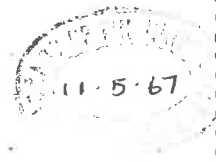


PLATELET KINETICS IN HEALTH AND DISEASE

THESIS

SUBMITTED FOR THE

DEGREE OF DOCTOR OF MEDICINE

IN THE

UNIVERSITY OF ADELAIDE

BY

RAELENE L. KINLOUGH

AUGUST, 1966

Dear Sir,

I am agreeable to my thesis "Peanut lectin in health and disease" being posted in the library and to be available loan and photocopying.

Yours sincerely,

CONTENTS

ACKNOWLEDGEMENTS

The work described in this thesis was carried out in the Department of Medicine at the University of Adelaide and was made possible by the generous support of the National Heart Foundation of Australia and its Medical Director, Dr. Ralph Reader. I am particularly grateful to Professor D.J. Deller and to Professor H.N. Robson for their support and encouragement, and to Dr. Harry Lander, who directed this work, for his energetic stimulus, valuable advice and helpful criticisms. My colleagues Dr. Colin Schwartz and Dr. Neville Ardlie of the Institute of Medical and Veterinary Science must receive particular mention as part of this work was carried out in close collaboration with them. My thanks are due also to Dr. James Bonnin, Director of the Institute of Medical and Veterinary Science, Adelaide, for permission to employ the facilities of his various laboratories; to the Honorary Staff of the Royal Adelaide Hospital for permission to study patients under their care; to members of the Norwood Apex Club who acted as normal volunteers for platelet survival studies; to the medical and laboratory staff of the Institute of Medical and Veterinary Science and the porters of the Royal Adelaide Hospital who participated in Chandler studies, and to all patients who kindly submitted to investigation.

For technical assistance I must record the able work carried out at various times by Mrs. D. Pyle, Miss M. Hall and Miss C. Hallsworth. Finally my sincere appreciation is extended to Mr. W. Nolan who has photographed the illustrations, and to Miss Joan Devaney who typed the thesis.

Declaration of Originality

I declare that this thesis is of my own composition and is a true record of work carried out on platelet behaviour in the Department of Medicine of the University of Adelaide. I have not submitted this material for any other degree and any work carried out in conjunction with colleagues has been fully acknowledged.

CHAPTER I

AN HISTORICAL REVIEW

platelets were in fact merely the results of fragmentation of leucocytes. Weigert (1887) claimed that the findings were artefactual and a result of vessel compression, circulation disturbance and the type of anaesthetic used. By repeating his experiments on the intact vessels of the wing of an unanaesthetized bat, Bizzozero confirmed his previous work. In 1885 his observations were reinforced by Eberth and Schimmelbusch and again in 1889 by Laker who studied the circulating blood in the vessels of living dogs and other mammals.

Counting Platelets

The first attempts to count the number of platelets in the blood were made by Reiss in 1872, but in many instances the results he obtained were erroneous as he found increased numbers of platelets in conditions which are now known to be accompanied by thrombocytopenia.

The first accurate counts of platelets were probably those of Hayem in 1878 and the present range of normal is not significantly different from that of Hayem.

As a result of Bizzozero's work showing platelets to play a considerable role in thrombus formation, Hayem postulated in 1882 that the platelet played an important part in haemostasis and that alteration in numbers of platelets, for example, a decrease or absence, should result in disorganization of the of the haemostatic mechanism.

Krauss (1883) and Denys (1887) in part supported this concept when they noted a dimunition in platelets in purpura haemorrhagica, followed by an increase after the arrest of the haemorrhage.

Source of Platelets

Throughout the nineteenth century controversy raged. On the one hand were those who would not allow that platelets even existed, and on the other the protagonists in conflict as to their source. Engel (1893) believed they arose from the nuclei of normoblasts, while Wlassov (1894) and Bremer (1894) held that they arose from disintegrated erythrocytes and would not allow them to be third elements of the blood.

Dominici remained an antagonist to the theory of the platelet being derived from red cell destruction, and in 1900 introduced the concept that platelets were "organites", that is, formed elements liberated by cells and lacking a nucleus. As the parent cell he described mononuclear cells with pedicles of protoplasm which, when broken off, made up the platelet.

Foa and Salvioli (1880) observed giant cells of bone

marrow (the megakaryocyte of Howell), and commented on its fragmentation into colourless hyaline bodies and thought these to be red cell precursors.

The consensus since Wright's work in 1906 and 1910, however, has been that platelets are produced by megakaryocytes. These cells are distributed mainly in the bone marrow in man while in rodents the spleen contains varying numbers. Platelet formation was described as follows:

> "All of the blood platelets are detached portions or fragments of the cytoplasm of the megakaryocytes; which are in such relation to the blood channels in the marrow that detached portions of their cytoplasm are quickly carried by the blood current into the circulation. The breaking up of the cytoplasm into the platelets occurs only in cells which have reached a certain stage of growth and development and is probably rapidly completed once begun. It takes place in various ways but usually by the pinching off of small rounded projections or pseudopods from the cell body or from larger pseudopods, or by the segmentation of slender pseudopods, or by the pinching off of longer or

shorter pesudopods which may or may not undergo segmentation later. All or most of the cytoplasm of the giant cell is given off to the blood stream and the nucleus degenerates. The more or less naked nucleus is often carried by the blood stream to the lungs where it lodges in the capillaries. Before the separation of a platelet takes place, the red to purple staining granules in that portion of the cytoplasm which is to form the platelet are separated from the rest by a zone of hyaline cytoplasm and this sharply outlined mass of granules becomes the central granular mass of the blood platelet which has been regarded by some workers as a nucleus."

Wright's conception of platelet formation was obtained from the study of stained sections of tissue and supravital preparations. The source and mechanism of platelet production proposed by Wright was met with some opposition initially and such workers as Brown (1913) proposed that under conditions of increased demand platelets could develop by cytoplasmic fragmentation of leucocytes thus disputing Wright's theory.

Bunting (1909) was one of the first to uphold

Wright's work experimentally and he did this by noting an increase in the number of megakaryocytes and the fragments issuing from their pseudopods coincident with the rise in the number of platelets induced by repeated bleeding or by the injection of haemolytic substances.

Downey (1913) and Smith et alii (1938) followed suit, but irrefutable evidence of the correct nature of origin of platelets was eventually put forward by Humphrey (1955). With the technique of using fluorescent antibodies devised by Coons and Kaplan (1950) for identifying substances of similar antigenic structure, he produced antisera against rabbit platelets and the bone marrow was then stained with the fluorescent antisera. Fluorescence was seen only in the cytoplasm of megakaryocytes and he deduced that megakaryocytes and platelets possess some characteristic antigenic structure which may be interpreted as direct evidence of their relationship.

Subsequently Kinosita et alii (1961) have developed a technique for placing windows in bone which permit the marrow to grow into the thin space between the windows. They then exteriorize the femur and observe platelet production directly from the megakaryocytes of bone marrow with an intact blood supply. This accumulated evidence

now proves that blood platelets do indeed arise from the megakaryocytes of the bone marrow.

The Fine Structure of Blood Platelets

The small dimensions of platelets have always limited their morphological study, but with the development of the electron microscope new possibilities were opened for their full examination in minute detail. The first description of blood platelets by this method was published by Wolpers and Ruska in 1939. As a result of this new technique, data concerning their morphology, physiology and pathology have been gathered. Platelets are now known to consist of a limiting membrane which is identical to the cell membrane. In the hyalomere several granulomere elements are dispersed. These consist of dense granules, mitochondria - some of which may be seen to have cristae, cytosomes, vacuoles, vesicles and tubules or canaliculi, and small granules. Glycogen granules have been identified either dispersed or forming aggregates in the hyalomere. The fine structure of the platelet has been fully reviewed by David-Ferreira (1964).

The Regulation of Platelet Production

Thrombopoiesis is influenced by several factors. The number of circulating platelets per se is of importance as acute stimulation of platelet production may be caused by

thrombopheresis (Finch, 1961) producing a release of megakaryocyte cytoplasm with an increase in platelet count within three or four days. Chronic stimulation of this type causes an increased number of megakaryocytes. Humoral substances capable of stimulating platelet production are known to occur and may be fractioned from plasma. Steinberg, Dietz and Martin (1959) fractionated normal human plasma and injected rabbits producing several different effects on the cellular content of the bone marrow and peripheral blood including megakaryocytic hyperplasia and thrombocytosis as well as effects on the granulocytic series and erythrocytes. They postulated that the fractions represented haemocytopoietins which regulated the haemopoietic mechanism. It is likely that a balance between plasma fractions is necessary normally to maintain platelet levels.

Linman (1962) in experiments on rats using anaemic rat plasma induced a leucocytosis and thrombocytosis and tended to support the theory that all aspects of haemopoiesis may be subject to humoral regulatory control. Other factors reported to mobilize platelet reserves are ACTH, epinephrine, serotonin and hypothermia, while pyridine, fats, batyl alcohol and citrovorum factor will also increase thrombopoiesis.

Platelet Metabolism

The platelet is now recognised as a highly metabolic functional unit with a complete biochemical and biophysical machinery. Its enzymatic activity was considered for some time to be rudimentary and Roskam (1924) and Tocantins (1938) afforded it brief mention only, while as recently as 1953 Tullis acknowledged it to have only a feeble metabolic function. However, with constant improvement in techniques it has been found to contain most of the enzymes and co-enzymes for the major pathways of intermediary metabolism, especially those involved in glycolysis. The greater part of the glucose metabolized is converted to pyruvate while a proportion is converted to glycogen, lipids and amino acids.

No demonstrable desoxyribonucleic acid has been found but small amounts of ribonucleic acid have been identified. A sulphated mucopolysaccharide is present (Odell and Anderson, 1957) but this is probably of a structural nature. There are also high concentrations of adenine and adenosine triphosphate (Born, 1956), almost all of the energy produced by adenosine triphosphate being used in structural metamorphosis and clot retraction.

This link between energy metabolism and the morphologic aspects of platelet function was established by Bettex-Galland and Lüscher (1959) with the isolation of a contractile

protein from human platelets. This material, named thrombosthenin, accounts for 15 per cent of the total protein and is an actomyosin, in many ways similar to muscle actomyosin. It contracts in the presence of magnesium and adenosine triphosphate and is an adenosine triphosphatase, breaking a phosphate group from adenosine triphosphate to form adenosine diphosphate.

The platelet lipids contain the thromboplastic activity of blood platelets (Monkhouse, 1960; Troup, 1961). Most of this activity has been found to be due to phosphatidyl serine and may be enhanced by lecithin. Phosphatidyl enthanolamine shows some activity only in the presence of the individually inactive lipids lecithin and sphingomyelin. Inositol phosphatide and cholesterol are also present in platelets but have no thromboplastic effect. All these lipids are present in approximately the same concentrations as in red cells, but there is somewhat more lecithin and slightly less phosphatidyl serine in platelets. Serotonin and histamine (Humphrey and Jaques, 1954) and the catechol amines (Hughes and Brodie, 1959) are present in significant quantities in platelets.

Platelet Lifespan

Platelet lifespan has been determined by a variety of

different methods over the years. Studies carried out have tended to fall into well defined categories.

1. Depletion studies
   (a) Animals have been made thrombocytopenic by chemical means and the rate of regeneration of the platelets determined.
   (b) Radiation studies.
2. Transfusion studies
3. Labelling of cells using radioactive isotopes

Depletion

Duke (1911) using dogs, and Firket (1922) using rabbits both found that after removing blood from the circulation, defibrinating it and returning it, one-fifth of the normal platelet mass was regenerated per day. Firket also observed a similar response to the injection of small amounts of saponin. Bedson (1923) induced thrombocytopenia in guinea pigs and again noted the rate of regeneration of platelets to be similar to that of Duke and Firket, while Tocantins (1936), using the same method to make dogs thrombocytopenic, found that preinjection platelet levels were reached 3 - 5 days after the last day of thrombocytopenia. Roy (1962) was able to observe the rate of regeneration of blood platelets in rats by infusions of bacterial endotoxin.

The behaviour of circulating platelets and marrow megakaryocytes after total body irradiation has also been studied for information about platelet survival. In 1931 Shouse et alii exposed dogs to large doses of irradiation and it was found that the platelets had disappeared after 7 - 8 days. These workers also observed that at autopsy the megakaryocytes of the dogs had been destroyed by the radiation and suggested that the platelet disappearance time indicated the life cycle of the platelets in circulation.

Lawrence and Valentine (1947) cross circulated a normal cat with a cat made thrombocytopenic by irradiation and found that the platelets derived from the normal cat disappeared from the thrombocytopenic cat 2 - 4 days after the cross circulation was severed. Further information has been gained from the examination of humans who have accidentally received single doses of total body irradiation.

In several criticality accidents (Andrews et alii, 1959; Hempelmann et alii, 1952; Mathe et alii, 1959; Jammet et alii, 1959) and in patients who did not have marrow disease who received doses of radiation of the order of 250 - 400r, a drop in the level of circulating platelets occurred after a latent period of about 10 days and reached low levels

within 21 days, a level which was maintained over a period of 10 - 14 days. Gradual recovery began between 31 and 35 days of the initial radiation. This has been interpreted as meaning that the pre-existing megakaryocytes continued to function soon after the irradiation but production of new megakaryocytes was suspended. When those megakaryocytes which were present initially were exhausted, there followed a phase of falling platelet levels which lasted about 10 days - commensurate with the life-span of normal platelets. In fatal accidents (Hempelmann et alii, 1952; Mathe et alii, 1959) resultant on larger doses of irradiation having been received there was a rapid fall in platelets due to immediate megakaryocyte inhibition. This fall in platelet levels took place over a period of 9 days, a time which once again would represent the lifespan of circulating platelets.

Transfusion Studies

There are numerous observations on the circulation of platelets transfused into thrombocytopenic subjects. In 1910 Duke performed direct (vein to vein) blood transfusions on three patients with thrombocytopenia. In two of the recipients the platelet count was increased for 3 - 4 days and the bleeding was controlled for this period in all

three. Stefanini and Chatterjea (1951) and Stefanini et alii (1952) transfused polycythaemic blood into patients with idiopathic thrombocytopenia and secondary thrombocytopenia. The platelets disappeared in 0 - 3 hours in the first group and in the latter 12 - 96 hours. Hirsh et alii (1950, 1951) transfused platelet rich blood into thrombocytopenic patients and found platelet survival time to vary between 1 and 8 days, depending on the type of thrombocytopenia from which the patient was suffering.

The Use of Radioactive Isotopes for Platelet Labelling

The introduction of radioisotopes as a clinical tool has been of considerable use. The advantages of radioactive isotopes are:

(a) the ease of performance;

(b) some information may be gained which is not readily available by other techniques;

(c) The functional state of organs or cells may be examined under physiological conditions;

(d) measurements may be made in vivo;

(e) experimental data obtained may be amenable to mathematical analysis; and

(f) they may allow a kinetic study of the turnover of cells etc.

The main disadvantage is that the fate of the label is not necessarily the fate of the cell and some elution of the label may take place or reutilisation occur with some labels. There has been a variety of different experimental methods using labelled platelets in the study of platelet lifespan. In the earlier studies performed, platelets were labelled in vitro by incubation with a radioisotope compound. After washing, often several times, the platelets were reinfused and their disappearance from circulation noted. In general this method suggested shorter platelet survival times than other isotope methods, varying from one hour to several days. Undoubtedly in these early experiments the damage caused by excessive in vitro handling was largely responsible for the shortened survival time and in addition some label may be eluted from the platelets after reinfusion. Isotopes that have been used in labelling of cells include:

Radioactive phosphorus - P^{32} a beta emitting isotope with a half life of 14.5 days. In 1952 Julliard et alii labelled human blood platelets in vitro with P^{32} and after injecting them into the rabbit were able to estimate their survival. Later using the same technique of labelling they estimated the survival of platelets in man. Mueller

in 1953 also utilised this isotope to label platelets and noted that the cells were rapidly removed from circulation. The evidence of his experiments suggested that damage to the cells had occurred during their manipulation prior to infusion. Estimates of platelet lifespan using this technique showed considerable variation as evidenced in the work of Desai et alii (1955) who tagged polycythaemic platelet rich plasma with P^{32} and found the apparant half life of platelets from polycythaemics to normal individuals to be only 35 - 50 hours. It was not until in vivo labelling with P^{32} became the practice that more exact values of platelet lifespan were obtained.

The first to use the in vivo technique of labelling with P^{32} were Adelson and Rheingold in 1956. They accomplished this by injecting a donor subject with P^{32} and one week later, following the incorporation of the isotope into platelets, they took 500 ml. of blood from the donor and transfused it into the recipient. This allowed a far more accurate determination of platelet lifespan than had previously been found possible, the average duration of platelet survival in circulation being 7 days.

As well as using P^{32} alone as a cell label, many workers have found that the isotope incorporated into diisopropyl fluorophosphate (DF^{P}) serves as a useful label

(Leeksma and Cohen, 1955, 1956; Polycove et alii, 1958; Alfos et alii, 1959; Zucker, Ley and Mayer, 1960). DPF is a potent esterase inhibitor which becomes rapidly and irreversibly bound to blood cells as well as to other tissues after injection. If DFP contains radioactive phosphorus, the survival within the circulation of a particular type of blood cell may be determined by measuring the disappearance rate of P^{32} activity from that particular type of cell. Using this method, platelet lifespan has been estimated as 8 - 9 days (Leeksma and Cohen, 1955, 1956) and 8 - 14 days (Zucker, Ley and Mayer, 1961).

Iodine - I^{131}. Radioactive iodine was used by Morgan et alii (1954) but following injection there was a rapid disappearance of platelet bound radioactivity and a subsequent rise in plasma radioactivity which indicated the possibility that the injected platelets did not survive the labelling procedure. This isotope has not been used for further platelet studies.

Gold - Au^{198}. Maupin and Loverdo (1959) found radioactive gold to be a stable platelet label but thought that the integrity of the cells was affected and did not recommend its further use as a cell label.

Carbon - C14, is a further isotope which has been

widely employed in studies of platelet survival in animals but to a lesser extent in man owing to its long half life (5760 years). In 1953 Odell et alii determined the life span of rat platelets following the administration of C^{14} formate and in 1961, Heysell, utilising C^{14}-labelled serotonin, determined the platelet survival in man and found a half life of 5 days.

Tritium - H^3. Adelson et alii (1964) have carried out platelet survival studies using tritiated diisopropylfluorophosphate (DFP-H^3) in dogs. The advantages of this technique are that the platelets are labelled in vivo and that the long half life of tritium (12.3 years) results in an additional two-fold increase in specific activity towards the later stages of the platelet survival. Survival curves obtained by this method are exponential.

Chromium - Cr^{51}, a gamma emitting isotope with a half life of 27.7 days, is one of the most frequently employed radioactive isotopes used in measurements of platelet life span. The technique here involves the in vitro labelling of cells and although the site of binding has not yet been determined with any certainty, attachment of the label to the cells occurs quite readily. Gray and Stirling first reported the use of chromium in the radioactive form as a cell label in 1950, and it was not until 3 years later (1953)

that Visek et alii attempted to use it as a platelet label by incorporating the isotope into chromium chloride ($Cr^{51}Cl_3$). After labelling with chromic chloride, however, there was a rapid loss of radioactivity from the labelled platelets following injection into the subject owing to the high affinity of the plasma proteins for the chromic ions.

In 1954 Robertson et alii, pursuing the idea that radioactive chromium may be a suitable platelet label, effectively utilised the chromate form in tagging rat platelets, and in 1955 Morgan et alii in experiments on rabbits showed that platelets survived for several days following reinjection and that they retained their function.

By 1956 Reisner et alii had reported the results of their investigations in both animals and man. In man they showed a platelet survival of 5 - 6 days, increasing to 8 - 11 days in splenectomised individuals and reduced in subjects with idiopathic thrombocytopenic purpura or hypersplenism. They also noted that the platelets disappeared immediately following injection and gradually reappeared, to reach a maximum the day after injection, suggesting that they were sequestered somewhere in the body and slowly released into the circulation. Lewis and Szeto (1959) also found radio-

active chromium to be a suitable label, and Aas and Gardner (1958) described in some detail their method of platelet preparation. The technique that they employed, or modifications of it, has been widely accepted (Najean, 1959; Davey and Lander, 1963, etc.). The lifespan of chromium^{51} labelled platelets is 9 - 11 days when this method of preparation is employed.

Radioactive sulphur - S^{35}. Dziewiatkowski (1949) while studying the rate of excretion of radioactive sulphur and its concentration in some tissues of the rat after intraperitoneal administration of labelled sodium sulphate noted that S^{35} activity reached a maximum in the bone marrow in 24 hours and then gradually declined. Lathja et alii (1953) noted S^{35} to be taken up by human bone marrow cells in vitro and Belanger (1954) observed that the megakaryocytes of the spleen of the rat and hamster readily took up S^{35} following the injection of S^{35}-labelled H_2SO_4. Odell and his workers (Tauche and Gude, 1955) further observed uptake of S^{35} by platelets, megakaryocytes and the myeloid elements of the bone marrow of rats and commented that their results provided additional evidence of the origin of platelets from megakaryocytes as well as giving a measure of platelet lifespan.

Vodopick and Knisley (1963) labelled human platelets with this isotope and determined platelet survival to be 9 days, which is in accordance with the results of other workers using different isotopes in man.

Platelet Lifespan and Kinetics in Circulation

It is now generally accepted that the lifespan of human platelets in circulation is 9 - 11 days in normal subjects, and figures of this order may be obtained using any of the techniques at present available which do not cause excessive damage to the cells. Divergent views, however, have been expressed in relation to the mode of destruction of normal platelets in health. Investigators using compounds of P^{32}, S^{35} and C^{14} as labels (Adelson et alii, 1961; Murphy and Mustard, 1961; Heysell, 1961; Battacharya and Stewart, 1964) have generally found that the fallout of platelet activity from circulation can be expressed as a simple first order function suggesting random destruction. However, the use of such labels has been criticised for they are subject to metabolic turnover and loss from the cell (Parker-Williams et alii, 1963; Grossman et alii, 1963). Using radiochromate, most workers have obtained rectilinear survival curves suggesting that platelets have a finite lifespan, death being the result

of aging processes (Aas and Gardner, 1959; Najean 1959; Firkin, 1963). Davey, Lander and Robson (1964) have devised a model which closely approximates their experimental data and have suggested that both mechanisms of platelet utilisation are occurring simultaneously.

The question could now be asked - "What is the fate of the platelet ultimately?"

Normally, platelets are used to maintain the integrity of capillary endothelium (Danielli, 1940; Woods et alii, 1953; Cronkite and Brecher, 1954). Autoradiograms of capillaries of X-irradiated rats that have been injected an hour prior to sacrifice with platelets heavily labelled with radioactive sulphur suggested that platelets (or S^{35}-labelled mucopolysaccharides derived from platelets) were deposited along the capillary walls (Cronkite et alii, 1957). The distribution of transfused labelled platelets has been studied by ashing or homogenizing various organs and counting their radioactivity. Rabbits or rats were killed after transfusion of platelets labelled with radioactive phosphorus or radioactive chromic chloride (Julliard et alii, 1952; Mueller, 1953). The greatest specific activity under these circumstances was found in the spleen and the largest percentage of the total recovered radio-

activity was found in the liver while smaller amounts were detected in the bone marrow, kidney and lungs. Reisner et alii (1958) using sodium chromate^{51}-labelled platelets noted, in rabbits, that significant amounts of radioactivity were detectable in the spleen only. Cronkite et alii (1957) demonstrated in rats that following transfusion of labelled platelets, radioactivity was confined almost exclusively to splenic macrophages. Najean et alii (1959) injected normal subjects with radioactive labelled platelets and found that the radioactivity in spleen and liver was proportional to the declining platelet activity over a period of 6 days. These findings all appear consistent with the removal of effete platelets from circulation by the spleen. Handling of platelets before transfusion, however, causes damage to the cells and results in the rapid removal of some platelets from circulation, possibly in an abnormal fashion. Nonetheless, results do seem to implicate the spleen and possibly other reticuloendothelial tissue as sites of removal of senescent and damaged platelets from circulation. In spite of this evidence it is of interest that splenectomy does not affect platelet survival times (Hjort and Paputchis, 1960). It is possible that other reticuloendothelial

tissue takes over the function of the spleen after its removal, and Aster and Jandl (1964) suggested that although cells damaged in handling may be removed from circulation initially by the spleen, the majority of platelets normally die in the reticuloendothelial cells of the liver. Knowledge of the fate of platelets is still rudimentary and the evidence for any one site of platelet destruction to take precedence over others is only fragmentary.

Platelet Transfusions in Haemorrhagic Disease

Richard Lower in 1665 performed the first recorded successful blood transfusion in animals. The first transfusion in man is said to have been carried out by Jean Baptiste Dennis (1667) who transfused a young man with the blood of a lamb. At this time there was no consensus as to the indications for transfusion, and it is not clear when blood transfusions were first advised for haemorrhagic disease. The Italian physicians in the early part of the 20th century are known to have employed blood transfusions for the treatment of thrombocytopenic purpura (cited by E.J. Cohn). At that time anticoagulants were not available and the transfusion was performed with paraffin coated syringes. Following the introduction of sodium citrate as an anticoagulant (Hustin, 1914; Lewisohn, 1915)

transfusion in thrombocytopenic states fell into disrepute as the trisodium citrate used damaged the platelets resulting in impairment of their survival and function.

Subsequently attempts were made to infuse platelet concentrates into patients with platelet deficiency states (Krasso, 1927; Fonio, 1936; Benhamou and Pugliese, 1945; Lawrence, Valentine and Adams, 1948) but with only moderate success. Further improvement, however, followed a series of advances in transfusion techniques. These included the introduction of silicones to provide non wettable surfaces for glass containers and needles, thus reducing platelet adhesion and loss (Jacques et alii, 1946) and the elaboration of techniques of differential centrifugation at low temperatures for the separation of blood components (Dillard et alii, 1951; Hirsch et alii, 1952). The use of surface active agents to facilitate platelet resuspension during these procedures was suggested by Minor and Burnett (1952) and non wettable plastic containers for handling blood were developed (Gardner et alii, 1954).

Not only have these adaptations to collection techniques been devised over the years, but recipients have received fresh whole blood collected into ethylene

diaminetetraacetic acid (EDTA) (Dillard, Brecher and Cronkite, 1951). Transfusions of fresh platelet rich plasma (Gardner, Howell and Hirsch, 1954; Stefanini, 1955), fresh platelet concentrates, platelets preserved in gelatin (Tullis et alii, 1959), lyophilised platelets (Klein et alii, 1956) and platelet suspensions derived from stored blood (Mustard, 1956; Tobin and Friedman, 1960) have been employed.

In many instances the response to platelet preparations has been assessed by clinical interpretation and generally the bleeding associated with thrombocytopenia has been corrected successfully only when viable platelets are circulating in the recipient. In order to be effective in haemostasis, platelets must be "viable" and capable of remaining in circulation for some time after infusion. The demonstration that a given platelet concentrate is active in a coagulation system in vitro does not necessarily mean that the platelets possess all their physiological properties and are therefore viable cells which will be effective in the entire haemostatic processes in vivo. Their viability has been defined (Baldini et alii, 1960) as "the ability to recirculate and to survive, that is, to carry on their normal functions after infusion into a

normal recipient". It is evident that platelets prepared by certain procedures are viable, as indicated by the circulation of platelets in the recipients. In contrast, transfusion of platelets prepared by other procedures may not result in circulation of platelets, or may even induce a further degree of thrombocytopenia. The methods employed which have changed the platelet environment considerably, such as trapping and elution, separation and storage in media of plasma, freezing and thawing or lyophilisation, have resulted in platelets whose morphology has been distorted and which are not viable as the platelet count of normal or thrombocytopenic subjects has not been elevated following their infusion. The infusion of fresh blood usually results in an increase in platelet count except in those conditions known to be associated with excessive platelet destruction, for example, idiopathic thrombocytopenic purpura.

In all cases in which infused platelets do not circulate, a clear separation of the responsible factors should be elucidated. It may be as previously stated that lack of circulation is inherent to the disease; that irreversible injury has occurred during separation or storage, or that sensitisation to human platelets due to

previous transfusion may have occurred (Stefanani et alii, 1952).

The development of the atomic bomb and the incidence of thrombocytopenic purpura as a potential component of modern warfare provided further incentive for research into the development of platelet preservation and efficient means of platelet transfusion.

Of the most recent developments in this field are the use of dimethyl sulfoxide as a preservative of frozen platelets by Djerassi and Roy (1963) and the use of a new anticoagulant for the preparation of platelet concentrates by Aster and Jandl (1964). This latter will be discussed in more detail in Chapter III.

The Platelet and Atheroma

Morphologically atherosclerosis has been defined as a disease of the intimal layers of the arterial wall, occurring focally and associated with intimal thickening and lipid deposition. The frequent sites of these thickenings are the aorta, coronary, cerebral and lower leg arteries.

Thickened arteries were first noted by Aretaeus of Capadocia nearly two thousand years ago. The term atheromatous was probably first coined in recent times by

Morgagni (1728) although according to Paré (1575) the word atheroma was first used in ancient Greek works for any cystic space containing gruel-like material. It was not until the Napleonic era that the French physicians developed some of the more modern ideas of the disease. Lobstein (1829) classified the disease as a definite entity and was the first to use the term arteriosclerosis. It was he who first postulated that the disease was related to vascular accidents. After Lobstein's description theories of the pathogenesis of the disease were developed. In Vienna Carl von Rokitansky described in detail pathological lesions in the arteries and in the heart. Rokitansky demonstrated lesions which he called fibrinous deposits on the arterial intima and which he considered to be the cause of various pathological changes. These included intimal hypertrophy, intimal vascularisation, calcification, atheroma formation and weakening of the media with aneurysm formation. Similar deposits of fibrin on the endocardium were described and he assumed they led to endocardial thickening and fibrosis. Rokitansky believed that the fibrinous deposits were produced by the interaction of an exudate released by local inflammatory processes in the vessel wall or of blood and mediated by a catalytic process.

Rokitansky's concepts were arrived at by brilliant intuition and were premature only because they were impossible to prove or disprove at the time because the physiological and biochemical background needed for this was non-existent. With the foundation of cellular pathology by Rudolph Virchow, Rokitansky's ideas were considered obsolete and erroneous and they soon ceased to form the basis of investigative work and over the years were nearly forgotten.

At the turn of the century it was discovered that early atherosclerotic lesions occur in children (Simnitsky, 1903) and this was later extended to infants (Strong et alii, 1958). The rapid progress in biochemistry and nutrition began to influence the work on atherosclerosis so that for a time most investigations centred around disturbances of lipid metabolism and their association with dietary fat. The accumulation of fatty substances in the atheromatous areas was good evidence that investigations on these lines would be profitable. Lemoine (1911) established that the serum cholesterol was elevated in patients with advanced atherosclerosis and it was also found that the serum is lipaemic in these subjects (Kusunoki, 1914). Further work elucidated other disturbances

of lipid metabolism in this disease. When these lipid disturbances were first examined investigators considered them a consequence rather than a cause of the lesions as many chronic diseases are accompanied by similar lipid changes. However, since Anitschkow's (1913) experiments with cholesterol feeding which resulted in atherosclerosis in animals, many leading investigators have been inclined to accept disturbances of lipid metabolism as aetiological factors in atherosclerosis and the "lipid imbibition" theory largely over-shadowed any other.

It was not until nearly a century had elapsed that Rokitansky's suggestion came to notice again when Duguid (1946) studied thrombosed and recanalised coronary arteries. He observed that at sites where the thrombus had not occluded the whole lumen of the vessel but had apparently formed a mural fibrin deposit on the intimal surface, the resulting pathological processes consisted of an intimal hyperplasia leading to intimal proliferation and intimal thickening. This resembled organisation of a parietal thrombus with connective tissue. The existence of several layers clearly separating or overlapping one another suggested the recurrent formation of fibrin deposits followed by fibroblastic proliferation and

organisation. When deeper layers of thickening occurred secondary intramural processes appearing as cellular necrosis and lipid deposition were observed. In such processes Duguid saw an aetiological factor in the development of the atherosclerotic lesion. In reaching this conclusion he revised the old suggestion of Rokitansky and laid the foundation for the thrombogenic theory of the pathogenesis of atherosclerosis.

There is good evidence that platelets are normally required to preserve an intact vascular tree. Danielli (1940) perfused the isolated hind limb of a frog with platelet-free fluid and this led to increased fluid loss to the tissues. This did not occur following perfusion with platelet-rich fluid. Furthermore, injection of fresh platelets into haemorrhaging rats or dogs that were thrombocytopenic after total body irradiation rapidly stopped the bleeding (Woods et alii, 1953; Cronkite and Brecher, 1954) . As well as the continuous need for platelets to maintain vascular integrity, increased deposition occurs if any local injury arises (Bizzozero, 1882) and any accumulation of material may become incorporated into the intima and be overgrown by endothelium (Duguid, 1946, 1948; Haust et alii, 1959; More

and Haust, 1961).

In 1958, Filshie and Scott showed that in rabbits, injection of thrombin into an isolated venous segment produced a mixed thrombus which was endothelialised over 28 days. Many of the organised thrombi contained foamy phagocytes and resembled the lesions found in the arteries of rabbits fed lipid enriched diets. Hand and Chandler (1962) produced atheromatous lesions, again rich in foam cells, by injecting homologous thrombi consisting mainly of platelets into the pulmonary circulation. These findings have led Mustard et alii (1962) to modify the more classical fibrin theory of encrustation and to relate the development of atheroma more directly to the blood platelet and to its role firstly in thrombogenesis and subsequently to atherogenesis. Study of this type has proved a complex problem, however, as there are many facets of platelet behaviour to be considered.

Undoubtedly one of the first considerations involves coagulation studies. These have been widely carried out in people suffering from the complications of atheroma (Mustard et alii, 1962; Spittel et alii, 1960; Mcdonald, 1957, 1959, and many others) and have revealed an increase in the activity of various coagulation factors in subjects

with occlusive vascular disease. Other workers (Hurn et alii, 1947; Merskey et alii, 1960) have failed to show any difference when comparing coagulation activity in normal and atheromatous individuals. McDonald (1957, 1959) reported that subjects who have had the complications of atheroma, for example, myocardial infarction, have more adhesive platelets than normal individuals and Horlick (1961) using a different technique, has confirmed this finding. Overall, to date it has been difficult to determine from in vitro tests how coagulation relates to atheroma formation and its subsequent complications.

Two in vitro methods of determining platelet behaviour have recently been developed which allow direct visualisation of the platelets throughout the study. One of these involves use of a spectrophotometric technique, and the other the formation of a true thrombus by artificial means.

A number of attempts have been made over the years to produce objects having the histological structure of a thrombus. For a variety of reasons the early methods failed to yield as much new information as could be hoped for. In 1958 Chandler showed that when blood is made to flow continuously round a closed circular loop of plastic

tubing mounted on a rotating turntable the blood eventually solidified. When the solidified blood was examined histologically it was found to contain areas which were similar in many ways to the appearances found in naturally occurring thrombi. In 1959, Poole, using a slight modification of the Chandler apparatus, was able to obtain conditions under which blood did not solidify completely. Instead a small firm object was formed just behind the advancing edge of the column of blood and which floated around independently after reaching maximum size, usually within a few minutes. Histological examination showed that the object so formed consisted of a white head composed of platelets and leucocytes and a red tail composed of fibrin, trapped red blood cells and a few scattered leucocytes reproducing many of the characteristic features of a natural thrombus. This technique obviously has many applications and has been used for a variety of purposes associated with determinations of platelet behaviour and thrombus formation. It has been used to study the effects of anticoagulants (Poole, 1960a); the incorporation of bacteria (Poole, 1960b); the effect of fatty acids (Connor and Poole, 1961) and the subsequent fate of the artificial thrombus when returned to

circulation (Hand and Chandler, 1962). Owing to the apparently direct relationship between thrombosis and atheroma formation, this provides an in vitro technique of determining abnormal platelet behaviour in subjects predisposed to atheroma or its complications.

The formation of the white head of a thrombus involves adhesion of platelets to each other. As platelets in the blood stream do not normally stick to each other, it is probable that the surface or environment of a platelet which has stuck must change rapidly because within seconds of platelets sticking more platelets stick to each other. The actual mechanisms involved in platelet adherence to each other and to endothelial surfaces are not fully determined. Platelet adhesion occurs when blood clots (Wright and Minot, 1917), when a vessel is injured (Bizzozero, 1882), when platelets are exposed to factors released from red cells (Hellem, 1960; Gardner et alii, 1961), and when platelets are in contact with connective tissue (Zucker and Borelli, 1962). Any local injury to the vessel wall will result in the accumulation of material which may become incorporated into the endothelium (Duguid, 1946, 1948). The spectrophotometric technique (O'Brien, 1962) which is dependent on increased transmission of light through a platelet suspension if

the platelets aggregate has provided a sensitive method for determining increased platelet adhesiveness which occurs in subjects with thrombotic tendencies.

The introduction of radioisotopes into clinical medicine has provided a further tool for the investigation of abnormal platelet behaviour. The advantage of studies of this type are that they give an index of platelet behaviour in vivo. Pursuing the encrustation theory of atherogenesis with particular emphasis on platelet utilisation, Murphy and Mustard (1962) have carried out a series of investigations on atheromatous and normal individuals using DFP^{32} labelled platelets. Results of their studies have revealed that the mean platelet survival is shorter and the mean platelet turnover greater in atheromatous subjects than in others. These studies were carried out in the quiescent phase of the disease. In an effort to obtain information on the effect of diet on platelet behaviour in vivo they also investigated the platelet behaviour of seven subjects on three different diets controlled in a metabolic ward. It was found that platelet survival was shortest when the diet was rich in dairy fat and eggs and longest when it contained little fat (Murphy and Mustard, 1962). There was also a tendency

for the platelet adhesive index to increase in subjects on dairy produce and these subjects also had the highest serum lipid levels. These findings tend to parallel, in part, those of McDonald and Edgill (1958) in which subjects on low fat diets were found to have less adhesive platelets. Further studies have been carried out on subjects after paying particular attention to factors which have been incriminated in atherogenesis or increased tendency to thrombotic episodes. Data have indicated a reduced platelet survival in patients who smoke (Mustard and Murphy, 1963b), and in gouty subjects (Mustard et alii, 1963). The effect of anticoagulant therapy on platelet survival and turnover in patients with atheroma has also been determined because of its wide therapeutic use. Dicoumarol in doses that prolong the prothrombin time to greater than two and one-half times normal, prolongs platelet survival and decreases platelet turnover (Murphy and Mustard, 1961). Inadequate doses of dicoumarol (prothrombin time less than one and one-half times normal) do not prolong platelet survival and,if anything, have the reverse effect. A similar effect is obtained with heparin (Mustard and Murphy, 1963a). Atromid has also been found to prolong platelet survival in "atheromatous"

individuals (Gilbert and Mustard, 1963).

O'Neill and Firkin (1964) performed platelet survival studies in a group of subjects with coagulation disorders, thrombocythaemia and conditions associated with atherosclerosis. Their results indicate that under most conditions random removal of platelets, in particular by the processes of continuous intravascular coagulation, does not play a significant part in the destruction of blood platelets in either normal subjects or in subjects prone to atherosclerosis. This is in contradistinction to the findings of Murphy and Mustard, and has raised some doubt as to the validity of their attractive hypothesis. On the whole, such divergence of opinion and multiplicity of theories on the pathogenesis of disease is usually a good indication that any one theory does not satisfy all the facts that are available. Such is the case with atheroma. Of all the factors incriminated in the aetiology of the disease, one factor which constantly recurs is the blood platelet and any elucidation of its role may permit the course of the disease to be influenced decisively in the future.

Purpose of the Present Study

The work in this thesis is related to a number of aspects of platelet behaviour in health and in disease.

1. The introduction of disodium ethylene diamine tetraacetate (Na_2EDTA) as an anticoagulant used for the collection of blood (Dillard, Brecher and Cronkite, 1951) led to its widespread use for platelet transfusion. The arguments in its favour were that it obviated platelet clumping and allowed platelets to be handled more easily. In 1961 Kissmeyer-Nielson and Madsen concluded from their studies of radiophosphorus labelled platelets that acid-citrate dextrose (ACD-USP formula A) was superior to EDTA and more recently Aster and Jandl claimed outstanding merit for a particularly acidic solution of sodium citrate and dextrose (ACD 'S").

Owing to the need for effective platelet transfusion in many haemorrhagic states and in view of these divergent claims, a comparative study was undertaken to assess the relative merits of anticoagulant solutions used in platelet transfusions. Studies had previously been carried out in this laboratory using ethylenediamine tetraacetic acid (EDTA) and ACD 'A'. Further studies were therefore planned using the newer formula (see Chapter II) of acid

citrate dextrose in an effort to determine the efficiency of this anticoagulant in relation to those previously recommended for use in platelet transfusion.

2. Study of the relationship of the platelet to the development of atheroma and its complications has been a difficult problem in the past. The introduction of isotope labelling of cells allowed the hope that this technique may be of use in determining platelet utilisation and behaviour in circulation in this condition. However, results of studies carried out by two major groups working in this field have been contradictory. It was with the hope of clarifying this problem that a systematic study was instituted to determine the behaviour and survival in circulation of blood platelets in persons suffering from atheroma or its complications. Furthermore, there appears to be a close relationship between thrombosis and atheroma. A study of platelet behaviour and of the tendency to thrombosis in subjects predisposed to vascular disease is warranted as of all the factors incriminated in the aetiology of the disease one factor which constantly recurs is the blood platelet. Any elucidation of its role in the pathogenesis of atheroma may eventually influence future management of the disease.

3. Until recently the surgical treatment of valvular heart disease has been concerned primarily with the relief of obstruction caused by stenosis. Diseased aortic and mitral valves, however, are frequently incompetent as well as stenosed since fibrosis and calcification render them rigid and unable to open and close efficiently. Thus, for a number of years attention has been focused on devising methods of valve replacement either by the use of homografts or of prosthetic material. In 1956 Murray reported the successful insertion of aortic valve homografts in dogs with mitral and aortic insufficiency, and he also described the insertion of aortic valves into the thoracic aorta of three patients, all of whom derived benefit from operation.

Another approach to valve prosthesis has been made by Starr and Edwards. In 1960 Starr reported the results of mitral valve replacement on 25 mongrel dogs. The valves consisted of a rigid frame that supported a variety of shapes of silicone rubber leaflets or enclosed ball of nylon, lucite or silicone rubber. Fixation was achieved by means of a teflon cloth. Thrombosis was a major problem in these animals. The thrombus appeared first at the zone of fixation to the mitral annulus and extended

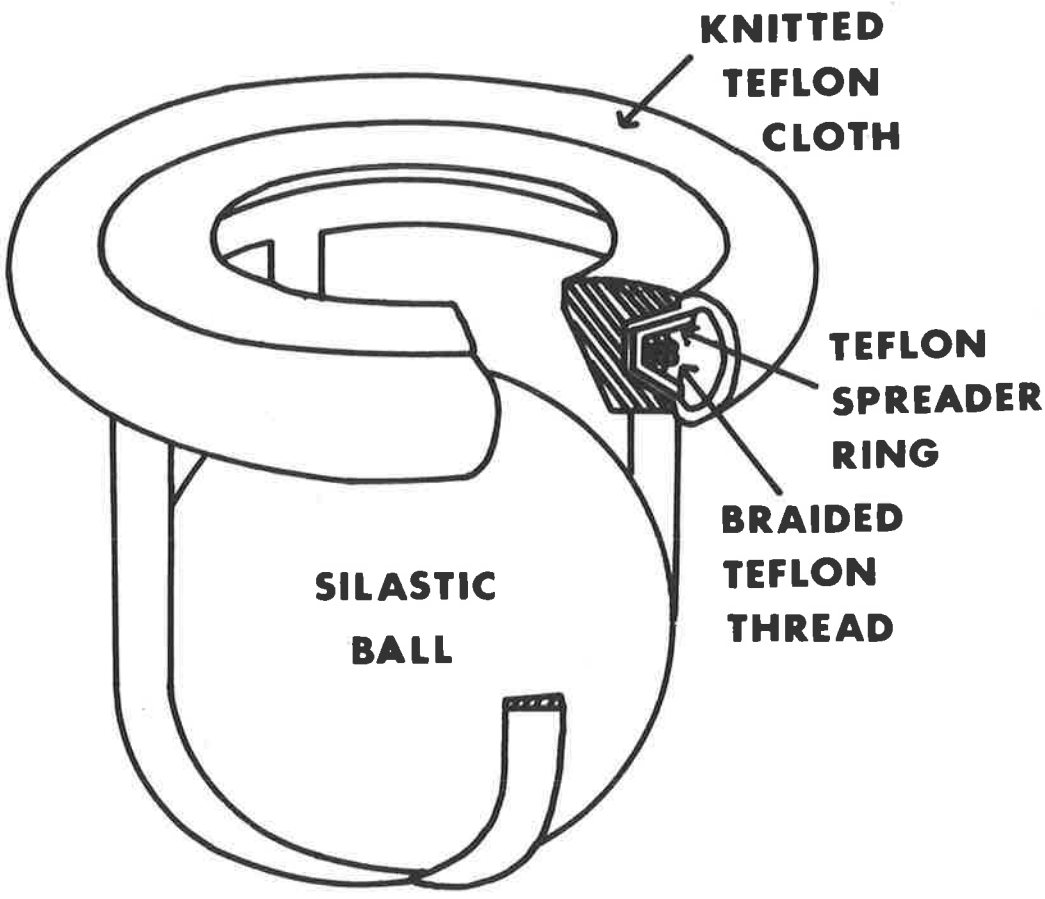


Figure 1: Diagrammatic representation of the Starr-Edwards ball-valve prosthesis.

over the valve ring despite changes in the materials used and despite anticoagulant drugs. This always interfered with leaflet function but was less likely to interfere with the ball valve. In 1961 Starr and Edwards reported eight cases who had undergone mitral replacement with the Starr-Edwards ball valve prosthesis (Figure 1). In humans clotting had not been a problem to that time (as had been the case with dogs) and no deaths or complications from thrombo-embolic disease, thrombotic occlusion of the prosthesis or haemorrhage from postoperative anticoagulant therapy had occurred.

