than that in normal subjects, but the percentage of RBC and WBC present compared to platelets was minimal.

The *in vitro* aggregation responses to 10 $\mu molL^{-1}$ ADP for PRP control and PRP test are shown in figure 3.1. The responses are similar which demonstrates that the labelled platelets are functioning normally.

When developing a platelet labelling method the most important consideration is the maintenance of a viable population of platelets. Platelet function is affected by physical manipulation, physiological conditions and the time period required for the procedure. Although tropolone will label platelets in plasma, the time required for this is

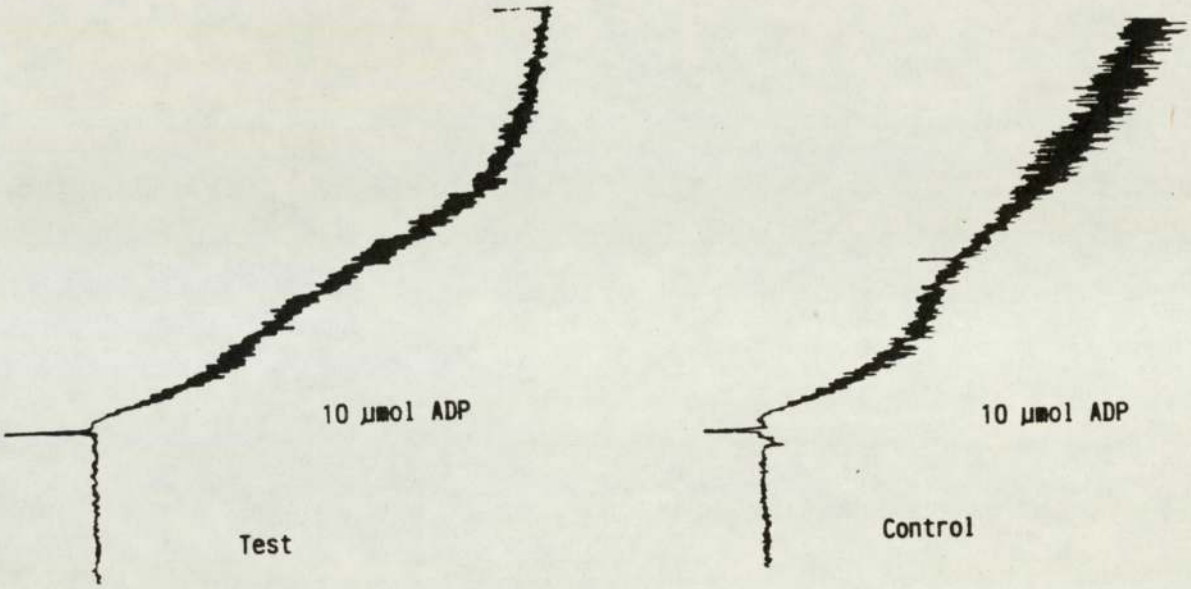


Figure 3.1 In Vitro Aggregation Responses to 10 µmol ADP for PRP test and PRP control in a patient with Inflammatory Bowel Disease

longer and physical handling more vigorous, than required for labelling with oxine. The L.E. obtained for each agent was similar. The comparatively low values obtained initially were subsequently improved by investigating various aspects of the labelling procedure, the incubation temperature being the most important factor in improving L.E. As a result of these investigations, ^{111}In oxine was adopted as the platelet labelling agent. To minimise platelet damage practice of the labelling technique was essential. Blood from healthy volunteers and patients with IBD was labelled and function of ^{111}In oxine labelled platelets was maintained as demonstrated by the *in vitro* aggregation tests.

3.2 LABELLING EFFICIENCY

L.E. was calculated for 21 patients, who took part in the study, and the results are shown in table 3.3

Table 3.3 Labelling Efficiency

Patient	Diagnosis	Platelet count (10^9L^{-1})	L.E. (%)
1	UC	388	34
2	ITP	51	39
3	DVT	229	35
4	C	99	60
5	DVT	226	45
6	DVT	281	60
7	C	117	51
8	C	313	44
9	ITP	60	76
10	UC	445	75
11	UC	325	76
12	DVT	201	57
13	ITP	33	57
14	UC	313	63
15	DVT	287	58
16	DVT	506	69
17	C	456	65
18	ITP	55	57
19	UC	366	70

20	C	380	63
21	UC	409	71
MEAN (SD)			58(13)

All L.E.'s were obtained under the same labelling conditions. There is no correlation between L.E. and disease state and L.E. and platelet count. The low L.E.'s shown here reflect the practice required for the labelling technique as the low values were obtained for patients investigated at the beginning of the study.

3.3 SPLENIC UPTAKE

3.3.1 Dynamic study

Dynamic splenic uptake is shown in figure 3.2. In each disease group, splenic uptake followed a similar pattern. The activity increased steadily reaching a plateau at approximately 20 minutes. This time was constant irrespective of the magnitude of uptake within the spleen.

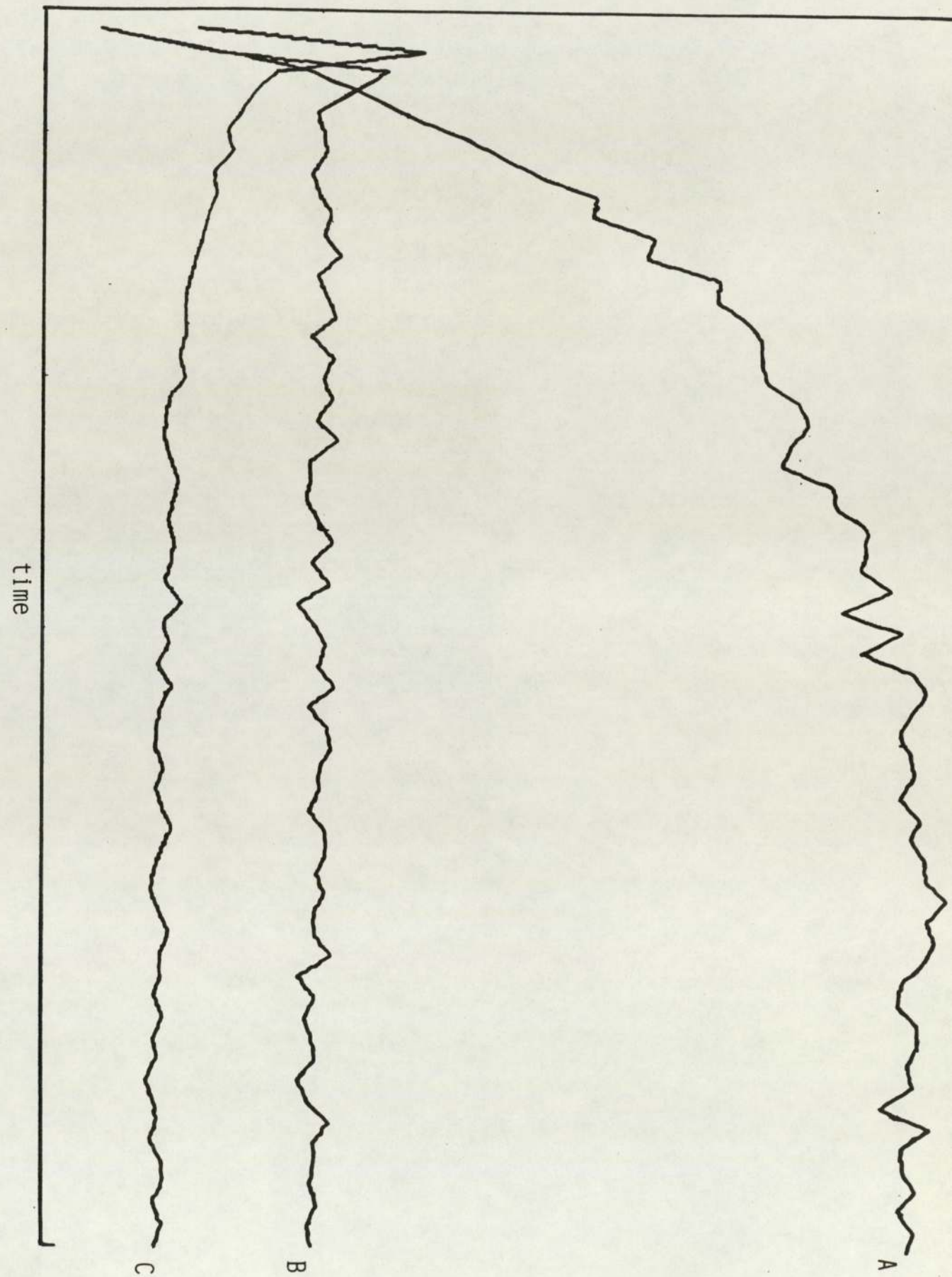
3.3.2 Static study

Splenic uptake calculated at 1, 4 and 24 hours was found to remain constant. An example of distribution of activity is shown in figure 3.3. A mean value for each patient was calculated and the results are shown in figure 3.4. The mean splenic uptake for each patient group is listed in table 3.4.