In 1962, Starr et alii, reporting on further results of mitral valve replacement, commented: "The use of foreign material in the heart, however, is associated with the danger of infection and thrombo-embolism", two of the patients in this series of 16 having suffered cerebro-vascular accidents postoperatively. Since this report and following the increased use of the Starr-Edwards prosthesis in patients with severe valvular disease, the number of persons who have had otherwise successful operations but who have experienced the sequelae of thrombo-embolism, is increasing. A study of the platelet behaviour in these individuals was undertaken as, at

autopsy, many of the thrombi were found adherent to the valve or in close proximity to it, and were composed almost entirely of platelets and fibrin.

4. Cohen (1961) suggested that the only satisfactory method of classifying the thrombocytopenias at present depends on whether the survival of platelets in circulation is short or normal. Najean et alii (1963), using radiochromate labelled platelets, found that splenic localisation of platelets in subjects with thrombocytopenia showed good correlation with the subsequent response to splenectomy. Castaldi and Firkin (1963) also noted that of six subjects responding to splenectomy for thrombocytopenia, five showed an uptake of labelled platelets by the spleen. Subjects with thrombocytopenia were studied therefore to determine whether surface scanning data obtained in conjunction with estimates of platelet lifespan using the chromium^{51} technique might provide a suitable method for the prediction of the outcome of splenectomy in these subjects.

5. The mechanism of platelet destruction and particularly the site of platelet destruction remain problems of considerable interest and to which no solution has yet been determined. Removal from

circulation has been attributed to reticulo-endothelial sequestration of effete platelets, the liver and spleen playing a major role in this respect. It remains uncertain, however, why in some instances the principal site of removal is the spleen, while under other circumstances most cells appear to be removed by the liver. Jacob and Jandl (1962), writing of red cells, postulated that the shift in site of red cell sequestration is due to alterations in cell membrane, a feature which has been adequately confirmed, most recently by Kimber and Lander (1965). To determine the sites of removal of platelets from circulation therefore, studies were devised in which the cells could be damaged to varying degrees and their removal from circulation traced.

A total of 87 platelet survival studies were carried out on 78 subjects. These could be grouped as follows.

1. Atheroma or its complications

   36 studies on 34 patients.

2. Valvular disease of the heart

   i. Preoperative studies - 6 subjects.

   ii. Following the insertion of Starr-Edwards prosthesis - 12 studies (10 subjects).

3. Thrombocytopenia

   14 studies on 9 subjects.

4. Postoperative patients

   6 studies on 6 subjects.

5. Following treatment of platelets with sulphydryl inhibitors

   6 studies on 6 subjects.

6. Normal healthy young controls

   7 studies on 7 subjects.

In addition, studies were carried out on the blood of 117 patients for a total of 746 days using the Chandler apparatus. This part of the work was carried out in collaboration with Dr. Colin Schwartz and Dr. Neville Ardlie of the Institute of Medical and Veterinary Science, Adelaide. All subjects studied were selected and personally interviewed by myself and full clinical documentation carried out including results of electrocardiographic examinations, recent blood pictures and serum enzyme levels, and results of relevant X-ray examinations particularly arteriography. The blood of all patients was taken daily by myself using the same techniques. Platelet counts were performed on every sample. Observations in the Chandler tube were performed by Dr. Schwartz and Dr. Adrlie, and the final evaluation and interpretation of results was carried out as a group correlating all available data.

Studies carried out using this technique may be sub-divided accordingly.

1. Healthy control subjects
   i. Subjects ≤ 40 years - 24 subjects studied on 142 days.
   ii. Subjects > 40 years - 28 subjects studied on 161 days.

   Total control subjects: 52 subjects studied on 303 days.

2. Patients with myocardial infarction
   i. Subjects treated with anticoagulants - 24 subjects studied on 148 days.
   ii. Subjects not treated with anticoagulants - 26 subjects studied on 169 days.

   Total myocardial infarction: 50 subjects studied on 317 days.

3. Patients with valvular disease of the heart
   I. Uncorrected valvular disease - 6 subjects studied on 41 days.
   ii. After valve replacement with Starr-Edwards prosthesis - 9 subjects studied on 85 days.

   Total valvular disease: 15 subjects studied on 126 days.

The assessment of the relative merits of anti-coagulant solutions used in platelet transfusions and the evaluation of the new Aster-Jandl anticoagulant were made possible through the help of Dr. Martin G. Davey. He had previously performed platelet survival studies using ethylene diamine tetraacetic acid and the standard formula of acid citrate dextrose (ACD'A'), making it possible to utilise some of the data he had obtained for the purposes of the comparative assessment of the new anticoagulant solution.

CHAPTER II

MATERIALS AND METHODS

A. MATERIALS USED

1. Polyvinyl chloride collection packs were used for the initial venesection. These were double packs consisting of a 550 ml. collection pack which contained the anticoagulant, connected to a second compartment of 300 ml. capacity by PVC tubing (Figure 2). Packs were supplied by Tuta Products Ltd. of Sydney.

2. Polyvinyl (PVC) coupler sets were used where required for the transfer of plasma or platelet suspensions (Figure 2).

3. Polypropylene centrifuge tubes (MSE) were used for all blood samples collected during the course of platelet survival studies. These were of 10 ml. or 15 ml. capacity and their use precluded the need for silicone treatment to reduce platelet adhesiveness to the surface.

4. Cellulose acetate tubes, flat bottomed with close fitting plastic stoppers, were employed for the storage of platelets isolated from the daily blood samples prior to final estimations of radioactivity in the specimen. Following use the tubes were discarded.

5. Blood for Chandler studies were collected into siliconised glass centrifuge tubes of 15 ml. total capacity.

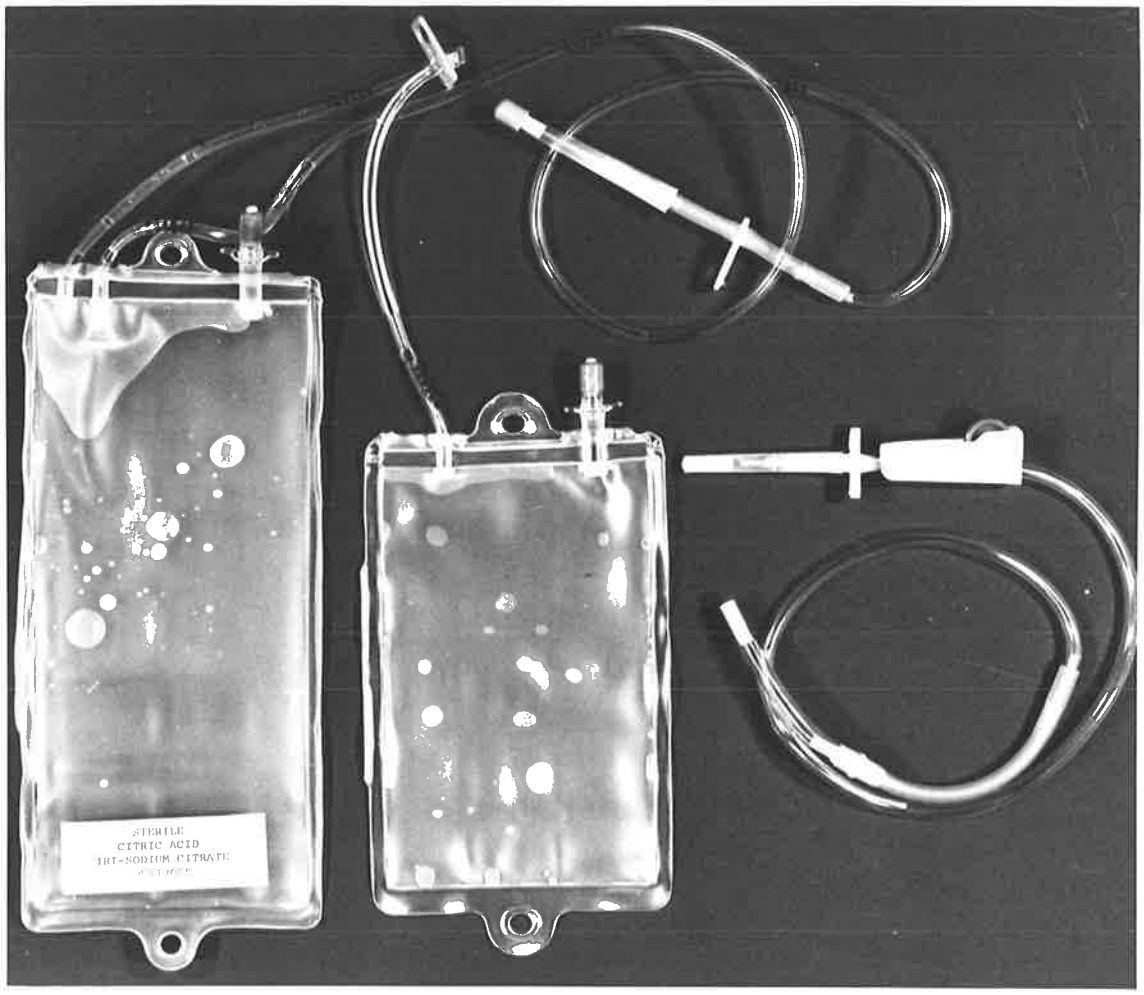


Figure 2: Double platelet pack.
Collection pack containing anticoagulant on the left and transfer pack to the right.
Coupler set also illustrated on far right.

6. Anticoagulants.

(1) The anticoagulant used for the initial collection of blood for platelet labelling was of two types.

(a) That used in the majority of studies was a special form of acid-citrate dextrose anticoagulant as described by Aster and Jandl (1963, 1964) and called here ACD 'S'. This formula consists of

i. 2G dextrose)
ii. 1.37G citric acid) per 100 ml.
iii. 2.5G trisodium citrate)

For each 100 ml. whole blood collected 18 ml. anticoagulant were required.

(b) EDTA - 1.5% (w/v) disodium dihydrogen EDTA. $2H_2O$ in 0.7% saline for which 1 volume of the anticoagulant solution is required for each 9 vols of whole blood. The third anticoagulant with which comparison has been made was acid-citrate dextrose (USP formula 'A') of which 15 ml. are required for addition to 100 ml. of whole blood.

(2) Anticoagulant for blood samples was composed of ethylene diamine tetraacetic acid (EDTA) 1.5% and superinone 1% in normal saline. Superinone is a nontoxic surface active substance which facilitates platelet resuspension.

2 ml. of this anticoagulant were used for each 10 ml. of whole blood collected.

7. Needles and syringes were coated with silicone. Gauge 19 needles were used for all venepunctures.

8. Silicones. The silicone compounds used were supplied by Dow Corning and the varieties employed were:

(1) A chlorosilane No. 1107 as a 1% solution in acetone to treat pipettes and glassware. This was later substituted by a further compound "siliclad".

(2) A lacquer No. 2210 in xylol to proof the needles used for venepuncture.

(3) A dimethyl fluid (200/200) to lubricate glass syringes.

(4) A mixture of a dimethyl and a phenylmethyl fluid (200/20 and 555) for the purpose of isolating platelets from samples of whole blood. Four parts of the 555 solution were added to one part of the 200/20 and adjusted to a final SG of 1.036-1.040 after thorough mixing.

9. Choice of isotope. Radioactive chromium^{51} was chosen for use in platelet labelling in this study. The

reasons for this choice were several.

(1) The isotope has a suitable half life of 27.7 days. Any isotope with an extremely short half life would be inefficient because of the short platelet lifespan (9-11 days), and isotopes of long half life are associated with an increased radiation hazard to the individual under investigation.

(2) There are no harmful side effects related to the use of chromium^{51}.

(3) It is relatively easy to label cells with radiochromate.

(4) Relabelling of platelets does not occur if any isotope is eluted following injection.

(5) The functional state of organs could be determined by using surface counting techniques. This is possible because radioactive chromium^{51} is a λ-emitting isotope whose rays are able to pass through the body without being absorbed by surrounding tissues and which may be measured by suitable detectors placed on the body surface.

(6) The technique of labelling with radioactive chromium had been widely used in this laboratory

previously and found to compare favourably and to be equally as accurate as measurements of lifespan using other isotopes.

10. Isotope supply. Radioactive disodium chromate ($Na_2Cr^{51}O_4$) was supplied by the Commonwealth X-Ray and Radium Laboratories, Melbourne, made up in a sterile solution of physiological saline and autoclaved to BP specifications. The specific activity of the chromium^{51} during the course of these investigations varied between 63 uc./ug. Cr and 225 uc./ug. Cr. The mean specific activity during this period was 104 uc./ug. Cr.

11. Plastic intravenous catheters used on the first day of each study when repeated sampling was required were "Bardic Intracaths" No. 1814. Needle gauge was 14 and the plastic catheters were 12 inches in length.

B. LABORATORY METHODS

1. Sterilisation procedures. Syringes and needles were sterilised following package in paper containers by placing in a hot air oven for at least 60 minutes at a temperature of 120^{o}C. Fluids for use in preparation of platelet suspensions, for example, 0.2% superinone in saline and 2% superinone in saline were sterilised in an autoclave at 15 lb. pressure for 30 minutes. Platelet

packs, coupler sets and intravenous catheters were provided by the manufacturers in a sterile form.

2. Technique of labelling platelets with Chromium^{51}. The method used for the labelling of platelets was that described by Davey and Lander (1963), a modification of the technique used by Aas and Gardner.

Procedure:

1. 300 ml. of whole blood (500 ml. in thrombocytopenic individuals) were collected from the antecutibal vein of the donor. Blood flowed into the sterile plastic bag containing the anticoagulant solution. Admixture of blood with the anticoagulant was ensured by gentle tilting or massage of the pack throughout the collection period.
2. Blood was then centrifuged in a refrigerated centrifuge (4 ^{o}C) at 300g for 20 minutes. The red cells were thus separated from the platelet-rich plasma. The supernatent platelet-rich plasma was then transferred to the adjoining smaller plastic bag by means of a spring press and 10 ml. of 2% superinone in saline added.
3. The platelet-rich plasma in this smaller bag was centrifuged at 4 ^{o}C at 1000g for 30 minutes. At

the end of this time platelets could be readily identified as a creamish button at the bottom of the plastic bag. Platelet-poor plasma was removed and stored under sterile conditions.

4. 300 uCi of sterile radioactive sodium chromate^{51} were added to the platelet button. The ampoules containing the radioactivity were well washed out with 5 ml. 0.2% superinone in saline and this solution was also added to the platelets. The contents of the plastic bag (platelets and radioactive solution) were gently but thoroughly mixed and allowed to incubate at room temperature for 30 minutes. Intermittent tilting to ensure equal suspension was carried out over this time.
5. At the end of the incubation period platelet-poor plasma was added to the plastic bag and centrifuged for 15 minutes at 1000g. Platelets were again concentrated at the bottom of the bag. The plasma containing radioactive chromium which had not been bound to platelets was decanted.
6. The labelled platelets were resuspended in a mixture of 10 ml. platelet-poor plasma (previously isolated from that used to wash platelets) and

10 ml. of 0.2% superinone in saline. 20 mg. of a 5% ascorbic acid solution was added to the suspension to reduce any free sodium chromate to the chromic form. In this form there is ready attachment of non-platelet bound radioactivity to plasma proteins and this excess radioactivity is readily excreted from circulation.

7. The final platelet suspension was infused into the patient through an antecubital vein approximately 2 hours after the initial collection of blood.

8. Blood samples, each of 10 ml. volume, were taken from the patient at frequent intervals for 4 hours following the infusion of the labelled platelet concentrate. This was facilitated by using a Bardic intracath in an antecubital vein to obtain samples. Subsequently blood samples of 20 ml. were taken at daily intervals for 10-12 days following infusion. These samples were collected into 1.5% EDTA and 1% superinone in saline. 2 ml. of this anti-coagulant were used for each 10 ml. of whole blood collected.

3. Preparation of platelets treated with N-ethyl-maleimide (NEM).

1. All stages in the preparation of platelets for treatment with the sulphydryl inhibitor NEM were as described for the usual preparation of radioactive labelled platelets to the point following incubation with 300 uCi of radioactivity.
2. Following incubation of platelets with radioactive chromium, a freshly-prepared Seitz-filtered solution of NEM was added to the platelets in the plastic bag. Quantities of NEM sufficient to result in final concentrations of 0.25 micromoles NEM/1 ml. platelets: 1.0 micromoles, 2.5 micromoles, 5.0 micromoles, 10.0 micromoles, 15.0 micromoles NEM/1 ml. of platelets were added. The mixture was allowed to incubate at room temperature for 30 minutes.
3. At the end of this time platelet-poor plasma was added once more to the plastic bag containing platelets and NEM. The plastic bag and contents were then centrifuged for

15 minutes at 1000 g following which the plasma containing any unbound radioactivity or free NEM was decanted and discarded.

4. Labelled NEM-treated platelets were re-suspended in a mixture of equal parts of platelet-poor plasma and 0.2% superinone in saline to a final volume of 20 ml. 20 mg. of 5% ascorbic acid solution were added and the platelets were ready for reinfusion into the subject.

5. Following platelet infusion, samples of blood were taken at 1, 2, 3, 5, 10, 15, 20, 30, 45 and 60 minute intervals from the plastic catheter placed in the antecubital vein of the forearm. The platelet radio-activity in each sample of blood was estimated as described following separation of the platelets from whole blood.

4. Separation of platelets from whole blood.

Separation of platelets from whole blood samples collected during the course of an experiment was carried out by the method of Morgan and Szafir (1961). 1-2 cc. of silicone of S.G. 1.036-1.040 were layered on the whole blood sample

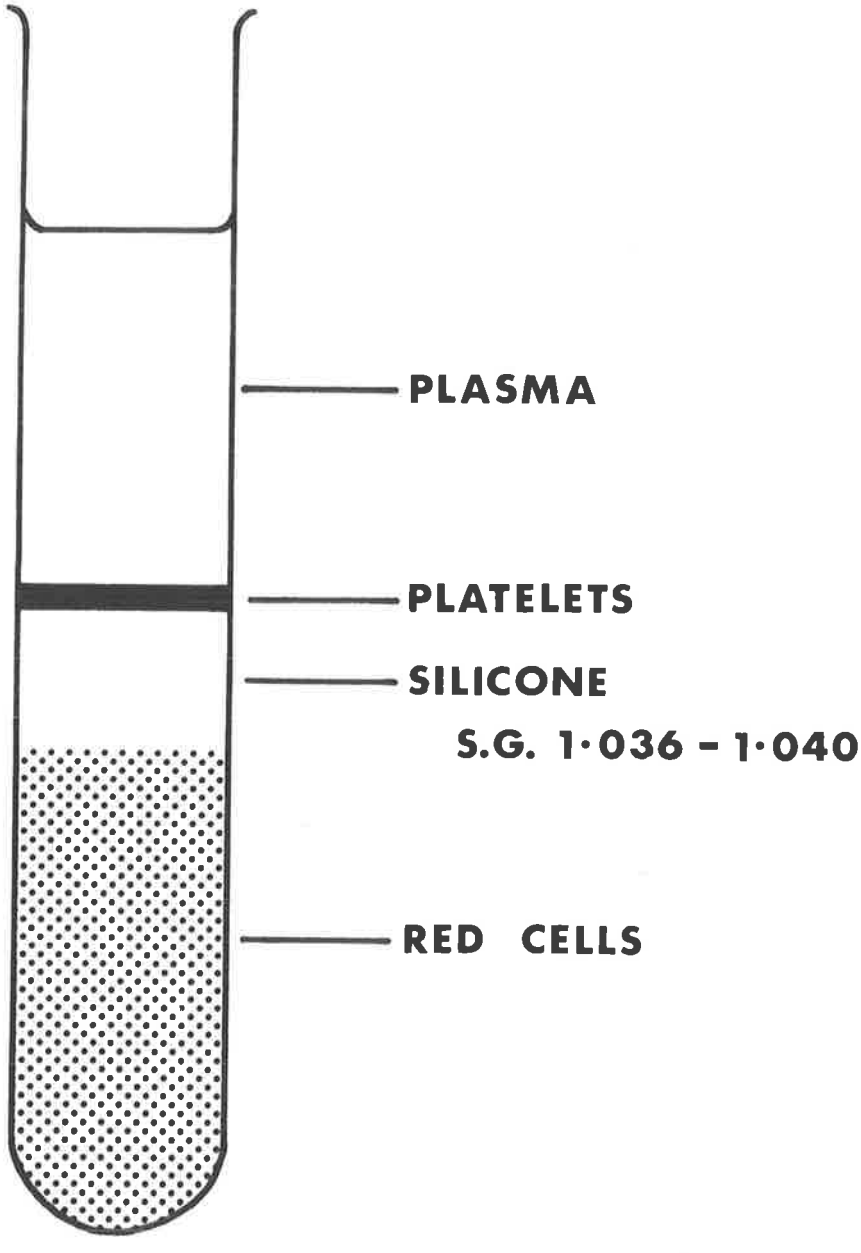


Figure 3: Diagrammatic representation of blood components following addition of silicone SG 1.036 - 1.040 and centrifugation. Clear separation of blood components occurs as shown.

which was then spun at 1000g in a refrigerated centrifuge at 4°C for 15 minutes. This resulted in a separation of the blood into its various components (see Figure 3). The platelet-poor plasma was removed with a Pasteur pipette to within 1 cm. of the silicone surface and discarded. It was then possible to pipette the remaining plasma and the platelets lying on the surface of the silicone into a separate polypropylene test tube. The platelets thus isolated were washed once in 0.2% superinone in saline and recentrifuged at 4°C for 15 minutes at 1000g. The supernatant fluid was pipetted away and the platelets transferred to a cellulose acetate tube. The volume in the cellulose acetate tube was adjusted to 4 ml. by the addition of 0.2% superinone in saline. Samples were sealed in these tubes by means of a plastic cap and stored in a refrigerator until the end of the experiment when the radioactivity in each specimen was determined.

5. Platelet counts were carried out using the method of Brecher and Cronkite (1951). Samples of whole blood were diluted 1:100 in red cell counting pipettes with a solution of 1% ammonium oxalate in distilled water. After mixing for 15 minutes, counts were carried out on a double Neubauer counting chamber using a Leitz microscope adapted

to phase contrast.

Counting of radioactive samples. Radioactivity was determined in platelet samples by a sodium iodide well crystal (EKCO N597) adapted to a Philips Universal gamma spectrometer. An automatic sample changer (Paton Industries Ltd.) was able to handle up to 50 4 ml. samples at a time (Figure 4). The detector crystal was placed in the centre of a circular sample storage platform which rotated around the detector. With this symmetrical construction there was no variation in the contribution of strong waiting samples to the background of the weak samples in the same batch. As all samples were counted at the end of each study the need to apply decay corrections for the isotope was obviated. The number of counts obtained in each specimen in a preselected time period was automatically printed by means of a Victor digitmatic apparatus.

Surface counting. Determinations of the radio-activity of organs were carried out using the Philips scintillation detector (PW4119) which was designed particularly for gamma spectrometry. The PW4119 contains a thallium activated sodium iodide crystal 1-3/4" diameter and 2" height and impulses received here are amplified by a photomultiplier tube. To prevent the photomultiplier

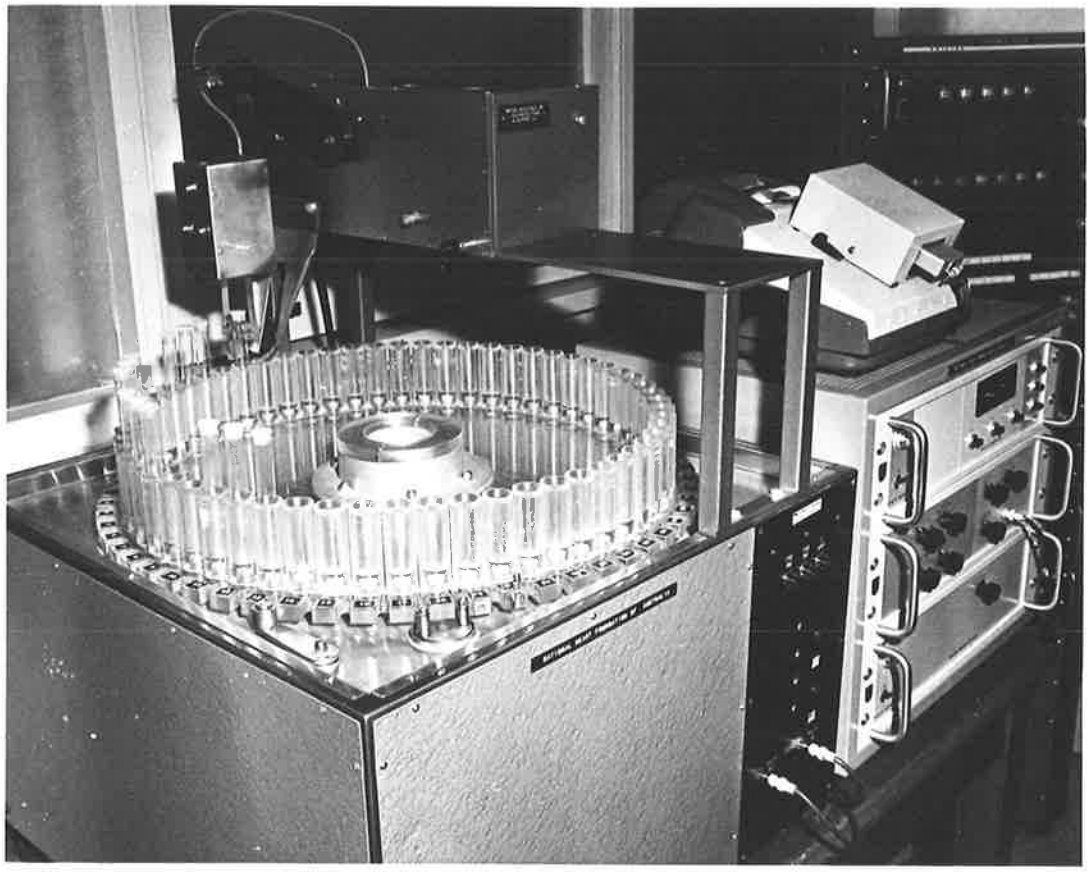


Figure 4: Automatic sample changer (Paton Industries Ltd.) capable of handling up to 50 specimens. The well crystal is situated at the centre of the rotating turntable.

from being influenced by magnetic fields, a heavy metal casing was provided. The collimator used has a large angle aperture which is suitable for measuring radioactive sources of larger surface area. The output measured by this system was further amplified and counted in a Philips Universal gamma spectrometer (PW4032) which was fitted with facilities for channel counting (Philips PW4082). Results representing the number of gamma emissions could be read directly from the counting panel (PW4032) at the end of a preset time sequence or from the ratemeter (PW4042). Results were also recorded graphically from an ink-writing electronic recording potentiometer (type PR2210A/21). Apparatus and detecting probe are seen in Figure 5. Decay corrections for the isotope were applied to the daily readings obtained by this method to account for disintegration of the isotope.

Counting efficiency.

1. Platelet samples. Throughout the course of each study background and counting efficiency of the apparatus were determined daily. The counting efficiency was determined by the use of a sealed natural uranium standard (uranyl nitrate). The background count rate was found to be 13 c.p.s.

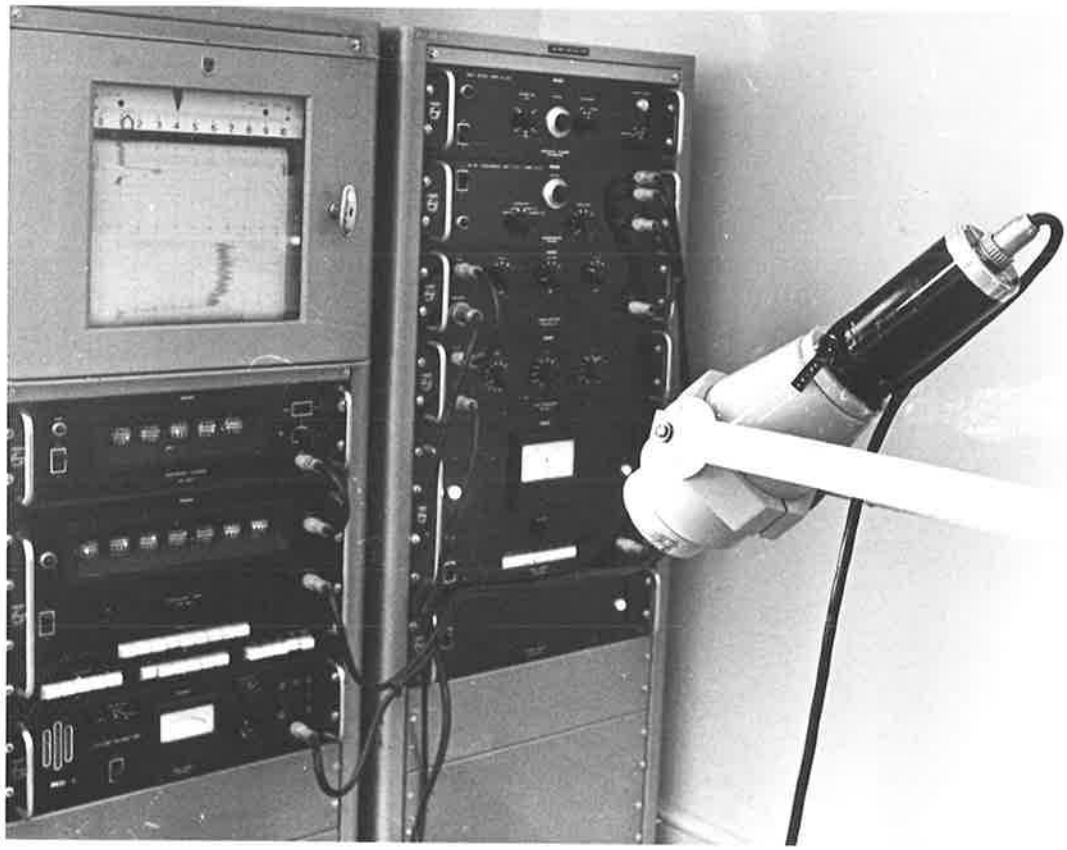


Figure 5: Scintillation counter and probe. Probe, on far right of picture, contains the detecting crystal and photomultiplier tube. Impulses are amplified and pass through panel on right which is set for optimal detection of gamma rays of $Na_2Cr^{51}O_4$ and recordings are made on the panel on the left of picture.

and the standard source 125 c.p.s. Whenever the background or standard count rate deviated by more than ± 15% of these values, the counting equipment was examined and any errors of setting corrected or faulty components replaced. The counting efficiency for a 4 ml. Cr^{51} standard solution was determined at the beginning of the study and found to be 4.3%.

2. Surface counting. The apparatus used in counting over surface projections of various organs would generally yield a background count of the order of 38 c.p.s, and the standard uranyl nitrate source a count rate of 123 c.p.s. The overall efficiency of surface counting with chromium^{51} was 1.1%. Surface counting differed from sample counting in that whereas the latter could be carried out at the completion of a study, it was necessary to estimate counts over the various surface projections on each day of study. This allows for greater variability in the results obtained owing to factors beyond control, for example, daily variations in electrical mains output. Corrections in the count rate over

surface projections could be made where required under these circumstances by applying a correction factor obtained from consideration of variations in the count rate emitted by the standard uranium source.

6. The Chandler apparatus. This consists of a circular disc of perspex attached to a larger perspex disc inclined at 30° from the horizontal and mounted on an electrically driven motor rotating at 9 r.p.m. The apparatus is pictured in Figures 6 and 7.

Technique using Chandler apparatus: 1 ml. of citrated whole blood or platelet-rich plasma was placed in a closed circular loop of polyvinyl chloride tubing (Portex NT/F) with an internal bore of 3 mm. This was recalcified by the addition of 0.1 ml. of M/4 calcium chloride solution and a stop watch started at the time of this addition. The tube was closed with a short cuff of plastic tubing of slightly greater diameter (Portex NT/K), and the closed loop fitted around the smaller perspex disc which had a small recess to accommodate the cuff. The motor was started. The length of each loop was 37.9 cm. and with rotation the column of whole blood and of platelet-rich plasma had a constant linear velocity of

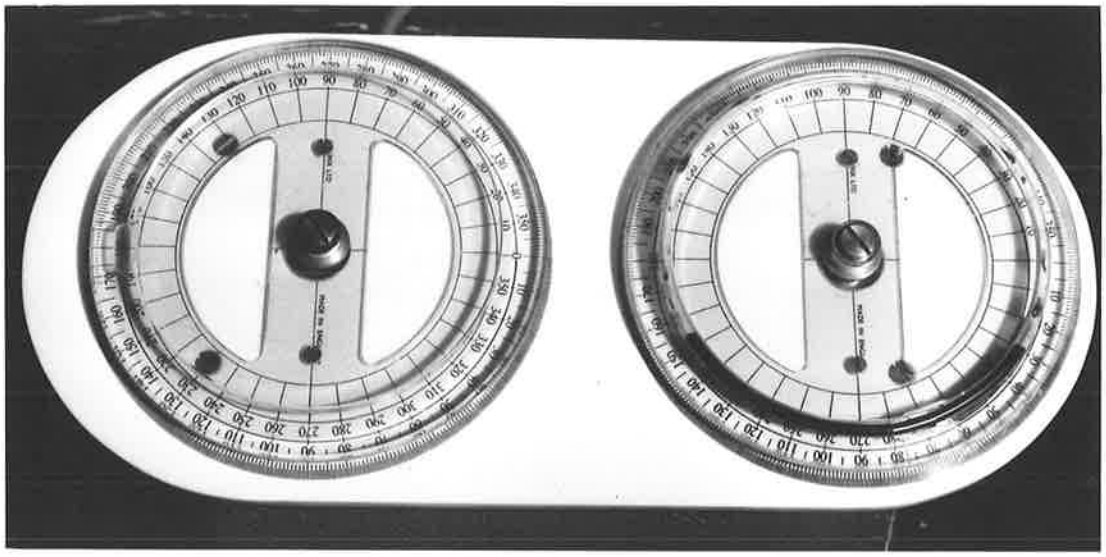


Figure 6: The Chandler apparatus.
The plastic tube containing the blood is fitted around the inner perspex disc.
The protractor for measuring the angle of the advancing head of blood is readily identifiable.

Figure 7: The Chandler apparatus shown with the dissecting microscope in place over the advancing head of the column of blood. Fitting attached to microscope is light attachment to provide adequate illumination.

340 cm./min. A fixed protractor was mounted beneath the perspex discs and the angle of the advancing column of blood or plasma was recorded at 30 second intervals. Changes occurring in the advancing edge were observed through a wide-angle stereoscopic dissecting microscope under bright oblique illumination. The following observations were made.

Thrombus formation time. This term was first coined by Connor and Poole (1961) and denotes the time at which the angle of the advancing edge of whole blood or platelet-rich plasma changes.

Whole blood thrombus length. In all cases rotation of the Chandler tube was allowed to continue for 7 minutes after an angle change had occurred. The thrombus formed was emptied into a Petri dish containing 0.9% saline and its length measured in millimetres. The mean thrombus length for each subject was the mean of the values obtained on each day of study.

Whole blood thrombus weight. The thrombi were fixed in 10% formalin and later weighed in milligrams after blotting dry between filter papers. The mean thrombus weight for each patient was again the mean of the values obtained on each of the days studied.

Platelet aggregation. Platelet-rich plasma was observed in the transparent plastic tube through a dissecting microscope at 12 magnifications. The level at which visible platelet aggregates formed, their number, size and time of persistence were recorded. Three patterns were observed.

(1) Normal pattern: the intermittent appearance and disappearance of visible platelet aggregates rarely exceeding 1-2 in number and persisting for 1 minute or less before a change in the angle of the advancing column of plasma occurred. Just before this angle change there was invariably a rapid and dramatic increase in the number of aggregates, which increased in size and coalesced to form a solid mass at the advancing edge.

(2) Persistence pattern: The continuous presence of visible platelet aggregates for a minimum of 4 minutes. In many cases aggregates formed within 1-2 minutes of re-calcification and persisted throughout the experiment.

(3) Snowstorm phenomenon: The formation of hundreds or thousands of small platelet aggregates often within 1-2 minutes of recalcification. These invariably persisted for at least 4 minutes. On a number of occasions the process was either completely or partially reversible.

This phenomenon is distinct from the large number of aggregates seen normally just prior to a change in the angle of the advancing column of plasma.

Special diagnostic procedures carried out in the laboratories of the Institute of Medical and Veterinary Science:

Complete blood pictures and tests of blood coagulation were carried out using standard techniques.

Platelet thromboplastic function was estimated by the method of Bonnin and Cheney (1961).

Plasma fibrinogen estimations were determined by the method of Coles and Roman (1957), and

Serum cholesterol levels were estimated with a Technicon autoanalyser using a modification of the method of Zlatkis, Zak and Boyle (1953).

7. Statistical methods. Results were analysed according to the statistical methods outlined by Bailey (1959).

List of symbols used in statistical formulae:

n, n_1, n_2 = Numbers of observations in samples.

P = Significance level achieved by data.

a, b = Numbers in one sample which have and have not a particular characteristic.

c, d = Numbers in second sample which have and have not a particular characteristic.

x^2 = Chi-squared.

r = Estimated correlation coefficient.

s = Estimated standard deviation.

Σ = Summation symbol.

t = "Student's" t.

x, x_i = Observed measurements, independent variables in regression.

$\bar{x}$ = Mean of sample of measurements x.

y, y_i = Observed measurements, dependent variables in regression.

$\bar{y}$ = Mean of sample of measurements y.

Statistical formulae:

1. Calculation of mean and standard deviation.

mean $(\bar{x}) = \bar{x} = \frac{1}{n} \Sigma x.$

standard deviation $(s) = \sqrt{\frac{1}{n} \Sigma (x - \bar{x})^2}$

2. Comparing means of two samples.

"Student's" t test.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

3. Comparing the percentage of two samples in 2 x 2 contingency tables using Yates' correction.

$$x^2 = \frac{n \{(ad - bc) - \frac{1}{2} n\}^2}{(a+b)(c+d)(a+c)(b+d)}$$

4. Calculation of the correlation coefficient (r)

$$r = \frac{\Sigma (x-\bar{x})(y-\bar{y})}{\sqrt{(x-\bar{x})^2 (y-\bar{y})^2}}$$

The significance levels for the various lists were obtained from published tables (Bailey, 1959). The following notations have been used in this thesis:

$p > 0.05$	not significant.
$p < 0.05$	significant at $p < 0.05$ or almost significant.
$p < 0.01$	significant at $p < 0.01$ or significant.
$p < 0.001$	significant at $p < 0.001$ or highly significant.

CHAPTER III

RESULTS

The following section includes the results of platelet studies using the technique of platelet survival in vivo as determined by the labelling of platelets with radioactive chromium. Furthermore the results of studies using the in vitro technique utilising the Chandler apparatus are included.

I. RESULTS OF STUDIES DESIGNED TO COMPARE THE EFFICIENCY OF ANTICOAGULANT SOLUTIONS USED IN THE PREPARATION OF PLATELET CONCENTRATES.

Subjects studied were healthy male volunteers between the ages of 18 and 47 years. None showed evidence of any haematological abnormality which might be expected to influence platelet survival.

Thirty-nine studies were performed using ACD 'S' as the anticoagulant into which the blood was collected. Use of this anticoagulant solution resulted in a final whole blood pH of 6.5.

Results were compared with those obtained in this laboratory previously. This included 23 studies with blood collected into ethylene diamine tetraacetic acid (EDTA) which conferred a whole blood pH of 7.3; and 6 studies in which acid-citrate dextrose formula A (ACD 'A') had been used, and which resulted in a final blood pH of 6.7.

Results

Irrespective of the anticoagulant used, the appearance of radioactivity in circulation after injection was followed by a phase of rapid clearance of the labelled platelets, a period of return of portion of the infused platelets to circulation, and a final decline of circulating platelet activity in a curvilinear fashion over the course of 9 to 11 days, an interval reflecting the lifespan of viable platelets.

Appearance of radioactivity in circulation. Following the infusion of labelled platelets, a procedure occupying approximately 60 seconds, frequent sampling of venous blood was carried out through the indwelling catheter and the radioactivity in each sample of blood determined. The peak of radioactivity generally occurred between 30 and 90 seconds of the completion of the infusion.

Clearance of platelets from circulation. This was studied in detail in 3 subjects with ACD 'S' anticoagulant and compared with the results that had been obtained similarly in 3 patients with EDTA platelets and 3 with ACD 'A' platelets (Figure 8). In all studies, platelet radioactivity fell rapidly after peak mixing values had been reached. The rate of platelet clearance was most rapid in

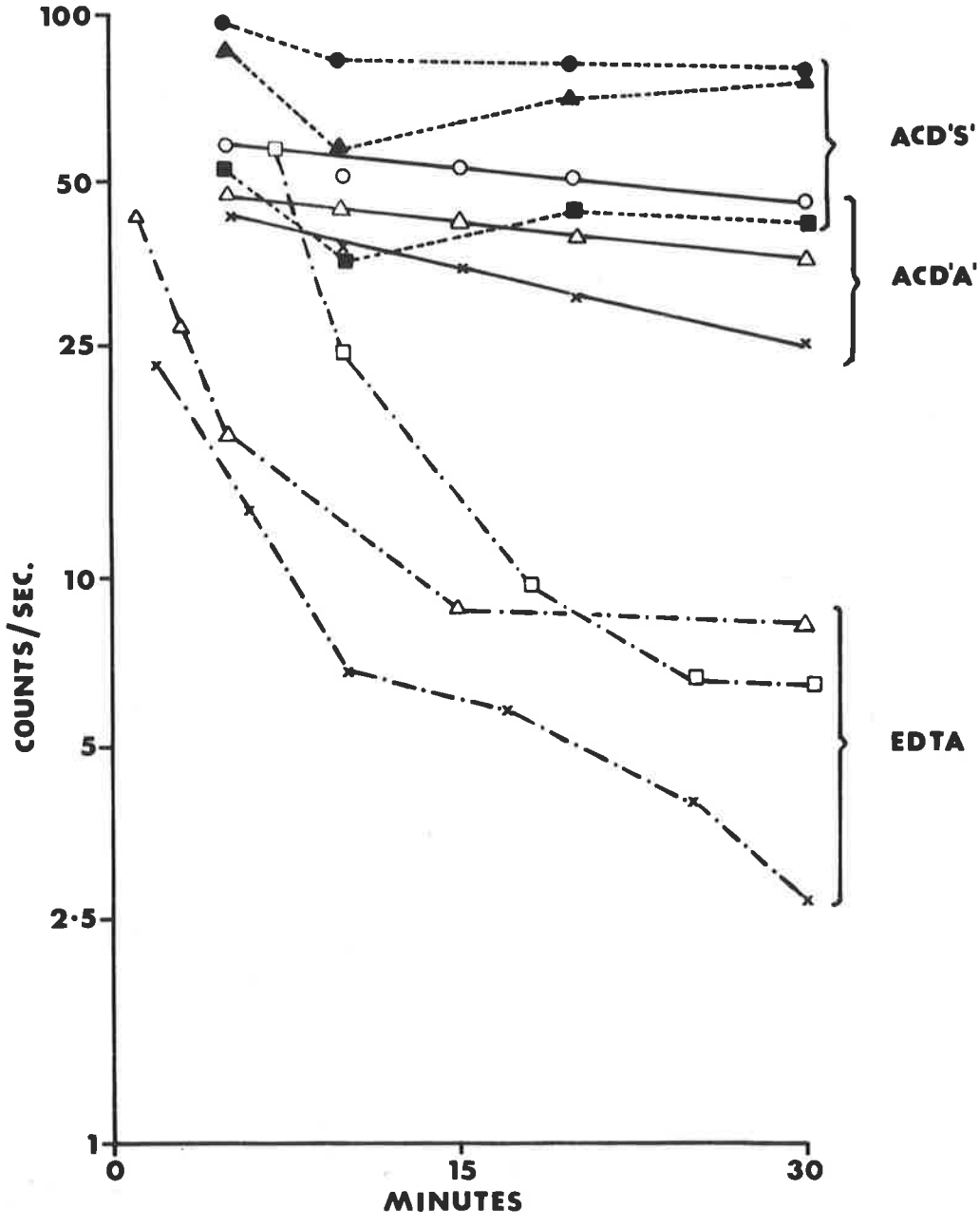


Figure 8: Comparison of the immediate clearance patterns of platelet radioactivity from circulation with three anticoagulant solutions.