Table 3.4 Mean Splenic Uptake

Diagnosis	Number in study	Splenic uptake %	SD
UC	6	29	(5)
C	6	30	(6)
ITP	5	30	(10)
DVT	4	28	(4)
Coeliac	1	23	

Figure 3.2 Time-activity Curves recorded over Spleen (A), Liver (B) and Cardiac Blood Pool (C)



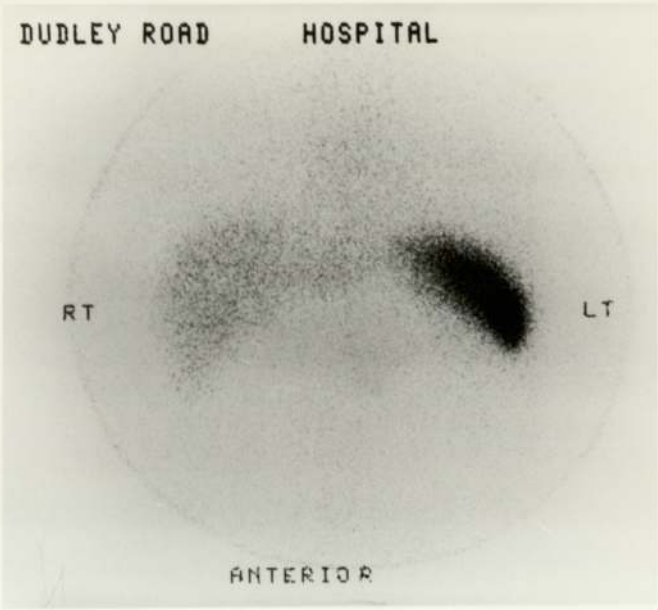


Figure 3.3 Anterior View of Lower Chest and Upper Abdomen at 20 minutes Post Injection showing Uptake in Spleen, Liver and Blood Pool

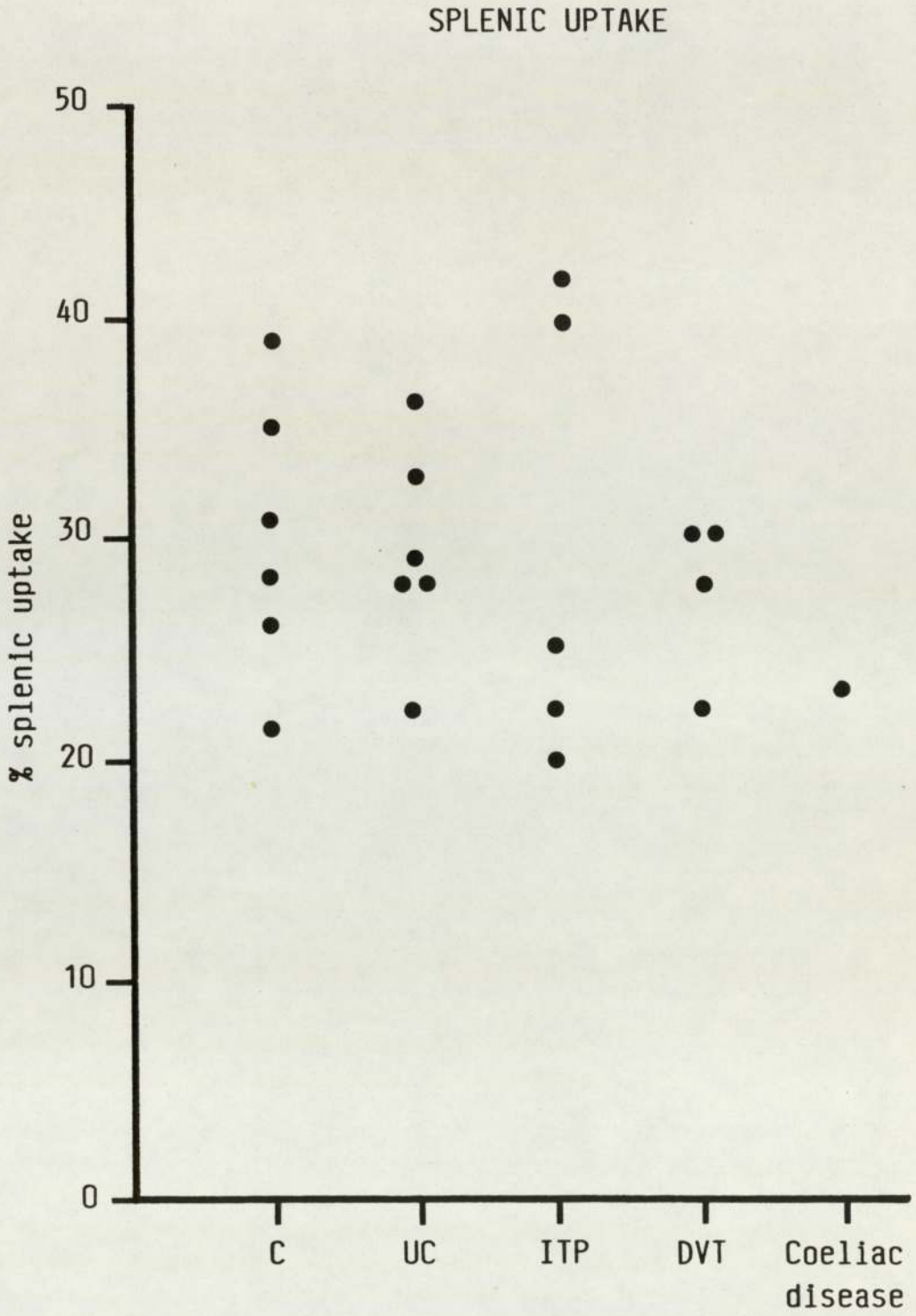

SPLENIC UPTAKE
% splenic uptake
50
40
30
20
10
0
C
UC
ITP
DVT
Coeliac disease

Figure 3.4

The values for patients with IBD (both UC and C) are close to the normally quoted value of 30% (32,34,36).

The value for patients with suspected DVT is also close to the normal value. It might be expected that patients with DVT are in hypercoaguable states and therefore have altered platelet function. However, no indisputable evidence has been presented to demonstrate this (82). One report has shown patients with DVT to have a normal splenic uptake pattern (26).

The mean value of 30% obtained for ITP is somewhat misleading. Looking at individual values, this group falls into 2 distinct categories. Two patients had increased splenic uptake (40%, 42%) and 3 patients had decreased splenic uptake (20%, 22%, 25%). On assessing the images this decrease appears to be due to an increased hepatic sequestration. An example of increased splenic uptake and decreased splenic uptake as a result of hepatic sequestration is shown in figure 3.5. These studies provide information that is of value in the clinical management of the patients as illustrated in the 5 cases described below:

Case 1

A 65 year old man was found to be thrombocytopenic whilst participating in a clinical drug trial. He was immediately withdrawn from the trial but his platelet count remained below 70 x $10^{9}L^{-1}$. A bone marrow aspirate indicated an immune related thrombocytopenia. However, platelet antibody screening proved negative. The patient remains thrombocytopenic but well and is being regularly monitored in clinic. Platelet kinetic studies showed splenic uptake increased to 42% suggesting splenectomy may be of value if symptoms appear.

Case 2

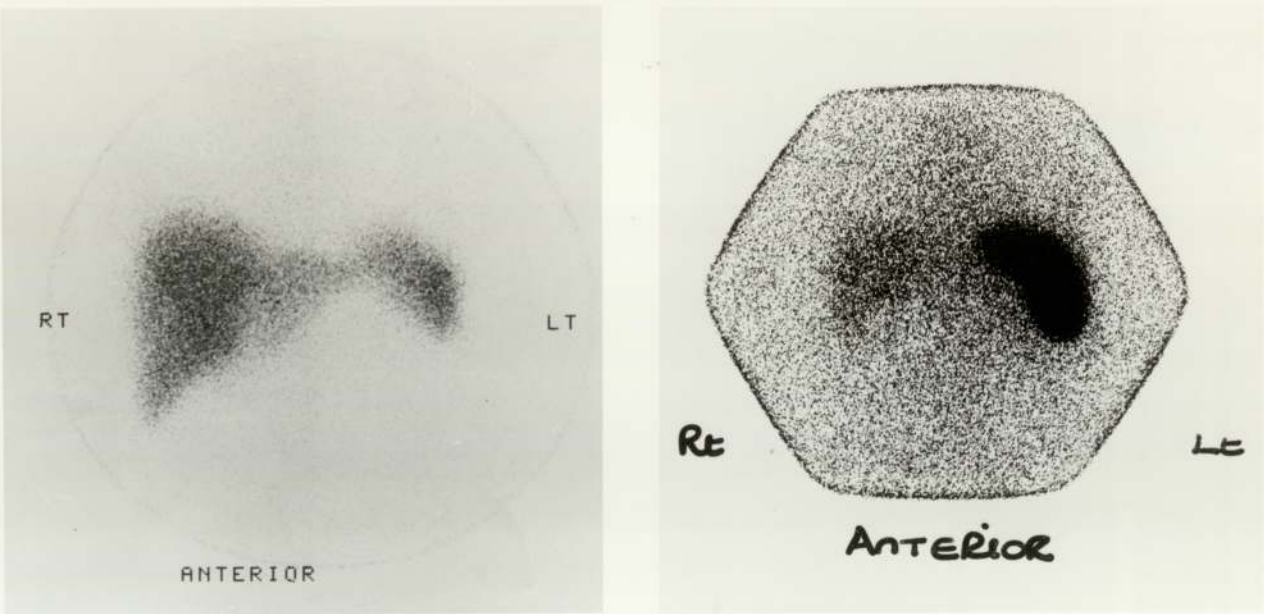


Figure 3.5 Anterior Views showing Uptake in Spleen and Liver of 2 patients with Idiopathic Thrombocytopenia
(The view on the right demonstrates increased splenic uptake, the view on the left demonstrates increased hepatic uptake)

A 39 year old lady was found to be thrombocytopenic, her platelet count having fallen to 51 x 10^9L^{-1}. She was started on steroid therapy to which there was no response. As the patient was asymptomatic no further therapy was indicated and she is currently attending clinic to be monitored. Platelet kinetic studies showed splenic uptake increased to 40% indicating that splenectomy may be of value if the patient becomes symptomatic.