Table 1 - Percentage platelet recovery in circulation at five and thirty minutes after infusion (maximum % recovery also shown).

		5 mins.	30 min.	Maximum
EDTA	Mean	24 ± 11	4.4 ± 3	24 ± 11
23 studies	Range	8 - 49	1 - 12	7 - 51
ACD 'A'	Mean	44 ± 7	37 ± 7	45 ± 6
6 studies	Range	36 - 52	28 - 45	39 - 51
ACD 'S'	Mean	30 ± 14	26 ± 12	32 ± 12
39 studies	Range	3 - 54	6 - 51	10 - 52

the EDTA studies, especially during the first 3 to 5 minutes. After 5 minutes, the rate of clearance slowed in all subjects and the minimum level ofcirculating platelet radioactivity was invariably found 15 to 20 minutes after injection. This nadir of circulating platelet radioactivity tended to occur earlier when the platelets were collected in ACD 'S', and the extent of platelet clearance was greatest when collected in EDTA. The proportions of labelled platelets injected and still present in circulation 5 and 30 minutes after injection are shown in Table 1.

Recirculation of platelets. The return of platelets to circulation was fully studied in 35 instances with platelets collected in ACD 'S'. The remaining subjects (4) were excluded from the study because of failure to obtain a 30 minute sample. The general pattern of behaviour with this anticoagulant is illustrated in Figure 9, and has been related to that found with EDTA platelets (19) and ACD 'A' platelets (5). Davey and Lander (1964) have shown that with EDTA studies circulating platelet activity increases from a nadir in a simple exponential fashion. Maximum levels of circulating activity were generally found within 24 hours when EDTA platelets were

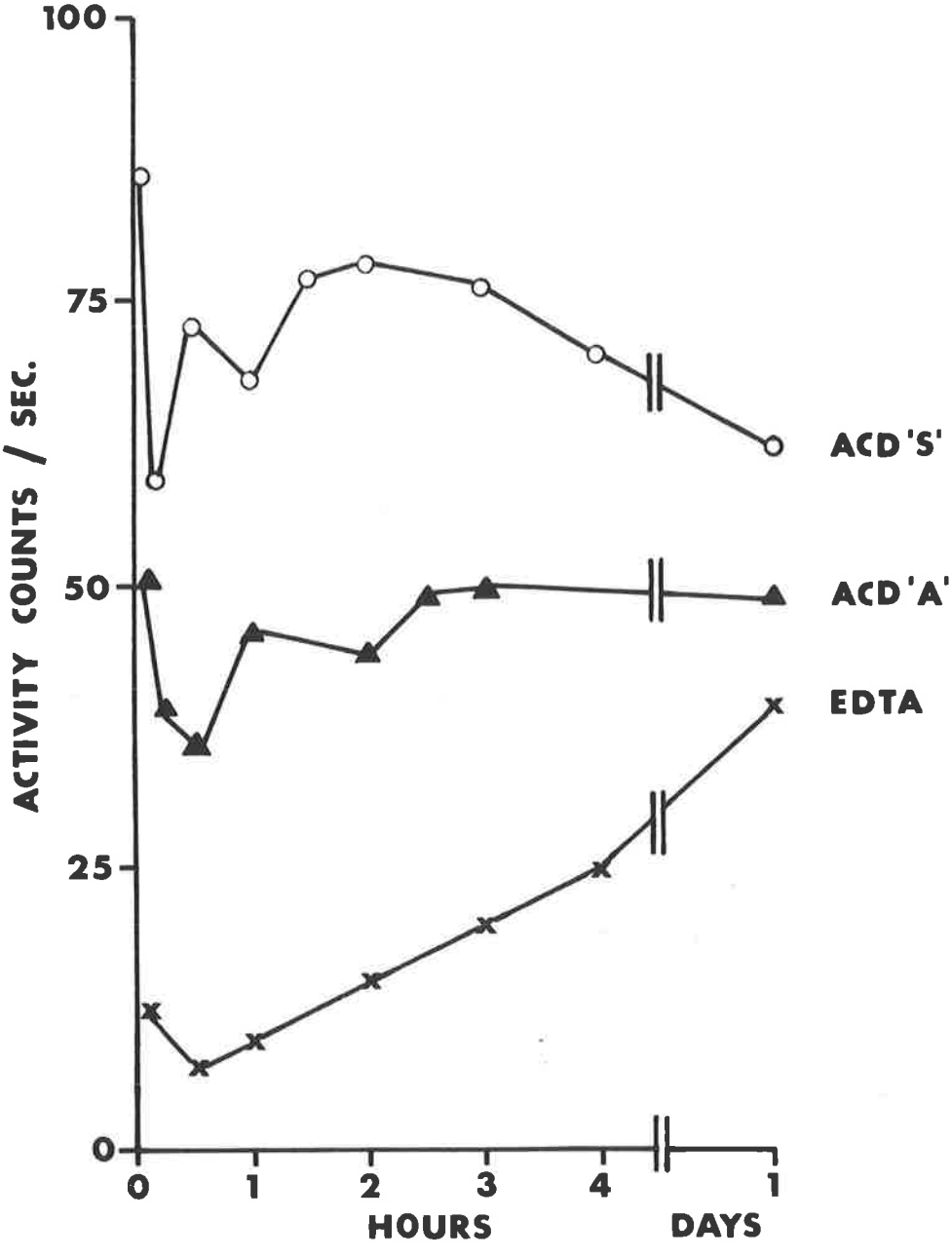


Figure 9: Comparison of patterns of return to circulation of Cr^{51}-labelled platelets.

Table 2 - Yield, recovery and efficiency of different platelet preparations

Anticoagulant		Platelet yield (%)	Platelet Recovery (%)	Efficiency index
EDTA	Mean	62 ± 17	24 ± 11	15
	Range	(20 - 90)	(7 - 51)	
ACD 'A'	Mean	49 ± 12	45 ± 6	22
	Range	(40 - 71)	39 - 51	
ACD 'S'	Mean	55 ± 16	32 ± 12	18
	Range	26 - 100	10 - 52	

used but were occasionally delayed for 48 hours, and in one study, for 72 hours after infusion. In studies with ACD 'S' platelets (and ACD 'A' platelets) the circulating platelet radioactivity always increased after 15 to 30 minutes but the pattern of platelet return was not as regular as with EDTA (Figure 9). With both solutions of ACD, maximum recirculation invariably occurred within 24 hours and usually within the first 4 hours following re-injection.

Maximum platelet recovery. For purposes of comparison, the recovery of platelets in circulation in each study was estimated as the maximum proportion of those platelets infused which reappeared in circulation after the nadir of activity had been reached, irrespective of the time at which the maximum occurred. For this calculation the subjects were assumed to have a total blood volume equivalent to 67 ml./kg. body weight. The maximum recovery in circulation was significantly lower when platelets were collected into EDTA than in either of the acid citrate dextrose solutions (Table 2.).

Platelet survival. Maximum recirculation was followed in all subjects by a slow decline in circulating platelet radioactivity over the course of 9 to 11 days, a period representing the lifespan of viable platelets.

Survival "curve". Because of differences in the amount of platelet-bound radioactivity infused into the subjects studied and the variations encountered in the time and extent of maximum platelet recirculation, an expression independent of these variables is required for comparison of platelet survival. This is obtained by considering the maximum value of platelet radioactivity appearing in circulation as the 100% reference to which all subsequent values are related, irrespective of when this maximum occurs.

The survival curve obtained with platelets collected in ACD 'S' is shown in Figure 10 and compared with that obtained with EDTA and ACD 'A' platelets. Statistical analysis showed no significant difference between the values obtained with the three anticoagulants. The common survival curve obtained is curvilinear and cannot be expressed aither as a linear or simple exponential function.

Reproducibility. The reproducibility of ACD 'S' platelet survival studies in normal subjects was tested by repetition of the autologous studies in 2 subjects using the same anticoagulant. In addition simultaneous determination of the survival of the same platelets in

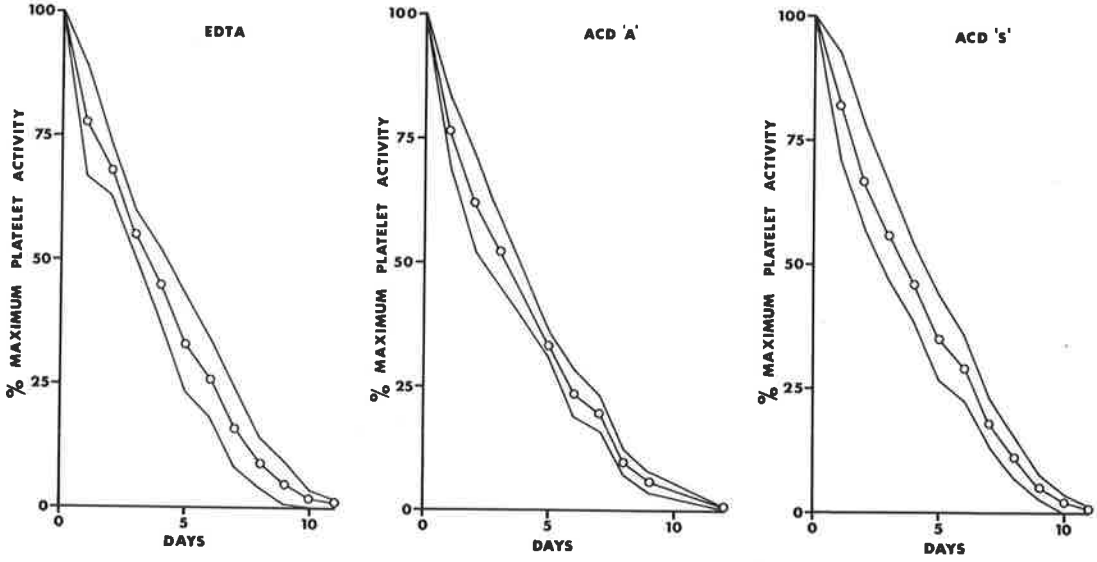


Figure 10: Mean platelet survival curves for each group of subjects using the three different anticoagulants.

their donor and in a normal ABO/Rh compatible subject, was carried out (1 study). Close correlation was demonstrated in each instance (Figure 11) as had previously been determined with both ACD 'A' and EDTA studies.

Surface counting. External scintillation counting over surface projections of the praecordium, liver, spleen, lungs and sacrum was performed in 36 subjects given ACD 'S' platelets and related to the findings in 22 subjects given EDTA platelets and 6 given ACD 'A' platelets. The results of these observations may be considered in two phases: (1) An early period occupying the first 24 hours after infusion, and

(2) A late period, occupying the remainder of the study.

The early phase. Distinct patterns were observed in the surface counts performed during the first 24 hours for each of the three groups studied. These are evident both in mean surface counts and, allowing for experimental variation, in individual studies. With EDTA platelets (Figure 12), the early phase was characterised by a marked initial uptake of activity in the liver which declined steadily in the first 4 hours, and generally continued to fall for 24 hours. This was in contrast to

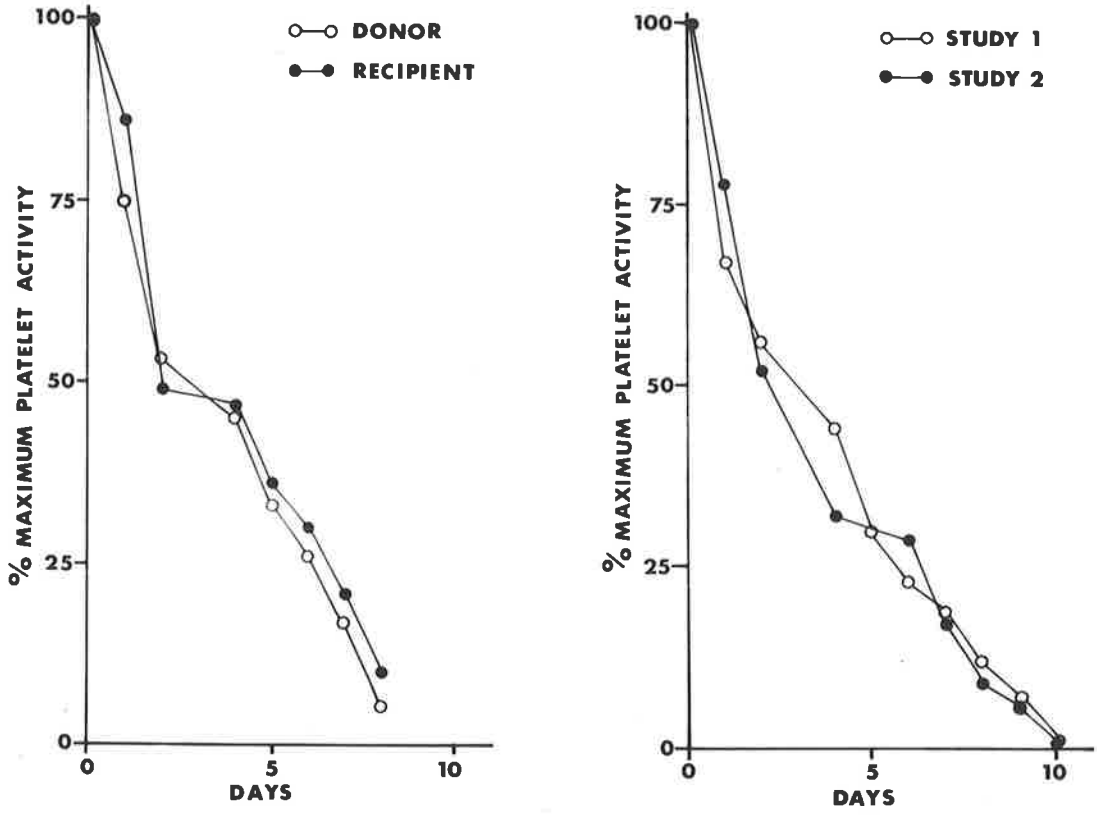


Figure 11: Results of a cross transfusion study showing identical platelet survival in both donor and recipient and the reproducibility of survival studies carried out on the one subject on different occasions (ACD 'S' anti-coagulant.

the situation with platelets collected in either of the ACD solutions, where hepatic activity usually approximated that over the praecordium throughout this period (Figures 13 and 14).

Splenic activity with EDTA platelets was generally low initially and tended to rise in association with the fall in hepatic activity. With ACD 'A' platelets (Figure 13) high initial splenic activity generally decreased markedly during the first few hours; while intermediate splenic activity with ACD 'S' platelets tended to remain at the same level or to increase only slightly (Figure 14).

Late phase. After the first 24 hours, activities over the spleen exceeded those over any other organ, irrespective of the anticoagulant in which the platelets had been collected. The spleen-praecordial ratio generally remained constant with EDTA and ACD 'A' platelets, but tended to rise progressively during the course of the studies with ACD 'S' platelets.

In all studies, there was a progressive uptake of activity in the liver as judged by increasing liver/praecordial ratios. This was most marked with ACD 'A' platelets and least with EDTA platelets.

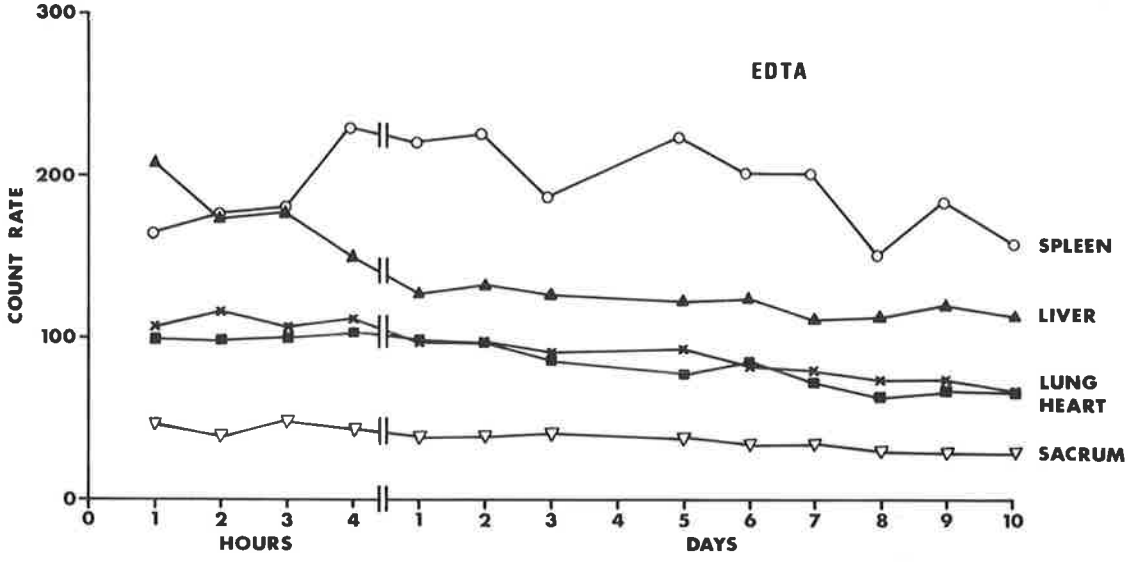


Figure 12: Mean surface activities with EDTA platelets showing an initial hepatic uptake and subsequent increase in splenic uptake of radioactivity.

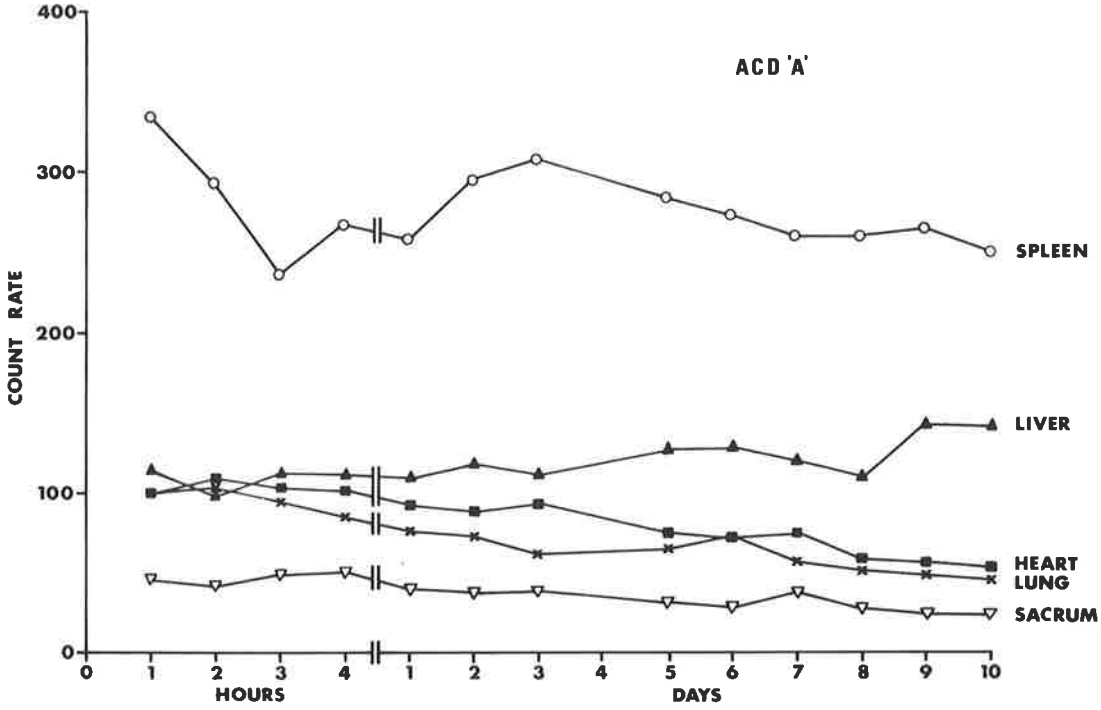


Figure 13: Mean surface activities obtained with ACD 'A' platelets.

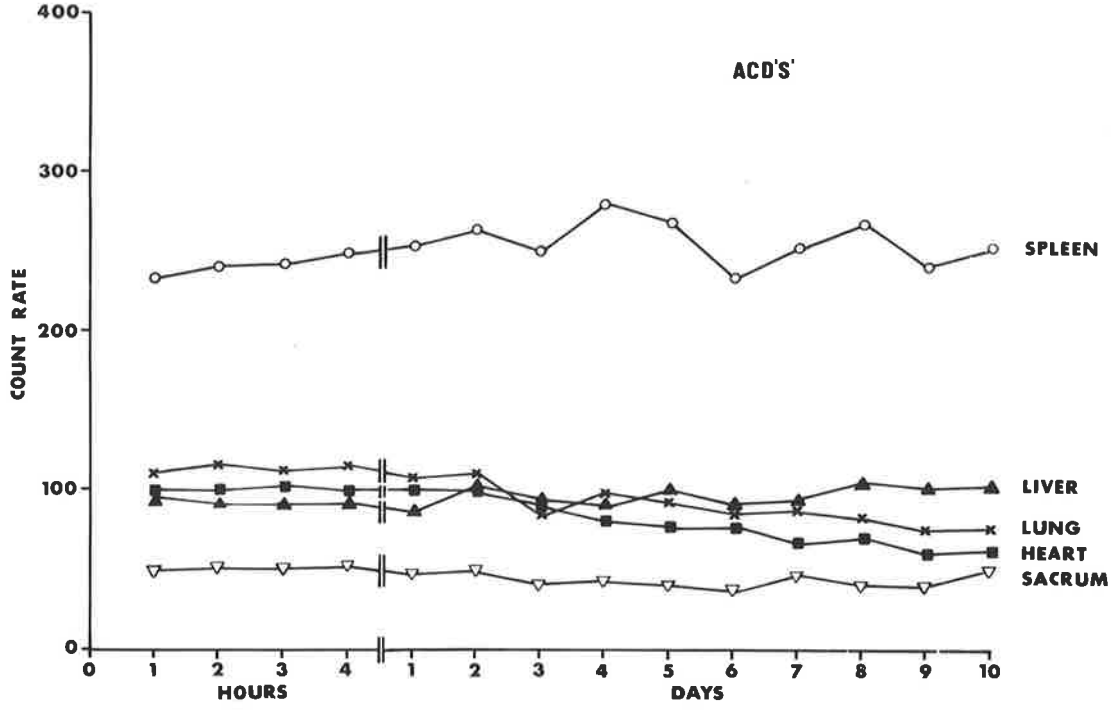


Figure 14: Mean surface activities obtained with ACD 'S' platelets.

Irrespective of the anticoagulant used, lung activities approximated those over the praecordium, while sacral activities generally remained at a low level throughout the studies.

The mean surface activities recorded for each group during this phase are illustrated in Figures 12, 13 and 14.

Platelet efficiency. To compare the relative merits of each of the anticoagulant solutions, three criteria were employed.

Platelet 'yield' - the proportion of platelets collected which remained available for transfusion after preparation of the platelet concentrate.

Platelet 'recovery'* - the maximum proportion of those cells injected which reappeared in circulation.

Platelet 'efficiency'* - an index of the maximum proportion of those platelets collected which finally reappeared in circulation and obtained from the formula

$$\frac{\text{platelet yield} \times \text{platelet recovery}}{100}$$

The values obtained for each anticoagulant are

---

*For the purposes of these calculations it was assumed that all platelets were equally labelled with radiochromate.

contained in Table 2. Although more platelets were available for transfusion when whole blood was collected in EDTA than in either ACD 'A' or ACD 'S', only a relatively small proportion of EDTA platelets survived in circulation as viable units. Thus, in terms of overall efficiency, these studies demonstrate that both solutions of citrate-dextrose are superior to EDTA.

II. RESULTS OF STUDIES CARRIED OUT IN PATIENTS SUFFERING FROM ATHEROMA OR ITS COMPLICATIONS

Platelet survival

Subjects studied: 36 studies were carried out in 34 subjects. Two subjects were studied both while receiving anticoagulant treatment and again at a later date when anticoagulant therapy had been discontinued. Phenindione was the therapeutic anticoagulant used in all cases (19) and prothrombin levels measured by Quick's one stage method were maintained between 15 and 25% activity.

Blood taken for labelling was collected into two anticoagulant solutions. In 12 subjects EDTA was used, and in 24 ACD 'S' was the anticoagulant employed.

Results

Following infusion of platelets into patients after

Table 3 - Daily mean percentage radioactivity in circulation of control subjects and of subjects with atheroma receiving and not receiving anticoagulant treatment - EDTA platelets only.

Day	Controls	'Atheroma" Anticoagulant Treatment	'Atheroma' No Anticoagulant
0	100	100	100
1	77.8 ± 11.2	76 ± 15.5	84.4 ± 11.7
2	68.0 ± 15.6	59.8 ± 11.1	76.8 ± 21.7
3	55.3 ± 14.7	55.0 ± 2.8	62.4 ± 11.8
4	45.1 ± 7.3	35.3 ± 6.5	47.3 ± 9.5
5	33.3 ± 9.7	34.3 ± 4.9	39.3 ± 5.7
6	26.3 ± 7.7	22.3± 8.2	29.3 ± 6.7
7	16.4 ± 7.7	17.0 ± 5.5	21.3 ± 8.8
8	8.7 ± 5.3	6.3 ± 3.4	12.2 ± 5.0
9	4.7 ± 4.6	3.5 ± 3.5	5.3 ± 5.9
10	1	2.0	4.7 ± 4.1

labelling with radioactive chromium the general pattern of behaviour did not differ from that described earlier. Infusion was followed by immediate clearance of a proportion of platelets. Gradual reappearance of some platelets occurred and maximum levels of circulating platelet radioactivity were found within the first four hours with acid citrate dextrose anticoagulants and within 24 hours in subjects whose blood had been collected in EDTA. This was again followed by the gradual fall off of platelet radioactivity over a period of days.

Results in patients with untreated vascular disease. The mean values of radioactivity for each day of study are represented graphically in Figure 15 and are also tabulated (Tables 3 and 4). In EDTA studies points fell within the range of normal as also occurred in ACD 'S' studies.

Results in patients with treated vascular disease. The mean platelet survival curves in this group are also illustrated in Figure 14. Again the curve falls within the range of normal and platelet lifespan in circulation was between 9 and 11 days. There is a tendency, however, for the curve in this group to be "shifted to the left", suggesting that anticoagulant therapy has a tendency to reduce platelet survival in circulation in subjects with

Table 4 - Daily mean percentage radioactivity in circulation of control subjects and of subjects with atheroma receiving and not receiving anticoagulant treatment - ACD 'S' platelets only.

Day	Controls	'Atheroma' Anticoagulant Treatment	'Atheroma' No Anticoagulant
0	100	100	100
1	79.8 ± 10.3	78.3 ± 11.9	85 ± 8.7
2	64.6 ± 9.9	65.7 ± 10.7	71.1 ± 10.6
3	57.7 ± 10.7	53.7 ± 10.2	57.7 ± 9.3
4	44.4 ± 8.1	46.1 ± 4.4	45.2 ± 10.8
5	33.1 ± 4.5	30.6 ± 4.6	42.4 ± 9.4
6	25.7 ± 6.2	27.6 ± 8.4	29.25 ± 6.2
7	14.3 ± 3.4	18.7 ± 5.3	19.5 ± 4.7
8	8.0 ± 1.4	10.8 ± 4.6	12.1 ± 4.4
9	4.3 ± 3.1	5.8 ± 2.5	6.2 ± 2.1
10	1.5	2.8 ± 0.9	3.2 ± 2.2

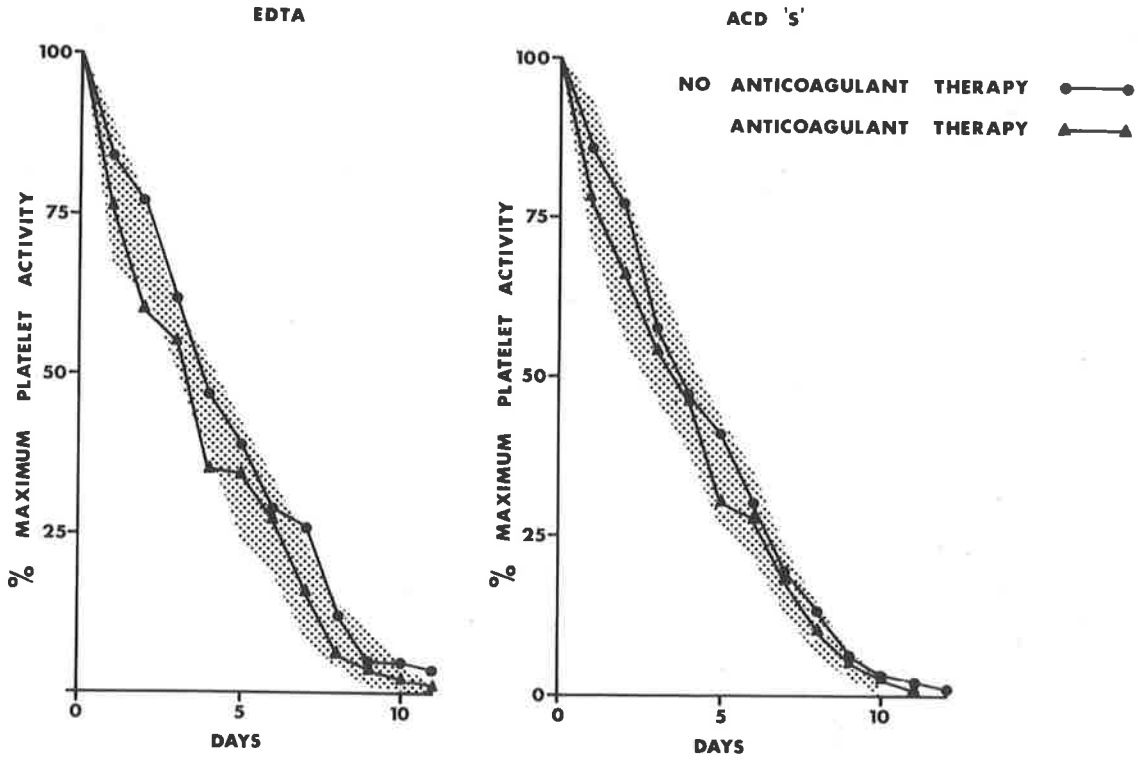


Figure 15: Mean platelet survival curves in patients with atheroma. Differences between anticoagulated and non-anticoagulated groups were not significant statistically.

Table 5 - Platelet turnover in subjects with atheroma

	Normals	"Atheroma" Anticoagulant	"Atheroma" No anticoagulant
A. EDTA			
Platelet life (days)	9 - 11	9 - 11	9 - 11
Platelet turnover % per die (range)	12 - 20	15 - 17	15 - 28
B. ACD 'S'			
Platelet life (days)	9 - 11	9 - 11	9 - 11
Turnover % per die (range)	15 - 23	15 - 35	12 - 28

Differences in platelet turnover were not statistically significant ($p > 0.05$).

atheroma. This is particularly obvious in those subjects who have been studied both while receiving anticoagulant therapy and again several months after cessation of phenindione.

Platelet turnover. Although the precise mode of platelet utilisation is still subject to some dispute, platelet turnover in individual subjects was calculated on an exponential basis assuming firstly that platelet production remained steady for the duration of the study and secondly, that the function used for these calculations is probably unimportant provided the same method is used throughout. This was subsequently expressed as a percentage of the total platelet count and the results are illustrated in Table 5. Statistically there was no difference in platelet turnover between groups.

Results in isolated studies not included above. O.G., a 57 year old female, was admitted to hospital with impending gangrene of the right foot following a 12 month history of intermittent claudication. A lumbar sympathectomy was planned and a preoperative electrocardiograph carried out. There was evidence of old myocardial infarction. On the seventh day after operation a platelet survival study was commenced in conjunction with

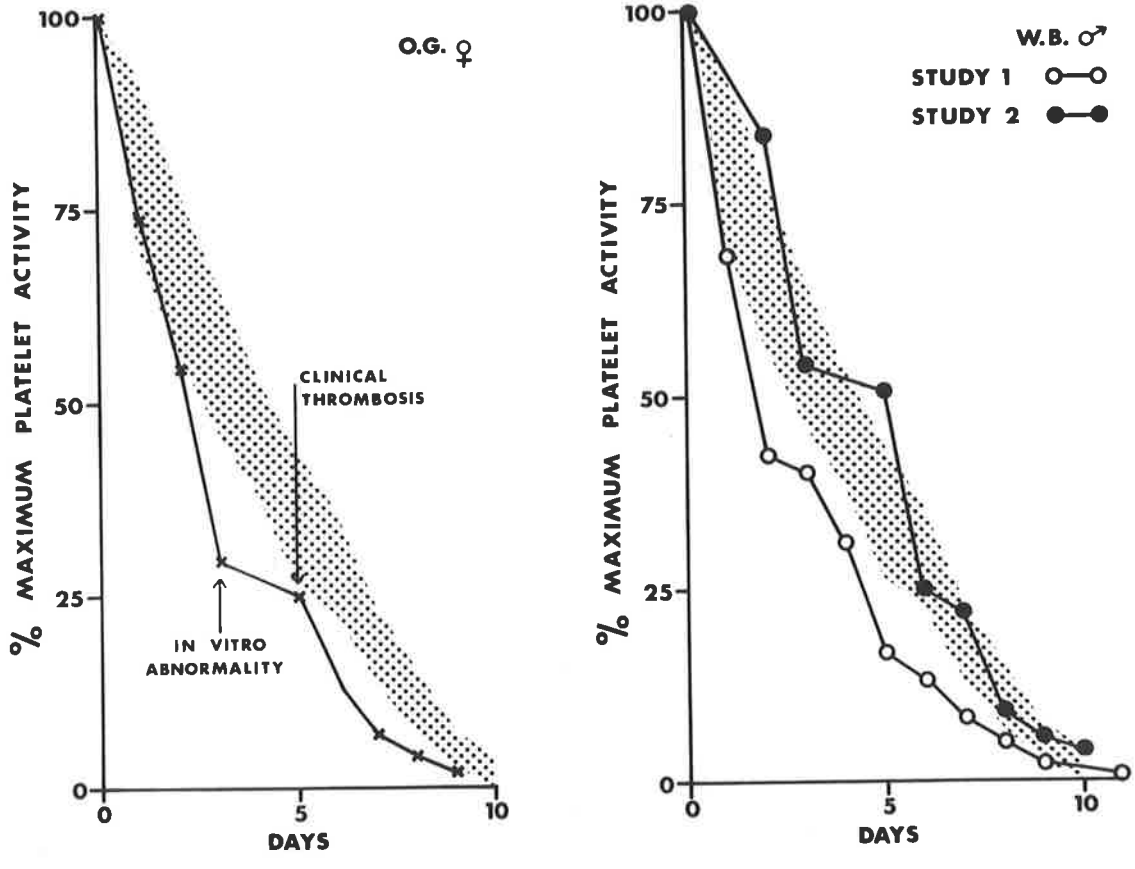


Figure 16: Platelet survival curves obtained in patients with proven vascular disease.
(a) deep vein thrombosis.
(b) aortic aneurysm resection.

Chandler studies. On the sixth day of study there was good clinical evidence of a deep vein thrombosis of the right leg. Calculation of platelet survival (Figure 16) revealed blood platelet radioactivity to have fallen markedly on days 4 - 6 corresponding with the clinical development of venous thrombosis. Platelet behaviour in the Chandler tube was grossly abnormal from the fourth day.

W.B., a 64 year old male, had an operation for resection of an aortic aneurysm. The aorta was atherosclerotic and the aneurysm, which was of 10 cm. diameter, was replaced with a dacron graft 19 cm. in length. Labelling of blood platelets with radioactive chromium was carried out on the seventh postoperative day and the study continued for 11 days thereafter. There was a reduction in platelet survival (Figure 16) and platelet behaviour in the Chandler tube was grossly abnormal throughout. A further survival study carried out three months postoperatively was completely normal, as were Chandler studies.

Chandler Studies

Subjects studied. The blood of 102 subjects was studied in the Chandler tube. Fifty-two subjects were

Table 6 - Age distribution of the 102 subjects studied with the Chandler apparatus. Patients with myocardial infarction have been subdivided into those receiving or not receiving anticoagulant therapy.

Subject Categories	Age Group (years)						
	11 - 20	21-30	31-40	41-50	51-60	61-70	71-80
Controls	3	9	12	15	10	3	-
Myocardial infarct. Anticoagulant	-	-	1	4	15	3	1
Myocardial infarct. No anticoagulant	1	-	-	1	4	14	6
Total Myocardial infarct.	1	-	1	5	19	17	7

healthy control subjects made up of colleagues, laboratory assistants and hospital porters, in all of whom there was neither electrocardiographic nor clinical evidence of myocardial infarction.

Fifty patients had suffered from myocardial infarction. These were selected from both hospital inpatient and outpatient services and all had clinical, laboratory and electrocardiographic evidence consistent with the diagnosis of myocardial infarction.

Six of the control subjects and 10 of the subjects with myocardial infarction were women. The age distribution of individuals studied is tabulated in Table 6.

Results

Subjects have been subdivided into four main categories. The control group was divided into two age groups - those of 40 years or less and those greater than 40 years of age. Patients with myocardial infarction have been subdivided into those either receiving or not receiving anticoagulant therapy.

Thrombus length and thrombus weight. Values obtained for mean thrombus length and mean thrombus weight are represented in Figures 17 and 18. There was a tendency for both thrombus length and weight to increase with age

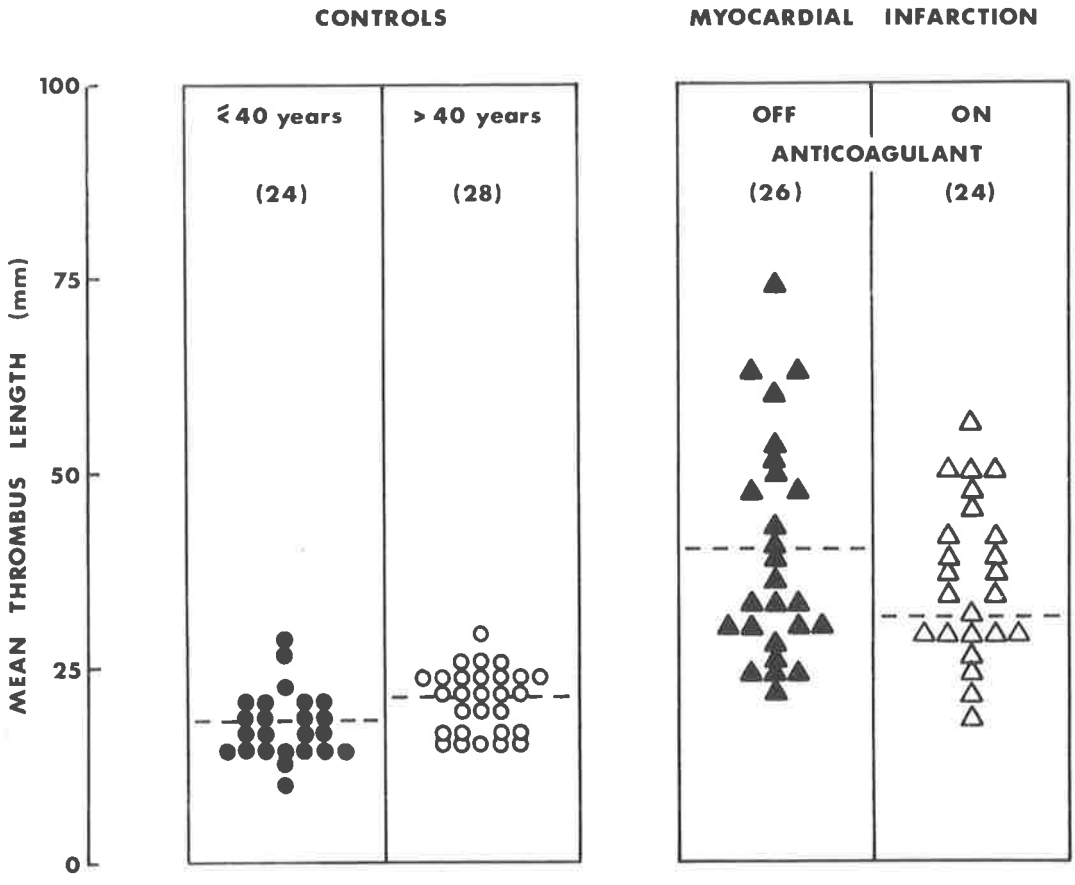


Figure 17: Mean thrombus length of control subjects and patients with myocardial infarction, obtained from blood in the Chandler tube.

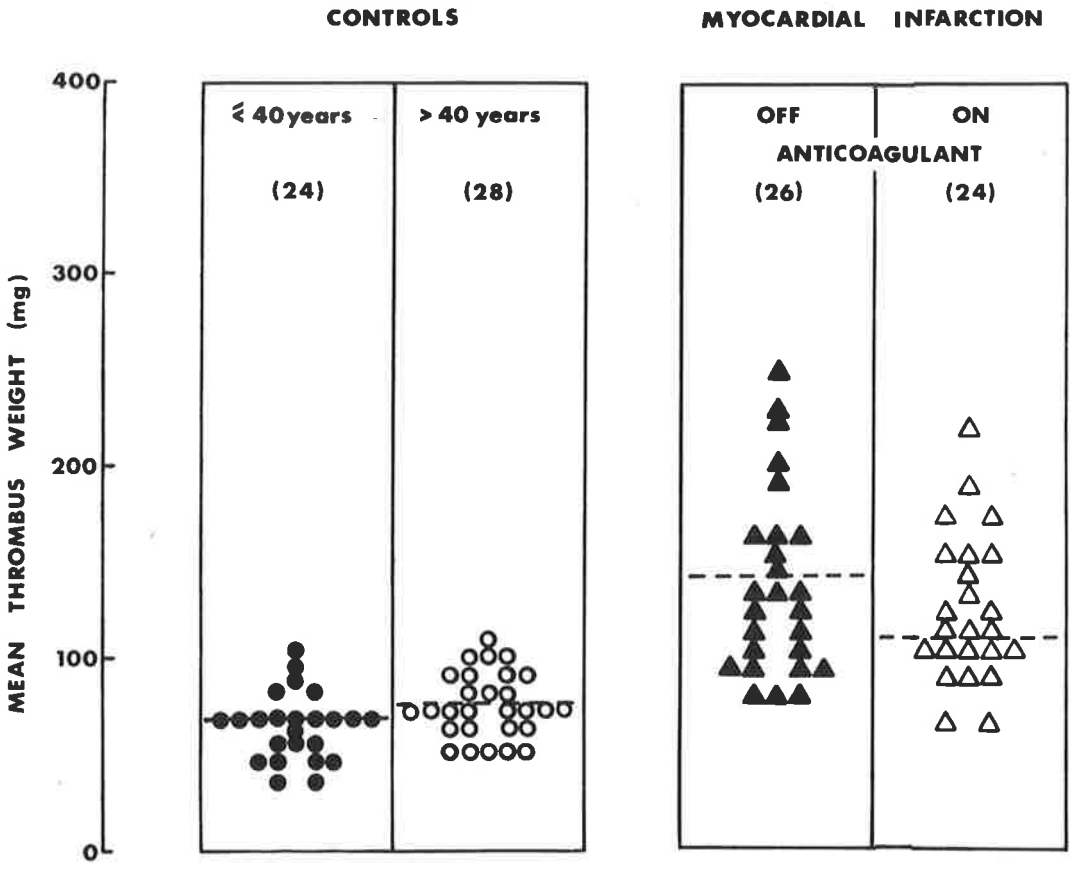


Figure 18: The mean thrombus weight in control subjects less than or equal to forty years of age (●) and of control subjects greater than forty years (o) compared with the mean thrombus weight in patients with myocardial infarction who are receiving anticoagulant treatment (△) and with those who are not anticoagulated (▲).

in control subjects. In patients with myocardial infarction the increase in both these parameters is even more obvious when compared with controls over the age of 40 years. It can also be seen that subjects who are receiving anticoagulant therapy following myocardial infarction produce thrombi which are both shorter and less heavy than in patients who are not being treated in this fashion.

Time after myocardial infarction. Mean thrombus weight and length have also been analysed according to the time after myocardial infarction. The subdivisions considered were 0 - 7 days, 8 - 42 days and greater than 42 days after myocardial infarction. A further subdivision was made according to the presence or absence of anticoagulant therapy. Figures 19 and 20 denote the results in these subjects. Thrombus weight and length decline steadily with the passage of time after myocardial infarction independently of anticoagulant therapy. In patients studied more than 42 days after the clinical event the values for thrombus weight and length had returned to within the normal range in only 7 of the 12 subjects studied.
It is again obvious that thrombus size in each of the subdivisions tends to be slightly less in subjects

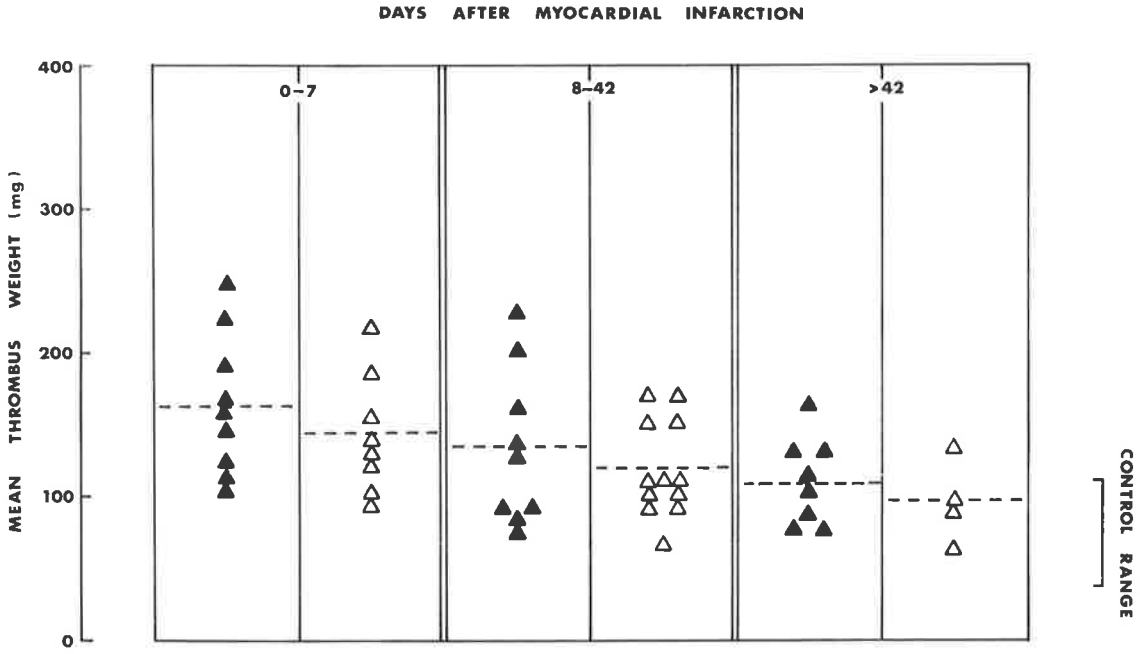


Figure 19: Mean thrombus weight in patients with myocardial infarction analysed according to time after infarction and presence (▲) or absence (△) of anticoagulant therapy.

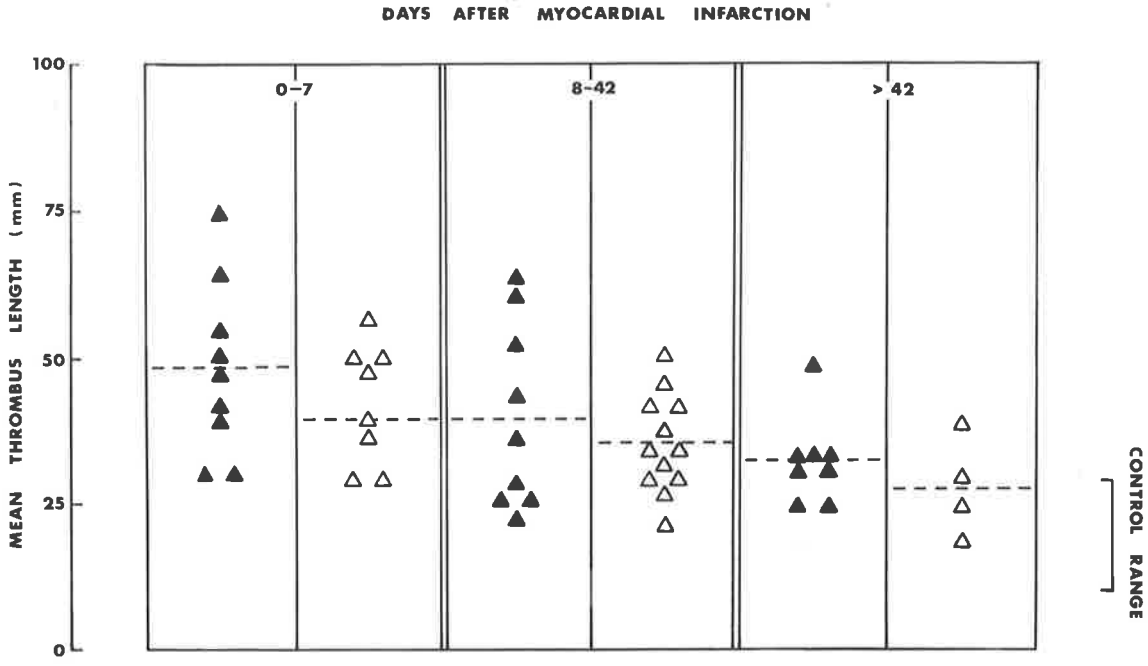


Figure 20 : Mean thrombus length in patients with myocardial infarction subdivided according to time after infarction and the presence (▲) or absence (△) of anticoagulant therapy.

who are receiving anticoagulant therapy.

Thrombus size and plasma fibrinogen. Plasma fibrinogen levels were determined in all subjects studied and were found to be elevated in patients following myocardial infarction (Figure 21). Both in control subjects and in patients with infarction there was a statistically significant correlation between thrombus weight and plasma fibrinogen levels (Figure 22). In healthy control subjects the correlation coefficient r was 0.4136 ($p < 0.01$) while in patients with myocardial infarction $r = 0.7180$ ($p < 0.001$).

Thrombus size and platelet count. The platelet count was determined daily for the duration of study in each patient. The mean platelet count was calculated for each subject and related to the mean thrombus weight determined over the same period. There was no statistically significant correlation between thrombus weight and platelet count in either healthy control subjects or in patients with myocardial infarction.

Total serum cholesterol. Total serum cholesterol (Figure 23) was estimated and found to be greater in healthy subjects over the age of 40 years than in younger control subjects. Cholesterol levels for patients with

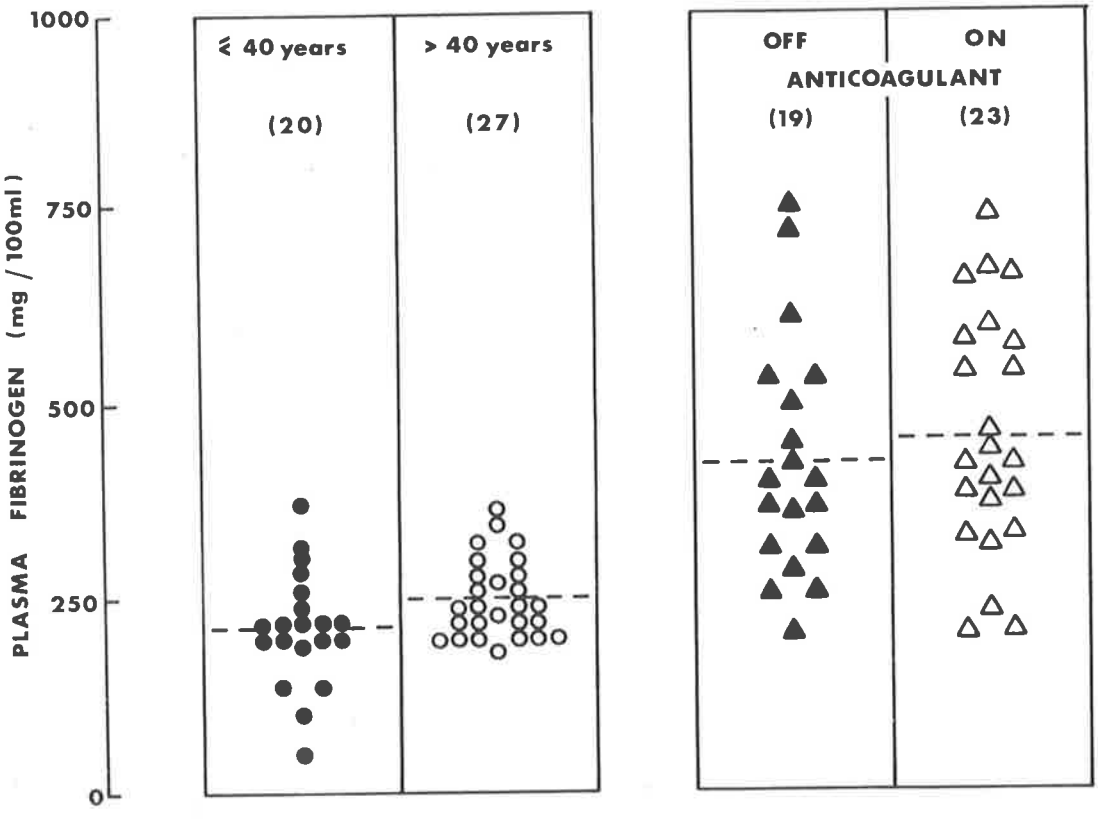


Figure 21: Plasma fibrinogen levels in control subjects and patients with myocardial infarction.

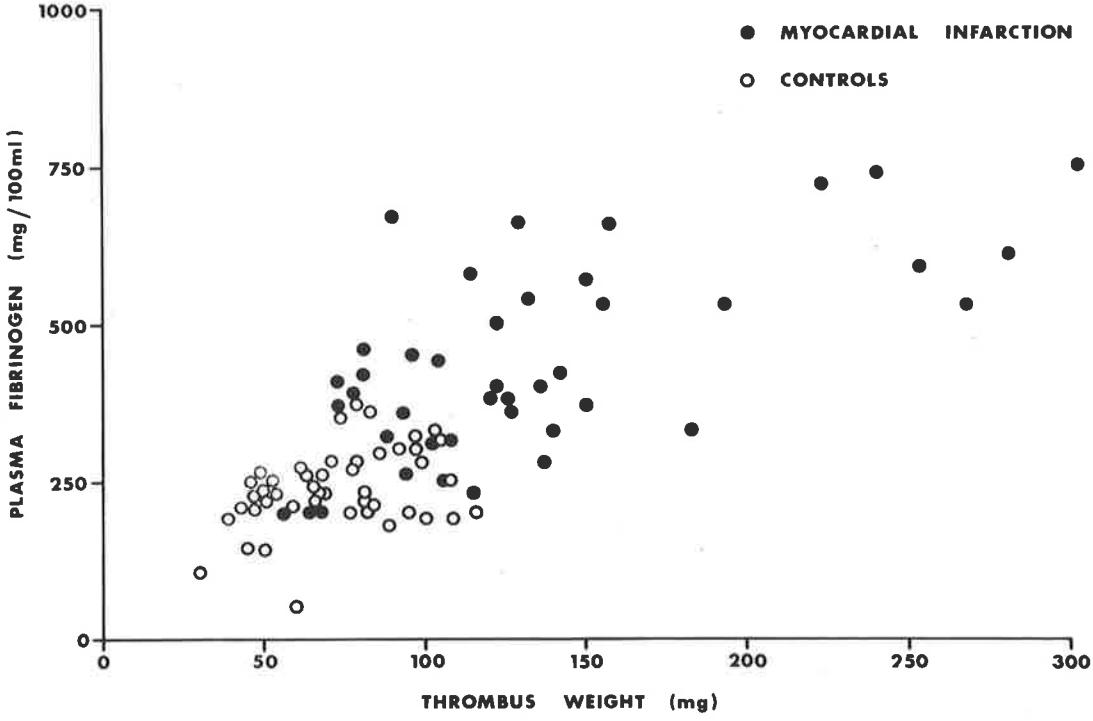


Figure 22: Thrombus weight plotted against plasma fibrinogen in control subjects (o) and in patients with myocardial infarction (●). There is a significant correlation in both the control group ($r = 0.4136$) and infarct group ($r + 0.718$).

myocardial infarction were of the same order as in controls over 40 years of age.

Total serum cholesterol and thrombus size. There was no significant correlation between thrombus weight and serum cholesterol levels neither in controls nor in patients with myocardial infarction (Figure 24).

Thrombus formation time. The range of values for whole blood (Figure 25) and platelet rich plasma (Figure 26) thrombus formation time did not differ significantly between controls over 40 years of age and patients with myocardial infarction. Thrombus formation time (except in one instance) was virtually unchanged in patients receiving oral anticoagulant therapy. This is contrary to the findings of Cunningham et alii (1965) who found platelet aggregation in the Chandler tube to be much delayed in patients receiving treatment with anticoagulants.

Platelet rich plasma. In healthy subjects less than 40 years of age the normal pattern of platelet aggregation was observed in 98.6% of days studied (Table 7). In none of the subjects in this age group was the snowstorm phenomenon of platelet aggregation observed. Thus abnormal platelet aggregation in the younger controls was found with an overall frequency of only 1.4% of the total

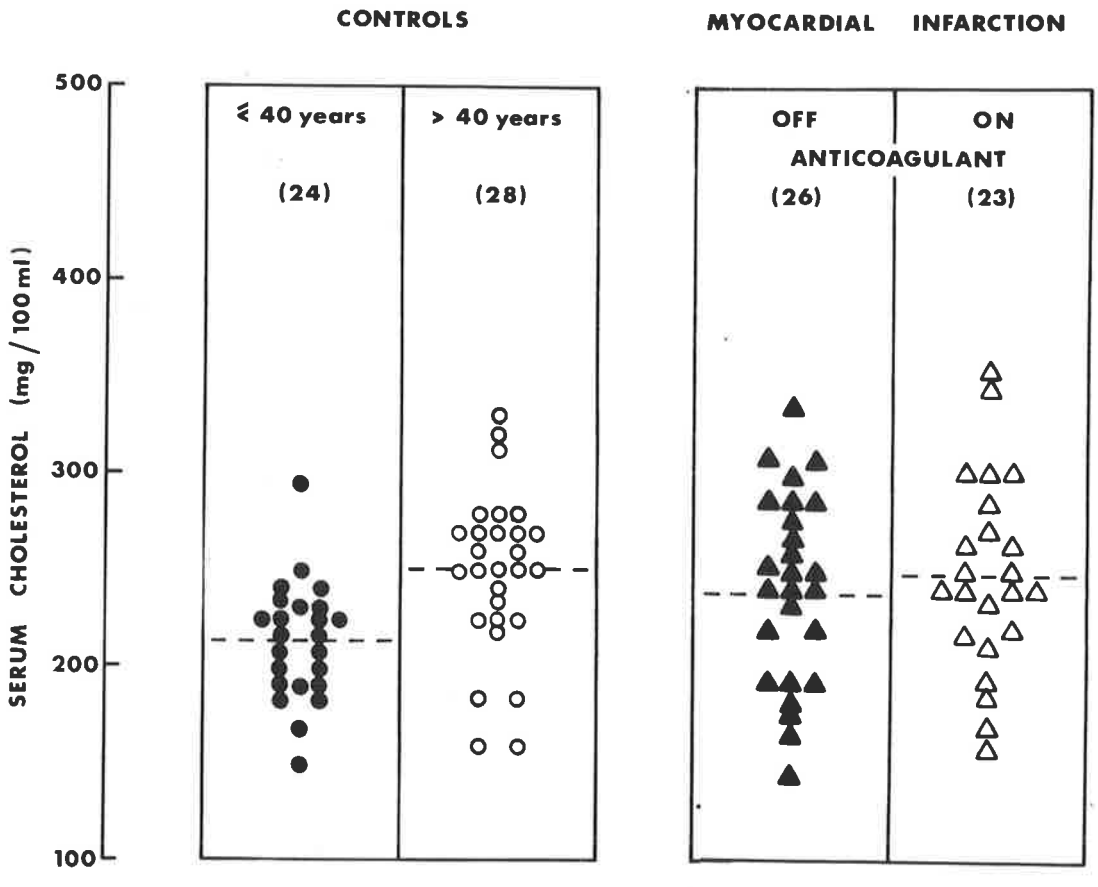


Figure 23: Serum cholesterol levels in control subjects and patients with myocardial infarction.

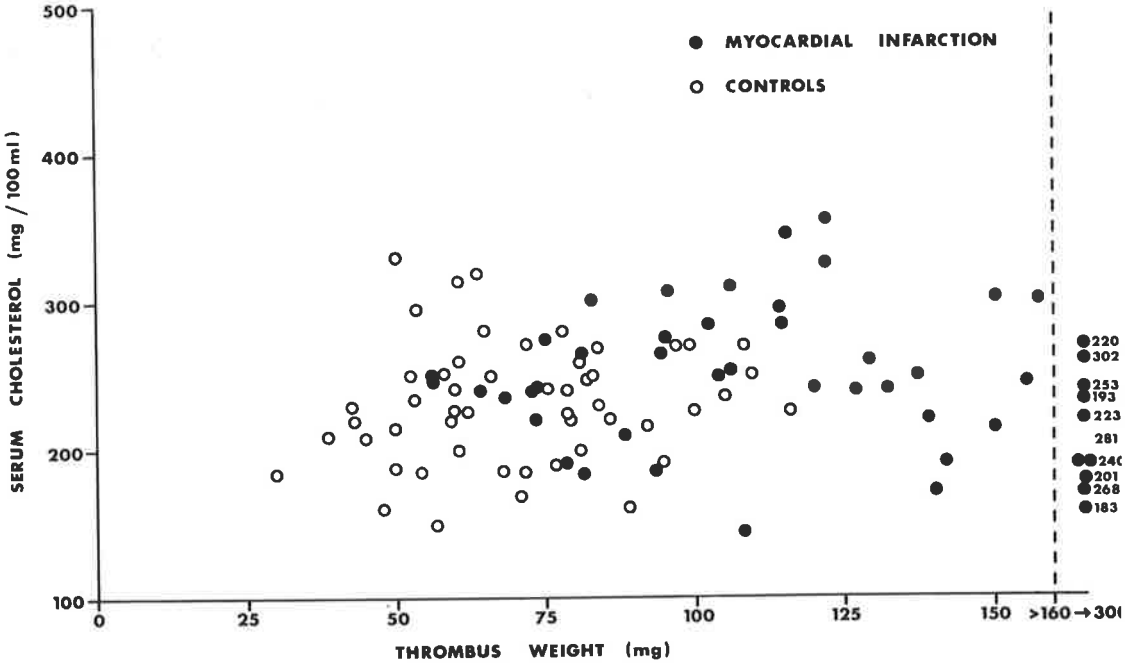


Figure 24: Mean thrombus weight plotted against serum cholesterol in patients with myocardial infarction (●) and in control subjects (o). There is no significant correlation.

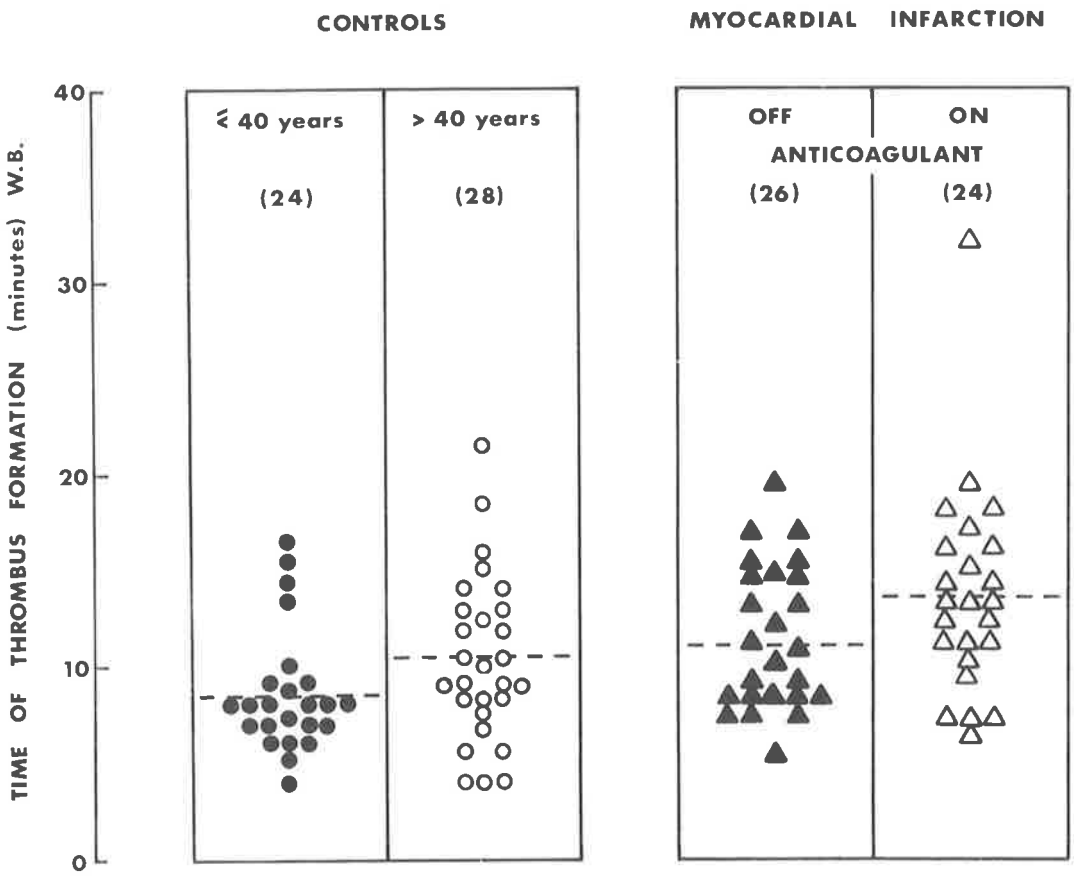


Figure 25: Time of thrombus formation of whole blood blood in controls and in patients with myocardial infarction. Time of thrombus formation is unaffected by anticoagulant therapy.

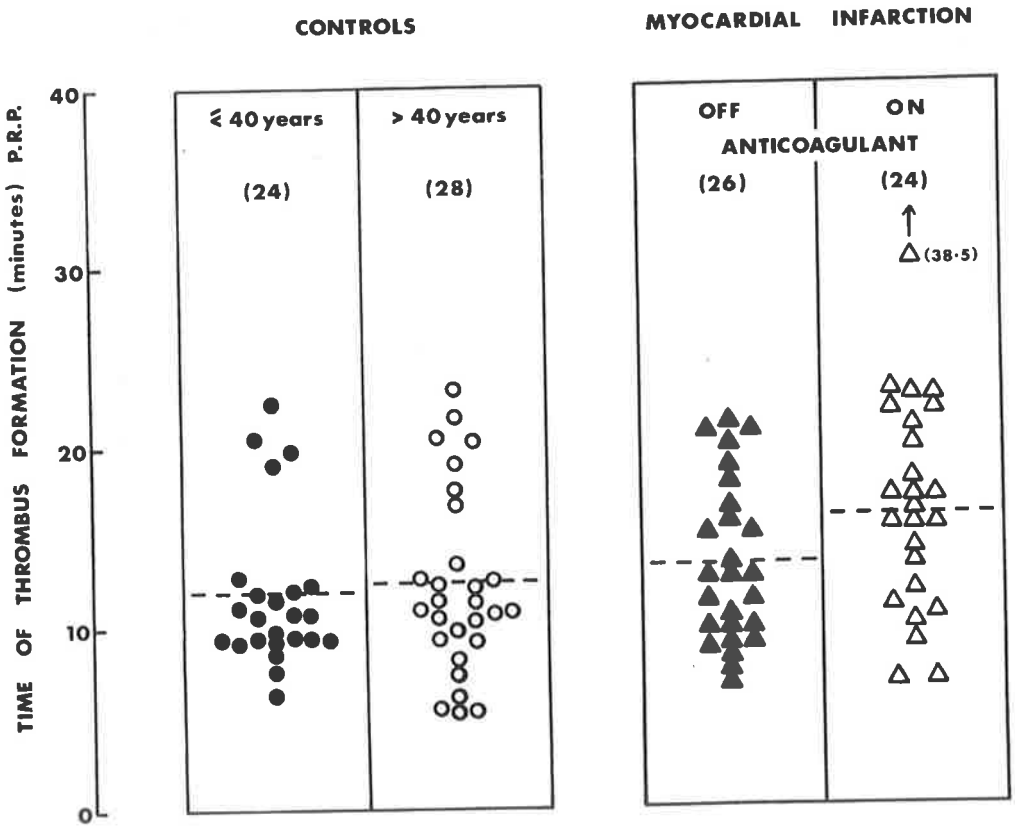


Figure 26: Time of thrombus formation of platelet rich plasma in control subjects and patients with myocardial infarction.

Table 7 - The frequency (expressed as percentage of the number of days studied) of abnormal platelet aggregation in control subjects and in patients with myocardial infarction.

Subject Categories	Days Studied	Persistence (%)	Snowstorm Phenomenon (%)	Total Platelet Abnormality (%)
Controls < 40 years	142	1.4	0.0	1.4
Controls > 40 years	161	4.3	2.5	6.8
Myocardial infarct. On anticoagulant	148	16.2	11.5	27.7
Myocardial infarct. No anticoagulant	169	20.7	4.7	25.4
Myocardial infarct. Combined	317	18.6	7.9	26.5

days (142) studied. In controls over 40 years of age abnormal platelet persistence was noted in 4.3% of 161 days studied. In addition the snowstorm phenomenon occurred on 2.5% of days studied giving a total of 6.8% of days showing abnormal platelet aggregation. There was therefore a tendency for the frequency of spontaneous platelet aggregation to increase with age in control subjects, and analysis has shown this difference to be statistically significant (p lies between 0.01 and 0.05).

In contrast to the findings in healthy controls, abnormal platelet aggregation was observed with an overall frequency of 26.5% of 317 days studied in patients with myocardial infarction. Persistence accounted for 18.6% and the snowstorm phenomenon for 7.9% of the days studied showing abnormal platelet aggregation (Table 7).

Table 7 also shows the percentage incidence of days of abnormality in patients with and without anticoagulant therapy. The overall frequency of platelet abnormality was found to be 25.4% and 27.7% in patients on and off anticoagulants respectively, a difference which is not statistically significant.

Persistence occurred less frequently in patients receiving anticoagulants (16.2%) than in those not

Table 8 - The frequency (expressed as a percentage of the number of days studied) of the patterns of abnormal platelet aggregation according to the time after myocardial infarction and the use of anticoagulant therapy.

Myocardial Infarct Category	TIME AFTER INFARCTION (DAYS) 0 - 7				8 - 42				>42			
	Days studied	Persistence (%)	Snowstorm Phenomenon (%)	Total Platelet Abnormality (%)	Days studied	Persistence (%)	Snowstorm Phenomenon (%)	Total Platelet Abnormality (%)	Days Studied	Persistence (%)	Snowstorm Phenomenon (%)	Total Platelet Abnormality (%)
On anticoagulant	47	21.3	14.9	36.2	76	18.4	13.2	31.6	25*	0.0*	0.0*	0.0*
No anticoagulant	52	21.2	1.9	23.1	51	25.5	3.9	27.5	66	18.2	7.6	25.8
Combined	99	21.2	8.1	29.3	127	20.5	9.4	29.9	91	13.2	5.5	18.7

*Only four patients in this group.

receiving anticoagulants (20.7%), but again this difference was not statistically significant. The snowstorm phenomenon however, was more frequent in patients on anticoagulants (11.5%) than in those not receiving anticoagulants (4.7%). This trend did not attain statistical significance.

The frequency of abnormal platelet aggregation was also considered in relation to the time after infarction (Table 8). The overall frequency of platelet abnormality was practically the same at 0 - 7 days (29.3%) and 8 - 42 days (29.9%), but in patients 42 or more days after infarction the number of days showing abnormality has decreased to 18.7%. When the two patterns of abnormal platelet aggregation are considered separately (Table 8) it is apparent that the frequency of persistence 0 - 7 days after infarction (21.3%) was similar to the frequency of 8 - 42 days (20.5%) after infarction, but in patients who had sustained infarcts more than 6 weeks before the time of study, only 13.2% of days studied showed this pattern.

The overall frequency of the snowstorm phenomenon varied from 8.1% at 0 - 7 days to 9.4% at 8 - 42 days, while in patients with infarcts over 6 weeks of age the number of days studied showing this phenomenon had

Table 9 - The frequency of abnormal platelet aggregation in vitro in 16 subjects studied simultaneously by Cr^{51} platelet survival and Chandler techniques.

Subject Category		Days Studied	Persistence (%)	Snowstorm Phenomenon (%)	Total Platelet Abnormality (%)
Healthy Controls	(52)	303	3.0	1.3	4.3
Myocardial Infarction - Anticoagulant	(11)	90	20.0	7.7	27.7
Myocardial Infarction - No anticoagulant	(5)	41	14.6	12.2	26.8

decreased to 5.5%. It is of interest to note that in anticoagulated patients 6 or more weeks after infarction neither persistence nor the snowstorm phenomenon was observed. As only 4 subjects were studied for a total of 25 days in this group, it is difficult to draw any conclusions from this latter observation.

Subjects with platelet survival and Chandler studies. Sixteen patients who had unequivocal evidence of myocardial infarction were studied by both the platelet survival and Chandler tube techniques. Eleven patients were not receiving anticoagulant treatment and the remainder were well anticoagulated throughout the course of the study with prothrombin values maintained between 15 and 25% activity.

Of the 11 subjects who were untreated by anticoagulants, only one had any evidence of reduction in platelet survival and this was due to a deep vein thrombosis which occurred during the study. Chandler studies were carried out on a total of 90 days and platelet abnormalities occurred on 27.7% of the days studied (Table 9). Of this persistence accounted for 20% of the abnormality and the snowstorm phenomenon occurred in 7.7% of days studied.

Table 10 - Details relating to patients studied prior to heart valve replacement.

Subject	Valve Affected	Anticoagulant Therapy	Embolism	Platelet Survival
1. R.C.	Aortic stenosis	Nil	-	Normal
2. E.C.	Mitral stenosis Mitral incompetence Aortic incompetence	Nil	-	Normal
3. G.S.	Mitral incompetence Aortic incompetence	Nil	-	Normal
4. J.deG.	Aortic incompetence	Nil	-	Normal
5. T.M.	Mitral stenosis Mitral incompetence Tricuspid incompetence	Nil	-	Normal
6. J.A.	Mitral incompetence	Nil	-	Normal

Chandler studies were carried out on 41 days in the 5 subjects with myocardial infarction who were being treated with anticoagulant therapy. Platelet abnormality occurred on 26.8% of days studied in which persistence accounted for 14.6% and the snowstorm phenomenon for 12.2% of days studied. One platelet survival study in this group was considered to be reduced slightly below the mean control range.

III. RESULTS OF STUDIES CARRIED OUT IN PATIENTS SUFFERING VALVULAR DISEASE OF THE HEART

Subjects studied. Eighteen platelet survival studies were carried out in 16 subjects. There were two categories of patient.

(a) Patients with valvular disease of the heart whose disease was of such severity that valve replacement was being seriously considered. In this group there were six subjects. There were five males and one female. Their ages ranged from 32 to 60 years. Details relating to the lesions encountered are included in Table 10. Blood of all subjects in this category was simultaneously investigated in the Chandler apparatus.

(b) The remaining 10 subjects were patients who had undergone successful valve replacement with Starr-Edwards

Table 11 - Details relating to patients studied following the insertion of Starr-Edwards prostheses.

Subject	Valve Replaced	Time After Operation	Anticoagulant	Embolism	Platelet Survival
1. A.T.	Aortic	17 weeks	Phenindione	Cerebral - postoperative	Normal
2. J.K.	Mitral	25 weeks	Phenindione	-	Normal
3. E.K.	Mitral	29 weeks	Phenindione	Cerebral - preoperative - early post-operative	Normal
4. S.R.	Mitral	32 weeks	Phenindione	-	Normal
5. E.C.	Aortic	45 weeks	Phenindione	-	Normal
6. R.J.	Aortic	45 weeks	Nil	Cerebral - postoperative	Normal
		48 weeks	Phenindione	-	
7. R.L.	Mitral	46 weeks	Phenindione	Cerebral - preoperative	Reduced
8. S.M.	Aortic	46 weeks	Nil	-	Reduced
		56 weeks	Phenindione	-	Reduced
9. P.B.	Mitral	52 weeks	Phenindione	-	Reduced
10. G.B.	Mitral	96 weeks	Phenindione	-	Reduced

prostheses. There were four males and six females. All had been considerably improved by operation and none was in cardiac failure. Details relating to these subjects are included in Table 11. Two patients were studied on two separate occasions. Chandler studies were carried out on the blood of all but two patients.

Results of Platelet Survival Studies

Results of studies in patients following the insertion of Starr-Edwards ball-valve prostheses. All points on the platelet survival curve in subjects 1 to 6 (Table 11 and Figure 27) fell within the range of normal. Subjects 1 to 5 were receiving oral anticoagulant therapy (phenindione) in doses adequate to maintain their prothrombin index between 15 - 25% of normal. Subject 6 had been placed on phenindione therapy 10 days after operation and this was discontinued after 34 weeks. Six weeks later he presented with a sudden onset of aphasia and a mild right hemiparesis. A platelet survival study was begun six days after this episode. At its completion phenindione therapy was recommenced and a second survival study was undertaken. On both occasions the platelet survival pattern fell within normal limits (Figure 28b). In subjects 7 - 10 the platelet survival was reduced, but

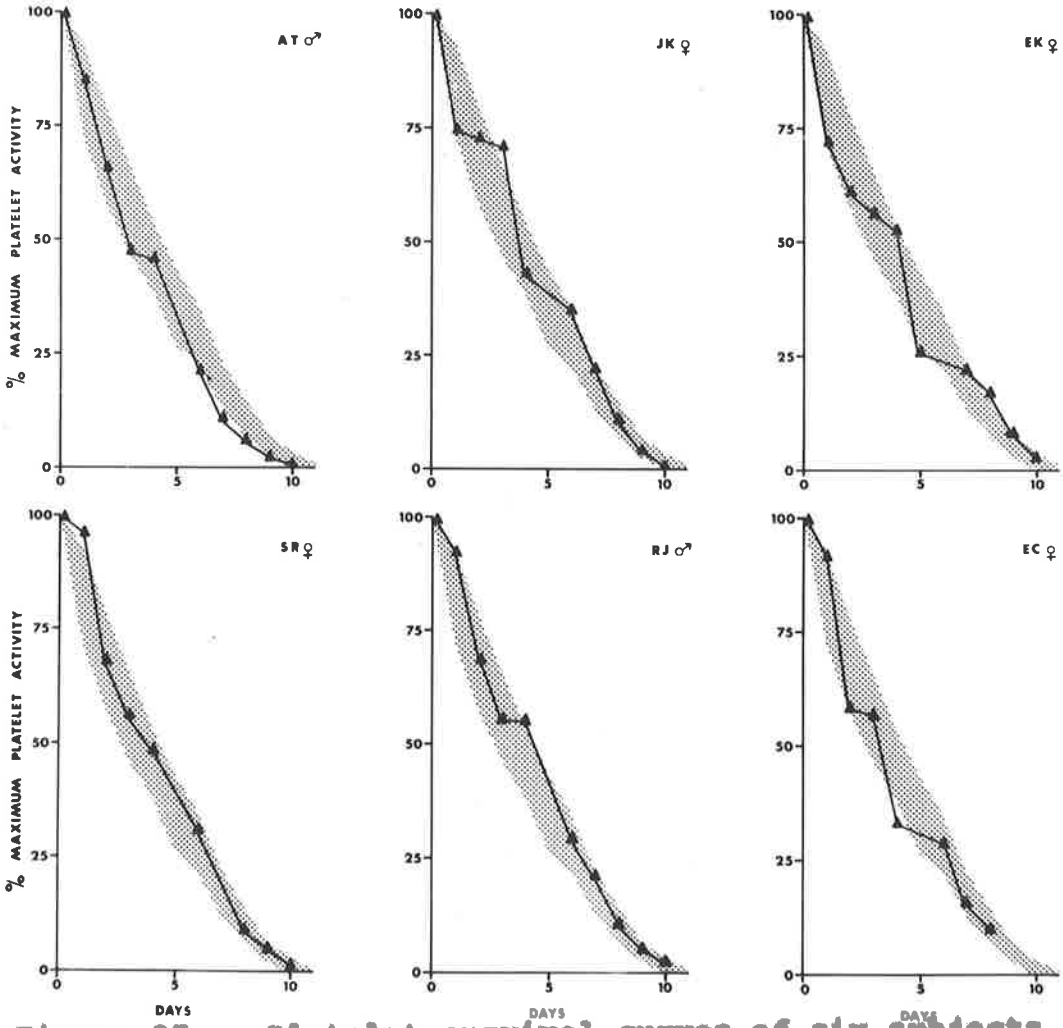


Figure 27: Platelet survival curves of six subjects studied less than 46 weeks after valve replacement with Starr-Edwards prostheses.

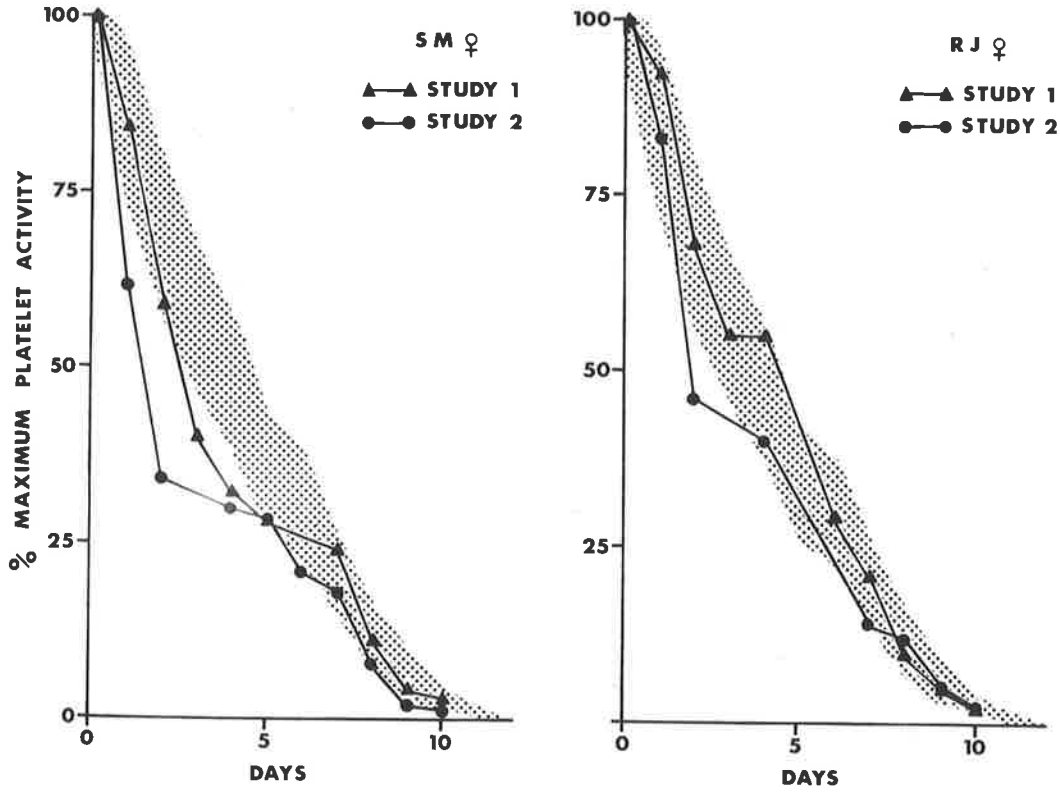


Figure 28: (a) Reduced platelet survival in one subject studied more than 46 weeks after valve replacement.
Study 1 - no anticoagulant therapy.
Study 2 - anticoagulant therapy.

(b) Normal platelet survival.
Study 1 - no anticoagulant therapy.
Study 2 - anticoagulant therapy.

Table 12 - Mean platelet count and platelet thromboplastic function in subjects studied following the insertion of Starr-Edwards prostheses.

Subject	Mean Platelet Count	Range of Platelet Count x 10^{-3}	Platelet Thromboplastic Function
A.T.	198,000	156 - 231	100%
J.K.	313,000	207 - 375	100%
E.K.	232,000	171 - 345	N.R.
S.R.	240,000	180 - 327	100%
E.C.	266,000	207 - 375	100%
R.J.	230,000 251,000	168 - 294 207 - 324	100%
R.L.	254,000	171 - 360	100%
S.M.	214,000 261,000	174 - 273 174 - 324	100%
P.B.	244,000	180 - 360	100%
G.B.	204,000	159 - 270	100%

in each instance platelet radioactivity persisted in circulation for the usual period of 9 to 11 days (Figure 29). Subjects 7, 9 and 10 were receiving anticoagulant therapy (phenindione) and subject 8 was studied both while being treated with phenindione and after it had been discontinued (Figure 28a).

External scintillation counting initially revealed a more marked uptake of radioactivity in the spleen and to a lesser extent the liver in those subjects with reduced survivals than in normal patients or in those with valvular prosthesis who manifested a normal survival pattern. However, as the number in the series increased, it became obvious that there was not a consistent pattern of organ uptake and that this factor was confined to certain individuals only. No patient was thrombocytopenic and platelet function was 100% in all subjects (Table 12).

Studies in patients with uncorrected valvular disease. Because of the possibility that turbulent flow arising as a result of severe valvular disorders may account for the platelet abnormality found, six studies were carried out in patients prior to operation. All points on the survival curve fell within the range of normal in all cases.

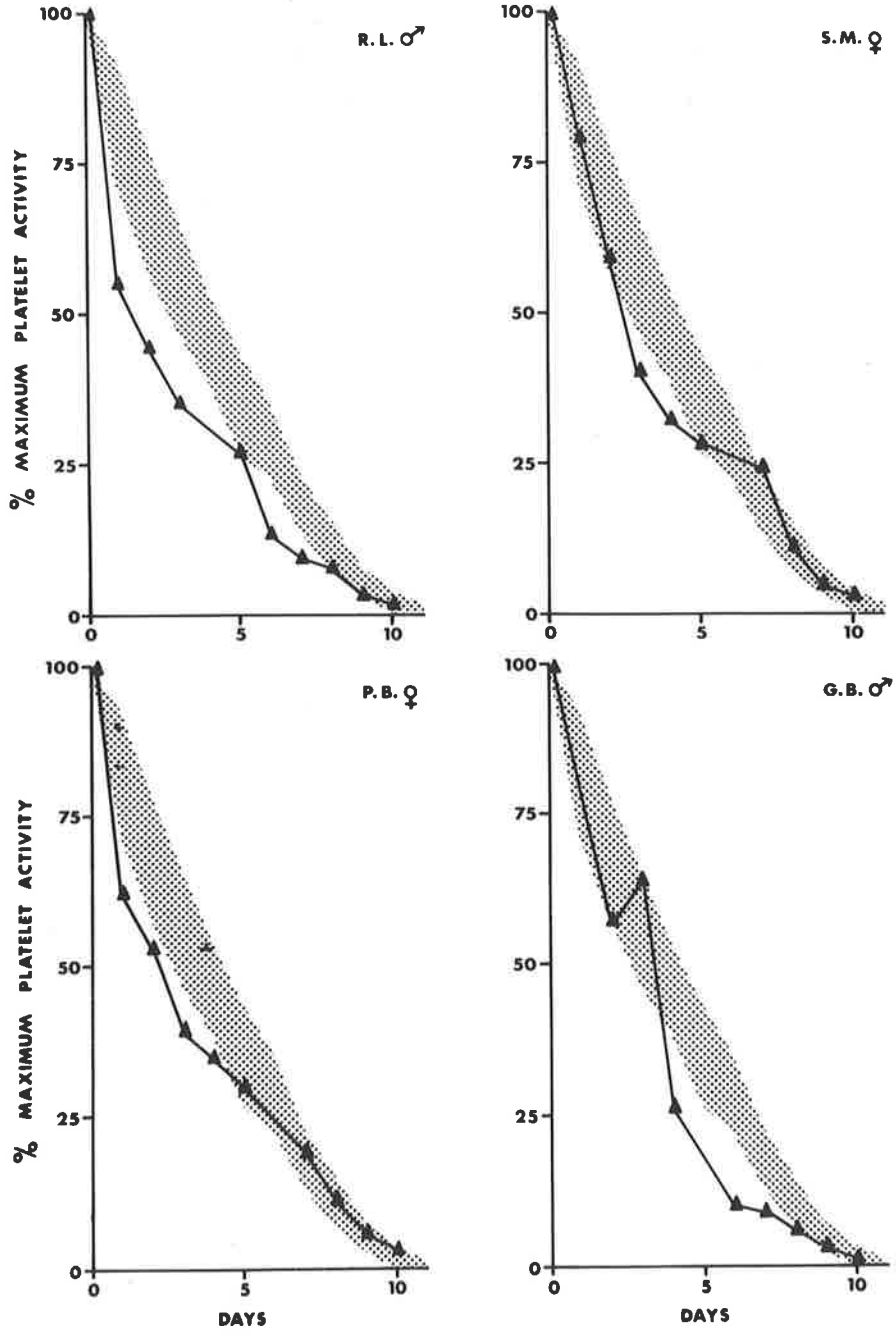


Figure 29: Reduced platelet survival in patients studied more than 46 weeks after valve replacement with Starr-Edwards prostheses.