Case 3

The patient presented with brusing and haematuria and was found to have a platelet count of 6 x 10^9L^{-1}. A high dose steroid course (prednisolone 60mg daily) was prescribed to which she responded, her platelet count rising to within the normal range. On discontinuing steroid therapy, her platelet count dropped to 6 x 10^9L^{-1} and she was prescribed a maintenance dose of prednisolone 5mg daily to which she initially responded. Unfortunately, 6 months later, the patient was once more symptomatic and her platelet count was 4 x 10^9L^{-1}. At this stage, a splenectomy was indicated. Platelet kinetic studies were performed one week prior to splenectomy. A low splenic uptake value of 22% was obtained and the images demonstrated high hepatic sequestration indicating that splenectomy might not be successful. The patient initially responded to splenectomy, her platelet count rising to 131 x 10^9L^{-1}. However, one month after the operation the platelet count had once again fallen to 20 x 10^9L^{-1} indicating that this patients thrombocytopenia may be due to abnormal hepatic sequestration.

Case 4

A 23 year old lady was found to have a platelet count of 66 x 10^9L^{-1}. She was asymptomatic so treatment with steroids was not thought to be necessary and she is currently attending clinic to be monitored. Platelet kinetic studies found splenic uptake to be only 20%, the images

demonstrated increased sequestration in the liver, suggesting that the thrombocytopenia is due to increased hepatic destruction.

Case 5

A 50 year old man presented with bruising and was found to have a platelet count of 26 x 10^9L^{-1}. The patient did not respond to steroid therapy. Platelet kinetic studies found this patient to have a slightly reduced uptake of 25% suggesting splenectomy might not be of value in this patient.

The patient with coeliac disease was found to have a low splenic uptake of 23%. Splenic atrophy has been associated with adult coeliac disease (80,83,84). Marsh and Stewart reported that almost all patients with coeliac disease have some degree of splenic hypofunction (83). Radioisotope studies using ^{51}Cr-labelled heat damaged red cells have shown no localization of radioactivity in the spleen, the diagnosis of splenic atrophy being subsequently confirmed at laparotomy (84). The platelet studies performed in this one patient showing reduced splenic uptake demonstrates that platelet studies are a useful experimental tool in the investigation of splenic function. Further work would be of benefit to correlate the 2 radioactive methods in order to elucidate the mechanisms involved in RBC clearance and platelet pooling within the spleen.

3.4 PLATELET SURVIVAL

The effect of RBC contamination on the determination of platelet survival was investigated by measuring the difference in count rate between PRP samples prepared with saline and those prepared with ammonium oxalate. These results are shown in table 3.5.

Table 3.5 Difference in Count Rate of PRP due to RBC Contamination.

Sample	Counts (min^{-1})	Average count	Background adjustment
background	44,46	45	-
PRP in saline	10576,11023	10780	10755
PRP in ammonium oxalate	10619,10632	10626	10581

Percentage difference in count rate = 1.6%

If the counting rate of the standards prepared with saline do not differ more than 10% from those of standards prepared with ammonium oxalate, labelled RBC contamination may be disregarded. Therefore, whole blood samples were used to calculate platelet survival as activity due to RBC contamination was <2%. As discussed in section 1.2.2, using whole blood samples is beneficial as any extra-centrifugation procedures required to separate platelets for counting are likely to cause lysis of the cells and increase the amount of ^{111}In released.

Platelet survival was calculated for 17 patients and the results are shown in figure 3.6. The mean value for each group is listed in table 3.6.

Table 3.6 Mean Platelet Survival

Diagnosis	Number in study	Platelet survival (days)	SD
UC	6	8.9	(0.9)
C	4	7.9	(1.2)
ITP	5	5.0	(1.0)
DVT	1	8.7	-
Coeliac	1	8.5	-

From these results, patients with IBD (both UC and C) were found to have normal platelet survival (range 8-10 days (2)). One report published in 1973 demonstrated a slightly decreased survival in these patients (78). It was calculated to be 7.0 days in UC and 7.2 days in C compared with a control value of 7.8 days. These values were statistically different both with a p value of <0.05. However, it is difficult to draw conclusions from these results as the calculation of TBV used in the determination of platelet survival has confidence limits of +/- 15% (85).

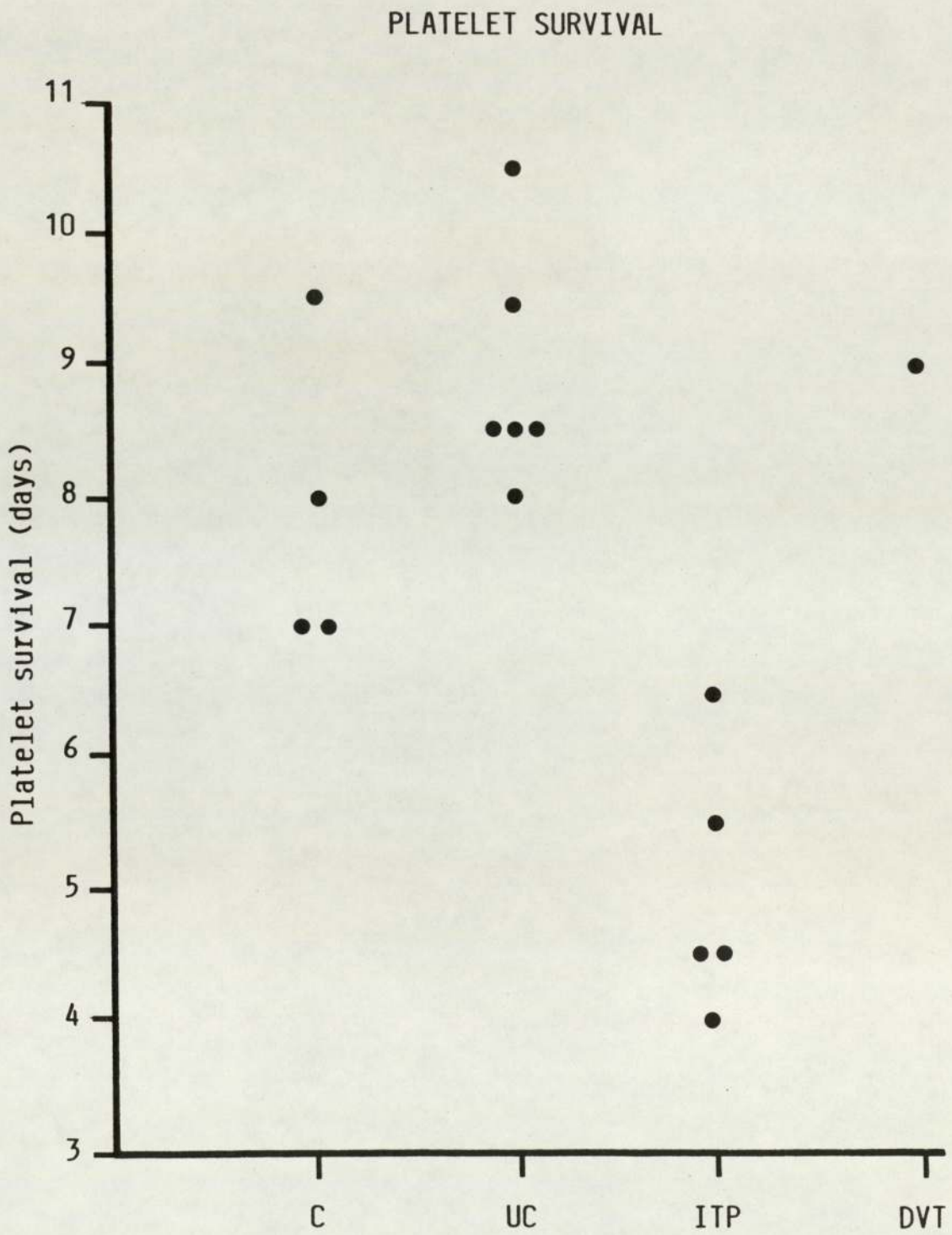

PLATELET SURVIVAL
Platelet survival (days)
11
10
9
8
7
6
5
4
3
C
UC
ITP
DVT

Figure 3.6

Platelet survival in all patients with ITP was reduced. This reduction was independent of the site of destruction.