Table 13 - Mean platelet count in subjects studied prior to heart-valve replacement.

Subject	Mean Platelet Count	Range of Platelet Count x 10^{-3}
1. R.C.	242,000	153 - 280
2. E.C.	177,000	120 - 240
3. G.S.	199,000	129 - 285
4. J. DeG.	195,000	144 - 252
5. T.M.	166,500	129 - 195
6. J.A.	400,000	270 - 498

Ultimate platelet lifespan was between 9 and 11 days. The mean platelet count and range of platelet counts are seen in Table 13 where it will be noted that none of the subjects was thrombocytopenic.

Results of Chandler Tube Studies

Whole blood thrombus length and weight. Figure 30 shows the mean thrombus length (mm.) and the mean thrombus weight (mg.) in patients with valvular disease of the heart studied prior to operation (Six subjects) and patients after the insertion of a Starr-Edwards prosthesis (10 studies in 9 patients). In only one case was there any deviation from the normal control range.

Platelet rich plasma. The overall incidence of abnormal platelet aggregation of healthy control subjects has been found to be 4.3% of the total days (303) studied. Contrasting with these findings are those in patients with valvular disease of the heart. In subjects studied following the insertion of Starr-Edwards prostheses, the overall frequency of abnormal platelet aggregation was 32.9% of total days (85) studied. Of this persistence accounted for 15.3% and the snowstorm phenomenon for 17.6% of days studied (Table 14). This tendency for the frequency of abnormal platelet aggregation to occur in

Table 14 - The frequency of abnormal platelet aggregation in subjects with valvular disease of the heart.

Subject Categories	Days Studied	Persistence (%)	Snowstorm Phenomenon (%)	Total Platelet Abnormality (%)
Healthy controls	303	3.0	1.3	4.3
Valvular disease of heart - preoperative	41	4.9	7.3	12.2
Starr-Edwards prostheses	85	15.3	17.6	32.9

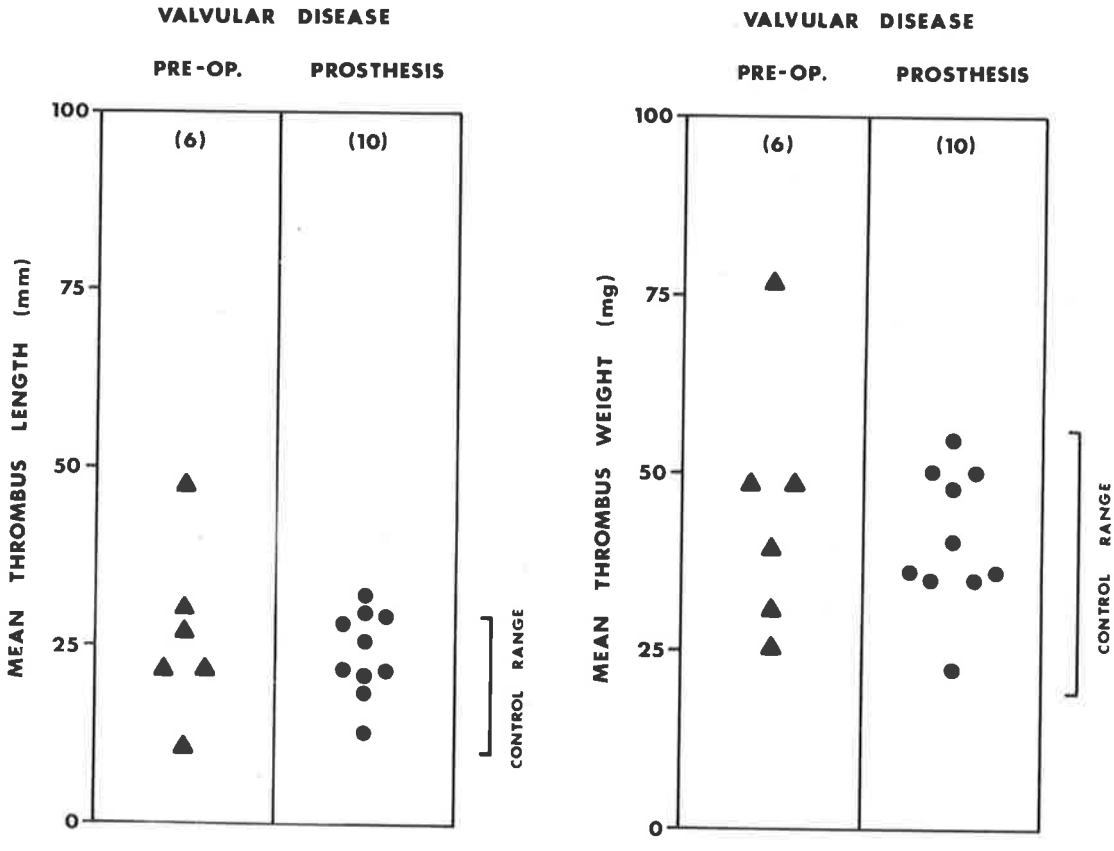


Figure 30: Mean thrombus length and mean thrombus weight in patients with uncorrected valvular disease and in subjects with Starr-Edwards prostheses.

patients with Starr-Edwards prostheses attained a statistical significance ($p < 0.001$) with both the persistence and snowstorm phenomena.

In subjects with uncorrected valvular disease platelet abnormality occurred in 12.2% of days (41) studied. Persistence accounted for 4.9% and the snowstorm phenomenon for 7.3% of the abnormalities (Table 14). It is not possible to draw any absolute conclusion as to the significance of results in these patients studied prior to operation, but there appears to be a greater frequency of abnormal spontaneous platelet aggregation in subjects with uncorrected valvular disease.

The incidence of platelet abnormality was also considered in relation to the time for which the prosthesis had been in situ. The overall frequency of platelet abnormalities in patients less than 46 weeks after replacement was 29.3% of days (41) studied and in subjects 46 weeks or longer after replacement, 36.4% of days (44) studied. This difference did not, however, attain statistical significance.

Patients with Chandler and platelet survival studies. Both Chandler and platelet survival studies were carried

Table 15 - Details relating to subjects studied in the postoperative period.

Subject	Days After Operation	Operation Performed	Platelet Survival	Mean Platelet Count	Mean Platelet Count for Group
F.W.	7	Hemicolectomy	Reduced	526,000)	
W.B.	7	Resection aortic aneurysm	Reduced	357,000)	476,000
O.G.	8	Bilateral lumbar sympathectomy	Reduced	544,000)	
P.S.	5	Femoro-popliteal by-pass	Normal	211,000)	
C.W.	9	Ileo-femoral by-pass	Normal	321,000)	267,000
T.L.	3	Femoro-popliteal by-pass	Normal	274,000)	

out in 10 instances. Four platelet survival studies were considered to be abnormal (reduced) to varying degrees and six were normal. Chandler studies were carried out on a total of 36 days in those with reduced platelet survival and an abnormal pattern was noted in 44.4% of the days studied. In the 49 days of Chandler investigation in those with normal platelet survival, abnormalities were noted in 24.5% of total days studied. This indicates that the frequency of abnormal platelet behaviour is greater in subjects with reduced platelet survival than in subjects whose survival curve showed a normal contour. This tendency was not found to be of statistical significance.

IV. PLATELET SURVIVAL STUDIES IN PATIENTS AFTER OPERATION

Platelet survival studies were carried out in six subjects following major operations (Table 15). In three subjects the platelet half life was reduced to a varying degree (Figure 31) and in three the contour of the platelet survival curve was normal. It is interesting to note that the platelet survival curve was most abnormal in subjects whose mean platelet count was highest (Table 15).

V. RESULTS OF STUDIES IN PATIENTS SUFFERING FROM THROMBOCYTOPENIA.

Subjects studied. Fourteen platelet survival

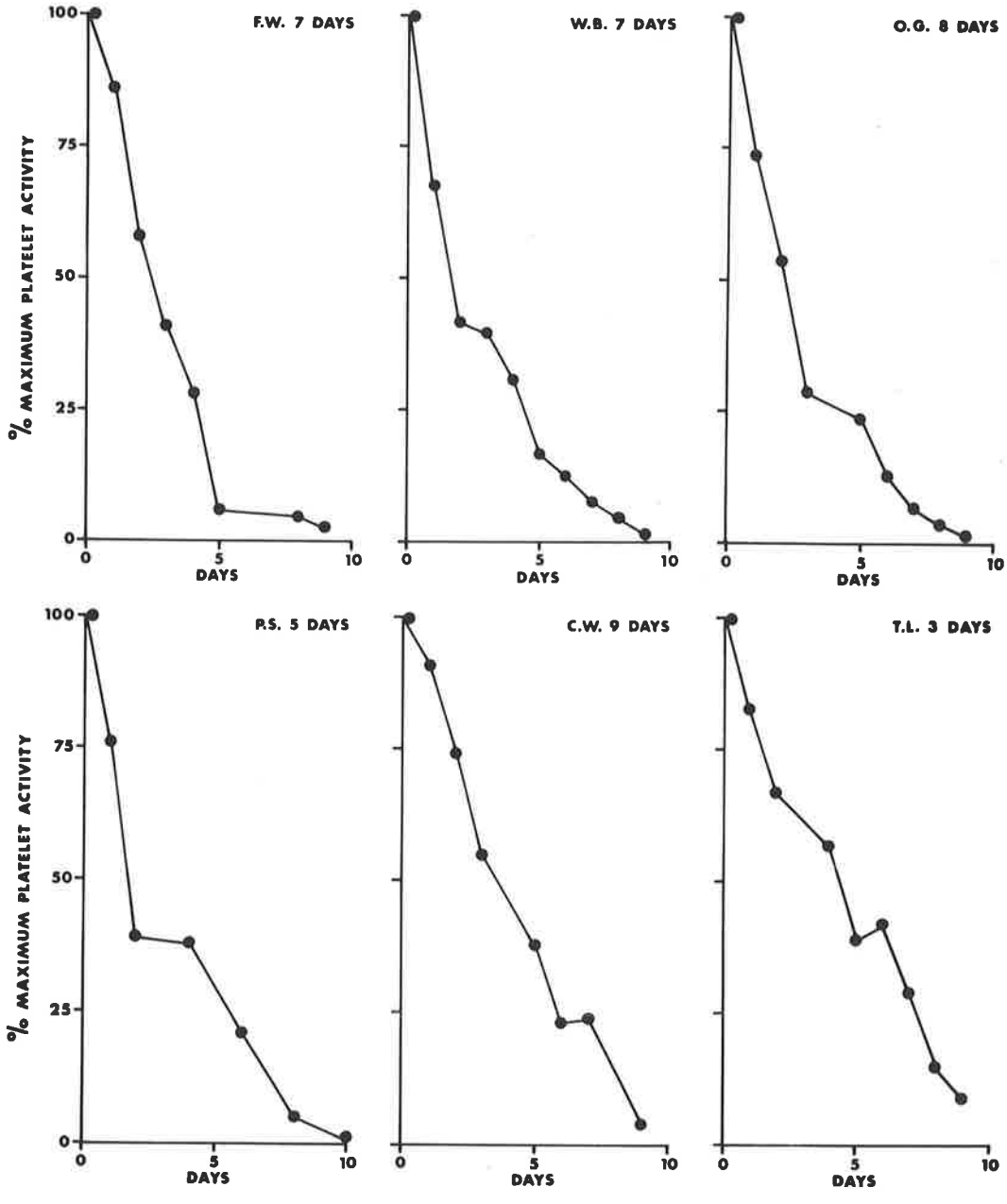


Figure 31: Platelet survival curves of six subjects studied in the postoperative period showing a varying reduction in platelet survival in the upper three studies.

Table 16 - Platelet survival studies in subjects with thrombocytopenia.

Subject	Diagnosis	Type of Platelets	Platelet Survival	Excessive Organ Uptake
M.S.	*Chronic I.T.P.	Autologous	Reduced	-
		Isologous	Reduced	Liver
P.R.	Chronic I.T.P.	Autologous	Reduced	-
		Isologous	Reduced	Liver
C.M.	Chronic I.T.P.	Autologous	Reduced	Spleen
		Autologous	Normal	-
M.S.	Chronic I.T.P.	Autologous	Reduced	-
		Isologous	Reduced	-
J.L.	Chronic I.T.P.	Autologous	Reduced	Liver
B.M.	Chronic I.T.P.	Isologous	Reduced	Spleen + Liver sl.
		Autologous	Reduced	Spleen
J.M.	Acute I.T.P.	Isologous	Reduced	-
E.G.	Lymphosarcoma	Autologous	Reduced	-
A.T.	Chronic lymphatic leukaemia	Autologous	Reduced	-

*Chronic I.T.P. has been defined as thrombocytopenia of unknown cause present for 3 months or more.

studies were carried out in 9 subjects suffering from thrombocytopenia. The clinical details relating to these subjects are represented in Table 16. There were seven cases of idiopathic thrombocytopenia, one of lymphosarcoma and one of chronic lymphatic leukaemia. Two patients were investigated both before and after splenectomy. Studies utilising autologous platelets were carried out in 9 instances, and in 5 it was necessary to use ABO Rh compatible platelets owing to the extremely low platelet counts in the subjects at this time.

Results

Platelet yield: The mean yield of platelets in patients with idiopathic thrombocytopenic purpura in whom autologous studies were carried out was 50% (range 24 - 100%) which compares favourably with that obtained in haematologically normal subjects.

Platelet recovery: The mean platelet recovery of autologous platelets in circulation in subjects with idiopathic thrombocytopenic purpura was 33% (range 7 - 55%). The mean recovery of isologous platelets in circulation in patients with idiopathic thrombocytopenic purpura was 24% (range 1 - 38%). Maximum recovery in the

Table 17 - Mean platelet count and platelet turnover in subjects with thrombocytopenia.

Subject	Mean Platelet Count	Range Platelet Count x 10^{-3}	Platelet Turnover (Normal = 55,000 ± 21,000/ cu.mm./die)
M.S.	135,000	40 - 177	134,000
	148,000	63 - 216	137,000
P.R.	106,000	10 - 183	141,000
	9,000	5 - 11	6,000
C.M.	91,000	23 - 130	126,000
	640,000	402 - 918	177,000
M.S.	34,000	27 - 41	19,000
	35,000	17 - 58	44,000
J.L.	45,000	26 - 57	62,000
B.M.	41,000	29 - 79	492,000
	217,000	168 - 300	100,000
J.M.	114,000	75 - 168	158,000
E.G.	61,000	41 - 83	57,000
A.T.	152,000	93 - 187	106,000

one subject with lymphosarcoma was 6% and in the patient with chronic lymphatic leukaemia 16%.

Platelet survival: Examples of platelet survival in circulation are illustrated in Figures 32 and 33. In all cases the general pattern of initial clearance followed by reappearance of a proportion of cells into circulation as in normal controls did not occur. Following infusion there was a sustained clearance of platelets from circulation throughout the course of the study. Platelet half life was markedly reduced in every subject with the exception of study 2 in C.M. Small amounts of circulating platelet radioactivity occasionally persisted for several days despite the markedly increased removal of platelets from circulation.

Platelet turnover: In 7 studies in patients with idiopathic thrombocytopenia platelet turnover per day was increased (Table 17). In 2 studies it was reduced and in 2 was within the normal range. In the patient with chronic lymphatic leukaemia platelet turnover was increased, and in the patient with lymphosarcoma was within the normal range.

Surface counting: There was clearcut evidence of splenic uptake of platelet radioactivity in only 3 studies

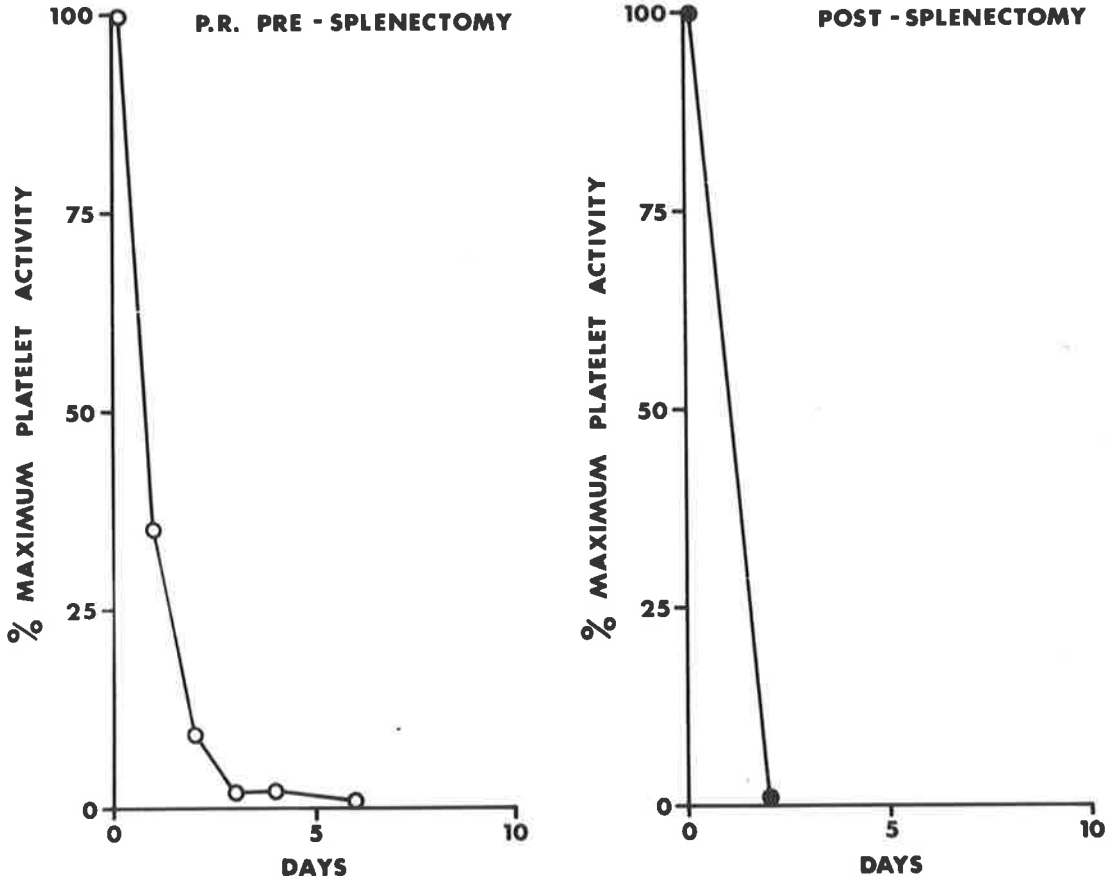


Figure 32: Platelet survival in a patient with idiopathic thrombocytopenia studied both before and after splenectomy. Survival is markedly reduced on both occasions.

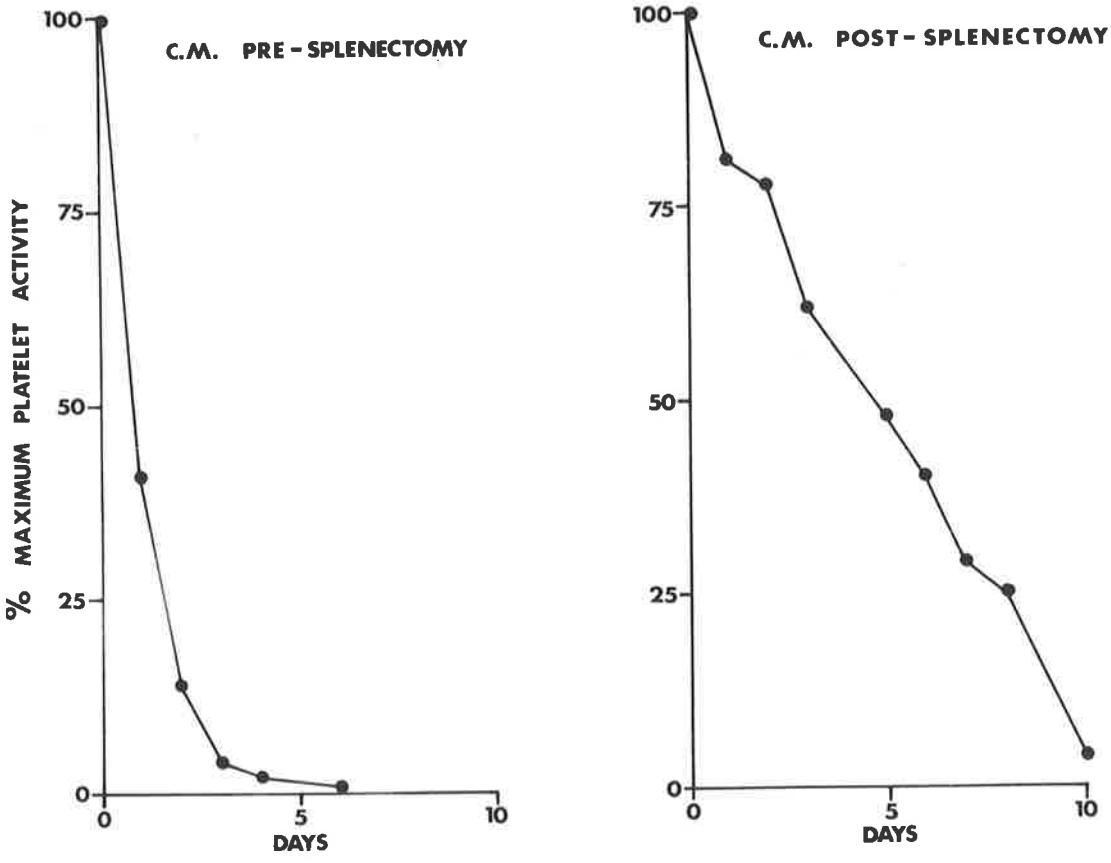


Figure 33: Platelet survival in a patient with idiopathic thrombocytopenia showing a markedly reduced platelet life span before splenectomy and a normal platelet survival after splenectomy.

(carried out on 2 subjects). In subject P.R. (Figure 34) following splenectomy, and in J.L. who had had a splenectomy some years previously, there was convincing hepatic uptake of platelet radioactivity with liver/heart ratios far in excess of that found in normal subjects. Both subjects had remained thrombocytopenic after splenectomy. One subject (M.S. - spleen intact) was studied on two occasions and marked differences in the surface counting data were detected. On the first occasion maximum uptake occurred in the spleen although this was not in excess of normal. On the second occasion there was a marked increase in the liver/heart ratio beyond the control range indicating excessive hepatic uptake of radioactive platelets (Figure 35). Subject C.M. showed a marked splenic uptake of platelets initially (Figure 36) but following splenectomy and a complete haematological remission, further study showed not only a completely normal platelet survival in circulation, but a normal pattern in surface counting data. Splenectomy in J.M. also resulted in complete haematological and clinical remission but studies were not repeated.

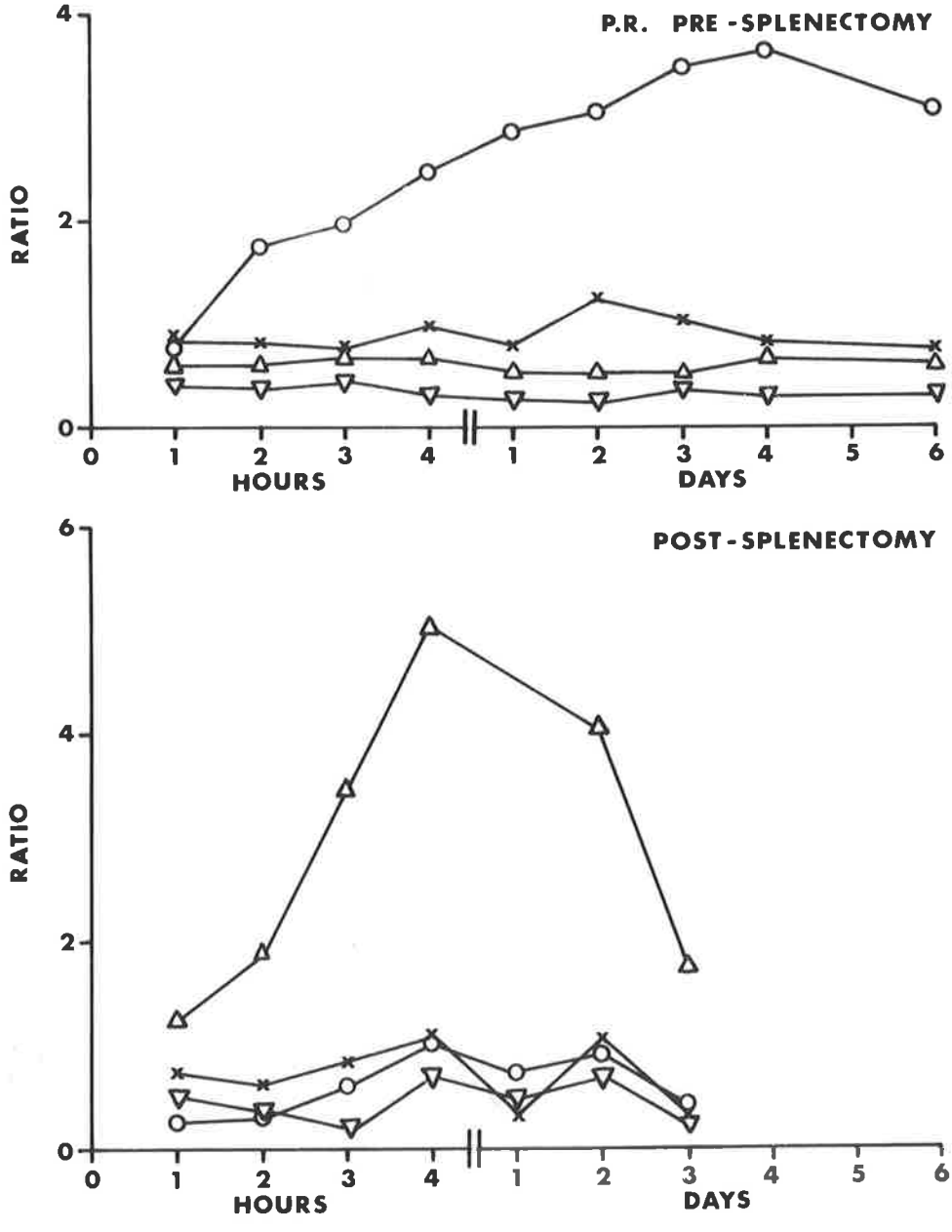


Figure 34: Surface activities in a patient with idiopathic thrombocytopenia (P.R.) showing an excess hepatic uptake following splenectomy.

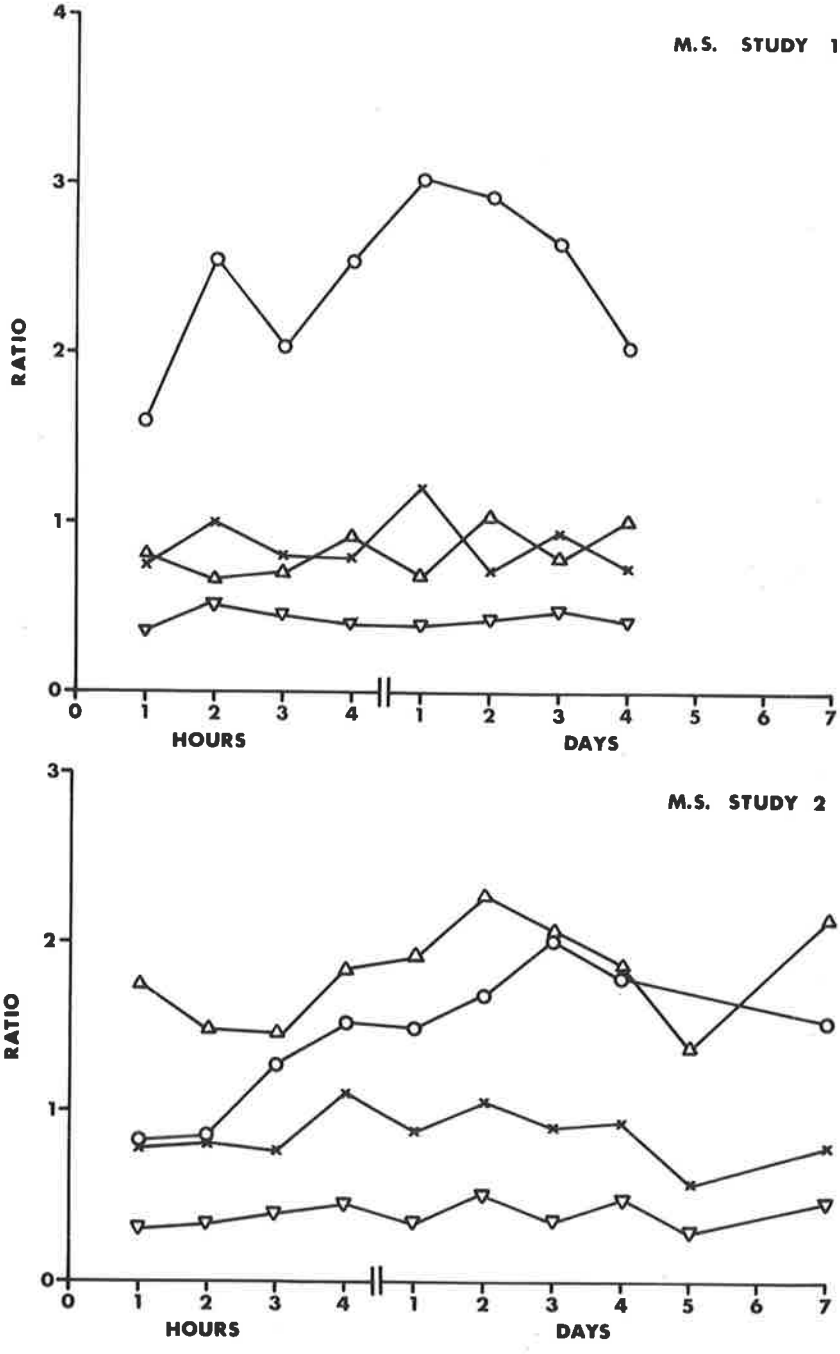


Figure 35: Results of surface studies in subject M.S. with idiopathic thrombocytopenia showing a normal uptake in Study 1 and excess hepatic uptake of platelets (isologous) in Study 2.

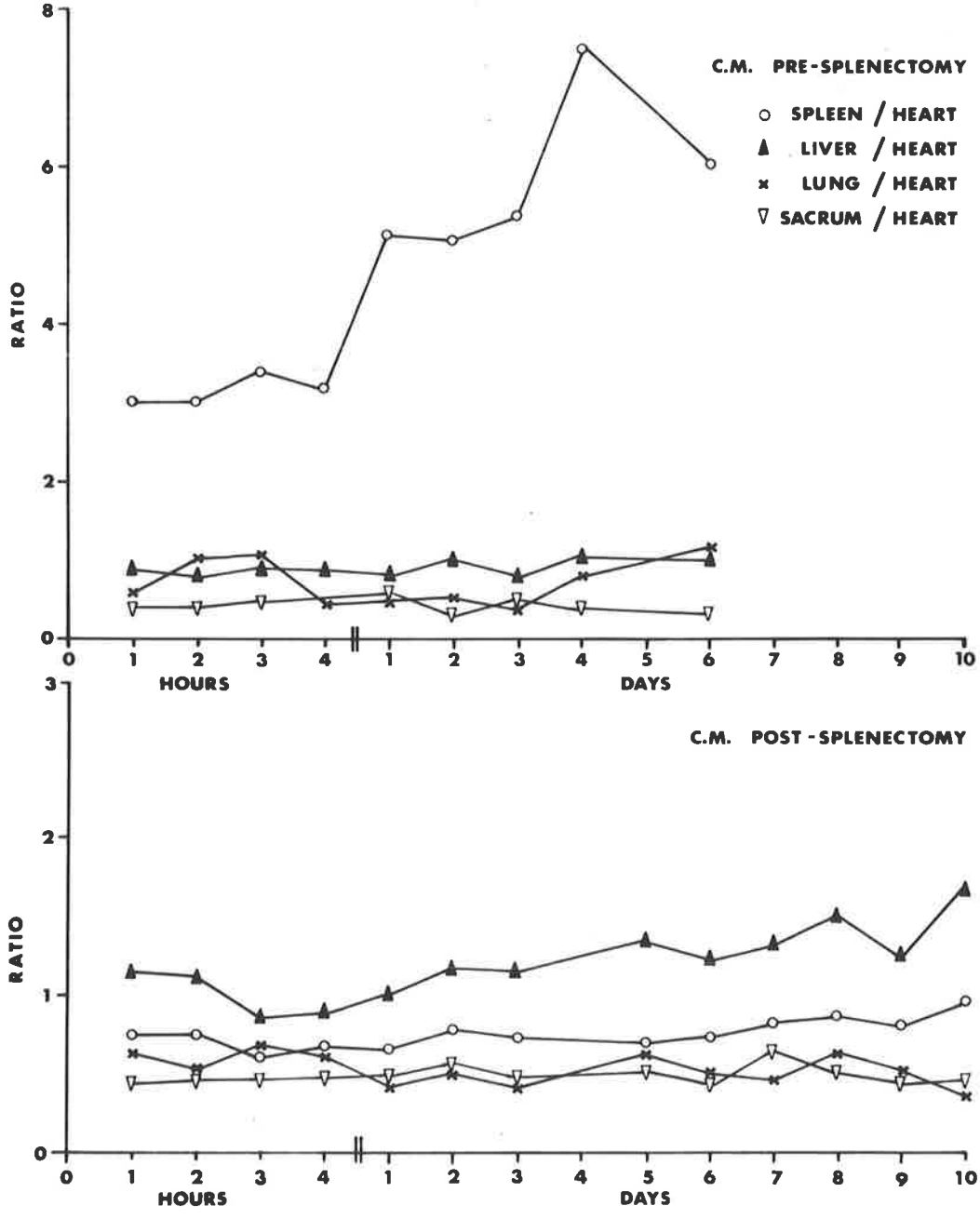


Figure 36: An example of excess splenic destruction of labelled platelets subsequently relieved by splenectomy. Post splenectomy pattern of uptake normal. Note scale differences.

VI. RESULTS OF STUDIES ON THE SEQUESTRATION OF PLATELETS TREATED WITH NOR-ETHYL MALEIMIDE - A SULPHYDRYL INHIBITOR

Subjects studied. A total of 6 studies were carried out in 6 subjects. The treatment of blood platelets with NEM was carried out as described in Chapter II. Final concentrations achieved were:

1. 0.25 uM NEM/ml. platelets
2. 1.00 uM NEM/ml. platelets
3. 2.5 uM NEM/ml. platelets
4. 5.0 uM NEM/ml. platelets
5. 10.0 uM NEM/ml. platelets
6. 15.0 uM NEM/ml. platelets

Following this cells were reinjected into the donor and continuous surface counting carried out with probes placed over the hepatic and splenic areas.

Results

Treatment of the platelet concentrate with 0.25 uM NEM/ml. platelets did not alter the behaviour of platelets in circulation. The usual segregation phenomenon occurred followed by the reappearance of a proportion of cells into the circulation and by a more gradual fall off of radio-activity over the course of several days. Treatment with

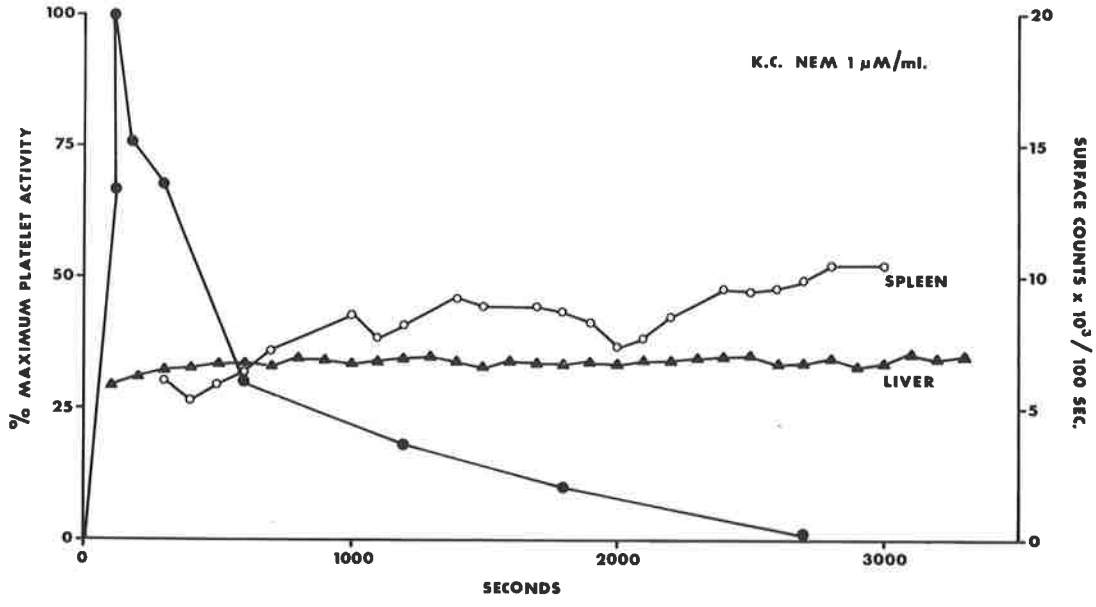


Figure 37: Sequestration of platelets principally in the spleen (o-o) following treatment with 1 µM NEM/ml. platelets. Note rapid clearance of labelled platelets from circulation (e-e).

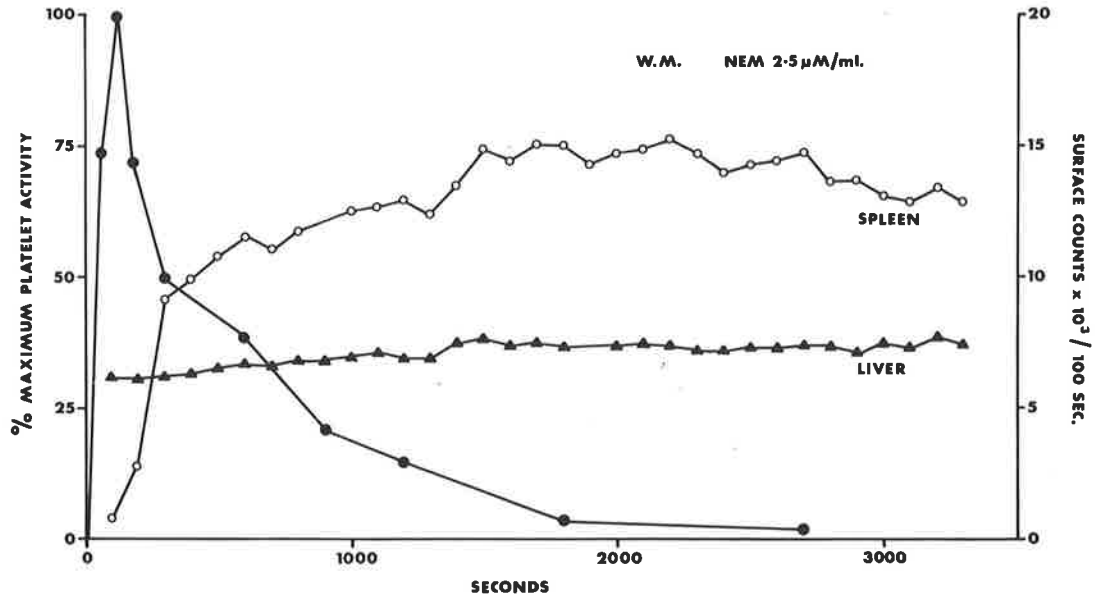


Figure 38: Sequestration of platelets in the spleen (o-o) after treatment with NEM 2.5 µM/ml. Rapid clearance of platelet radioactivity (e-e) from circulation again obvious.

1 uM NEM/ml. of platelets resulted in a rapid clearance of radioactivity from circulation in a matter of minutes. Scintillation counting carried out simultaneously showed the count rate to be equal in both hepatic and splenic regions followed by a gradual increase in the splenic area throughout. Radioactivity was completely cleared from circulation and did not follow the usual pattern of behaviour following reinjection (Figure 37). Treatment with 2.5 uM NEM/ml. again revealed the phenomenon of complete rapid platelet clearance from circulation associated with splenic uptake (Figure 38), but treatment with 5.0 uM NEM/ml. platelets, although following the same trend of clearance of radioactivity from circulation within minutes plus some splenic uptake, also showed a tendency towards slight gradual increase in hepatic uptake (Figure 39). This trend for increasing concentrations of the sulphydryl inhibitor to be associated with changes in the site of organ uptake persisted with increasing the concentration of NEM to 10.0 uM and 15.0 uM/ml. platelets (Figures 40 and 41). Under these circumstances, although the rate of clearance of radioactivity from blood was unchanged, maximum uptake occurred in the hepatic area with only slight

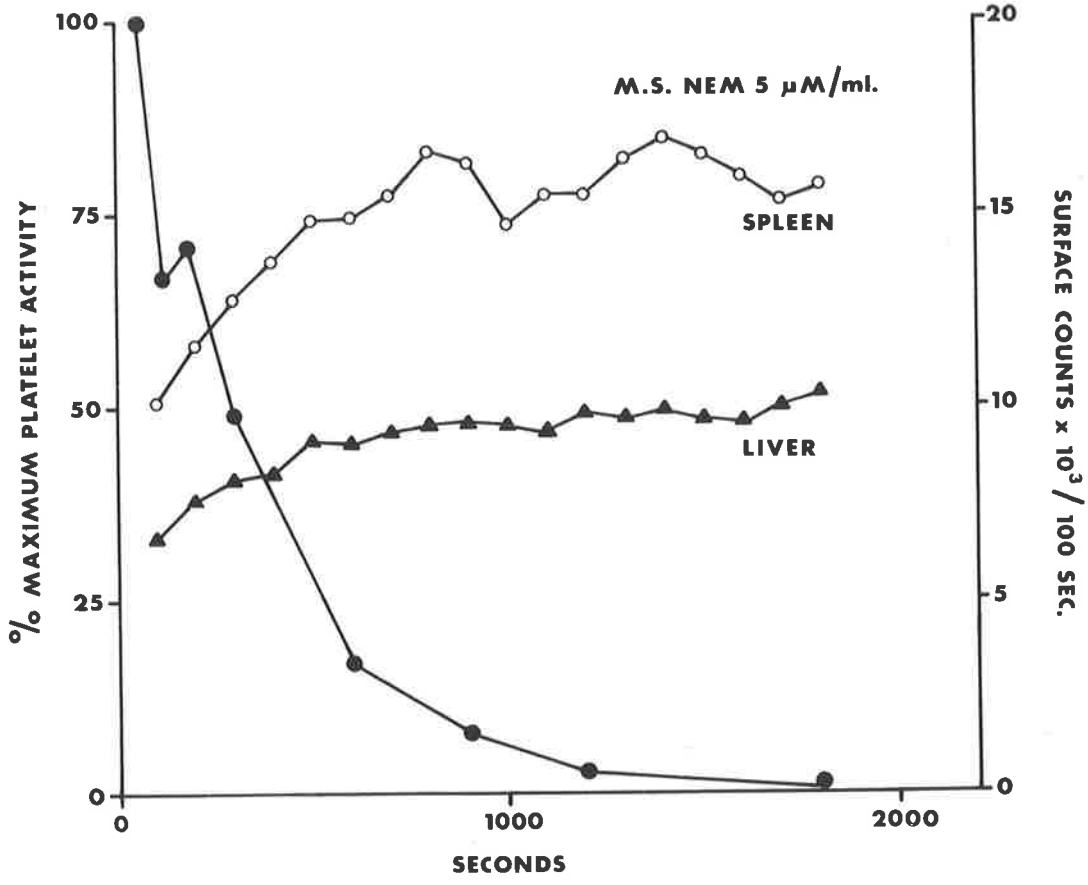


Figure 39: Splenic (o-o) and slight hepatic (Δ-Δ) sequestration of NEM treated platelets (5 µM NEM/ml. platelets).

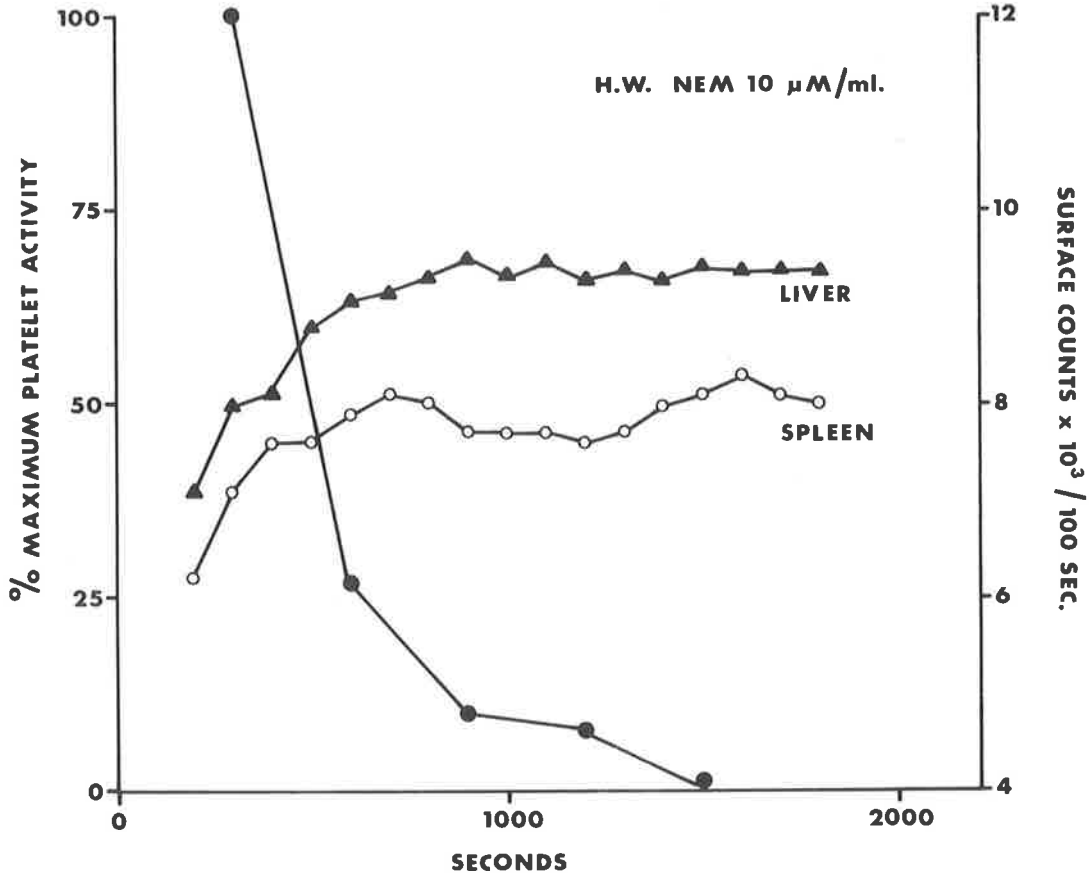


Figure 40: Hepatic (▲-▲) and some splenic (o-o) sequestration of damaged platelets.

splenic uptake with 10 uM concentration and no increase in splenic activity when 15 uM NEM/ml. platelets had been added.

Platelet morphology. As determined by phase contrast microscopy platelets appeared to be discrete, circular or ovoid discs and no overt morphological abnormality could be detected. There was no adverse reaction on the part of the patient following injection of the damaged labelled platelets into circulation.

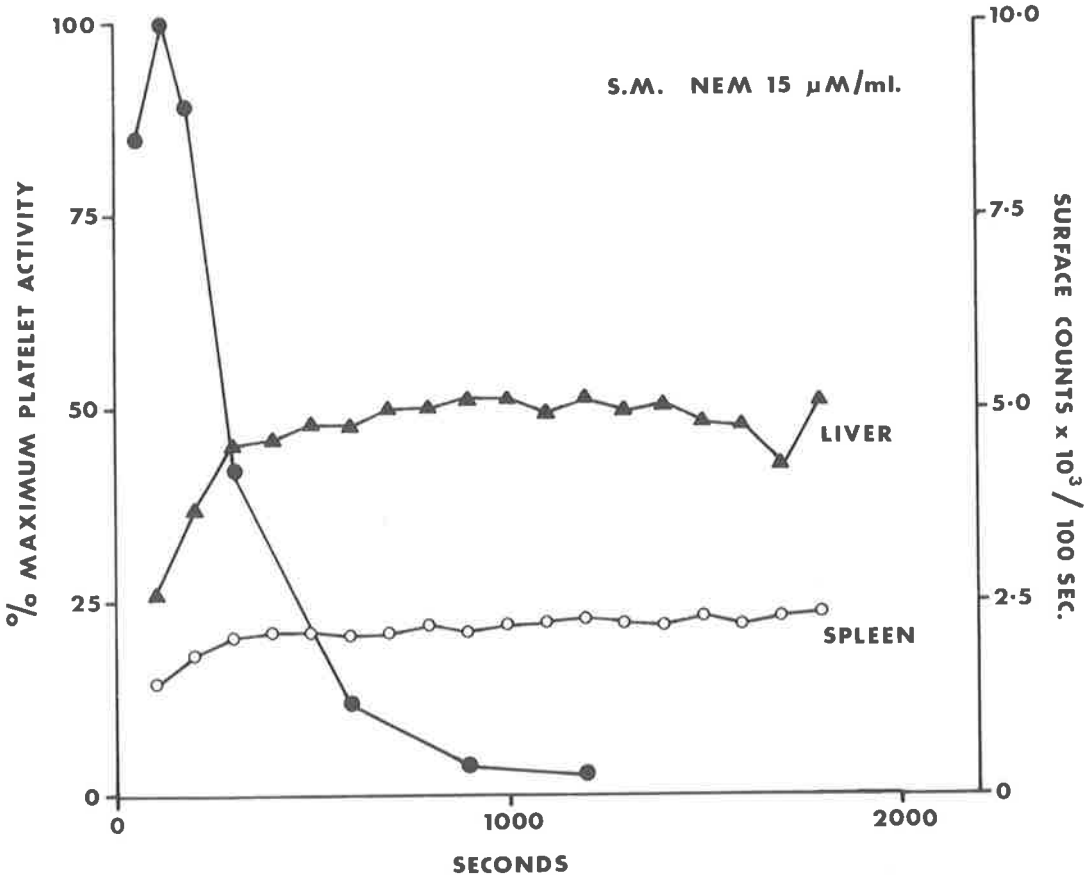


Figure 41: Sequestration of platelets in the liver (▲-▲) following damage with NEM in a final concentration of 15 µM NEM/ml. platelets.

CHAPTER IV

DISCUSSION

An elucidation of many facets of platelet behaviour has been made possible in this study by the use of both in vivo and in vitro techniques. Some aspects of the study confirm the findings of other workers while others have served to throw a new light on platelet behaviour and altered platelet kinetics in some disease states. In certain instances the findings have not been described previously. It has been of particular importance to evaluate the efficiency of the new anticoagulant of Aster and Jandl (1964). Obviously an anticoagulant which has claims to such an efficiency warrants full study as the therapeutic implications will be unbounded. The ultimate criterion of platelet viability following handling in vitro is the ability of the platelet to circulate and behave in a physiological fashion in vivo following reinfusion.

When normal platelets were reinfused into their donors or normal ABO/Rh compatible subjects, a variable but generally substantial proportion disappeared from circulation within 15 to 30 minutes. From these and previous studies (Davey and Lander, 1964; Aster and Jandl, 1964), it appears that some of the cleared platelets are permanently removed from circulation - the sequestered

fraction; and that the remainder - the segregated fraction - return to circulation after a variable period, usually within a few hours. Following maximum platelet recirculation, the cells which survive in the blood stream as viable units are slowly removed from circulation over the course of 9 - 11 days, a period commensurate with their life span.

The sequestered fraction. The extent of the initial platelet loss which is irreversible may be derived from consideration of the maximum proportion of platelets infused which can be recovered in circulation. It will be seen from Figure 42 that approximately three-quarters of all EDTA platelets infused in these studies were irreversibly lost soon after infusion. Fewest platelets were lost when ACD 'A' was used as anticoagulant, nevertheless over one-half of such platelets failed to survive in circulation as viable units. Intermediate values were obtained with ACD 'S' platelets, where a mean of approximately two-thirds of those injected failed to survive.

Surface counting studies indicate quite clearly that the spleen is the major site of sequestration of such platelets, irrespective of the anticoagulant used.

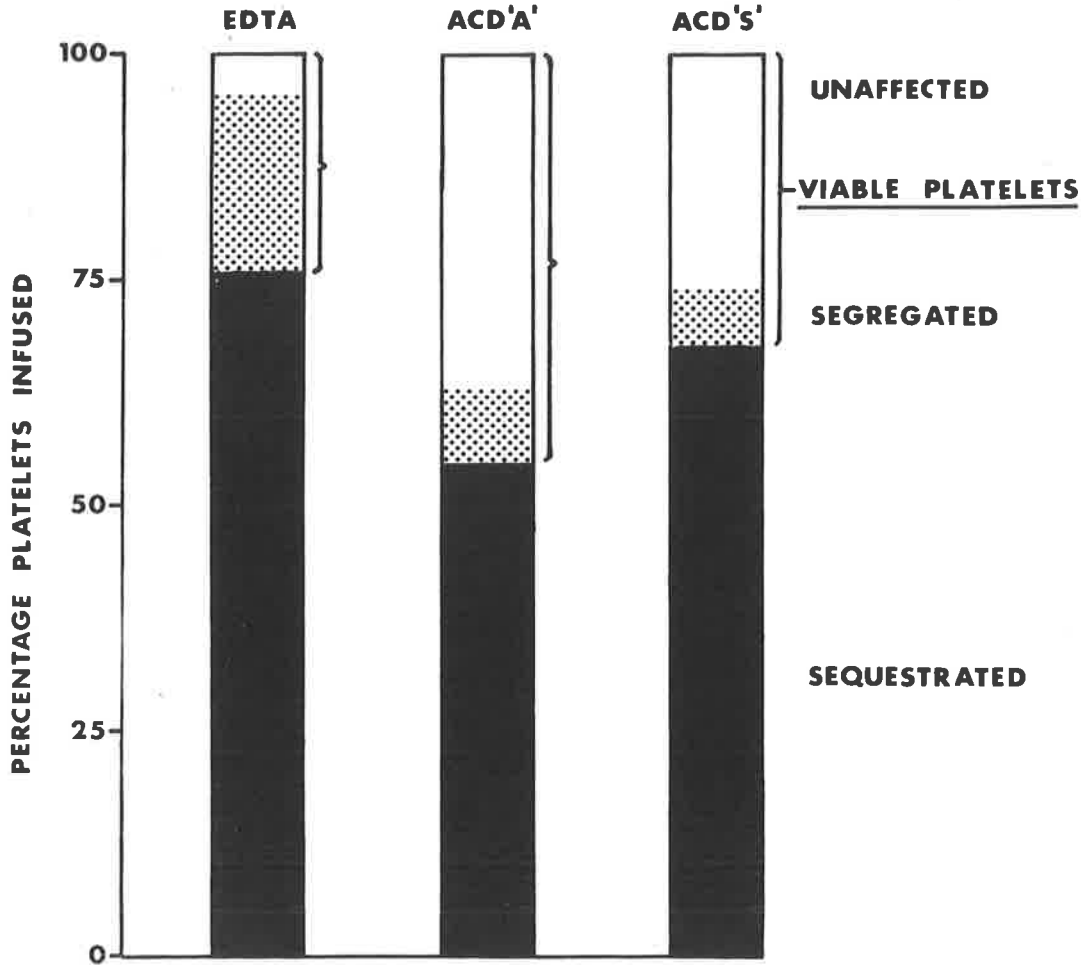


Figure 42: The sequestration ■ and segregation ▨ of platelets collected into each of the anticoagulant solutions. The small proportion of "viable" platelets with EDTA is clearly shown.

The segregated fraction. The phenomenon of transient disappearance followed by return to circulation of a proportion of injected platelets has been termed "segregation" by Davey and Lander (1964), and the degree of reversible platelet segregation may be gauged from the difference between the maximum and minimum levels of platelet radioactivity found in circulation during the initial stages.

Once again the proportion of EDTA platelets involved in this process far exceeded that found with platelets collected in either of the citrate dextrose solutions. Furthermore, segregation and re-entry into circulation was almost invariably complete within a few hours with platelets collected in citrate dextrose: completion of the phenomenon was generally delayed for 24 to 48 hours when EDTA was used.

The mechanism responsible for the transient segregation of platelets from circulation is not known at present and is open to conjecture. The suggestion that some platelets are damaged during handling and following reinjection are immediately destroyed with release of the label which then becomes attached to circulating platelets, does not appear tenable. It has been shown (Davey and

Lander, 1964) that only an extremely small proportion of circulating activity at any time is not bound to platelets. Further, Baldini et alii (1960) have shown that radioactive chromium liberated from labelled platelets does not reattach to platelets in vitro, and Ebbe et alii (1965) have shown conclusively that in vivo as well as in vitro chromium^{51} is released from destroyed platelets in a form incapable of attaching itself to other platelets. The evidence at present available suggests that the phenomenon is due to the transient adhesion of a proportion of the infused platelets to vascular endothelium throughout the body (Cronkite et alii, 1957) and it was suggested by Davey and Lander (1964) that the removal of platelets from the body for any time is associated with metabolic changes within the platelet, possibly as the result of the actions of coagulation factors and changes in the ionic composition of the milieu. Such alterations may be reversible in some platelets (segregated fraction) and irreversible in others (sequestrated fraction). Those in which the reaction is reversible are capable of apparently normal survival within the body along with the proportion of cells which is seemingly unaffected by these changes and

which remains in circulation following injection.

With EDTA platelets, the high initial activity recorded over the liver which subsequently declines over the course of 4 to 24 hours, suggests that segregated platelets are particularly concentrated in this organ. However, their release from this organ is generally associated with an increasing count rate over the spleen, which would indicate that at least some of the platelets temporarily segregated in the liver are released from this organ only to be permanently sequestered in the spleen.

With ACD 'A' platelets, high initial activity over the spleen is followed by a decline to a steady level within a few hours and would suggest that the spleen is the predominant site of both segregation and sequestration of such platelets. With ACD 'S' platelets, the proportion segregated is small and no particular organ could be implicated in the process.

Platelet lifespan and kinetics in circulation. These studies indicate that the lifespan of human platelets in circulation is in the vicinity of 9 - 11 days. Although it may be argued that those platelets which ultimately survive sequestration and segregation to circulate as

viable units may not be representative of the original population sampled, it must be emphasised that the life-span determined from these studies is not influenced by the particular anticoagulant employed, and is in agreement with that observed by other workers using similar and different techniques.

Divergent views have been expressed only in relation to the mode of destruction of normal platelets in health.

It has been noted previously that investigators using compounds of P^{32}, S^{35} and C^{14} as platelet labels have generally found the fallout of platelet activity from circulation to be a simple first order function suggesting random destruction. The use of these labels has been criticised, however, as they are prone to metabolic turnover and loss from the cell (Parker-Williams et alii, 1963; Grossman et alii, 1963). Most workers who have used radioactive chromium as the platelet label have obtained rectilinear survival "curves" suggesting that platelets have a finite lifespan and that death is a result of senescence. More recently it has been suggested that linear and random processes are involved in the removal of platelets from circulation (Davey et alii, 1964; Ebbe et alii, 1965). The platelet survival

curves obtained in this study support the contention that both linear and random processes are operating simultaneously. By applying the mathematical model devised by Davey et alii (1964) analysis of the platelet survival curves conforms to the theory of a finite lifespan being operative and upon which is superimposed a random loss of approximately 10 - 12% of platelet radioactivity per day.

Site of destruction. The site of destruction of normal platelets is also open to question. The present studies, which show a progressively rising liver/praecordial ratio with time irrespective of the anticoagulant used, support the conclusion of Aster and Jandl (1964) that "although areas such as the marrow and lymph nodes may play a role in the removal of effete platelets from the circulation, in man the majority of platelets seem to normally die in the reticuloendothelial cells of the liver." However, the slight increase in spleen/praecordial ratios frequently observed in individual studies and reflected particularly in the mean values obtained with ACD 'S' platelets, show that the spleen may play a secondary, but not unimportant, role in this respect.

Choice of anticoagulant. There can be little doubt from the results of the present studies and those of

Aster and Jandl (1964) that in terms of platelet viability following infusion, the use of EDTA as an anticoagulant for platelet collection and preservation leaves much to be desired. Certainly it is inferior to either of the solutions of citrate dextrose employed. The present studies would indicate that there is probably little to choose between ACD 'A" and ACD 'S' for the preparation of platelet concentrates. However, ACD 'A' does result in platelet aggregation in vitro and consequently handling of the platelets and their resuspension is more difficult.

With ACD 'S', platelets retain their normal shape, aggregation does not occur in vitro and, as with EDTA, the suspensions are easy to handle. Upon infusion segregation is minimal or absent, yet the proportion of platelets which survive in circulation is no greater than with ACD 'A' and the overall efficiency of the transfusion is lower. In an early communication, Aster and Jandl (1963) reported "recoveries" of 80 to 100% of labelled platelets in circulation. Later, presenting their results in 23 studies, they noted a mean recovery of 67% with a range of 36 to 85%. Although almost complete recovery has occasionally been noted in recently splenectomised patients, it has not been possible in the present study to

achieve a recovery of better than 52% in normal spleen-intact subjects using this anticoagulant. It appears that while the low environmental pH achieved with ACD 'S' may reduce platelet aggregation and the degree of segregation, the proportion sequestered is greater than with ACD 'A'. The overall conclusion is that platelet concentrates prepared from blood collected into ACD 'A' and infused into recipients are slightly superior in terms of platelet viability than those collected into ACD 'S' and are considerably superior to those prepared from blood collected into EDTA.

Results of studies on patients suffering disorders known to predispose to thrombosis or to be associated with increased atherogenesis demonstrated no significant deviation from those found in normal healthy subjects. There was no significant increase in the calculated turnover of platelets and the contour of the platelet survival curve did not deviate from that found in normal subjects. Platelet lifespan remained between 9 and 11 days as with healthy controls. This is contrary to the findings of Murphy and Mustard but is in accordance with the findings of O'Neill and Firkin (1964). The use of phenindione as a therapeutic anticoagulant in subjects with vascular

disease was found to exert no beneficial effect in decreasing platelet utilisation. Platelet survival in individual subjects who were studied both while receiving anticoagulant therapy and later following its withdrawal was found to be either unchanged or appeared to be slightly reduced. Any reduction which occurred was not however, of statistical significance and was not of sufficient magnitude to be reflected in turnover data. The constancy of this trend is of some interest, however. It may be postulated that subjects who are studied in the early stages after myocardial infarction may have a more severe form of the disease (on clinical grounds) than those who are not anticoagulated. The tendency for the survival curve to be shifted to the left under these circumstances would indicate an increased random consumption of platelets as a result of the disease rather than to any effect of the anticoagulant. However, analysis of this problem is complex as although platelet survival appears to be slightly less in subjects who are anticoagulated than in subjects who are not receiving anticoagulants, there is no difference between the curve obtained in the former and that observed in normal, healthy control subjects.

It is acknowledged that platelets are utilised during thrombosis and it has been shown by Adelson et alii (1961) and by O'Neill and Firkin (1964) that platelet survival is reduced in subjects studied during thrombotic episodes. In subject O.G., the clinical manifestations of venous thrombosis were associated not only with a sudden reduction in circulating platelet radioactivity in vivo but were also preceded by marked abnormalities in platelet behaviour in the in vitro Chandler tube system. Similarly, with subject W.B., both in vivo and in vitro abnormalities in platelet behaviour once more coincided. It seems likely that in this case the changes were associated in part with post-operative deposition of platelets in considerable numbers during endothelialisation of the dacron graft and post-operative thrombosis of damaged vessels, as further studies carried out three months later showed a completely normal behaviour pattern. It may be concluded from these findings that increased platelet utilisation may be detected by the chromium^{51}-labelling technique, but the degree of thrombosis is highly significant. Any acute thrombotic episode must be of some magnitude to alter the contour of the platelet survival curve.

Although it is an attractive theory that platelet encrustation is a contributing factor to the processes resulting in the development of atheroma, the results of platelet survival studies using the chromium^{51} technique do not support this contention. This may indeed be due to a deficiency in the sensitivity of the technique rather than to the fact that platelets are not being incorporated into the vascular endothelium during atherogenesis. The accuracy to which some workers have expressed their survival data in these conditions known to be associated with atheroma formation has been defined to within an hour of platelet viability in some cases, an exactitude which cannot be obtained using this method, and indeed is unlikely with any of the other methods at present used for determination of platelet survival.

The thrombi produced in the Chandler apparatus with blood from patients after myocardial infarction were found to be longer and heavier than the thrombi of control subjects having neither clinical nor electrocardiographic evidence of myocardial infarction. Thrombus size was clearly found to decrease with the passage of time after infarction and had a tendency to be less in patients being treated with oral anticoagulants. Thrombus weight and plasma fibrinogen level showed statistically significant

correlation in control subjects ($p < 0.01$) and in patients with myocardial infarction ($p < 0.001$). It has previously been determined that the plasma fibrinogen level is increased in patients who have suffered myocardial infarction (Meyers, 1948) and this finding has been adequately confirmed in this study. Losner (1956) also demonstrated that the plasma fibrinogen level remains elevated during the period of healing of the infarcted area. This alteration of the plasma fibrinogen could either wholly or partly account for the gradual return of these parameters towards the normal range with the passage of time. The possibility remains, however, that factors other than plasma fibrinogen may determine thrombus size.

Connor and Poole (1961) have shown that if long chain saturated fatty acids are added to blood in the Chandler tube the length of the thrombus tended to increase markedly and the time taken for the thrombus to form was reduced. Unsaturated fatty acids had little or no effect. They suggested that the greater thrombus length produced by the "active" fatty acids may indicate that the conversion of fibrinogen into fibrin had been altered in some way so that a loose, more strung-out configuration of fibrin resulted.

A similar mechanism could be implicated in the formation of the large thrombi found in patients with myocardial

infarction, particularly as abnormal lipid metabolism has been widely incriminated as an aetiological factor in this disease. It was not possible to demonstrate any significant correlation between thrombus weight and total serum cholesterol levels either in healthy control subjects or in patients with myocardial infarction. Elevated serum cholesterol levels are however only one parameter of abnormal lipid metabolism and do not preclude the presence of other lipid abnormalities as aetiological factors in the formation of these abnormal thrombi. In this study serum cholesterol levels were more a function of age than of presence of overt myocardial disease (Figure 23).

It is not possible to determine whether the factor or factors responsible for the development of these large thrombi in patients with myocardial infarction were present prior to infarction or are merely the result of changes occurring subsequent to the clinical event. It appears that the latter is more likely. This tendency towards abnormal thrombosis in vitro following myocardial infarction may in part account for the thrombotic episodes which are known to occur from time to time in the post-infarction period.

The results of these studies also clearly indicate

that there is a significant increase in the frequency of abnormal, spontaneous platelet aggregation in patients with myocardial infarction compared with healthy control subjects greater than 40 years of age. It is again not possible to state whether these findings relate to a state existing prior to infarction or are the sequel of myocardial necrosis, but it has been demonstrated that there is a definite tendency for the frequency of spontaneous platelet aggregation and thrombus size to increase with age in apparently healthy subjects. This suggests that the factor or factors responsible for these phenomena are present to some extent and that coronary thrombosis may result from an exaggeration of this trend.

Anticoagulant therapy in patients with myocardial infarction had no effect on the overall incidence of platelet abnormality. Two explanations for this finding may be tenable. Firstly, it could be that in anticoagulated patients, the initial overall frequency of platelet abnormality was greater and that this frequency has in fact been reduced by therapy. Alternatively, anticoagulant therapy may have little or no effect on platelet aggregation. On the other hand, there was a trend, although not statistically significant, for the snowstorm phenomenon to

occur more frequently in anticoagulated patients (11.5%) than in those not receiving anticoagulant therapy (4.7%). Has anticoagulant therapy enhanced platelet aggregation and is this merely a reflection of the fact that those patients selected for treatment by anticoagulants differed from those not given anticoagulants, probably on the basis of more extensive infarction? It is apparent that there is no answer to this question on the evidence available and that the effect of oral anticoagulants on platelet aggregation in an in vitro system of this type is inconclusive.

Results of studies in patients who have had both platelet survival studies and Chandler tube studies carried out show that the likelihood of detecting abnormal platelet behaviour is greater using the Chandler tube technique than using the Cr^{51} method of labelling platelets. Major episodes of thrombosis can be detected using both methods but the Chandler technique provides a more sensitive measure of abnormal behaviour and defects which cannot be measured in the survival technique may be obvious in the in vitro system.

Having determined that it is possible to detect active thrombosis of any degree in estimates of platelet survival and that the Chandler apparatus provides a useful and informative measure of abnormal platelet behaviour in vitro, studies were carried out on patients with valvular disease

of the heart. It has been noted previously that the number of patients who have had otherwise successful replacement of diseased heart valves with Starr-Edwards prostheses but who have subsequently suffered thrombo-embolism and its sequelae is increasing. At autopsy many of the thrombi have been found adherent to, or in close relation to the valve prosthesis and have been composed almost entirely of platelets and fibrin.

The pattern of the platelet survival curves in subjects 7 to 10 (Table 11) who have had valve replacement with Starr-Edwards prostheses suggests that although many of the labelled platelets survived for their natural lifespan of 9 to 11 days, a considerable number of platelets was being prematurely removed from circulation in a random fashion. This increased platelet loss was presumably matched by increased production, for none of the patients was thrombocytopenic. There was no suggestion that these abnormal findings were related to either age or sex or to the use of phenindione therapy. The particular valve replaced also could not be incriminated as a major factor influencing the alteration in contour of the survival curve. The major factor which evolved in the consideration of these results was that abnormal results were obtained in those subjects in whom prostheses had been in place for the longest period

(Table 11).

The premature removal of platelets in these subjects may be due to their being consumed in thrombus formation. Adelson et alii (1961), Firkin (1963) and O'Neill and Firkin (1964) have observed similar curves in association with intravascular thrombosis and a similar pattern of behaviour has also been demonstrated in subjects in this laboratory. It is conceivable that in these subjects the abnormal platelet survival patterns are an indication that continuing platelet deposition and thrombus formation are occurring in the region of the prosthesis. Figure 43 illustrates the autopsy findings in a man who had had a successful aortic valve replacement but who subsequently died following a mesenteric artery thrombosis secondary to gross atheromatous changes in his aorta and mesenteric vessels. The valve had remained well situated and there was no disruption of its attachment. In the region of the teflon ring obvious thrombi were present. These were of both ante and post-mortem types and had in no way interfered with valve function.

It could also be postulated that platelets are being damaged in circulation by the Starr-Edwards valve. Cellular damage following the insertion of valve prosthesis has been

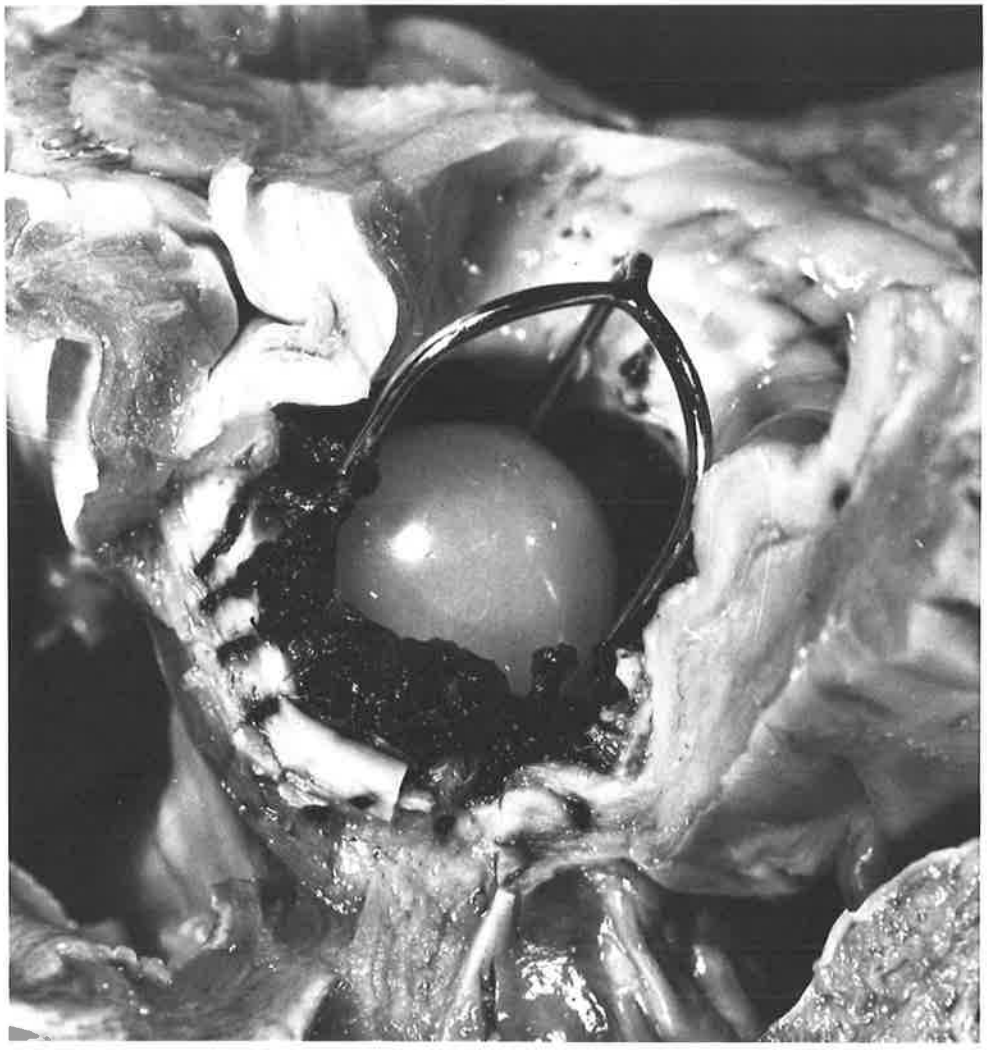


Figure 43: A mixture of both antemortem and post-mortem thrombi in the region of the teflon cuff of a Starr-Edwards ball valve prosthesis in the aortic region.

well documented. In 1954 Rose et alii reported anaemia following the insertion of prosthetic material, and in 1956 Stohlman et alii reported red cell destruction in dogs following the insertion of a Hufnagel valve. Cell destruction was accompanied by haemoglobinaemia, haemoglobinuria, anaemia, reticulocytosis and renal haemosiderosis. Chromium^{51} red cell survival was markedly reduced.

The first reports of haemolytic anaemia of mechanical origin after open heart surgery in humans were presented by Neill et alii (1961) and by Sayed et alii (1961), and subsequently reports of this phenomenon have continued to appear (Verdon et alii, 1963; Sigler et alii, 1963). Stevenson and Baker (1964) and Marsh (1964) simultaneously noted severe intravascular haemolysis following the insertion of Starr-Edwards prostheses and postulated that this type of haemolytic anaemia with fragmentation of the red cells was due to turbulent blood flow and the presence of a rigid prosthesis in circulation.

No morphological abnormality of the subjects' platelets or red cells was observed in the studies at present under discussion. However, it has been noted in individual studies that platelet radioactivity may accumulate in the spleen (and liver). This could suggest that in the affected

subjects a proportion of their platelets is being damaged in circulation and subsequently sequestered in reticulo-endothelial tissue.

A further possibility for the altered survival curve pattern arises from the recent studies of Pirofsky et alii (1965) who demonstrated the existence of an auto-immune phenomenon associated with the haemolysis which may complicate aortic stenosis or the insertion of a Starr-Edwards prosthesis. Extension of this argument to the present situation would suggest that the platelet surface membrane may be so altered by mechanical trauma that the modified platelet appears foreign to the host and antibodies against it are produced. Once again platelet aggregation could result. Since such "damaged" platelets might be more liable to local deposition as well as reticulo-endothelial sequestration, both these mechanisms of removal could operate simultaneously. Facilities for the detection of platelet antibodies being unavailable, it has not been possible to explore this further.

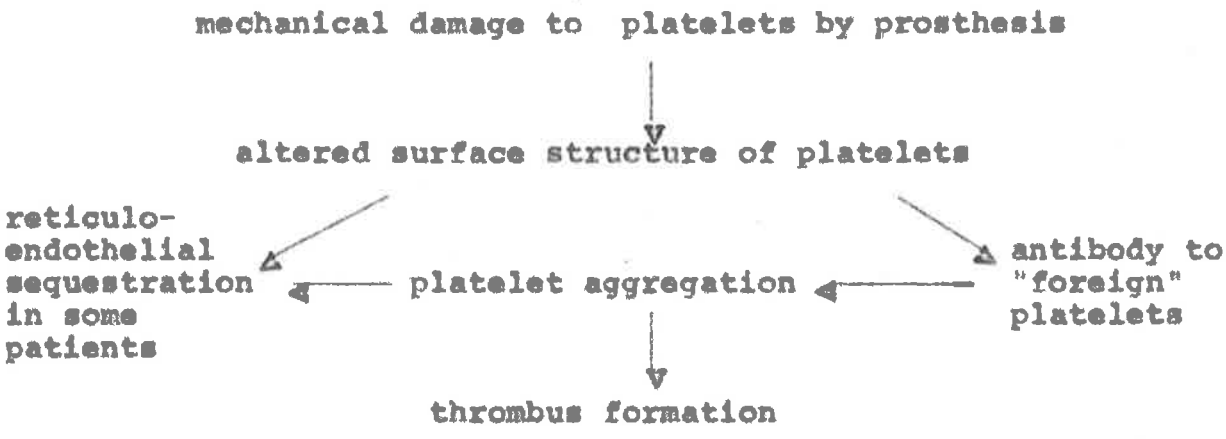
The results of studies in the Chandler tube indicate that there is a significant increase in the frequency of abnormal platelet aggregation in patients with Starr-Edwards prostheses compared with healthy control subjects ($p < 0.001$). Whether these findings are related solely to the presence of

the artificial valve or to a state existing prior to valve replacement is difficult to assess. It has been shown that there is a tendency for the frequency of spontaneous aggregation to increase in subjects who have valvular disease of the heart but in whom valve replacement has not been carried out. However, it should be pointed out that the majority of days of abnormal platelet aggregation in this section was found in the one subject and did not reflect an even scatter throughout the group. Exclusion of this subject would result in a frequency of abnormal platelet aggregation comparable with that in healthy normal subjects.

The thrombi produced in the Chandler apparatus from the blood of subjects with heart disease were neither longer nor heavier than those of control subjects. This may indicate that despite the platelet abnormalities detected, thrombosis was not active to a major degree during the period of study.

In subjects who have had platelet survival studies and Chandler studies carried out simultaneously, abnormal platelet aggregation determined by the Chandler method occurred more frequently in subjects with reduced platelet survivals than in subjects with normal survival curves. This

correlation between two different measurements of platelet behaviour indicates that the factors which may be responsible for the abnormalities of platelet survival in patients with Starr-Edwards prostheses are also the factors which cause the increased platelet abnormalities in the Chandler apparatus and that the same phenomenon is being measured by these two quite different methods of study. These factors are not necessarily exclusive and it is possible that they may occur either independently or simultaneously to varying degrees. The various hypotheses may be integrated under the following scheme:



In 1926 Huek demonstrated a postoperative rise in the level of blood platelets. These findings have been subsequently confirmed and it is now apparent that the postoperative rise in the platelet count is most marked between

10 and 12 days after operation, a time which corresponds with the tendency to thrombo-embolic complications. There have been varying reports on the immediate effect of operation on platelet levels. Mustard (1957) found that patients who had not been transfused showed a rise in platelet count immediately after operation and that a proportion of patients receiving up to 6 transfusions of bank blood showed no fall in platelet count. Other workers (Warren et alii, 1953) have demonstrated an immediate fall in platelets in the early post-operative period and it has been well documented that greater than eight transfusions are associated with thrombocytopenia in the early stages (Desforges et alii, 1954; Krevans and Jackson, 1955). The first suggestion that platelet behaviour as well as platelet number may be affected in the postoperative period was made by Payling-Wright (1942) who demonstrated that there was an increased adhesiveness of platelets to glass at this time.

Leeksma and Cohen (1956) carried out platelet survival studies in patients after operation and, although results were difficult to interpret, were not inconsistent with normal lifespan. Further platelet survival studies were carried out in the early postoperative period by Zucker et alii (1961) following pre-operative platelet labelling and

in only two subjects was there a decrease in platelet survival. In the remaining subjects the contour of the survival curve was normal and compatible either with a pattern totally unaffected by surgery or with a reduced survival combined with continued re-entry of labelled platelets into circulation. The latter feature was a probability owing to the use of DFP^{32} as the labelling isotope. In the subjects in this study relabelling has not been a problem as $chromium^{51}$ has previously been shown not to relabel platelets in circulation. Three subjects showed a normal platelet survival curve and the other three showed some reduction in platelet survival. None of the patients had received massive blood transfusion. An interesting feature of this study was that platelet survival was most abnormal in subjects whose mean platelet count was highest. This indicates that increased platelet utilisation in these subjects was well compensated and had been matched by either increased production or release of platelets from megakaryocytes, or by mobilisation from other platelet pools or depots. The decrease in platelet survival with a shift of the curve to the left is compatible with increased random removal from circulation. This may be accounted for by increased utilisation during the thrombosis of vessels

damaged during the course of the operation. Furthermore, it is a possibility that one influencing factor could be the release of red cell or tissue factors, for example, adenosine diphosphate or tissue thromboplastin which may promote platelet clumping with possible ready adhesion to vascular endothelium and consequent removal from effective circulation. These factors in turn may be associated with the known tendency to thrombo-embolism that occurs in the postoperative period. Two of the three subjects who had reduced platelet survivals had previously suffered from myocardial infarction and their reduction in platelet life-span may be a further reflection of an abnormal tendency to' thrombosis in these subjects.

Platelet survival studies using radioisotopes have been widely carried out in cases of thrombocytopenia. The mechanisms in the pathogenesis of idiopathic thrombocytopenic purpura are disputed and the presence of thrombocytopenia in the blood associated with normal or increased numbers of megakaryocytes in the bone marrow has been explained by a number of contrasting theories. The findings may be explained on the basis of a defect of platelet formation, or of excessive disposal or destruction in the peripheral circulation, or in the peripheral organs (Hayem, 1895). Frank (1915, 1916)

attributed the thrombocytopenia of idiopathic thrombocytopenic purpura to decreased formation by the megakaryocytes and suggested that this mechanism arose as a result of megakaryocyte inhibition by "noxious" substances from the spleen. Kaznelson's suggestion (1916) that the removal of the spleen could be of value was based on the concept that the spleen may be a thrombocytolytic organ. This idea was extended (Doan et alii, 1949) with the concept of selective splenic sequestration of circulating platelets. A number of significant contributions to the mechanism of thrombocytopenia have been made in recent years with the elucidation of the mechanism of sedormid (Ackroyd, 1949); the thrombocytopenic activity of plasma from individuals with idiopathic thrombocytopenic purpura when injected into normal subjects (Harrington et alii, 1951); the effect on the lifespan of platelets of healthy subjects following their incubation in the plasma of patients with idiopathic thrombocytopenic purpura (Penny et alii, 1965); the rate of survival of normal platelets transfused into patients with thrombocytopenic purpura (Stefanini and Chatterjea, 1951); the frequent coexistence of idiopathic thrombocytopenic purpura and acquired haemolytic anaemia (Fisher, 1947; Evans et alii, 1949) and studies on the beneficial effect of ACTH and

cortisone in the disease (Robson and Duthie, 1950).

It is at present generally accepted that idiopathic thrombocytopenic purpura is a disease with an autoimmune background but the site of the immune damage remains uncertain. Difficulties have arisen with the identification of platelet antibodies and no reliable in vitro technique is available at present. Cohen (1961) has suggested that the only satisfactory method of classifying thrombocytopenias at present depends on whether the platelet survival is short or normal and, using radiochromate labelled platelets, devised a reclassification scheme. Najean et alii (1963), also using radioactive chromium-labelled platelets, attempted to correlate platelet survival with the clinical picture in thrombocytopenic purpura and with the results of splenectomy. Probably the most noteworthy finding arising from his studies was that the presence of splenic localisation showed good correlation with subsequent response to splenectomy. In patients whose splenic localisation was less pronounced or absent, the results of splenectomy were less certain. Results of surface counting data in this series have tended to support this conclusion. Two subjects with splenic uptake of radioactivity responded to splenectomy but one subject who did not have a splenic uptake passed into a

complete haematological remission immediately after splenectomy. Although this suggests that splenic uptake may allow greater predictive value to be placed on the likely outcome of splenectomy, such a factor should probably not be the only criterion for operative intervention. In two patients who were studied following splenectomy, surface counting data showed an hepatic uptake of platelets. It has been known for some time that the spleen is important in removing platelets which have been damaged immunologically. More recently, however, Aster and Jandl have suggested that the liver plays an important role in this respect also, and that although platelets which have been slightly damaged by antibody may be removed in the spleen, with increasing damage induced by higher concentrations of antibody, the site of sequestration of platelets is predominantly in the liver. The occurrence of hepatic localisation suggests that in some subjects, despite splenectomy, some abnormality persists which affects the platelets and results in their sequestration. It may be inferred from this that the spleen does not contribute to any degree to the elaboration of anti-platelet antibody, at least in some patients.

It has been widely demonstrated (Laurence et alii, 1946; Maupin, 1956, and others) that transfused platelets are

removed from circulation very rapidly in patients suffering from idiopathic thrombocytopenia. This rapid removal of platelets under these circumstances is not necessarily entirely due to platelet injury during handling but is probably an inherent part of the disease. In some instances previous sensitisation to human platelets by blood transfusion cannot be excluded as a contributing factor. In these studies platelets were rapidly removed from circulation in each patient resulting in a reduced platelet half-life in each instance and confirming the findings of previous investigators.

Turnover data in patients with idiopathic thrombocytopenia in this series show increased turnover associated with reduced platelet survival in the majority suggesting that peripheral destruction is a major aetiological factor in the thrombocytopenia. Of these patients, three (C.M., J.M., B.M.) responded to splenectomy and two of them had shown an increased splenic uptake of radioactivity. In two subjects (M.S. Study 1; P.R. Study 2) total platelet turnover was reduced and both had a reduced platelet survival. Bone marrow examination showed that there was no reduction in megakaryocytes. This suggests that there was, in these patients, some failure of release of platelets into circulation coupled with an increased peripheral destruction. Factors such as

splenic inhibition of platelet release could not be incriminated here as both subjects had had their spleens removed. Two patients (B.M. Study 2, and M.S. Study 2), show evidence of partly compensated thrombocytolysis - a normal mean platelet count has been maintained despite evidence of enhanced destruction mirrored both in reduced platelet survival and in excessive organ uptake. It is probable that this is a steroid induced phenomenon as both patients were being treated with prednisolone throughout the study.

What is it that determines the sites of uptake of cells? Previously it has been shown that when erythrocytes are mildly damaged by heat (Harris et alii, 1957; Wagner et alii, 1962; Kimber and Lander, 1965) or by low concentrations of p-chloromercuribenzoate (PCMB) or n-ethyl-maleimide (NEM) (Jacob and Jandl, 1962; Wagner et alii, 1962; Kimber et alii, 1965) both of which are sulphydryl inhibitors, they are removed in man mainly by the spleen. The same workers have demonstrated that when erythrocytes are damaged by higher concentrations of sulphydryl inhibitors or by higher degrees of heat they are removed principally by the liver. Lander et alii (1965) have similarly shown that subjecting blood platelets to varying degrees of heat prior to re-injection results in a similar pattern of behaviour.

Platelet survival in circulation was considerably reduced when platelets were subjected to 45°C heat and no labelled platelets reappeared in circulation after heating at 56°C. These studies indicate that treatment of platelets with sulphydryl inhibitors, in this case NEM, results in a similar pattern of platelet behaviour in vivo following reinjection. The site of sequestration of labelled platelets changed from spleen to liver as increasing damage with higher concentrations was effected.

These results are analogous to those obtained by Aster and Jandl (1964) who observed that transfused platelets reacted with injected iso-antibody and that minimal sensitisation caused a lesion detectable only by the spleen. Greater sensitisation caused platelets to be sequestered in the liver. It has been postulated (Jacob and Jandl, 1962) that the shift in the site of red cell sequestration is due to alterations in the cell membrane and it appears likely from the results of Lander et alii (1965) using heated platelets, which have also been examined electromicroscopically, that similar changes in the platelet membrane could induce altered sites of platelet sequestration. The same mechanism would operate with NEM-treated platelets. Added evidence that alterations in the degree of damage cause changes in the

sites of sequestration of platelets may be obtained from the results of surface counting data obtained when platelets are handled in the various anticoagulant solutions. Ethylene diamine tetra-acetic acid is a powerful chelating agent and its use in platelet preparation results in the immediate sequestration and segregation of platelets in the liver. Its effect, however, is incomplete and partially reversible as many of the platelets are subsequently released from the liver and a proportion of them is then permanently sequestered in the spleen. The remainder circulate and live a normal life-span. The acid-citrate dextrose anticoagulants alter the platelet less than EDTA and because of this milder action, most platelets which are sequestered are trapped in the spleen.

The sites of destruction of normal platelets are difficult to ascertain. Aster and Jandl (1964) feel that the majority of platelets dies in the reticulo-endothelial system, particularly in the liver, and to a lesser extent the spleen. As stated previously, the results of studies in this laboratory indicate that both liver and spleen may be important in the removal of effete platelets from circulation under normal circumstances. Having ascertained the normal pattern of platelet behaviour, and having determined

that platelets damaged by either physical or chemical agencies are sequestered in various sites depending on the degree of damage, it may be possible to relate such findings to those found in a variety of disorders, but particularly to states found in individuals suffering from thrombocytopenia.