Although conclusions cannot be drawn from the single studies of a patient with DVT and a patient with coeliac disease, the calculated platelet survival of each patient was in the normal range.

3.5 HEPATIC UPTAKE

Time course activity curves, where each 1 minute value is expressed as a percentage of the 20 minute value, over the spleen, liver and cardiac blood pool following injection of labelled platelets in 5 patients with UC and 5 with C were similar to those illustrated in figure 3.7. Composite time activity curves, where the counts in each 1 minute frame were expressed as a percentage of the counts in the 2nd 1 minute frame, demonsrated that there was some degree of hepatic sequestration (figure 3.8). Values of > 100% are representative of platelet damage. This hepatic sequestration can be quantified by expressing the liver activity at 10 minutes as a percentage of the activity at which completion of uptake in the liver has occurred. This has been reported to be at 2 minutes (43) and at 6 minutes (41,42). Therefore, both these values were used to calculate hepatic sequestration. Using the 2 minute activity, sequestration of platelets was 116± SD13%. When the 6 minute activity was used it was calculated to be 100 ± SD3%. Thus, the time chosen to represent the completion of hepatic uptake made a significant difference ($p < 0.01$) when calculating the extent of hepatic sequestration. At the 2 minute value, the result calculated is comparable to that reported by Peters *et al* who reported hepatic sequestration to be 113 ± SD 7.6% (n = 5) for platelets labelled with ^{111}In oxine in saline (43).

It is difficult to interpret the results obtained in this study as the time at which completion of uptake has occurred is subjective assessment. It is now generally accepted that *in vivo* evidence of disturbed bio-distribution is a more sensitive indicator of cell activation than *in vitro* parameters eg) aggregation tests. It is possible to estimate the degree of platelet damage by comparing the shapes of the blood and hepatic time activity curves. Figure 3.7 shows the liver curve to parallel blood pool activity.

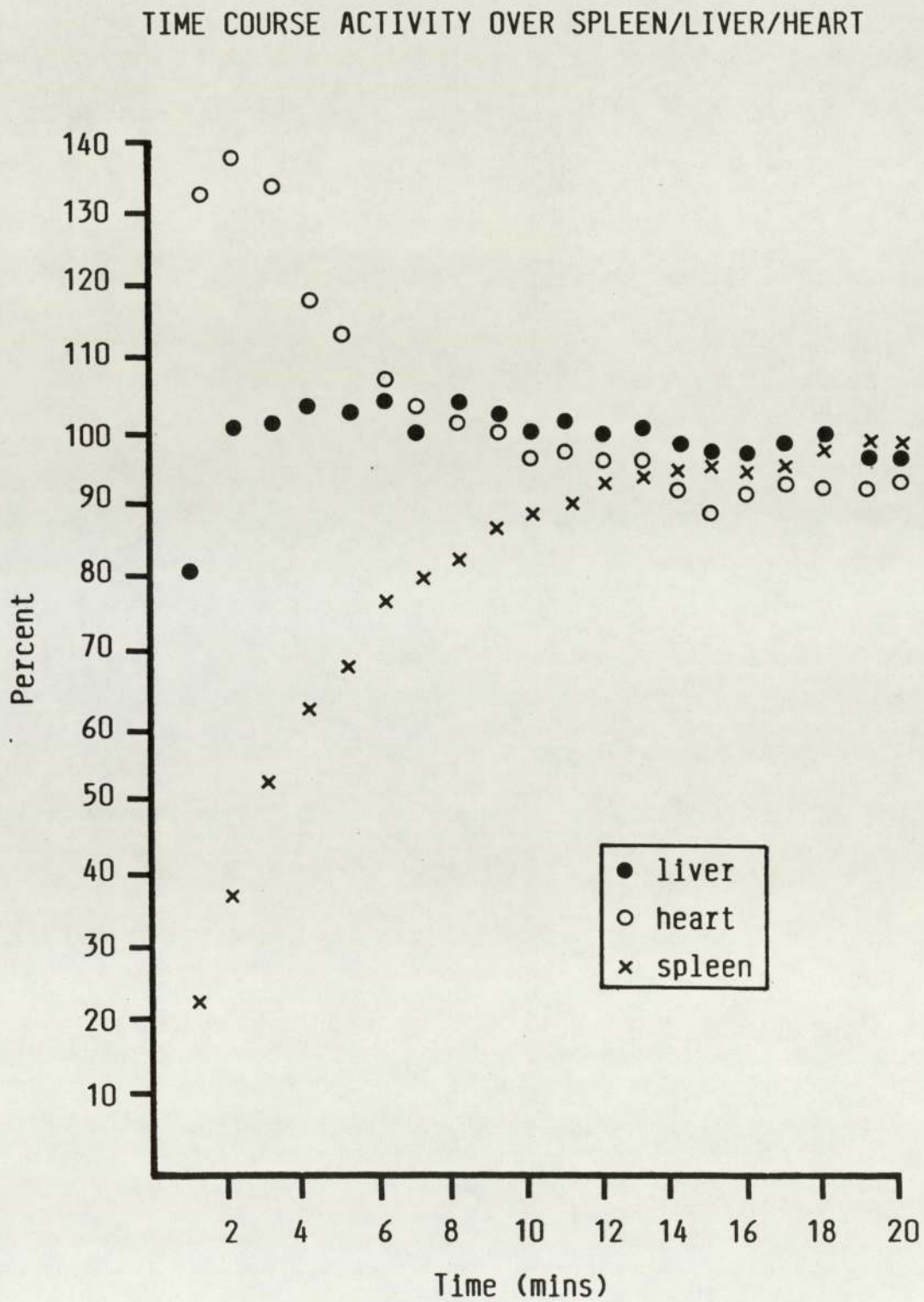

TIME COURSE ACTIVITY OVER SPLEEN/LIVER/HEART
Percent
140
130
120
110
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
Time (mins)
liver
heart
spleen

Figure 3.7

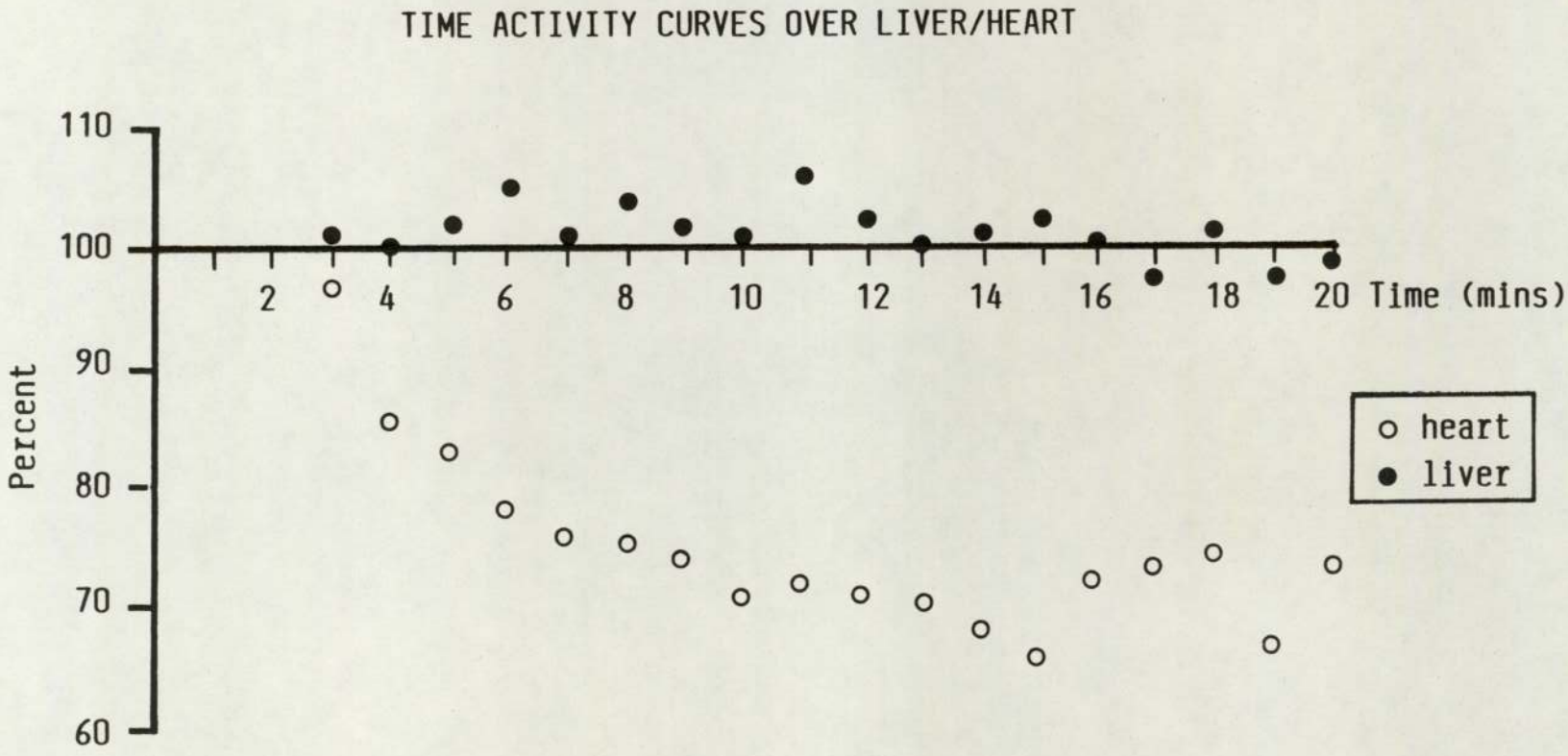

TIME ACTIVITY CURVES OVER LIVER/HEART
110
100
90
80
70
60
Percent
2
4
6
8
10
12
14
16
18
20
Time (mins)
heart
liver

Figure 3.8

3.6 SPLENIC BLOOD FLOW

The rate constant (Ks) of ^{111}In labelled platelet uptake by the spleen was calculated by the extrapolating technique described by Peters *et al* (42). This requires a subjective estimate of the asymptote value on the activity curve. This value was taken to be the point at which the splenic uptake curve reaches a plateau. This was difficult to determine in certain cases where uptake in the spleen was slow to reach equilibrium. In addition, fluctuations in the splenic uptake curve made it difficult to determine the asymptote. Such fluctuations were reduced by using a computer protocol to smooth the dynamic curve. Thus, an estimate of Ks for each patient (5 UC, 5 C, 3 ITP) was determined by 3 independent observers. The results are listed in table 3.7.

The mean value of Ks for each patient was used to calculate the mean values for SBF and tp in each disease group. The results are listed in table 3.8.

Table 3.8 Mean SBF and tp Values

Disease	No. in study	SBF (SD) (% TBV min^{-1})	tp (SD) (min^{-1})
UC	5	14.0 (2.3)	11.6(2.8)
C	5	9.0 (2.2)	14.8 (5.1)
ITP	3	8.3 (1.5)	37.7 (4.9)

The normal values for SBF and tp reported in the literature are listed below.

Table 3.7 Estimates of Ks and SBF by 3 independent Observers

DISEASE	OBSERVER 1			OBSERVER 2			OBSERVER 3		
	Asymptote value	Ks	SBF	Asymptote value	Ks	SBF	Asymptote value	Ks	SBF
UC	49	0.003	13	50	0.003	13	49	0.003	13
UC	155	0.003	11	154	0.0033	15	147	0.0059	27
UC	75	0.002	7	70	0.0033	12	67	0.0047	16
UC	75	0.0024	8	72	0.0034	16	69	0.0049	17
UC	40	0.0021	10	39	0.0027	12	37	0.0038	17.5
C	35	0.0016	6	35	0.0016	6	31	0.0041	14
C	135	0.0035	11	136	0.0031	9	132	0.0051	16
C	70	0.0013	5	61	0.0027	9.5	57	0.0047	16
C	40	0.0017	7	53	0.0020	9	36	0.0019	8
C	72	0.0016	5	72	0.0016	5	68	0.0031	9
ITP	70	0.0009	3.5	56	0.0019	9	47	0.0044	17
ITP	40	0.0016	3	37	0.0024	10	34	0.0045	7.5
ITP	60	0.001	3	48	0.0027	8	44	0.0043	12

SBF = % TBV min^{-1}

Ks = sec^{-1}

Author	SBF (SD)	tp (SD)	Ref
Peters	3.8 (0.9)	6.5 (-)	(52)
Peters	4.8 (0.3)	9.5 (-)	(55)
Wadenvik	4.8 (1.9)	9.7 (1.6)	(56)

Each value was calculated using the same extrapolating technique as described in Appendix I. Various disease states have been reported to produce wide ranging values of SBF, from 2.4 %TBV min^{-1} in a patient with spurious polycythaemia to 30% TBV min^{-1} in a patient with cirrhosis of the liver with portal hypertension (56).

It has been demonstrated that platelet kinetics in patients with IBD are normal. Therefore, it was expected that SBF and tp would be within the normal range. However, the values obtained were much higher than the reported normal values. One explanation could be the error involved in calculating SBF due to the subjective estimation of the asymptote value. There are 2 other potential sources of error. Firstly, other sites of platelet pooling in addition to the spleen eg) lung and liver and secondly, as the labelling agent is non-specific, labelling of RBC and WBC could occur. However, both of these would alter the distribution pattern to thus underestimate SBF.

Hyposplenism has been described in IBD but only occurring in patients with extensive colonic involvement and suffering either a relapse or persistenly active course (80,81). Splenic function in these patients was calculated by measuring the rate of clearance of ^{51}Cr labelled heat damaged red cells. A marked prolongation in clearance was demonstrated representing a decrease in spleen function (values ranging from 20.4 to > 171 minutes compared to controls in the range 9.0 to 17.5 minutes). The mechanisms of splenic pooling are unknown. To draw any conclusions of SBF and tp in IBD, investigations at DRH on healthy volunteers and patients

with known altered splenic function are required.

There have been 2 reports in the literature demonstrating a significant positve correlation between SBF and spleen size but lack of correlation between tp and spleen size (55,56). One report used matrix points to determine spleen size (55) but one used a more accurate method for the measurement of spleen size (56). This was calculated by the use of $^{99}Tc^{m}$ labelled stannous colloid to allow spleen edge detection. In this study, no significant correlation has been found between SBF, tp and spleen size in 10 patients with IBD as shown in table 3.9.

Table 3.9 Correlation between SBF, tp and Spleen Size

Disease	SBF (%TBV min^{-1})	tp (min 1^{-1})	Spleen size (no. pixels)
C	9	11	292
C	8	23	296
UC	18	10	324
C	6	15	360
UC	13	8	368
UC	12	15	384
C	10	15	424
C	12	10	492
UC	13	11	520
UC	14	14	536

Causes of ITP are numerous and varied. One cause can be due to

increased pooling of platelets in the spleen which could be demonstrated by ^{111}In uptake as described earlier. An increase in pooling would produce an increase in tp. The results obtained for 3 ITP patients in this study demonstrate an increased tp. However, only 1 of these patients was found to have an increased splenic uptake as shown in table 3.10.

Table 3.10 SBF and tp in 3 Patients with ITP

Patient	Splenic uptake (%)	SBF (%TBV min^{-1})	tp (min^{-1})
1	42	7	41
2	22	8	40
3	20	10	32

3.7 DIAGNOSIS OF DEEP VEIN THROMBOSIS

Seven patients were investigated for DVT using the protocol described. Their case histories are listed below.

Case 1

This patient had complained of pain in the left calf for 2 years, which radiated to the thighs and was associated with parathesing of the feet. She was not on anticoagulant therapy. Twelve days after admission her platelets were labelled with ^{111}In. Imaging did not detect thrombus formation. The patient subsequently underwent exploratory surgery. Pathology was shown to be normal although the patient was reported to be subsequently relieved of pain after surgery. A venogram was not performed.

Case 2

An 82 year old lady presented with a 4 day history of a painful swollen left calf. She was immediately started on heparin therapy and on day 3 of her admission a venogram was performed and reported filling of superficial veins which shows multiple areas of thrombosis. On day 7 her platelets were labelled with ^{111}In and the scans obtained 24 hours after re-injection showed a major concentration of label in the left calf; corresponding to the thrombus detected by venography (figure 3.9).

Case 3

This patient had complained of a swollen leg 2 weeks previously and now presented with a 2 day history of a swollen left calf. He was immediately started on heparin. His platelets were labelled on the 2nd day of his admission and scans 24 hours later showed abnormal accumulation of activity in the lower left calf. The venogram performed 3 days later was consistent with these findings showing a small clot in the lower left calf.