It will be recalled that subject M.S. with idiopathic thrombocytopenia was studied on two occasions and that on the first most platelets accumulated in the spleen, and on the second the majority was taken up by the liver. It is possible that this patient is suffering from thrombocytopenia associated with circulating anti-platelet antibodies. On the first occasion the degree of platelet damage may have been only mild and resulted in maximum counts being obtained over the spleen. Some months later when the study was repeated a greater degree of damage may have arisen, possibly due to an increased antibody titre, and accounted for the excessive hepatic uptake of labelled platelets. However, before such an explanation is taken as convincing evidence of this eventuality, it must be noted that on the second occasion isologous platelets were employed and this, coupled with the fact that she had had previous blood transfusions, may represent merely a similar phenomenon but one which is related rather to a state induced by previous transfusion of

foreign platelets than to one arising from her original disease.

Criticism of the Techniques Employed

As with most techniques of medical investigation, those employed in the present study may be criticised.

Firstly, platelet survival studies may not give a true representation of platelet behaviour inside the body. Not only are the platelets labelled outside the body, but the technique involves a moderate degree of handling of the cells which is reflected in the permanent clearance of a large proportion of them from circulation, presumably due to damage during labelling. Interpretation of this feature is made even more difficult by the variations in this phenomenon which appear to be dependent in part on the anticoagulant into which the blood is collected. It may well be argued that chromium^{51} platelet survival studies concern only the fixed population of cells which have survived the handling and sequestration, and may not give a true representation of the whole population of platelets in circulation. Argument against this being so, however, is the close correlation in overall survival data between platelets prepared by the chromium-^{51} method and those prepared by labelling in vivo with P^{32}. As a further criticism, it may be suggested that following platelet labelling it is possible that a change may occur in the

body which will affect other platelets as they are being released into circulation but have little or no effect on those cells which have previously been labelled. Changes in platelet behaviour may then take place which would not be reflected in the survival data.

It has been demonstrated that any change in platelet' utilisation or in platelet destruction must be of some magnitude to be detected in platelet survival data. Further, it appears from the results of studies in the Chandler tube that changes in platelet behaviour may be episodic and in a proportion of cases reversible, and under these circumstances, those defects which may be obvious in the Chandler tube may not be detectable in platelet survival studies. Indeed in many ways the Chandler tube technique appears to provide a more sensitive index of abnormal platelet behaviour at least in thrombotic disease. Furthermore, the Chandler technique is far easier to perform than platelet survival studies and this is especially so when the subjects studied are particularly ill. The disadvantage of the Chandler technique is that it is an in vitro test. Interpretation of results obtained in an in vitro system must be made with reservation as they need not necessarily represent the situation occurring in vivo. The chromium^{51} technique is probably most useful

in subjects who have thrombocytopenia where it may be used not only to determine platelet lifespan but also to identify sites of platelet destruction by the aid of external scintillation counting. Again there are some limitations with this category of patient as the technical difficulties are great if the subjects' platelet count falls below 60,000 per cu.mm.,and the difficulties increase with increasing degrees of thrombocytopenia. Frequently there is difficulty in the interpretation of surface counting data, and even in those cases who appear to have a specific organ uptake by the spleen in some cases of thrombocytopenia, it is not possible to predict confidently the outcome of splenectomy. Suffice it to say that in subjects who on clinical grounds should respond favourable to splenectomy and whose platelet studies clearly show a splenic uptake, the likelihood of success of operation is greater than in subjects who exhibit no splenic uptake of radioactivity during platelet survival studies.

CHAPTER V

SUMMARY

1. Radiochromate-labelled platelet concentrates were prepared following collection of blood into a solution of acid-citrate dextrose anticoagulant (ACD 'S') which resulted in a final whole blood pH of 6.5 - a more acidic form of anticoagulant than that customarily employed. Results were compared with those previously obtained in this laboratory using ACD 'A' and EDTA as anticoagulants for collection of blood. Platelet yield was greatest when EDTA was used but after transfusion relatively few platelets survived in circulation as viable units. In terms of overall efficiency both solutions of ACD were superior to EDTA. Platelet lifespan in circulation was unaffected by the anticoagulant used for the collection of blood. External scintillation counting showed that in man the majority of effete platelets is normally removed by the liver and that the spleen plays a secondary, but not unimportant role in this process.

2. It is an attractive theory that platelet encrustation is a contributing factor in the processes resulting in the development of atheroma. However, using chromium^{51}-labelled platelets, results of investigations carried out on 34 subjects with atheroma do not support the view that platelet lifespan is reduced and platelet turnover increased in these subjects. Furthermore, platelet survival was not prolonged

by the administration of the anticoagulant phenindione. Although platelet deposition on vascular endothelium may be a contributing factor in the pathogenesis of this disorder it is concluded that the techniques at present available are not sensitive enough to detect this phenomenon.

3. Studies using the Chandler apparatus have shown that the blood of patients suffering from myocardial infarction shows an increased tendency to abnormal spontaneous platelet aggregation and thrombosis in vitro. Thrombus size tended to be less in patients treated with anticoagulants and decreased with the passage of time after myocardial infarction. There was a direct relation between thrombus size and the plasma fibrinogen level in both healthy controls and in patients studied after myocardial infarction. There was no such relation between serum cholesterol and thrombus size. The incidence of abnormal platelet aggregation tended to increase with age in healthy controls. In patients with myocardial infarction, treatment with anticoagulants caused no significant decrease in the incidence of abnormal platelet aggregation. No conclusions can be drawn as to the significance of these findings. They may be the result of infarction rather than reflect a state existing prior to the clinical event. There is some evidence to suggest that there

is an increase in abnormal platelet behaviour with age and myocardial infarction may be the result of an exaggeration of this trend in some subjects. It is likely that the frequency for thrombosis to occur in subjects following such episodes may be in part related to this increased thrombotic tendency.

4. It has been demonstrated that reduced platelet survival indicative of increased random loss of a proportion of platelets from circulation occure in some subjects following the insertion of Starr-Edwards prostheses for correction of valvular disease of the heart. This abnormality in platelet survival is directly related to the time after operation and occurs in those in whom the prosthesis has been in place the longest. Studies in subjects with uncorrected valvular disease were completely normal.

5. The results of studies in the Chandler tube in patients following the insertion of Starr-Edwards prostheses show that there is a significant increase in the frequency of abnormal spontaneous platelet aggregation in these subjects compared with normal control subjects. Furthermore, the frequency of abnormal spontaneous platelet aggregation was found to be greater in subjects with reduced platelet survival than in

subjects whose survival curve showed a normal contour. These findings in patients with Starr-Edwards prostheses had not previously been described by other workers.

6. Platelet survival in circulation was found to be reduced in some patients studied in the postoperative period and normal in others. Of the three subjects with reduced platelet survival two had suffered myocardial infarction some years previously, a factor which may be taken as additional evidence of an abnormal thrombotic tendency in these individuals.

7. Platelet survival studies in patients with thrombocytopenia showed a marked reduction of platelet lifespan in all cases studied. Surface counting data revealed that a more reliable prediction of the outcome of splenectomy may be made if there is an increased splenic uptake of labelled platelets. However, results of uptake studies should not be the only criterion to suggest operative intervention as favourable results may be obtained in subjects who show no increase in splenic localisation of radioactivity. Numbers in this series, however, are small - a limiting factor in the overall interpretation of data.

8. Studies on platelet sequestration using the sulphydryl inhibitor N-ethyl maleimide (NEM) have demonstrated that the site of sequestration of platelets is determined by the degree of damage they have sustained. Results are analogous to those found when red cells have been damaged by chemical and physical agencies and are comparable with the results of studies in which platelets have been damaged by either heat or immunological means. Minor damage causes platelets to be removed, principally by the spleen, and more severe damage results in platelet removal, mainly by the liver. These findings may be related to those in a number of disease states associated with increased platelet removal from circulation.

APPENDICES

APPENDIX A

A. Normal Healthy Control Subjects

1. Platelet Survival

	Age	Sex	Source
John Curtin	27	M	Norwood Apex
Jamie Shepherd	26	M	Norwood Apex
John Green	33	M	Norwood Apex
Barry Chambers	28	M	Norwood Apex
Rod Boswell	19	M	Norwood Apex
Gary Feutrill	27	M	Glenelg Apex
Cynthia Knight	30	F	Prof. Robson

2. Chandler Studies

Tom White	44	M	I.M.V.S.
Ian Robertson	38	M	I.M.V.S.
Michael Addison	41	M	I.M.V.S.
Martin Hansen	45	M	I.M.V.S.
Eric Hardy	55	M	I.M.V.S.
Richard Ibbotson	38	M	I.M.V.S.
Bill Howarth	40	M	I.M.V.S.
Douglas Hardy	42	M	I.M.V.S.
Alan Banks	47	M	I.M.V.S.
Heidi Taylor	42	F	I.M.V.S.
Bob Sheppard	42	M	I.M.V.S.

Sue Dixon	37	F	I.M.V.S.
Raelene Kinlough	28	F	Dept. Medicine
Geoff Glew	27	M	Student
Peter Davis	37	M	Dept. Medicine
Colin Luke	23	M	R.A.H.
Ward Derrington	39	M	I.M.V.S.
Kevin Clapp	38	M	I.M.V.S.
George Davies	49	M	I.M.V.S.
John Lloyd	25	M	Dept. Medicine
Nancy Mitchell	43	F	I.M.V.S.
George Gormley	47	M	I.M.V.S.
Earle Hackett	44	M	I.M.V.S.
Bill Nolan	49	M	Dept. Medicine
David Newman	45	M	I.M.V.S.
Eve Looke	28	F	I.M.V.S.
Tony Basten	25	M	R.A.H.
Joseph Barta	38	M	I.M.V.S.
Percy Watts	57	M	I.M.V.S.
Elizabeth Irving	37	F	I.M.V.S.
John Kleine	26	M	I.M.V.S.
Timothy Murrell	31	M	Dept. Medicine
Kevin Mattschoss	19	M	I.M.V.S.
David Howell	23	M	I.M.V.S.
Graham Meyer	20	M	I.M.V.S.
Stanley Hooper	19	M	I.M.V.S.

Barry Gormley	22	M	I.M.V.S.
Roy Pain	32	M	I.M.V.S.
Alan Addis	37	M	I.M.V.S.
Jack Edwards	59	M	R.A.H. Porter
Jack Nicholls	62	M	R.A.H. Porter
Charles Martin	55	M	R.A.H. Porter
John E. Lloyd	64	M	R.A.H. Porter
Bill Gittins	62	M	R.A.H. Porter
Horace Jackson	58	M	R.A.H. Porter
Edwin Chadwick	57	M	R.A.H. Porter
Francesco Rocca	56	M	R.A.H. Porter
Petro Scalzi	53	M	R.A.H. Porter
Archibald Dorsett	50	M	R.A.H. Porter
Pasquale Silvestri	57	M	R.A.H. Porter
Alan Hutton	62	M	R.A.H. Porter
Harold Bain	50	M	R.A.H. Porter
Archibald Heron	58	M	R.A.H. Porter

B. Subjects with Myocardial Infarction

1. Chandler Studies

	Age	Sex	Unit No.
Martha McKeough	80	F	002015
Walter Edwards	57	M	049335
Myrtle Stacey	64	F	048277

Violet Mulliner	71	F	050627
Fred Rogers	57	M	051153
Bert Franklin	69	M	051886
Keith Bowley	54	M	004404
Annie Waters	73	F	016943
William Bennett	66	M	052855
Thomas Crummey	58	M	053399
Ray Harvey	59	M	053387
Alice Woodcock	72	F	057658
Ted Varga	54	M	062818
Gwen Pash	54	F	064860
William Abel	63	M	037839
Murray Pearce	55	M	068003
Edmund Kas	45	M	036162
Robert Findlay	56	M	059460
Leslie Badman	75	M	067845
Lawrence Weller	63	M	
Iola Allen	66	F	068368
Albert Waddle	69	M	000125
Matthew Carragher	43	M	068310
Errol Winton	62	M	046250
Roy Till	75	M	023727
Oswald Ragless	54	M	068987

Cyril Rush	57	M	022892
Cyril Noble	68	M	051336
Albert Deverill	50	M	069258
Harding Tilley	70	M	068527
Bruce Swan	39	M	069715
Marguerita Hoffman	70	F	069149
Henry Fletcher	78	M	035296
Albert Lee	54	M	-

C. Subjects with "Atheroma" or Complications

1. Platelet Survival Studies (*indicates Chandler studies also)

David Thomas (1)	54	M	17838/62
Frederick Gabell	57	M	19140/62
Harold Ryles	51	M	010585
Ralph Cock	51	M	21125/62
David Thomas (2)	54	M	17838/62
Alice Brodie	63	F	027703
George Moir	50	M	028770
Oscar Peto	52	M	002377
Eric Stanley (1)	64	M	032822
Frank Stedt	69	M	033770
Roy Rogers	46	M	032715
Joyce Walton	52	F	035520
Arthur Johnston (1)	54	M	034492

William Bowmann	62	M	012194
Frank Meissenger	58	M	039242
Alfred Ronitis	60	M	010636
Roswald Kennett	45	M	039690
*Frank McDonald	61	M	030513
Richard Hocking	62	M	040492
*Alice Heatley	53	F	005499
Rita McGough	49	F	020540
Sidney Corbett	62	M	042006
*Arthur Johnston (2)	54	M	034492
Gilbert Johnson	61	M	Private patient
*Olive Goodger	57	F	028269
*George Colvill	51	M	043647
*John Karavenkas	57	M	043253
*Douglas Ball	50	M	044008
*Harold Martens	55	M	045065
Alfred Farson	49	M	044416
*Eric Stanley (2)	65	M	032822
*Walter Bullen	64	M	011190
Efim Konovaloff	62	M	059914
*George Freeman	54	M	001412
*Terence Dodd	16	M	060990
*William Moller	61	M	058097
*Wilfred Pavlovich	67	M	065934

D. Patients with Valvular Disease of the Heart

1. Uncorrected Valve Lesions

*Reginald Casey	59	M	049048
*Eric Cheney	44	M	011898
*Gordon Semple	58	M	043654
*John de George		M	068199
*Thelma Malin	31	F	067650
*John Anspach	67	M	071837

2. Corrected Valve Lesions - Starr-Edwards Prostheses

Phyllis Bellhouse	52	F	008366
Rudolph Janezic (1)	64	M	018724
*Ethel Kenney	59	F	027774
*Janet King	41	F	H.D.S.
*Selma Mundy (1)	51	F	015610
*Raymond Leighton	37	M	025480
*Rudolph Janezic (2)	64	M	018724
*Sandra Reidy	24	F	005424
*Selma Mundy (2)	51	F	015610
*George Bulatavas	39	M	014175
*Alan Tossel	50	M	048796
*Eleanor Conry	41	F	003213

E. Subjects Studied Postoperatively

Percy Smith	70	M	-
Olive Goodger	57	F	028269

Walter Bullen	65	M	011190
Charles Wickens	67	M	041124
Florence Wallis	59	F	000409
Theodore Lazdins	61	M	008997

F. Subjects with Thrombocytopenia

Maria Stergiou (2)	26	F	020370
Pauline Russ (2)	32	F	043642
Eric Gibson (1)	54	M	Private patient
Betty Mahoney (2)	30	F	Private patient
Clyda May (2)	14	F	005913
Mavis Smith (2)	52	F	Private patient
John Langman (1)	41	M	Repatriation Hosp.
Albert Thomas (1)	45	M	036233
Joan Mortimer (1)	24	F	066358

G. Subjects given NEM-treated Platelets

Henry Wells	42	M	054276
Milvert Smith	67	M	054401
Stanley Millis	67	M	047012
William Moller	61	M	058097
Kardy Czokas	47	M	East Side Clinic
Robert Gunter	26	M	060362

APPENDIX B

Copy of letter to patients with uncorrected valvular disease of the heart.

Dear ,

I am writing to ask you if you would be able to come to the hospital to have a further blood test done before your operation. I have spoken with the Cardiac and Thoracic Clinics about this and we are all anxious to do this test before the operation.

It is really only a blood test, possibly a little more complicated than those you usually have done, but it should cause you no trouble at all. This investigation takes about 10 days to complete. On the first day we should need you here for the greater part of the day but after that only for half an hour a day to have a specimen of blood taken. Could you get in touch with me either by phoning me at 23 4333 extension 429 or by coming in to see me when I could explain more fully what this test entails and arrange a suitable time for you to come in to have the investigation completed?

Yours sincerely,

Copy of letter to patients with Starr-Edwards prostheses.

Dear ,

Dr. Waddy and Dr. McPhie suggested that I get in touch with you. Following your operation there is another test we should like you to have done to judge your response to the operation.

Would you be able to get in touch with me, either by ringing 23 4333 extension 429, or by calling in to see me at the above address when I could explain to you what this would involve and arrange a convenient time for you to have the test carried out?

Yours sincerely,

Copy of letter to Lay Superintendent, Royal Adelaide Hospital.

August 23, 1965

The Lay Superintendent,
Royal Adelaide Hospital,
Adelaide.

Dear Mr. Kelly,

Dr. Schwartz and I should like to express our gratitude to you for allowing the Porters of the Royal Adelaide Hospital to take part in the investigations we are carrying out on vascular disease.

Everyone was extremely helpful and we have gained information which is valuable, not only at present, but which we expect to be of considerable use in the future.

Yours sincerely,

Copy of letter to Head Porters, Royal Adelaide Hospital.

August 23, 1965

The Head Porters,
Royal Adelaide Hospital,
Adelaide.

Dear Mr. Bain and Mr. Heron,

On behalf of Dr. Schwartz and myself I should like to thank you and the men of your staff for taking part in the project we are carrying out on vascular disease. The participants were particularly willing and several in fact came in on their days off duty to have specimens taken.

I think I can say without any reservations that we have gained much valuable information from the investigations performed and expect the results to be of considerable use in the future. We should like you to convey our appreciation to all those who took part.

Yours sincerely,

APPENDIX C

Publications

1. Lander, H., Kinlough, Raelene L., and Robson, H.N. Reduced platelet survival in patients with Starr-Edwards prostheses. Brit. Med. J. 1: 688 (1965).

2. Kinlough, Raelene L., Davey, M.G., and Lander, H. An evaluation of anticoagulant solutions used in the preparation of platelet concentrates. Transfusion, 6: 213 (1966).

3. Ardlie, N.G., Kinlough, Raelene L., and Schwartz, C.J. In vitro thrombosis and platelet aggregation in myocardial infarction. Brit. Med. J. 1: 888 (1966).

4. Ardlie, N.G., Kinlough, Raelene L., Glew, G., and Schwartz, C.J. Fatty acids and in vitro platelet aggregation. Aust. J. Exp. Biol. 44: 105 (1966).

5. Kinlough, Raelene L., Lander, H., Ardlie, N.G. and Schwartz, C.J. Platelet behaviour in patients with Starr-Edwards prostheses. Submitted to American Heart Journal.

6. Kinlough, Raelene L., Davey, M.G., and Lander H. Platelet survival in atheroma. Submitted to Aust. Ann. Med.

APPENDIX D

Lectures to Scientific Societies

The Haematology Society of Australia

"An assessment of the relative merits of anticoagulant solutions used in the preparation of platelets for transfusion."
Annual Meeting, Adelaide, April 1965.

"Platelet survival in sheep." (with H. Lander and B.G. Schultz - presented by B.G. Schultz).
Annual Meeting, Adelaide, April 1965.

Medical Sciences Club of South Australia

"Anticoagulant solutions used in the preparation of platelet concentrates."
April, 1965.

The Cardiac Society of Australia

"Platelet kinetics in patients with Starr-Edwards prostheses."
Annual Meeting, Melbourne, May 1965.

The Royal Australasian College of Physicians

"In vitro thrombosis and platelet aggregation in patients with myocardial infarction." (with N.G. Ardlie and C.J. Schwartz - presented by C.J. Schwartz).

XIth Congress of the International Society of Haematology

"Studies of the effects of heat and of n-ethyl maleimide on the sequestration and survival of platelets."

Accepted for presentation, Sydney, August 1966.

REFERENCES

Aas, K., and Gardner, F.H. (1958), Survival of blood platelets labelled with chromium^{51}. J. Clin. Invest. 37: 1257.

Ackroyd, J.F. (1949), The pathogenesis of thrombocytopenic purpura due to hypersensitivity to sedormid (allyl-isopropyl-acetyl-carbamide). Clin. Sci. 7: 249.

Adelson, E., Kaufman, R.M., Dimassis, S., Lear, A.A. and Rheingold, J. (1964), Platelet survival studies using tritium labelled diisopropylfluorophosphate. Proc. Xth Cong. Int. Soc. Haemat. Stockholm, 1964.

Adelson, E., and Rheingold, J. (1956), Studies of platelet survival by in vivo tagging with phosphorus^{32}. Proc. 6th Cong. Int. Soc. Haemat. (1956), Grune & Stratton, N.Y. (1958) p. 293.

Adelson, E., Rheingold, J.J. and Crosby, W.H. (1961), The platelet as a sponge: A review. Blood, 17: 767.

Adelson, E., Rheingold, J.J., Parker, O., Buenaventura, A. and Crosby, W.H. (1961), Platelet and fibrinogen survival in normal and abnormal states of coagulation. Blood 17: 267.

Alfos, L.G., Field, E.O., Ledlie, E.M. (1959), Clinical studies with DF^{32}P on the lifespan of platelets. Lancet, 2, 941.

Andrews, G.A., Sitterson, B.W., Kretchmar, A.L. and Brucer, M. (1959), Accidental radiation excursion at the Oak Ridge Y-12 plant IV. Health Phys. 2: 134.

Anitschkow, N. and Chalatow, S. (1913), Über experimentelle Cholesterinsteatose und ihre Bedeutung für die Entstehung einiger pathologischer Prozesse. Zbl. allg. Path. Anat. 24: 1.

Aster, R. and Jandl, J.H. (1963), Sequestration of human platelets. Clin. Res. 11: 189.

Aster, R.H. and Jandl, J.H. (1964), Platelet sequestration in man. I. Methods. J. Clin. Invest. 43: 843.

Aster, R.H., Jandl, J.H. (1964), Platelet sequestration in man. II. Immunological and clinical studies. J. Clin. Invest. 43: 856.

Bailey, N.T.J. (1959), Statistical methods in Biology. The Universities Press Ltd. (London) 1959.

Baldini, M., Costea, N., and Dameshek, W. (1960), Viability of stored human platelets. Blood 16: 1669.

Battacharya, D.K., and Stewart, J.W. (1964), The determination of the lifespan of the blood platelets by simultaneous use of two radioactive isotopes. Proc. Xth Cong. Int. Soc. Haematology, Stockholm, 1964.

Bedson, S.P. (1923), An enquiry into the genesis of the mammalian blood platelets. J. Path. Bact. 26: 145.

Bélanger, L.F. (1954), Autoradiographic visualisation of S^{35} incorporation and turnover by the mucous glands of the G.I. tract and other soft tissues of the rat and hamster. Anat. Rec. 118: 755.

Benhamou, E., and Pugliese, J. (1945), Les Plaquettes sanguines en suspensions concentrées. Compt. Rend. Soc. Biol. 139: 307.

Bettex-Galland, M., Luscher, E.F. (1959), Extraction of an actomyosin-like protein from human thrombocytes. Nature (Lond.) 184: 276.

Bizzozero, G. (1882), Über einen neuen Formbestandteil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung. Virchows Arch. path. Anat. 90: 261.

Blood Cells and Plasma Proteins - Their State in Nature (1953). Acad. Press. Inc. N.Y. Edited Tullis, J.L.

Bonnin, J.A. and Cheney, K. (1961), The PTF test: An improved method for the estimation of platelet thromboplastic function. Brit. J. Haemat. 7: 512.

Born, G.V.R. (1956), Adenosine triphosphate (A.T.P.) in blood platelets. Biochem. J. 62: 33P.

Brecher, G., and Cronkite, E.P. (1950), Morphology and enumeration of human blood platelets. J. Appl. Physiol. 3, 365.

Bremer, L. (1894), Ueber die Herkunft und Bedeutung der Blutplättchen. Centralbl. med. Wissensch. Berl. 32, 338.

Brown, W.H. (1913), The histogenesis of blood platelets. J. exper. Med. 18: 278.

Bunting, C.H. (1909), Blood platelet and megalokaryocyte reactions in the rabbit. J. exper. Med. 11: 541.

Castaldi, P.A. and Firkin, B.G. (1963), Studies of the life-span and fate of platelets. Aust. Ann. Med. 12: 333.

Chandler, A.B. (1958), In vitro thrombotic coagulation of the blood: A method for producing a thrombus. Lab. Invest. 7: 110.

Cohen, P., Gardner, F.H. and Barnett, G.O. (1961), Re-classification of the thrombocytopenias by the Cr^{51}-labeling method for measuring platelet lifespan. New Engl. J. Med. 264: 1294.

Coles, M. and Roman, W. (1957), A rapid method for estimating fibrinogen in plasma. J. Clin. Path. 10: 282.

Connor, W.E. (1962), The acceleration of thrombus formation by certain fatty acids. J. Clin. Invest. 41: 1199.

Connor, W.E. and Poole, J.C.F. (1961), The effect of fatty acids on the formation of thrombi. Quart. J. Exp. Physiol. 46: 1.

Coons, A.H. and Kaplan, M.H. (1950), Localization of Antigen in tissue celss. II. Improvements in a method for the detection of antigen by means of fluorescent antibody. J. Exper. Med. 91: 1.

Cronkite, E.P. (1958), Regulation of Platelet Production, in "Haemostatic Mechanism" - Brookhaven Symposium in Biology, No. 10, 96.

Cronkite, E.P., Bond, V.P., Robertson, J.S. and Paglia, D.E. (1957), The survival, distribution and apparent interaction with capillary endothelium of transfused radiosulfate-labeled platelets in rats. J. Clin. Invest. 36: 881.

Cronkite, E.P. and Brecher, G. (1954), The experimental therapy of the haemorrhagic phase of the radiation syndrome with platelet transfusions. Acta Radiol. Suppl. 116: 376.

Cunningham, G.M., McNichol, G.P. and Douglas, A.S. Effect of anticoagulant drugs in platelet aggregation in the Chandler tube. Lancet, 1: 729. (1965).

Danielli, J.F. (1940), Capillary permeability and oedema in the perfused frog. J. Physiol. (Lond), 98: 109.

Davey, M.G. and Lander, H. (1963), The labelling of human platelets with radiochromate. Aust. J. exp. Biol. 41: 581.

Davey, M.G. and Lander, H. (1964), The behaviour of infused human platelets during the first twentyfour hours after infusion. Brit. J. Haemat. 10: 94.

Davey, M.G., Lander, H. and Robson, H.N. (1964), A model for the analysis of platelet survival. Proc. Xth Cong. Int. Soc. Haemat. Stockholm, 1964.

David-Ferreira, J.F. (1964), The blood platelet: Electron microscope studies. In: International Review of Cytology (1964).

Dawbarn, R.Y., Earlam, F., Evans, W.H. (1928), The relation of the blood platelets to thrombosis after operation and parturition. J. Path. Bact. 31: 833.

Dennis, J.B. Philosph. Trans. Royal Soc. London 1: 617, 1665-1666; 2: 453 (1667); 3:710 (1668). Cited by E.P. Cronkite and D. Jackson in Progress in Haemat. Vol. II, 19, edited L.M. Tocantins.

Denys, J. (1887), Etude sur la coagulation de sang. La cellule, 3: 445.

Desai, R.G., Small, W., and Mednicoff, I. (1955), Studies on the survival and metabolic activity of platelets in humans utilising radioactive platelets. J. Clin. Invest. 34: 930.

Desforges, J.F., Bigelow, F.S. and Chalmers, T.C. (1954). The effect of massive gastrointestinal haemorrhage on haemostasis. I. The blood platelets. J. Lab. Clin. Med. 43: 501.

Dillard, G.H.L., Brecher, G., and Cronkite, E.P. (1951), Separation, Concentration and Transfusion of Platelets. Proc. Soc. exp. Biol. (N.Y.) 78: 796.

Djerassi, I., and Roy, A. (1963), A method for preservation of viable platelets: combined effects of sugars and dimethyl sulfoxide. Blood, 22: 703.

Doan, C.A. (1949), Hypersplenism. Bull. N.Y. Acad. Med. 25: 625.

Dominici, H. (1900) Arch. Med. Exp. 12: 733. Cited by L.M. Tocantins in Medicine 17, 155 (1938).

Donné, A. (1842). De l'origine des globules du sang, de leur mode de formation et leur fin. Compt. Rend. Acad. Sci. 14: 366.

Downey, H. (1913), The origin of blood platelets. Folia Haematol. 15: 25.

Duguid, J.B. (1946), Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. J. Path. Bact. 58: 207.

Duguid, J.B. (1948). Thrombosis as a factor in the pathogenesis of aortic atherosclerosis. J. Path. Bact. 60: 57.

Duke, W.W. (1910), The relation of blood platelets to haemorrhagic disease: Description of a method for determining the bleeding time and coagulation time and

report of three cases of haemorrhagic disease. J. Amer. Med. Ass. 55: 1185.

Duke, W.W. (1911), The rate of regeneration of blood platelets. J. exp. Med. 14: 265.

Dziewiatkowski, D.D. (1949), Rate of excretion of radioactive sulphur and its concentration in some tissues of the rat after intraperitoneal administration of labelled sodium sulfate. J. Biol. Chem. 178: 197.

Ebbe, S., Baldini, M. and Donovan, J. (1965). Comparative studies of platelet survival by different methods in the rabbit. Blood 25: 548.

Eberth, J.C. and Schimmelbusch, C. (1885), Experimentelle untersuchungen uber Thrombose. Fortschr. Med. 3: 379.

Engel, C.S. (1893) Zur Enstehung der Köperlichen Elementes des Blutes. Arch. Mikr. Anat. 42: 217. Cited by Tocantins L.M. Blood 3: 1073 (1948).

Evans, R.S. and Duane, R.T. (1949), Acquired haemolytic anaemia. I. The relation of erythrocyte antibody production to activity of the disease. II. The significance of thrombocytopenia and leucopenia. Blood 4: 1196.

Filshie, I. and Scott, G.B.D. (1958), The organisation of experimental venous thrombi. J. Path. Bact. 76: 71.

Finch, C.A. (1960), Thrombokinetics. In "Blood Platelets" Henry Ford Hospital International Symposium (1960), Little, Brown & Co., Boston, p. 629 (1961).

Firket, J. (1922), Recherches sur la régéneration des plaquettes dans l'intoxication par la saponine et après defibrination du sang. Arch. Biol. (Paris), 32: 539.

Firkin, B.G. (1963), The Platelet. Aust. Ann. Med. 12: 261.

Fisher, J.A. (1947), The cryptogenic acquired haemolytic anaemias. Quart. J. Med. 16: 245.

Foa, P., and Salvioli, G. (1880), Sull' origine dei globuli rossi del sangue. Arch. p. Sci. Med. 4: 1.

Fonio, A. (1936), Über fractionierte Bluttransfusion. Schweiz. Med. Wchnschr. 14: 338.

Frank, E. (1915), Die essentielle Thrombopenie (Konstitutionelle Purpura, Pseudo-Hämophilie). I. Klinisches Bild. Berl. klin. Wchnschr. 52: 454.

Frank, E. (1915), Die essentielle thrombopenie (Konstitutionelle Purpura, Pseudo-Hämophilie). II. Pathogenese. Berl. klin. Wchnschr. 52, 490.

Gaardner, A., Johnsen, J., Laland, S., Hellem, A. and Owren, P.A. (1961), Adenosine diphosphate in red cells as a factor in the adhesiveness of human blood platelets. Nature (Lond.), 192: 531.

Gardner, F.H., Howell, D. and Hirsch, E.D. (1954), Platelet transfusions utilising plastic equipment. J. Lab. Clin. Med. 43: 196.

Gilbert, J.B. and Mustard, J.F. (1963), Effects of Atromid on platelet economy and blood coagulation. J. Atheroscl. Res. 3: 623.

Gray, S.J. and Sterling, K. (1950). The tagging of red cells and plasma protein with radioactive chromium. J. Clin. Invest. 29: 1604.

Grossman, C.M., Kohn, R. and Koch, R. (1963), Possible errors in the use of P^{32}-orthophosphate for the estimation of platelet lifespan. Blood, 22: 9.

Hand, R.A. and Chandler, A.B. (1962), Atherosclerotic metamorphosis of autologous pulmonary thrombo-emboli in the rabbit. Amer. J. Path. 40: 469.

Harrington, W.J., Hollingsworth, J.W., Minnich, V. and Moore, C.V. (1951), Demonstration of a thrombocytopenic factor in the blood of patients with idiopathic thrombocytopenic purpura. J. Clin. Invest. 30: 646.

Harrington, W.J., Minnich, V., Hollingsworth and Moore, C.V. (1951), Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura. J. Lab. Clin. Med. 38: 1.

Harris, I.M., McAlister, J.M. and Prankherd, T.A.J. (1957), The relationship of abnormal red cells to the normal spleen. Clin. Sci. 16: 223.

Haust, M.D., More, R.H. and Movat, H.Z. (1959), The mechanism of fibrosis in arteriosclerosis. Amer. J. Path. 35: 265.

Hayem, G. (1878), Recherches sur l'evolution des hématies dans le sang de l'homme et des vertebrates. Arch. de Phys. norm et Path. 5: 692.

Hayem, G. (1882), Comp. Rend. Acad. Sci. 94: 200. Cited by L.M. Tocantins (1948) Blood 3: 1073.

Hayem, G. (1882), Sur la mecanisme de l'arrête des hemorrhagies. Comp. Rend. Acad. Sci. 95: 18. Cited by L.M. Tocantins (1948) Blood 3: 1073.

Hayem, G. (1895), Du Purpura. Presse Med. 3: 233.

Hellem, A.J. (1960), The adhesiveness of human blood platelets in vitro. Scand. J. Lab. Invest. 12: suppl. 51.

Hempelmann, L.H., Lisco, H. and Hoffman, J.G. (1952), The acute radiation syndrome: A study of nine cases and a review of the problem. Ann. Intern. Med. 36: 279.

Heysell, R.M. (1961), Determination of human platelet survival utilizing C^{14}-labelled serotonin. J. Clin. Invest. 40: 2134.

Hirsch, E.O., Favre-Gilly, J. and Dameshek, W. (1950), Thrombopathic thrombocytopenia: Successful transfusion of blood platelets. Blood 5: 568.

Hirsch, E.O. and Gardner, F.H. (1951). The lifespan of transfused human platelets. J. Clin. Invest. 30: 649.

Hirsch, E.O., Gardner, F.H. and Thomas, E.D. (1952), Isolation and concentration of human blood platelets: Their properties in vitro and in vivo. J. Clin. Invest. 31: 638.

Hjort, P.F. and Paputchis, H. (1960), Platelet lifespan in normal, splenectomised and hypersplenic rats. Blood 15: 45.

Horlick, L. (1961), Platelet adhesiveness in normal persons and subjects with atherosclerosis. (Effect ofhigh fat meals and anticoagulants on the adhesive index.) Amer. J. Cardiol. 8: 459.

Howell, W.H. (1890), Observations upon the occurrence, structure and function of the giant cells of the marrow. J. Morphol. 4: 177.

Huek, H. (1926). Munch. med. Wschr. 73: 173. Cited by Payling Wright, J. Path. Bact. 54: 461 (1942).

Hughes, F.B. and Brodie, B.B. (1959), The mechanism of serotonin and catecholamine uptake by platelets. J. Pharmacol. Exper. Therap. 127: 96.

Humphrey, J.H. and Jaques, R. (1954), Histamine and serotonin content of platelets and polymorphonuclear leucocytes of various species. J. Physiol. 124: 305.

Humphrey, J.H. (1955), Origin of blood platelets. Nature 176: 38.

Hurn, M., Barker, N.W. and Mann, F.D. (1947), Variations in prothrombin and antithrombin in patients with thrombosing tendencies. Amer. J. Clin. Path., 17: 709.

Hustin, A. (1914), Principe d'un nouvelle méthod de transfusion muqueuse. J. Méd. Brux. 12: 436.

Jacob, H.S. and Jandl, J.H. (1962), Effects of sulphydryl inhibition on red blood cells. II. Studies in vivo. J. Clin. Invest. 41: 1514.

Jacques, L.B., Fidlar, E., Feldsted, E.T. and MacDonald, A.G. (1946). Silicones and blood coagulation. Canad. Med. Ass. J. 55: 26.

Jammet, H., Mathé, G., Pendic, J.-F., Duplan, J.-F., Maupin, B., Latarjet, R. and Kalic, B. (1959). Étude de six cas d'irradiation totale aiguë accidentelle. Rev. Franc ét. clin. et biol. 4: 210.

Julliard, J., Maupin, B., Chary, R., Theilleux, R., Nau, P. and Loverdo, A. (1952), Transfusion au lapin de leucocytes et de plaquettes du sang humain après marquage par le radiophosphore. Comp. Rend. Soc. Biol. 146: 211.

Julliard, J., Maupin, B., Loverdo, Bernard, J. Colvez, P. and Lecomte, M. (1952), Premiers essais de transfusion à l'homme de leucocytes et de plaquettes marqués au radio-phosphore. Presse Méd. 60: 518.

Kaznelson, P. (1916), Versch winden der hämorrhagischen Diathese bei einem Falle von "essentieller Thrombopenie" (Frank) nach Milzexsterpation. Splenogene thrombolytische Purpura. Wein. klin. Wschr. 29: 1451.

Kinosita, R. and Ohno, S. (1960), Biodynamics of Thrombopoiesis. In "Blood Platelets", Henry Ford Hospital International Symposium (1960). Little, Brown & Co. Boston, 1961, p. 611.

Kimber, R.J., Lander, H. and Robson, H.N. (1965), The sequestration of NEM-treated red cells in normal and abnormal subjects: A test of splenic uptake function. J. Lab. Clin. Med. 65: 951.

Kissmeyer-Nielsen, F. and Madsen, C.B. (1961), Platelets in blood stored in untreated and siliconed glass bottles and plastic bags. II. Survival studies. J. Clin. Path. 14: 630.

Klein, E., Toch, R., Farber, S., Freeman, G. and Fiorentino, R. (1956), Haemostasis in thrombocytopenic bleeding following infusion of stored, frozen platelets. Blood, 11, 693.

Krasso, H. (1927), Über den Wert und die Wirkungsweise der Bluttransfusion bei der thrombopenischen Purpura. Wein. Arch. Inn. Med. 14: 377.

Krauss, E. (1883), Ueber Purpura. C. Winter, Heidelberg, 1883.

Krevans, J.R. and Jackson, D.P. (1955), Haemorrhagic disorder following massive whole blood transfusions. J. Amer. Med. Ass. 159: 171.

Kusunoki, M. (1914), Lipoidsubstanzen in der Milz und im Leichenblut. Beitr. path. Anat. all. Path. 59: 564.

Laker, C. (1889), Die Blutscheibchen sind constante Formelemente des normal circulinrenden Säugethier-blutes. Virchows Arch. 116: 28.

Lander, H., Davey, M.G. and Rogers, G. and Lloyd, J.V. (1965), Unpublished observations.

Lathja, L.G., Ellis, F. and Oliver, R. (1953), Isotope uptake of individual cells: Uptake of S^{35} sulphate by human bone marrow cells in vitro. Brit. J. Cancer, 7: 401.

Lawrence, J.S. and Valentine, W.N. (1947), The blood platelets. The rate of their utilization in the cat. Blood, 2: 40.

Lawrence, J.S., Valentine, W.N. and Adams, W.S. (1948), Thrombopenic purpura: The failure of direct blood transfusion to raise the platelet level. J. Lab. Clin. Med. 33: 1077.

Leeksma, C.H.W. and Cohen, J.A. (1955), Determination of the lifespan of human blood platelets using labelled diisopropyl-fluorophosphonate. Nature 175: 552.

Leeksma, C.H.W. and Cohen, J.A. (1956), Determination of the lifespan of human blood platelets using labelled di-isopropylfluorophosphonate. J. Clin. Invest. 35: 964.

Lemoine, G. (1911), Rôle de la cholésterine dans le developpement de l'arteriosclerose. Vigot, Paris, 1911.

Lewis, J.H. and Szeto, I.L. (1961), Survival of Cr^{51} labelled platelets in thrombocytopenia. Proc. 7th Congr. Europ. Soc. Haemat., London, 1959, p. 694. S. Karger, Basel, 1961.

Lewisohn, R. (1915), A new and greatly simplified method of blood transfusion. Med. Rec. 87: 141.

Linman, J.W. (1962), Factors controlling haemopoiesis: Thrombopoietic and leukopoietic effects of 'anaemic' plasma. J. Lab. Clin. Med. 59: 262.

Lobstein, J, -G, -C, -F, -M. (1829), Traités d'anatomie pathologique, Levrault, Paris. Cited by H. Kaunitz Nature 192:9 (1961)

Losner, S. and Volk, B.W. (1956), Fibrinogen concentration in various clinical conditions. Amer. J. Med. Sci. 232: 276.

Lower, R. (1665), Philosoph. Trans. Royal Soc. London, 1: 128 and 352 (1665) cited by E.P. Cronkite and D. Jackson in Progress in Haematology, 2: 19. Ed. L.M. Tocantins.

Lowit, M. (1889), Ueber die Präexistenz der Blutplättchen und die Zahl der weissen Blutkörperchen im normalen Blute des Menschen. Virchows Arch. Path. Anat. 117: 545.

McDonald, L. and Edgill, M. (1957), Coagulability of blood in ischaemic heart disease. Lancet 2: 457.

McDonald, L. and Edgill, M. (1959), Changes in the coagulability of the blood during various phases of ischaemic heart disease. Lancet 1:1115.

Marsh, G.W. (1964), Intravascular haemolytic anaemia after aortic-valve replacement. Lancet 2: 986.

Martelli, C. (1915), Su la genesi ed importanza delle piastrini Pathologica, 7: 77 (1915). Cited by G.-F. Saltzman, Acta med. Scand., Suppl. 221, 1948.

Mathé, G., Jammet, H., Pendic, B., Schwarzenberg, L., Duplan, J.-F., Maupin, B., Latarjet, R., Larrieu, M.-J., Kalic, D. and Djukic, Z. (1959), Transfusions et greffes de moelle osseuse homologue chez des humains irradiés à haute dose accidentellement. Rev. Franc Ét. Clin. et Biol. 4: 226.

Maupin, B. (1956), Indications des dérivés du sang dans les affections de la série plaquettaire. Sang, 27: 29.

Maupin, B. and Loverdo, A. (1959), Contrôle de la stabilité du marquage in vitro des leucocytes et des plaquettes sanguines par le P^{32}, le Cr^{51} et l'Au^{198} colloidal. Rev. Franc Études Clin et Biol. 4: 173.

Merskey, C., Gordon, H. and Lackner, H. (1960), Blood coagulation and fibrinolysis in relation to coronary heart disease. A comparative study of normal white men, white men with overt coronary heart disease and normal Bantu men. Brit. Med. J. 1: 219.

Meyers, L. (1948), Blood fibrinogen in myocardial infarction. Arch. intern. Med. 82: 419.

Minor, A.H. and Burnett, L. (1952), A method for separating and concentrating platelets from normal human blood. Blood 7: 693.

Monkhouse, F.C. (1960), Blood coagulation and lipid metabolism. Amer. J. Clin. Nutrition 8: 1.

More, R.H. and Haust, M.D. (1961), Atherogenesis and plasma constituents. Amer. J. Path. 38: 527.

Morgagni, G.B. (1728). Epist. Anat. 27 (Lugdum Bathavorum Apud Joannim á Kerkhem 1728). Cited by H. Kaunitz, Nature 192: 9 (1961).

Morgan, M., Keating, R.P. and Reisner, E.H. (1954), Labelling of rabbit platelets with I^{131} (1954). Proc. Soc. Exp. Biol. Med. 85: 420.

Morgan, M.C., Keating, R.P. and Reisner, E.H. (1955), Survival of radiochromate labelled platelets in rabbits. J. Lab. Clin. Med. 46: 521.

Morgan, M.C. and Szafir, J.J. (1961), Separation of platelets from whole blood by the use of silicone liquids. Blood 18: 89.

Mueller, J.F. (1953), Pathologic physiology of mammalian blood platelets utilizing P^{32} tagged rabbit platelets. Proc. Soc. Exper. Biol. Med. 83: 551.

Murphy, E.A. and Mustard, J.F. (1961), Platelet economy during moderate and intensive dicoumerol therapy. Lancet 2: 960.

Murphy, E.A. and Mustard, J.F. (1961), Dicoumerol therapy and platelet turnover. Circulation Res. 9: 402.

Murphy, E.A. and Mustard, J.F. (1962), Coagulation tests and platelet economy in atherosclerotic and control subjects. Circulation 25:114.

Murray, G. (1956), Homologous aortic valve segment transplants as surgical treatment for aortic and mitral insufficiency. Angiology 7: 466.