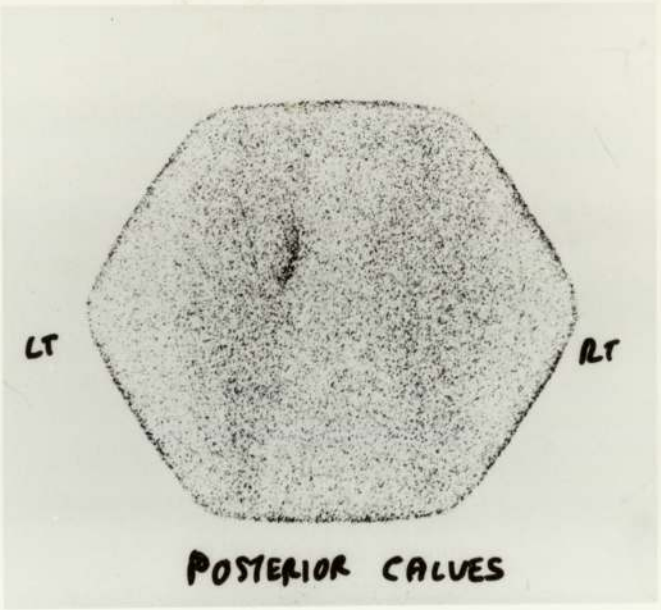


Figure 3.9 Posterior View of Lower Legs at 24 hours showing Abnormal Accumulation of Activity in Lower Left Calf

Case 4

This patient was admitted complaining of a tender left calf. It was not swollen and no skin changes around the tender area were seen. A venogram was not performed because of the patients allergic condition. Ten days after admission her platelets were labelled with ^{111}In. Imaging did not detect a thrombus formation.

Case 5

A 33 year old man with a 10 year history of recurrent DVT following an industrial injury presented with a swollen, tender left leg. He had previously been on anticoagulant therapy but had stopped 2 months ago. He was immediately started on heparin and 7 days after admission his platelets were labelled with ^{111}In. Imaging did not detect thrombus formation. A venogram was not performed.

Case 6

This patient with a previous history of DVT, presented with swelling in the left leg. He was immediately started on anticoagulant therapy. A venogram demonstrated thrombus in the deep veins of the calf. Two days after his admission his platelets were labelled with ^{111}In but imaging did not detect thrombus formation.

Case 7

After an operation for a torn achilles tendon, this patient presented with a 2 week history of a swollen left calf. It was diagnosed as cellulitis and she was started on a course of antibiotics. The swelling persisted and the medical team requested an ^{111}In labelled platelet scan for diagnosis of DVT. There was no abnormal accumulation on the images. A venogram was not performed.

^{111}In labelled platelets as a diagnostic test for DVT is not routinely used. Successful use of the technique requires prompt action. If an accurate diagnosis is to be obtained the investigation must be

performed as soon as there is a clinical suspicion of DVT and preferably before anticoagulant therpay is initiated. As can be seen from the case reports, the conditions under which the technique was performed were not ideal. Firstly, 4 out of 7 patients were started on anticoagulant therapy before the investigation was performed. Although this will have no effect on the platelet function, it will inhibit further propagation of the clot (65). Secondly, it was 7 days or longer between admission and the investigation for 5 patients. This would reduce the accuracy of the technique as the older the thrombi, the longer the delay between injection of radiolabelled platelets and first visualisation by scanning (65). These factors may help to explain the negative results obtained in this section.

$^{99}Tc^{m}$ HMPAO seems to be a promising platelet label for imaging thrombotic lesions. Unlike, ^{111}In oxine labelled platelets, thrombotic lesions can be localised 0.5-1.0 hours after re-injection of the cells. This reduces the possibility of obtaining false negative results due to the decrease in time taken between clinical suspicion and commencement of the test. In addition $^{99}Tc^{m}$ has superior imaging properties, making the lesion easier to visualise. This technique may also be more acceptable to the clinician as the test could be performed and anticoagulant therapy commenced on the basis of the results.

4.0 CONCLUSIONS

4.1 PLATELET LABELLING

The 2 most commonly used chelating agents for labelling platelets are oxine and tropolone. Both agents have been used successfully using simple laboratory techniques (6,10,11,12,18). However, experience of platelet labelling with ^{111}In tropolone is mainly isolated to one department (11), where as ^{111}In oxine, being commercially available, has been used by many departments adopting a variety of laboratory procedures. Adopting any cell labelling procedure requires practice to achieve successful results. The ideal technique must not only maintain the viability of platelets but also achieve high labelling efficiency. Several reports have shown ^{111}In tropolone achieves higher L.E.'s when compared to ^{111}In oxine but maintenance of platelet function with each chelating agent was similar (12,13,14). The L.E. obtained for ^{111}In tropolone in this work was comparable to that with ^{111}In oxine. However, it proved very difficult to adopt the procedure for labelling with ^{111}In tropolone. The samples were difficult to manipulate and platelet function was not maintained. Attempts to label platelets with ^{111}In oxine were successful. Platelet function, as demonstrated by *in vitro* aggregation tests and initial *in vivo* distribution, was maintained and it was decided that ^{111}In oxine was the superior platelet labelling agent. It was therefore subsequently used in the clinical and experimental investigations.

Studies of platelet kinetics have progressed since the introduction of ^{111}In as a platelet labelling agent. Although the mechanisms of platelet kinetics are not fully understood, the distribution, survival and sites of destruction have been determined using isotope techniques. These techniques have been modified for use and computer programs to determine

splenic uptake and platelet survival are now available (Appendices II and IV). It has been shown that abnormal platelet distribution and survival can be identified using these programms.

A protocol has also been defined to estimate hepatic activity. From the dynamic uptake pattern, platelet viability can be assessed. For normally functioning platelets, hepatic activity should parallel the time course of blood activity indicating that the platelets were reversibly pooling and not being irreversibly sequestrated. It is therefore important in kinetic studies to determine the extent of platelet damage using this *in vivo* technique. Normal *in vivo* platelet function was demonstrated in this study from the shape of the curves. Hepatic activity has also been quantified. The results obtained indicate that some hepatic sequestration had occurred but was comparable to that reported in the literature (43). Further work is however required in this area since determination of the time at which hepatic uptake is completed is subjective and markedly affects quantification of hepatic sequestration (41,42,43).

SBF has been calculated in 3 reports using the ^{111}In platelet technique to be 3.8, 4.8 and 4.8 %TBV min^{-1} in normal subjects (52,55,56). SBF was calculated for 13 patients in this study (10 IBD, 3 ITP) using the same techniques. The results were found to be considerably higher for patients with IBD than normal reported values (UC mean = 14.0 C mean = 9.0). The patients with ITP were found to have a mean SBF value of 8.3. It was demonstrated that error in the calculation of SBF could be introduced due to the subjective estimation of the asymptote value. In addition, the information in the literature on SBF has mainly come from one laboratory (42,43,52,55). This could help magnify the error present in the calculation from the subjective estimation. It will be necessary to investigate SBF in healthy volunteers at DRH in order to obtain "in

house" control values before any conclusions can be drawn from the results obtained from patients with IBD and ITP.

4.2 CLINICAL INVESTIGATIONS USING LABELLED PLATELETS

Venography of the leg remains the first line investigation for suspected thrombosis. As a result, patients referred for ^{111}In labelled platelet imaging for diagnosis of DVT are likely to have had a negative venogram or the technique was contra-indicated due to their clinical condition. These factors are likely to lengthen the time between clinical suspicion of a DVT and the request for the investigation. This time delay will decrease the possibility of successful diagnosis. In addition, the patients are likely to have started anticoagulant therapy. Although this will have no effect on the platelet function, it will inhibit further propagation of the clot (65). Since this is the point at which the platelets are accumulating to produce an area of increased uptake it is important that the investigation is performed immediately after clinical suspicion of a DVT. Because, in the majority of cases, it remains a second line investigation, it may be several days before the investigation is performed which reduces the chances of detecting a clot. These factors may contribute to the reasons for the ^{111}In labelled platelet technique remaining an alternative investigation in the diagnosis of DVT.

Platelet kinetic studies in ITP have been shown to be a useful investigation. The mechanisms of ITP are numerous. Imaging has demonstrated various patterns of sequestration of the platelets. Splenic, hepatic and combinations of sequestration site have been reported (1). The site of destruction is important when reaching a decision with respect to splenectomy. It has been demonstrated from the 5 cases presented that platelet kinetic studies provide information that is of value in the clinical management of these patients. A patient may be prevented from undergoing unnecessary surgery if the cause of ITP is found to be due to increased hepatic sequestration.

The development of ^{111}In as a platelet labelling agent at DRH allowed the technique to be used in the investigation of platelet function in IBD. The *in vitro* tests on such patients had demonstrated abnormal platelet function eg) the platelets were "hypersensitive" to aggregating agents. It was thought that this abnormality may contribute to the increase in thrombotic complications reported in IBD (79). Thus, platelet kinetic studies were performed to determine whether this abnormal *in vitro* platelet function was reflected in abnormal distribution, survival and destruction. The results obtained were all within the normal range. Imaging of the lower abdomen did not demonstrate abnormal accumulation of activity in the bowel wall. An increase in activity would demonstrate thrombosis of the bowel wall which has been observed after surgery.

The discrepancy between the *in vitro* and *in vivo* results may be explained by the sensitivity of the isotope technique. Due to the errors incorporated within the technique eg) determination of spleen size and depth in body for calculating splenic uptake and estimating TBV for calculating platelet survival, a large variation in platelet kinetics would be required to demonstrate any abnormality. It was hoped to improve the sensitivity of the technique when imaging the bowel by imaging the diseased bowel removed after surgery. This work still needs to be completed.

Appendix I

Method of calculating Splenic Blood Flow (SBF) and Intrasplenic Platelet Transit time (tp)

BLOOD $\rightleftharpoons$ SPLEEN

K_1 = fraction of total blood volume supplying spleen per minute

K_2 = rate constant of clearance of platelets from spleen

If: Qs = quantity of ^{111}In in spleen

Qb = quantity of ^{111}In in blood

Then: $\frac{dQs}{dt} = K_1Qb - K_2Qs$ (eqn 1)

And: $\frac{dQb}{dt} = K_2Qs - K_1Qb$ (eqn 2)

If total ^{111}In in blood is Qo

Then: $Qo = Qb + Qs$

Integrating for eqn 1 and eqn 2

1) $$\int_o^{Qs} dQs = \int_o^t dt$$

$$Qs = Qo \frac{K_1}{K_1 + K_2} \cdot [1 - e^{-(K_1 + K_2)t}]$$

2) $$\int_{Qo}^{Qb} dQb = \int_o^t dt$$

$$Qb = Qo \frac{K_2}{K_1 + K_2} \cdot [1 - e^{-(K_1 + K_2)t}] + Qo e^{-(K_1+K_2)t}$$

As the values are at equilibrium, the equations will simplify to:

$$\frac{Qb}{Qs} = \frac{\frac{QoK_2}{K_1 + K_2}}{Qo\ \frac{K_1}{K_1 + K_2}}.$$

And therefore:

$$\frac{Qb}{Qs} = \frac{K_2}{K_1}.$$

As: $Ks = K_1 + K_2$

Ks = rate constant of equilibrium between splenic and blood pool compartments

And: $SBF = K_1 \times TBV$

TBV = total blood volume

Substituting: $K_2 = Ks - \frac{SBF}{TBV}$

And: $K_2 = \frac{K_1 Qb}{Qb}.$

Then: $\frac{K_1 Qb}{Qs} = Ks - \frac{SBF}{TBV}$

$$\frac{SBF}{TBV}\ \frac{Qb}{Qs} = Ks - \frac{SBF}{TBV}$$

$$SBF = Ks \times \frac{TBV}{(1 + \frac{Qs}{Qb})}.$$

And: $\frac{1.}{K_2} = \frac{1}{(Ks - \frac{SBF}{TBV})}.$

$\frac{1.}{K_2}$ = tp = intrasplenic platelet transit time

```
 C£ PSPLAT 06/05/88H  SPLENIC ACTIVITY UPTAKE, PART 1; CALCULATION
C
C Program originally developed at the Physics Dept., Q.E.H. Birmingham,
C
C Modified for use at D.R.H. by:
C Nicholas Tulley. Physics Department, Dudley Road Hospital, Birmingham.
C
C Revisions:
C    June 87  2.0  To calculate liver uptake also.
C    June 87  2.1  To put correct factors in DISP2 and DISLV1
C                  and add revision letter to program.
C    June 87  2.2  Changed factors from .051 & .102 to .057 & .114
C    May  88  2.3  Removed all references to liver calculations.
C
      NAME PSPLAT
      LOGICAL CFV
      COMMON XABS,XPBS,XAS,XPS,ITME(12),NSET,REV
      COMMON SP1(12),SP2(12),UPTKS(12)
      DIMENSION SP3(12),NM(35)
      DIMENSION IVN(2,12),NRG(2,12),CTS(16),NPIX(16)
      DATA FMU, PIXSZ, PF1, PF2,   CMSEN/
     +    .114,  .625, .89, .78, 121453./
C
C Introduction
C
      CALL GNAME(NM,35)
      CALL $MOVE(1H?,NM(35),1)
      CALL TQUEST(' ?',0,I)
      CALL TQUEST(NM,0,I)
      CALL TQUEST(' ?',0,I)
      CALL TQUEST('ASSUMES IN EACH ROI GROUP:?',0,I)
      CALL IQUEST('1=SPLEEN, 2=BACKGROUND.  OK [Y]?',IQ,217,-1)
      IF (IQ .EQ. 206) STOP
      REV = 2.3
C
C Define body and spleen edges, from keyboard
C
      CALL TQUEST(' ?',0,I)
      CALL TQUEST('FROM THE LEFT LATERAL (IN 128 COORDS): ?',0,I)
      CALL TQUEST(' ?',0,I)
   10 CALL FQUEST('              ANTERIOR BODY COORD ?',XABS,0.)
      IF (XABS .LE. 0.) GOTO 10
   11 CALL FQUEST('            POSTERIOR BODY COORD ?',XPBS,0.)
      IF (XPBS .LE. 0.) GOTO 11
   12 CALL FQUEST('           ANTERIOR SPLEEN CORRD ?',XAS,0.)
      IF (XAS .LE. 0.) GOTO 12
   13 CALL FQUEST('          POSTERIOR SPLEEN CORRD ?',XPS,0.)
      IF (XPS .LE. 0.) GOTO 13
      GOTO 70
C
   70 DIFSP1 = ABS(XPBS - XABS)/2. * PIXSZ * PF1
      DIFSP2 = ABS(XPS  -  XAS)/2. * PIXSZ * PF2
C
      CALL FQUEST('    INJECTED ACTIVITY (MBQ) [3.0] ?',ACTY,3.0)
      CALL IQUEST('         IMAGED THROUGH COUCH [Y] ?',ICOU,217,-1)
```

```
      F9 = 1.168
      IF (ICOU .EQ. 206) F9 = 1.0
      CALL IQUEST('COUNTS FROM VIEWS OR KEYBOARD [V] ?',JQ,214,-1)
      CFV = .TRUE.
      IF (JQ .EQ. 203) CFV = .FALSE.
C
C Calculation
C
      ISET = 1
   42 CALL TQUEST(' ?',0,I)
      CALL IQUEST(
     +' TIME AFTER INJECTION (MINS) [FINISHED] ?',ITME(ISET),0,0)
      IF (ITME(ISET) .EQ. 0) GOTO 44
      IF (.NOT.CFV) GOTO 45
C
C ... Read anterior image and region numbers
      CALL TQUEST('ANTERIOR?',0,I)
      IVFAB = MFILE('V',0,IVN(1,ISET),IFF,IFL,MSIZ,ITYP)
      CALL CLOSE(IVFAB)
      IRFAB = MFILE('R',0,NRG(1,ISET),I,I,MSIZ,ITYP)
      CALL CLOSE(IRFAB)
C
C ... Read posterior image and region numbers
      CALL TQUEST('POSTERIOR?',0,I)
      IVFAB = MFILE('V',0,IVN(2,ISET),IFF,IFL,MSIZ,ITYP)
      CALL CLOSE(IVFAB)
      IRFAB = MFILE('R',0,NRG(2,ISET),I,I,MSIZ,ITYP)
      CALL CLOSE(IRFAB)
      CALL TQUEST(' ?',0,I)
      GOTO 40
C
   45 CALL FQUEST(' CORRECTED ANTERIOR SPLEEN COUNTS ?',SP1(ISET),0.)
      IF (SP1(ISET) .EQ. 0.) GOTO 45
   46 CALL FQUEST('CORRECTED POSTERIOR SPLEEN COUNTS ?',SP2(ISET),0.)
      IF (SP2(ISET) .EQ. 0.) GOTO 46
   40 ISET = ISET + 1
      GOTO 42
C
C OK, view and region numbers read ... read the counts:
C
   44 NSET = ISET - 1
      CALL TQUEST(' ?',0,I)
      IF (.NOT.CFV) GOTO 50
      CALL TQUEST('WAIT, READING COUNTS FROM VIEWS: ?',0,I)
      CALL TQUEST(' ?',0,I)
      CALL TQUEST(' --- VIEW NUMBER --?',0,I)
      CALL TQUEST(' ANTERIOR POSTERIOR?',0,I)
   50 CALL TQUEST(' ?',0,I)
      DO 20 ISET=1,NSET
      IF (.NOT.CFV) GOTO 60
      CALL DISPNN(IVN(1,ISET),2)
C ... Spleen counts
      CALL DCOUNT(IVN(1,ISET),1,1,NRG(1,ISET),CTS(1),NPIX(1))
      SP1(ISET) = CTS(1)-(FLOAT(NPIX(1))*CTS(2)/FLOAT(NPIX(2)))
      CALL DCOUNT(IVN(2,ISET),1,1,NRG(2,ISET),CTS(1),NPIX(1))
```

```
      SP2(ISET) = CTS(1)-(FLOAT(NPIX(1))*CTS(2)/FLOAT(NPIX(2)))
C
   60 TM = 0.01031 * FLOAT(ITME(ISET)) / 60.
      TDC = EXP(TM)
      ANTSP = SP1(ISET) * TDC
      PSTSP = SP2(ISET) * F9 * TDC
      SP3(ISET) = SQRT(ANTSP * PSTSP)
      S4 = (EXP(.057 * DIFSP2) - EXP(-.057 * DIFSP2)) / (FMU * DIFSP2)
      UPTKS(ISET) = SP3(ISET)*100./(CMSEN*ACTY*EXP(-.057*DIFSP1)*S4)
   20 CONTINUE
C
      CALL CHAIN('PSPLT2')
      STOP
      END
```

```
C£ PSPLT2 06/05/88F  SPLENIC ACTIVITY UPTAKE, PART 2; PRINTOUT
C
C Program originally developed at the Physics Dept., Q.E.H. Birmingham,
C
C Modified for use at D.R.H. by:
C Nicholas Tulley. Physics Department, Dudley Road Hospital, Birmingham.
C
      NAME PSPLT2
      COMMON XABS,XPBS,XAS,XPS,ITME(12),NSET,REV
      COMMON SP1(12),SP2(12),UPTKS(12)
      DIMENSION NM(35)
C
      CALL GNAME(NM,35)
      WRITE(1,80)NM,REV
      WRITE(9,80)NM,REV
   80 FORMAT(/1X,35A2//' (PROGRAM REV.',F5.1,')')
C
      WRITE(1,10)XABS,XPBS,XAS,XPS
      WRITE(9,10)XABS,XPBS,XAS,XPS
   10 FORMAT(/
     +' LEFT LATERAL BODY LIMITS:',2(F7.1,','),5X,
     +'SPLEEN LIMITS:',2(F7.1,','))
C
      DO 20 ISET=1,NSET
      IF (ISET .EQ. 1) WRITE(1,15)
      IF (ISET .EQ. 1) WRITE(9,15)
   15 FORMAT(/5X,
     +10X,'---------- SPLEEN ----------'/
     +' TIME (MINS)   ANTERIOR  POSTERIOR   UPTAKE'/)
      WRITE(1,30)ITME(ISET),SP1(ISET),SP2(ISET),UPTKS(ISET)
   20 WRITE(9,30)ITME(ISET),SP1(ISET),SP2(ISET),UPTKS(ISET)
   30 FORMAT(I7,5X,2F11.0,F7.1,' %')
      CALL EXITS
      STOP
      END
```

Appendix III

Method of Calculating Weighted Mean Survival

Platelet survival can be determined from the counts obtained at time, ti, after re-injection for each blood sample using a weighted mean value of the linear and logarithmic estimate.

a) to calculate deviation from the linear curve

$$\Sigma \frac{ti}{m} = \bar{t}$$

ti = time at which sample was taken
m = number of samples

$$\Sigma ti^2 - \frac{(\Sigma ti)^2}{m} = \Sigma (ti - \bar{t})^2 = F$$

F = deviation from $\bar{t}$

$$\Sigma \frac{Ni}{m} = \bar{N}$$

Ni = counts

$$\Sigma Ni^2 - \frac{(\Sigma Ni)^2}{m} = \Sigma (Ni - \bar{N})^2 = G$$

G = deviation from $\bar{N}$

$$\Sigma (Ni\ ti) - \frac{(\Sigma Ni)(\Sigma ti)}{m} = \Sigma [(Ni - \bar{N})(ti - \bar{t})] = H$$

H = sum of deviations of count and time

b) to calculate deviation from the logarithmic count

$$\sum \frac{yi}{m} = \bar{y}$$

yi = logarithmic count

$$\sum (yi\ ti) - \frac{(\sum yi)(\sum ti)}{m} = \sum [(yi - \bar{y})(ti - \bar{t})] = M$$

M = sum of deviations of log count and time

Then $A = \bar{t} - \bar{N}F/H$

A = time of linear platelet survival

$B = F/M$

B = time of logarithmic platelet survival

$S_A = G - H^2/F$

S_A = sum of residual squares of linear estimate

$C = \bar{y} - \bar{t}/B$

and Then

$$S_B = (e^{C - ti/B} - Ni)^2$$

S_B = sum of residual squares of logarithmic estimate

The weighted average estimate of mean survival, M_w, is given by

$$M_w = \frac{A\ S_B + B\ S_A}{S_B + S_A}$$

Appendix IV

```
C£ PLTSVL 18/05/88B  PLATELET SURVIVAL DETERMINATION (APM)
      PROGRAM PLTSVL
      DIMENSION ITIME(10),COUNTS(10)
      CALL SETAR(ITIME(1),10,0)
      CALL SETAR(COUNTS(1),10,0)
C
C  This program calculates a weighted mean estimate of
C the platelet survival time using a fit to the linear
C estimate of survival and an estimate derived from an
C exponential fit.
C
C Method of Calculating weighted Mean Survival.
C
C First get the data....
C
      CALL IQUEST('HOW MANY DATA POINTS [STOP] ?',NPTS,0,0)
      IF (NPTS .EQ. 0) STOP
      CALL INPUT(INPT)
      IF ( INPT .EQ. 1 ) CALL TQUEST('TYPE IN YOUR TIMES ?',0,I)
      READ(INPT,*)(ITIME(I),I=1,NPTS)
      IF ( INPT .EQ. 1 ) CALL TQUEST('TYPE IN YOUR COUNTS ?',0,I)
      READ(INPT,*)(COUNTS(I),I=1,NPTS)
      AM=FLOAT(NPTS)
C
C Next calculate average time and sum of time squared
C
      SUMTME=0
      STMSQD=0
      DO 100 I=1,NPTS
      STMSQD=FLOAT(ITIME(I))*FLOAT(ITIME(I))+STMSQD
  100 SUMTME=FLOAT(ITIME(I))+SUMTME
      TBAR=SUMTME/AM
      SQSUMT=SUMTME*SUMTME
C
C and average counts....
C
      SUMCTS=0
      SCTSQD=0
      DO 110 I=0,NPTS
      SCTSQD=COUNTS(I)*COUNTS(I)+SCTSQD
  110 SUMCTS=COUNTS(I)+SUMCTS
      CBAR=SUMCTS/AM
      SQSUMC=SUMCTS*SUMCTS
C
C Next calculate sums of count time product
C
      CTSTME=0
      DO 120 I=1,NPTS
  120 CTSTME=COUNTS(I)*FLOAT(ITIME(I))+CTSTME
C
C Now calculate sum of logs of counts
C
      SUMLOG=0
      SUMLTM=0
```

```
      DO130 I=1,NPTS
      SUMLTM=ALOG(COUNTS(I))*FLOAT(ITIME(I))+SUMLTM
  130 SUMLOG=ALOG(COUNTS(I))+SUMLOG
      ALBAR=SUMLOG/AM
C
C That done we can now calculate a linear platelet survival time 'A'
C using N(bar)=c-kt(bar). A logarithmic estimate 'B' is also
C obtained from ln(Ni)=c'-k't(i) where B=1/k'
C
      F=(STMSQD-(SUMTME*SUMTME/AM))
      G=SCTSQD-(SQSUMC/AM)
      H=CTSTME-((SUMCTS*SUMTME)/AM)
      FM=SUMLTM-(SUMLOG*SUMTME/AM)
      A=TBAR-(CBAR*F/H)
      B=F/FM
C
C Now the residual sum of squares 'SA' associated with A
C which we use to determine the weighting factors for our
C weighted mean, can be calculated.
C
      SA=G-(H*H/F)
C
C SB is associated with estimate B is calculated here...
C
      C=ALBAR-(TBAR/B)
      SB=0
      DO 150 I=1,NPTS
      SQRED=EXP(C-FLOAT(ITIME(I))/B)-COUNTS(I)
  150 SB=SQRED*SQRED+SB
C
C Which leaves just the weighted mean to be calculated....
C
      WMEAN=((A*SB)+(B*SA))/(SB+SA)
      WRITE(1,200)WMEAN
  200 FORMAT(' WEIGHTED MEAN (MINS) = ',G12.4)
      DWMEAN=WMEAN/(24*60)
      WRITE(1,210)DWMEAN
  210 FORMAT(' WEIGHTED MEAN (DAYS) =',G12.4)
      STOP
      END
```

Appendix V

PATIENT INFORMATION SHEET

Assessment of blood cell function in patients with inflammatory bowel disease.

You will be asked to attend the Physics & Nuclear Medicine Department at Dudley Road Hospital on each of 5 days starting on a Monday. On the first day a blood sample will be taken to which a radioactive material will be added. This will be injected into your arm. The amount of radioactivity involved is much lower than having a barium investigation. There are no unpleasant or after effects of the injection and you may continue to eat and drink normally. After the injection a series of pictures showing the distribution of the injection throughout your body will be taken. To obtain these pictures, all you have to do is lie on a couch and a camera will be placed in appropriate positions. This process will take approximately one hour. The pictures will be repeated on Monday afternoon. You will be asked to remain in the hospital all day.

On Tuesday the pictures will be repeated and another blood sample will be taken. The whole procedure will take 30 minutes.

On Wednesday, Thursday and Friday a blood sample only will be taken.

Would you please attend the Physics and Nuclear Medicine Department at 9.00 am on ..... for the test.

The Nuclear Medicine Department is at the front of the hospital. Take the right hand corridor after the tea bar which will take you to the X-ray reception, then follow the blue line which will take you to the Department.

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