Mustard, J.F. (1956), Platelets in stored blood. Brit. J. Haemat. 2: 17.

Mustard, J.F. (1957), Changes in the platelet levels of non transfused patients following surgical operations. Acta Haemat. 17: 257.

Mustard, J.F. (1957), The effect of stored blood transfusions on the platelet levels in patients undergoing surgical procedures. Acta Haemat. 18: 80.

Mustard, J.F. and Murphy, E.A. (1962), Effect of different dietary fats on blood coagulation, platelet economy and blood lipids. Brit. Med. J. 1: 1651.

Mustard, J.F., Murphy, E.A., Rowsell, H.C. and Downie, H.G. (1962), Factors influencing thrombus formation in vivo. Amer. J. Med. 33: 621.

Mustard, J.F. and Murphy, E.A. (1963a), Blood platelet economy during moderate and intensive heparin therapy. Blood 22: 1.

Mustard, J.F. and Murphy, E.A. (1963b), Effect of smoking on blood coagulation and platelet survival in man. Brit. Med. J. 1: 846.

Mustard, J.F., Murphy, E.A., Ogryzlo, M.A. and Smythe, H.A. (1963), Blood coagulation and platelet economy in subjects with primary gout. Canad. Med. Ass. J. 89: 1207.

Najean, Y., Ardaillou, N., Caen, J., Larrieu, M.-J., and Bernard, J. (1963), Survival of radiochromium-labelled platelets in thrombocytopenias. Blood 22: 718.

Najean, Y., Larrieu, M.-J., and Bernard, J. (1959), Technique d'étude de la survie des plaquettes par la méthode de marquage au Radio-chrome. Rev. Franc. Études Clin. et Biol. 4: 1071.

Neill, C.A., Forman, E.N. Sigler, A.T. and Bahnson, H.T. (1963), Hemolytic anaemia following surgical repair of endocardial cushion defects. - Presented at 31st Annual Meeting of Society for Pediatric Research Program, Atlantic City, New Jersey, 1961. Cited by Verdon et alii, New Engl. J. Med. 269.

O'Brien, J.R. (1962), Platelet aggregation II. Some results from a new method of study. J. Clin. Path. 15: 452.

Odell, T.T. (1956), Production, lifespan, and fate of blood platelets: Studies with radioisotope labelling techniques. In Proc. VIth Congr. Int. Soc. Haemat. (Boston). Grune & Stratton, N.Y. (1958), p. 294.

Odell, T.T. Jr., and Anderson, B. (1957), Isolation of a sulfated mucopolysaccharide from blood platelets of rats. Proc. Soc. Exp. Biol. Med. 94: 151.

Odell, T.T., Gamble, F.N. and Furth, J. (1953), Lifespan of naturally labelled platelets of rats. Fed. Proc. 12: 398.

Odell, T.T., Tauche, F.G. and Gude, W.D. (1955), Uptake of radioactive sulphate by elements of the blood and bone marrow of rats. Amer. J. Physiol. 180: 491.

O'Neill, B. and Firkin, B. (1964), Platelet survival studies in coagulation disorders, thrombocythemia and conditions associated with atherosclerosis. J. Lab. Clin. Med. 64: 188.

Osler, W. (1883), The third corpuscle of the blood. Med. News, 43: 701.

Ozge, A.H. and Mustard, J.F. (1962), The effect of repeated adrenaline injection on blood coagulation and platelet economy in rabbits. In Proc. IXth Congr. Int. Soc. Haemat. Part II, p. 35 (1964).

Paré, A. (1575), Cited by Long, E.R. in "Arteriosclerosis" (1933) ed. E.V. Cowdry, p. 28. Macmillan, N.Y.

Parker-Williams, E.J., Cohen, P., Watrouse, P. and Gardner, F.H. (1963), Platelet preservation studies using platelets labelled with C^{14}-5-hydroxytryptamine. J. Clin. Invest. 42: 963.

Payling Wright, H. (1942), Changes in the adhesiveness of blood platelets following parturition and surgical operations. J. Path. Bact.54: 461.

Penny, R., Rozenberg, M.D. and Firkin, B.G. (1965), The effect of incubation of normal platelets with plasma from patients with idiopathic thrombocytopenic purpura on the platelet's lifespan. Aust. Ann. Med. 14, 214.

Pirofsky, B., Sutherland, D.W., Starr, A. and Griswold, H.E.

(1965), Hemolytic anemia complicating aortic valve surgery - an autoimmune syndrome. New Engl. J. Med. 272: 235.

Pollycove, M., Dalsanto, G. and Lawrence, J.H. (1958), Simultaneous measurement of erythrocyte, leukocyte and platelet survival in normal subjects with DFP^{32}. Clin. Res. Proc. 6: 45.

Poole, J.C.F. (1959), A study of artificial thrombi produced by a modification of Chandler's method. Quart. J. Exper. Physiol.44: 377.

Poole, J.C.F. (1960a), The effect of anticoagulants on artificial thrombus production. Proc. VIIth Congr. Europ. Soc. Haemat. (1959), Part II, p. 646.

Poole, J.C.F. (1960b). In "Pathogenesis and treatment of occlusive arterial disease." Ed. L. McDonald, Pitman (London), 1960, p. 40.

Ranvier, L. (1873), Du mode de formation de la fibrine dans le sang extrait des vaisseaux. Comp. Rend. Soc. Biol. 5: 46 (1873).

Reisner, E.H., Keating, R.P., Friesen, C. and Loeffler, E. (1956), Survival of sodium $chromate^{51}$ labelled platelets in animals and man. Proc. VIth Congr. Int. Soc. Haemat. (1956) Boston, Grune & Stratton, N.Y. 1958, p. 292.

Reiss, L. (1872). Arch. Anat. Phys.237. Cited by A.H.T. Robb-Smith, Brit. Med. Bull. 11: 70 (1955).

Robertson, J.S., Milne, M.L. and Cohn, S.H. (1954), Labelling and tracing rat blood platelets with chromium^{51}. Radioisotope Conference 1954, 1: 205. Butterworth Scientific Press, London, 1954.

Robson, H.N. and Duthie, J.J.R. (1950), Capillary resistance and adrenocortical activity. Brit. Med. J. 2: 971.

Rokitansky, C. Handbuch der Pathol Ogischen Anatomie. Braunmüller und Seidel, Vienna (1841 - 1846).

Rose, J.C., Hufnagel, C.A., Freis, E.D., Harvey, W.P. and Partenope, E.A. (1954), The hemodynamic alterations produced by a plastic valvular prosthesis for severe aortic insufficiency in man. J. Clin. Invest. 33: 891.

Roskam, J. (1924), Les globulins (plaquettes de Bizzozero) contiennent - ils des ferments protéolytiques ou lipolytiques? Comp. Rend. Soc. Biol. 91: 373.

Roy, A.J., Djerassi, I., Neitlich, H. and Farber, S. (1962), Effects of Escherichia coli endotoxin on rat platelets. Amer. J. Physiol. 203: 296.

Sayed, H.M., Dacie, J.V., Handley, D.A., Lewis, S.M. and Cleland, W.P. (1961), Haemolytic anaemia of mechanical origin after open heart surgery. Thorax 16: 356.

Schmidt, A. (1882), Recherches sur le rôle physiologique et pathologique des leucocytes du sang. Arch. de Physiol. Nom. et Path. 9: 513.

Schultze, M. (1865). Arch. Mikr. Anat. 1: 36, 1865. Cited by A.H.T. Robb Smith, Brit. Med. Bull. 11: 70, 1955.

Shouse, S.S., Warren, S.L., Whipple, G.H. (1931), Aplasia of marrow and fatal intoxication in dogs produced by roentgen radiation of all bones. J. Exper. Med. 53, 421.

Sigler, A.T., Forman, E.N., Zinkham, W.H. and Neill, C.A. (1963), Severe intravascular hemolysis following surgical repair of endocardial cushion defects. Amer. J. Med. 35: 467.

Simnitsky, V. (1903), Z. Heilkunde, 24: 79. Cited by H. Kaunitz, Nature 192: 9, (1961).

Smith. C., Robinson, M. and Tyson, R. (1938), The oxydase reactions as applied to the mega karyocyte and blood platelet of the rat. Anat. Rec. 70: 139.

Spittel, J.A., Pascuzzi, C.A., Thompson, J.H. and Owren, C.A. (1960), Acceleration of early stages of coagulation in certain patients with occlusive arterial or venous diseases: use of a modified thromboplastin generation test to evaluate clot acceleration. Proc. Staff Meetings Mayo Clinic, 35: 37.

Starr, A. (1960), Total mitral valve replacement: Fixation and thrombosis. Surgical Forum, 11: 258.

Starr, A. and Edwards, M.L. (1961), Mitral replacement: Clinical experience with a ball-valve prosthesis. Ann. Surg. 154: 726.

Starr, A. and Edwards, M.L. (1961), Mitral replacement: The shielded ball valve prosthesis. J. Thoracic Cardiovasc. Surg. 42: 673.

Starr, A., Edwards, M.L. and Griswold, H. (1962), Mitral replacement: Late results with a ball valve prosthesis. Progress in Cardiovasc. Dis. 5: 298.

Stefanini, M., and Chatterjea, J.B. (1951), Rate of platelet survival in thrombocytopenia. J. Clin. Invest. 30: 676.

Stefanini, M., Chatterjea, J.B., Dameshek, W., Zannos, L. and Santiago, E.P. (1952), Studies on platelets II. The effect of transfusion of platelet-rich polycythaemic blood on the platelets and haemostatic function in 'idiopathic' and 'secondary' thrombocytopenic purpura. Blood 7: 53.

Stefanini, M., Dameshek, W. and Adelson, E. (1952), Platelets VII. Shortened 'platelet survival time' and development of platelet agglutinins following multiple platelet transfusions. Proc. Soc. Exp. Biol. N.Y. 80: 230.

Steinberg, B., Deitz, A.A. and Martin, R.A. (1959), Mechanism of haematopoiesis: Haemacytopoietic factors in human plasma. Acta Haemat. (Basel) 21: 78.

Stevenson, T.D. and Baker, H.J. (1964), Haemolytic anaemia following insertion of Starr-Edwards valve prosthesis. Lancet 2: 982.

Stohlman, F., Sarnoff, S.J., Case, R.B. and Ness, A.T. (1956), Hemolytic syndrome following the insertion of a Lucite ball valve prosthesis into the cardiovascular system. Circulation 13: 586.

Strong, J.P., McGill, H.M., Jejada, C. and Holman, R.L. (1958), The natural history of atherosclerosis. Comparison of the early aortic lesions in New Orleans, Guatemala and Costa Rica. Amer. J. Path. 34: 731.

Tobin, J.R. and Friedman, I.A. (1960), Platelet transfusion with use of blood in plastic bags from routine storage. J. Amer. Med. Ass. 172: 50.

Tocantins, L.M. (1936), Experimental thrombopenic purpura: Cytological and physical changes in the blood. Ann. Intern. Med. 9: 838.

Tocantins, L.M. (1938), The mammalian blood platelet in health and disease. Medicine 17: 155.

Tocantins, L.M. (1948), Historical notes on blood platelets. Blood 3: 1073.

Troup, S.B., Reed, C.F., Marinetti, G.C. and Swisher, S.N. (1960), The platelet lipids: Their identification, quantification and behaviour in clotting systems in vitro. In "Blood Platelets", Henry Ford Hospital International Symposium 1960. Little, Brown & Co. Boston (1961) p. 265.

Tullis, J.L., Surgenor, D.M. and Baudanza, P. (1959), Preserved platelets: their preparation, storage and clinical use. Blood 14: 456.

Verdon, T.A., Forrester, R.H. and Crosby, W.H. (1963), Hemolytic anaemia after open-heart repair of ostium-primum defects. New Engl. J. Med. 269: 444.

Visek, W.J., Whitney, I.B., Kuhn, U.S.G. and Comar, C.L. (1953), Metabolism of Cr^{51} by animals as influenced by the chemical state. Proc. Soc. Exper. Biol. Med. 84: 610.

Vodopick, H.A. and Knisley, R.M. (1963), $Sulphur^{35}$ studies in man: Platelet survival and plasma and urinary radio-activity assayed by beta liquid scintillation spectrometry. J. Lab. Clin. Med. 62: 109.

Wagner, H.N., Razzak, M.A., Gaertener, R.A., Caine, W.P. and Feagin, O.T. (1962), Removal of erythrocytes from the circulation. Arch. Intern. Med. 110: 90.

Warren, R., Amdur, M.O., Belko, J.S. and Baker, D.V. (1950), Postoperative alterations in the coagulation mechanism of the blood. Observations on circulating thromboplastin. Arch. Surg. 61: 419.

Warren, R., Lauridsen, J. and Belko, J.S. (1953), Alterations in numbers of circulating platelets following surgical operation and the administration of adrenocorticotrophic hormone. Circulation 7: 481.

Weigert, C. (1887), Bemerkungen über den weissen Thrombus (Zahn). Fortschr. Med. 5: 193.

Weissbach, H. and Redfield, B.G. (1960), Studies on the uptake of serotonin by platelets. In "Blood Platelets", Henry Ford Hospital International Symposium (1960), Little, Brown & Co. Boston, p. 393, 1961.

Wlassow, K. (1894), Untersuchungen über die histologischen Vorgänge bei der Gerinnung und Thrombose mit besonderer Berücksichtlgung der Enstehung der Blutplättchen. Beitr. z. path. Anat. Path. 15: 543.

Wolpers, C. and Ruska, H. (1939), Strukturunkersuchungen zur Blutgerinnung. Klin. Wschr. 18: 1077.

Woods, M.C., Gamble, F.N., Furth, J. and Bigelow, R.R. (1953), Control of the post irradiation haemorrhagic state by platelet transfusions. Blood 8: 545.

Wright, J.H. (1906), The origin and nature of the blood plates. Boston Med. & Surg. J. 154: 643.

Wright, J.H. (1910), The histogenesis of the blood platelets. J. Morphology, 21: 263.

Wright, J.H. and Minot, G.R. (1917), The viscous metamorphosis of the blood platelets. J. Exper. Med. 26: 395.

Zlatkis, A., Zak, B. and Boyle, A.J. (1953), A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 41: 486.

Zucker, M.B. and Borelli, J. (1962), Platelet clumping produced by connective tissue suspension and by collagen. Proc. Soc. Exper. Biol. Med. 109: 779.

Zucker, M.B., Ley, A.B. and Mayer, K. (1960), Studies on platelet lifespan and platelet depots by use of DFP^{32}. J. Lab. Clin. Med. 58: 405.

Reprinted from the BRITISH MEDICAL JOURNAL
13 *March* 1965, 1, 688–689

Reduced Platelet Survival in Patients with Starr–Edwards Prostheses*

HARRY LANDER,† M.R.A.C.P.
RAELENE L. KINLOUGH,‡ M.B., B.S.
H. N. ROBSON,§ F.R.C.P., F.R.A.C.P.

It is well recognized that patients with valvular disease of the heart are particularly susceptible to the complications of thrombosis and embolism, especially if the mitral orifice is stenosed. In recent years valvular repair by a variety of prosthetic procedures allowed the hope that such complications might be avoided. Unfortunately, thrombosis and embolism have continued to occur, often at a long interval after successful operations of this type. The finding of thrombi attached or in close proximity to artificial prostheses has led to the continued modification of such prostheses and operative techniques and to the extensive use of long-term anticoagulant therapy postoperatively (*Lancet*, 1962).

As many such thrombi have been composed mainly of platelets and fibrin, we have investigated the behaviour and survival of radiochromate-labelled platelets in patients who have undergone successful valve replacement with Starr-Edwards ball-valve prostheses (Starr and Edwards, 1961 ; Starr *et al.*, 1962).

Subjects Studied and Methods

Eight studies have been carried out in seven subjects. Two were males and five females. Their ages ranged from 24 to 64 years. In each instance the operation was performed by Mr.

* Supported by a grant-in-aid (G251/43) from the National Heart Foundation of Australia.
† Reader in Medicine, Department of Medicine, University of Adelaide, South Australia.
‡ Research Assistant, National Heart Foundation of Australia.
§ Professor of Medicine, Department of Medicine, University of Adelaide, South Australia.

H. D'Arcy Sutherland with the assistance of members of the Cardiac Surgery Unit of the Royal Adelaide Hospital. In five subjects the mitral valve had been replaced and in two the aortic. All had been considerably improved by operation, and none was in cardiac failure. One subject (R. J.) was studied twice. Details relating to each subject are included in the Table.

Platelets from each subject were labelled with 70–100 microcuries of radiochromate *in vitro* and reinfused into the donor, in whom their behaviour, survival, and distribution in the body were studied.

Details of the procedure employed have been described previously (Davey and Lander, 1963), except that the blood from which the platelets were separated was collected in the more acid solution of acid-citrate-dextrose (A.C.D. "S") suggested by Aster and Jandl (1964), instead of in a solution of ethylenediaminetetra-acetic acid (E.D.T.A.).

Platelet radioactivity was measured in samples of blood collected at frequent intervals for four hours following infusion of the labelled platelets and at daily intervals for at least 10 days. In addition, activity over the heart, lungs, liver, spleen, and sacrum was determined daily by external scintillation counting (Lander and Davey, 1964a). Platelet counts were performed on all samples of blood by the method of Brecher and Cronkite (1950).

Results

In all subjects infusion of the labelled platelets was followed by the normal phenomenon of transient "segregation" and subsequent re-entry into circulation of a proportion of the labelled platelets (Davey and Lander, 1964). However, as found by Aster and Jandl (1964), the degree of segregation is less when A.C.D. "S" is used as anticoagulant than when E.D.T.A. is used.

The survival curves obtained in the eight studies are illustrated in the Figure.

All points in the survival curves of subjects J. K., E. K., and S. R. fell within the curvilinear range found in normal subjects. All three subjects were receiving phenindione in doses adequate to maintain their prothrombin index between 10 and 25% of normal.

Subject R. J. had been placed on phenindione therapy 10 days after operation, and this was discontinued after 34 weeks. Some six weeks later he presented with a sudden onset of aphasia and a mild right hemiparesis. A platelet-survival study was begun six days after this episode. After its completion, phenindione therapy was recommenced and a second survival

Data Relating to Patients Studied

Subject	Sex	Age	Valve Replaced	Time After Operation (Weeks)	Anticoagulant	Thrombosis or Embolism	Platelet Count (per c.mm.) Mean and Range	Platelet Survival
J.K.	F	49	Mitral	25	Phenindione	Cerebral; pre-operative and early post-operative	313,000 (207,000–369,000)	Normal
E.K.	F	59	Mitral	29	Phenindione		232,000 (183,000–345,000)	Normal
S.R.	F	24	Mitral	32	Phenindione		240,000 (180,000–327,000)	Norma
R.J.	M	64	Aortic	45	Nil	Cerebral; post-operative*	230,000 (168,000–294,000)	Normal
				48	Phenindione		251,000 (207,000–324,000)	Normal
S.M.	F	51	Aortic	46	Nil		214,000 (180,000–273,000)	Reduced
R.L.	M	37	Mitral	46	Phenindione	Cerebral; pre-operative*	254,000 (207,000–360,000)	Reduced
P.B.	F	52	Mitral	52	Phenindione		244,000 (180,000–360,000)	Reduced

* See text.

study was undertaken. On both occasions the platelet-survival pattern fell within normal limits.

In subjects S. M., R. L., and P. B. the platelet half-life was reduced, but in each instance platelet radioactivity persisted in circulation for the usual period of 9 to 11 days.

External scintillation counting revealed a more marked uptake of radiocativity in the spleen and to a less extent in the liver in those subjects with reduced survivals than in normal persons or in those with valvular prostheses who manifested a normal survival pattern.

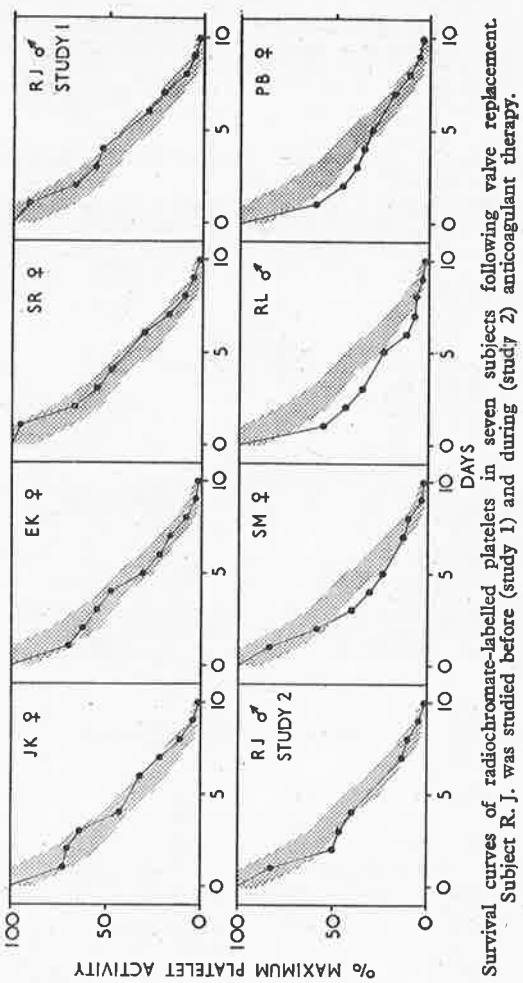
Discussion

The pattern of the survival curves in subjects S. M., R. L., and P. B. suggests that, although many of the labelled platelets survived for their natural life-span of 9 to 11 days, a considerable number of platelets were being prematurely removed from circulation in a random fashion (Lander and Davey, 1964b ; Davey, Lander, and Robson, 1964). This increased platelet loss was presumably matched by increased production, for none of the patients was thrombocytopenic. There was no suggestion that these abnormal findings were related either to replacement of a particular valve or to the administration of phenindione. However, it is interesting to note that abnormal results were obtained in those subjects in whom prostheses had been in place for the longest period (see Table).

The premature removal of platelets in these subjects could be due to their being consumed in thrombus formation, for similar survival curves have been observed in association with intravascular thrombosis (Adelson, Rheingold, Parker, Buenaventura, and Crosby, 1961 ; Firkin, 1963 ; O'Neill and Firkin, 1964). It is possible that in these three individuals the abnormal platelet-survival patterns are an indication that continuing platelet deposition and thrombus formation are occurring in the region of the prosthesis.

It could also be postulated that platelets are being "damaged" in circulation by the Starr-Edwards valve. Although no morphological abnormality of the subjects' platelets or red cells was observed in these studies, external scintillation counting showed that much more radioactivity accumulated in the spleen (and liver) of those cases with reduced survivals than in normal subjects or in those with valve prostheses and normal survivals. This could suggest that in the affected subjects a proportion of their platelets are being damaged in circulation and then sequestered in reticuloendothelial tissue.

However, since such "damaged" platelets might well be more liable to local deposition as well as to reticuloendothelial



Survival curves of radiochromate-labelled platelets in seven subjects following valve replacement. Subject R. J. was studied before (study 1) and during (study 2) anticoagulant therapy.

sequestration, both these mechanisms of removal could be operating simultaneously.

The significance of this finding of an abnormal platelet situation in some individuals, present long after apparently successful valve replacement, would seem to merit further examination.

Summary

Radiochromate-labelled platelet-survival studies were carried out on eight occasions in seven subjects following successful insertion of Starr-Edwards prostheses.

Abnormal survival, suggestive of increased random loss of a proportion of circulating platelets, was found in three subjects.

The possible significance of these findings in relation to post-operative thrombosis and embolism is discussed.

Our thanks are due to those physicians and surgeons of the Cardiac Investigation and Surgery Unit of the Royal Adelaide Hospital who allowed us to study patients under their care, and in particular to Mr. H. D'Arcy Sutherland and Dr. J. L. Waddy for the assistance and encouragement they gave us. We wish to thank Mrs. D. Pyle and Miss J. Devaney for technical and secretarial assistance.

References

Adelson, E., Rheingold, J. J., Parker, O., Buenaventura, A., and Crosby, W. H. (1961). *Blood*, 17, 267.
Aster, R. H., and Jandl, J. H. (1964). *J. clin. Invest.*, 43, 843.
Brecher, G., and Cronkite, E. P. (1950). *J. appl. Physiol.*, 3, 365.
Davey, M. G., and Lander, H. (1963). *Aust. J. exp. Biol.*, 41, 581.
—— —— (1964). *Brit. J. Haemat.*, 10, 94.
—— —— and Robson, H. N. (1964). Proceedings of the X Congress of the International Society of Haematology, Stockholm.
Firkin, B. G. (1963). *Aust. Ann. Med.*, 12, 261.
Lancet, 1962, 2, 763.
Lander, H., and Davey, M. G. (1964a). *Aust. Ann. Med.*, 13, 207.
—— —— (1964b). Proceedings of the III Asian-Pacific Congress of Cardiology, Kyoto.
O'Neill, B., and Firkin, B. (1964). *J. Lab. clin. Med.*, 64, 188.
Starr, A., and Edwards, M. L. (1961). *Ann. Surg.*, 154, 726.
—— —— and Griswold, H. (1962). *Progr. cardiovasc. Dis.*, 5, 299.

Printed in Great Britain by Fisher, Knight and Co., Ltd.,
Gainsborough Press, St. Albans.

Ardlie, N. G., Kinlough, R. L., Glew, G. & Schwartz, C. J. (1966). Fatty acids and in vitro platelet aggregation. *Australian Journal of Experimental Biology and Medical Science*, *44*(2), 105-110.

Reprinted from the BRITISH MEDICAL JOURNAL
9 *April* 1966, **1**, 888–891

In Vitro Thrombosis and Platelet Aggregation in Myocardial Infarction

N. G. ARDLIE,* M.B., B.S.
RAELENE L. KINLOUGH,† M.B., B.S.
C. J. SCHWARTZ,* M.D., M.R.A.C.P., M.C.P.A., M.C.PATH.

In 1958 Chandler showed that when recalcified citrated whole blood is made to flow in a closed rotating circular plastic loop it does not clot but forms a discrete mass with the histological features of a thrombus. This observation, confirmed by Poole (1959), prompted us to employ the Chandler tube in an attempt to determine if the circulating blood of patients with, after, or prone to thrombosis shows any propensity to abnormal thrombosis *in vitro*. As part of such a study we report our observations on 50 patients with myocardial infarction and 52 healthy controls.

Materials and Methods

Subjects Studied.—The age distribution of the 102 subjects studied is detailed in Table I. Control subjects consisted of our colleagues, laboratory assistants, and volunteer hospital porters in whom there was neither clinical nor electrocardiogaphic evidence of myocardial infarction. Patients with myocardial infarction were selected from both hospital in-patient and out-patient clinics, and all had clinical, laboratory, and electrocardiographic evidence consistent with this diagnosis. Ten of the 50 patients with myocardial infarction and 6 of the 52 controls were women, numbers which were too small to permit a separate sex analysis. Most subjects were studied for six or seven consecutive days.

Blood Samples.—Antecubital venous blood was collected each morning, with the aid of venous occlusion and mild forearm exercise, by means of siliconized glass syringes and 19-gauge needles. Nine parts of blood were added to one part of 3.8% trisodium citrate in siliconized glass centrifuge tubes, and were

* Department of Pathology, Institute of Medical and Veterinary Science, Adelaide, South Australia.
† Department of Medicine, University of Adelaide, South Australia.

mixed gently by inversion. Citrated platelet-rich plasma was prepared by centrifuging at 350 *g* for 15 minutes. On the first day of study samples for total serum cholesterol and plasma fibrinogen were collected.

Total serum cholesterol was estimated with a Technicon Auto-Analyzer, a slight modification of the method described by Zlatkis, Zak, and Boyle (1953) being used.

Plasma fibrinogen was determined by the method of Soles and Roman (1957).

The Chandler apparatus was essentially similar to that first described by Chandler in 1958 and subsequently modified by Poole (1959). A circular disk of perspex was attached to a larger perspex disk inclined at 30 degrees from the horizontal and mounted on an electrically driven motor rotating at 9 r.p.m.

One millilitre of citrated whole blood or platelet-rich plasma was placed in a closed circular loop of polyvinyl chloride tubing (Portex, NT/F) having an internal bore of 3 mm. This was recalcified by the addition of 0.1 ml. of M/4 calcium-chloride solution, at which time a stopwatch was started. The tube was closed with a short cuff of slightly larger tubing (Portex, NT/K), the closed loop fitted around the smaller perspex disk, and the motor started. The latter disk had a short recess to accommodate the cuff. Each loop had a length of 37.9 cm. ($r=6$ cm.), and with rotation the column of blood or plasma had a constant linear velocity of 340 cm./min. A fixed protractor was mounted beneath the perspex disks, and the angle of the advancing column of blood or plasma was recorded at 30-sec. intervals. Changes occurring at the advancing edge were observed through a wide-angle stereoscopic dissecting microscope under bright oblique illumination.

All experiments were performed at room temperature maintained within the range 22–25° C.

Thrombus Formation Time.—This term, coined by Connor and Poole (1961), relates to the time at which the angle of the advancing edge of blood or plasma changed.

TABLE I.—*Age Distribution of the 102 Subjects Studied. Patients with Myocardial Infarction are Subdivided Into Those Receiving or not Receiving Anticoagulant Therapy*

Subject Categories	Age Group (years)						
	11–20	21–30	31–40	41–50	51–60	61–70	71–80
Controls	3	9	12	15	10	3	—
Myocardial infarct:							
Anticoagulant ..	—	—	1	4	15	3	1
No anticoagulant ..	1	—	—	1	4	14	6
Total myocardial infarct	1	—	1	5	19	17	7

Whole-blood Thrombus Length.—In all cases rotation continued for seven minutes after an angle change had occurred. The thrombus formed was emptied into a Petri dish containing 0.9% saline, and its length was measured to the nearest millimetre. Mean thrombus length for each patient was the mean of the values obtained on each of the days studied.

Whole-blood Thrombus Weight.—After fixation in 10% formalin the thrombi were blotted dry between filter papers and weighed to the nearest milligram. Mean thrombus weight for each patient was again the mean of the values obtained on each of the days studied.

Platelet Aggregation.—Platelet-rich plasma was observed in the transparent plastic tube through a dissecting microscope at 12 magnifications. The time at which visible platelet aggregates formed, and their number, size, and time of persistence, were recorded. Three patterns of spontaneous platelet aggregation were recognized:

(*a*) *Normal pattern* was defined as a sporadic appearance and disappearance of visible aggregates rarely exceeding one or two in number and persisting only for one minute or less before a change in the angle of the advancing column of plasma occurred. Just before this angle change there was invariably a rapid and dramatic rise in the number of aggregates, which increased in size and coalesced to form a solid mass at the advancing edge.

(*b*) *Persistence* was defined as the continuous presence of visible platelet aggregates for a minimum of four minutes. In many cases aggregates formed within one or two minutes of recalcification and persisted throughout the experiment.

(*c*) *"Snowstorm"* phenomenon was defined as the formation of hundreds or thousands of small platelet aggregates often within one to two minutes of recalcification. These invariably persisted for at least four minutes. On a number of occasions the process was either completely or partially reversible. This phenomenon should not be confused with the large number of aggregates seen normally just prior to a change in the angle of the advancing column of plasma.

Results

Whole-blood Thrombus Length and Weight.—Figs. 1 and 2 show the mean thrombus length (mm.) and mean thrombus weight (mg.) in control subjects and patients with myocardial infarction. The control subjects were divided into two age groups, under and over 40 years, while patients with myocardial infarction were subdivided into those receiving or not receiving anticoagulant therapy.

Thrombus length and weight tended to increase with age in healthy subjects. Further, there was a marked increase in both thrombus length and weight in patients with myocardial infarction when these were compared with controls over 40 years of age. It can also be seen that, in patients with myocardial

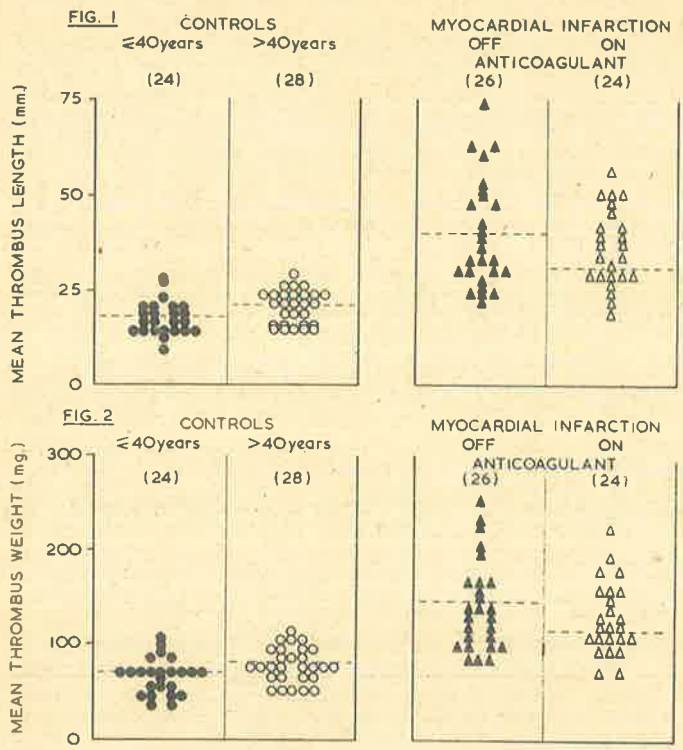


FIG. 1.—Mean thrombus length (mm.) in control subjects aged 40 or less and over 40, and in patients with myocardial infarction subdivided into those receiving and not receiving anticoagulant therapy. The number of subjects in each group is indicated at the top of each column. FIG. 2. —Mean thrombus weight (mg.) in control subjects aged 40 or less and over 40, and in patients with myocardial infarction subdivided into those receiving and not receiving anticoagulant therapy. The number of subjects in each group is indicated at the top of each column.

infarction, thrombus weight and length showed a tendency to be less in those receiving anticoagulant therapy.

Effect of Time After Infarction.—Fig. 3 A and B shows mean thrombus length (mm.) and mean thrombus weight (mg.) in patients with myocardial infarction analysed according to the time after infarction and the presence or absence of anticoagulant therapy. Thrombus length and weight clearly declined with the passage of time after infarction, irrespective of the presence or absence of anticoagulant therapy. In patients six or more weeks after the clinical event the values for thrombus length and weight returned to within the normal range in only 7 of the 12 subjects studied. Further, thrombus length and weight in each of the main subdivisions were consistently, though only slightly, less in those patients receiving anticoagulant therapy.

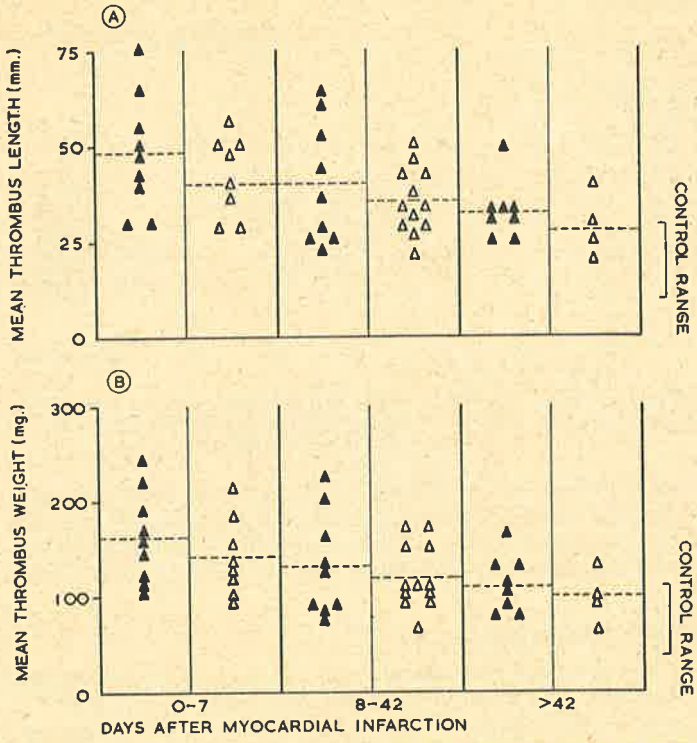


FIG. 3.—(A) The mean thrombus length (mm.) and (B) the mean thrombus weight (mg.) in patients with myocardial infarction are analysed according to time after infarction and the presence (△) or absence (▲) of anticoagulant therapy. The range of values for thrombus length and weight in control subjects over 40 years of age is indicated on the right of each figure.

Plasma Fibrinogen and Thrombus Size.—Plasma fibrinogen levels were found to be raised in patients with myocardial infarction. Both in these patients and in control subjects we found a statistically significant correlation between the plasma fibrinogen level on the one hand and thrombus weight on the other. In the case of patients with myocardial infarction the calculated correlation coefficient r was 0.7180 ($P<0.001$), and in the controls r was 0.4136 ($P<0.01$).

Total Serum Cholesterol and Thrombus Size.—Neither in patients with myocardial infarction nor in healthy controls (Fig. 4) did we find a significant correlation between thrombus weight and the total serum cholesterol level.

Thrombus Formation Time.—In patients with myocardial infarction both the whole blood and plasma thrombus formation times did not differ significantly from those of controls over

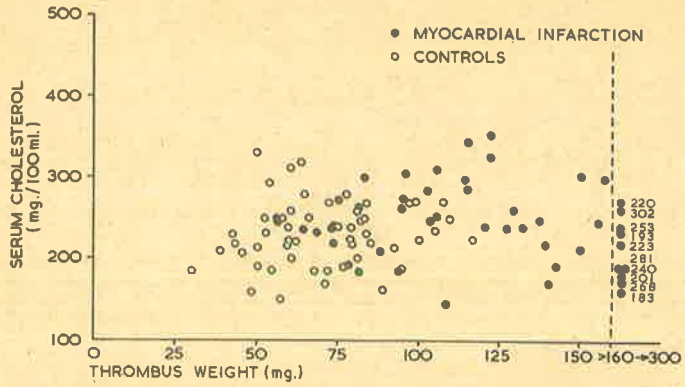


FIG. 4.—Mean thrombus weight (mg.) plotted against total serum cholesterol (mg./100 ml.) in patients with myocardial infarction (●) and in control subjects (○).

the age of 40 years. There was no prolongation of the thrombus formation time in patients with myocardial infarction receiving oral anticoagulant therapy, a finding which is not in agreement with the results of Cunningham, McNicol, and Douglas (1965).

Platelet-rich Plasma

In control subjects aged 40 and less the normal pattern (see Methods) of platelet aggregation was observed in 98.6% of days studied (Table II). In none of the subjects in this age group was the snowstorm phenomenon of platelet aggregation observed. Abnormal platelet aggregation in the young controls was found therefore with an overall frequency of only 1.4% of the total days (142) studied. In controls over 40 years of age abnormal persistence of platelet aggregates was noted in 4.3% of 161 days studied. In addition, the snowstorm pheno-

TABLE II.—*Frequency (Expressed as a Percentage of the Number of Days Studied) of the Two Patterns of Abnormal Platelet Aggregation Considered Separately and Together, in Controls Aged 40 or Less and Those Over 40, and in Patients with Myocardial Infarction Subdivided Into Those Receiving and Not Receiving Anticoagulant Therapy, and Combined*

Subject Categories	Days Studied	Persistence (%)	Snowstorm Phenomenon (%)	Total Platelet Abnormality (%)
Controls ≤ 40 years	142	1·4	0·0	1·4
Controls > 40 years	161	4·3	2·5	6·8
Myocardial infarct:				
On anticoagulant	148	16·2	11·5	27·7
No anticoagulant	169	20·7	4·7	25·4
Myocardial infarct combined ..	317	18·6	7·9	26·5

menon occurred on 2.5% of days studied, giving a total of 6.8% of days showing abnormal platelet aggregation. This tendency for the frequency of spontaneous platelet aggregation to increase with age in control subjects was found to be statistically significant ($0.01>P<0.05$).

In contrast to the findings in control subjects, abnormal platelet aggregation was observed in patients with myocardial infarction with an overall frequency of 26.5% of 317 days studied (Table II). Persistence accounted for 18.6%, and the snowstorm phenomenon for 7.9% of the days studied showing abnormal platelet aggregation. Table II also shows the percentage prevalence of days of abnormality in patients with and without anticoagulant therapy. The overall frequency of abnormal platelet aggregation was found to be 27.7% and 25.4% in patients on and off anticoagulants respectively, a difference which was not statistically significant. Persistence occurred less often in patients receiving anticoagulants (16.2%) than in those not receiving anticoagulants (20.7%), a difference which did not attain statistical significance. The snowstorm phenomenon was more frequent in patients on anticoagulants (11.5%) than in those not receiving anticoagulants (4.7%). This trend did not, however, attain statistical significance.

The frequency of abnormal platelet aggregation has also been considered in relation to the time after infarction (Table III). It can be seen that the overall frequency of platelet abnormality was practically the same at 0–7 days (29.3%) and 8–42 days (29.9%), but in patients six or more weeks after infarction the number of days showing abnormality had decreased to 18.7%. When the two patterns of abnormal platelet aggregation are considered separately (Table III) it is apparent that the frequency of persistence 0–7 days after infarction (21.2%) was similar to the frequency at 8–42 days (20.5%) after infarction, but in patients who had sustained infarcts more than six weeks before the time of study only 13.2% of days studied showed this pattern. The frequency of the snowstorm phenomenon varied from 8.1% at 0–7 days to 9.4% at 8–42 days, while in patients with infarcts over six weeks old the number of days studied showing this phenomenon decreased to 5.5%. It is of interest to note that neither persistence nor the snowstorm phenomenon was observed in anticoagulated patients six or more weeks after infarction. In this group only four subjects were studied for a total of 25 days. We are therefore reluctant to draw any final conclusions from this latter observation.

Discussion

We have found that the thrombi produced in the Chandler apparatus with blood from patients after myocardial infarction are both longer and heavier than the thrombi of control subjects

TABLE III.—*Frequency (Expressed as a Percentage of the Number of Days Studied) of the Two Patterns of Abnormal Platelet Aggregation (Considered Separately and Together), According to Time After the Event, in Patients with Myocardial Infarction, the Latter Being Subdivided Into Those Receiving and Not Receiving Anticoagulant Therapy, and Combined*

Myocardial Infarct Category	Time After Infarction (Days)											
	0–7				8–42				> 42			
	Days Studied	Persistence (%)	Snow-storm Phenomenon (%)	Total Platelet Abnormality (%)	Days Studied	Persistence (%)	Snow-storm Phenomenon (%)	Total Platelet Abnormality (%)	Days Studied	Persistence (%)	Snow-storm Phenomenon (%)	Total Platelet Abnormality (%)
On anticoagulant	47	21·3	14·9	36·2	76	18·4	13·2	31·6	25*	0·0*	0·0*	0·0*
No anticoagulant	52	21·2	1·9	23·1	51	25·5	3·9	27·5	66	18·2	7·6	25·8
Combined	99	21·2	8·1	29·3	127	20·5	9·4	29·9	91	13·2	5·5	18·7

* Only four patients in this group.

having neither clinical nor electrocardiographic evidence of infarction. Thrombus size clearly decreases with the passage of time after infarction, and tends to be less in those patients receiving anticoagulant therapy (Fig. 3 A and B). Both in the control subjects ($P<0.01$) and in patients with myocardial infarction ($P<0.001$) thrombus weight and the plasma fibrinogen levels showed statistically significant correlations. It is well known that the plasma fibrinogen level is elevated in patients after myocardial infarction (McDonald and Edgill, 1959), a finding which we have confirmed in this study. This elevation could either wholly or partly account for the longer and heavier thrombi found in patients with myocardial infarction.

We do not know whether the factor or factors responsible for the development of large thrombi were present before infarction or merely reflect the results of infarction. It appears that the latter is more likely. Nevertheless, it is interesting to speculate whether this propensity to abnormal *in vitro* thrombosis after myocardial infarction might be associated with the tendency of these patients to subsequent thrombotic episodes.

Our results clearly indicate that there is a significant increase in the incidence of platelet abnormalities in patients with myocardial infarction when compared with control subjects over the age of 40 years. Again we cannot be sure whether these findings relate to a state existing before infarction or are wholly or in part a sequel to myocardial necrosis. However, we have demonstrated that there is a definite tendency for both the frequency of spontaneous platelet aggregation and thrombus size to increase with age in apparently healthy subjects. These findings suggest that the factor or factors responsible for these phenomena are present to some extent before the clinical event, and that coronary thrombosis could result from an exaggeration of this trend.

The overall frequency of platelet abnormality was similar in patients with myocardial infarction whether or not they were receiving anticoagulant therapy (Table II). Two explanations for this finding should be considered. First, it could be that in anticoagulated patients the initial overall frequency of platelet abnormality was greater, and that this frequency has in fact been reduced by therapy. Alternatively, anticoagulants may have had little or no effect on platelet aggregation. On the other hand, there was a trend, though not statistically significant (Table II), for the snowstorm phenomenon to occur more often in anticoagulated patients (11.5%) than in those not receiving anticoagulant therapy (4.7%). At first sight this might suggest that anticoagulant therapy had enhanced platelet aggregation. This trend may, however, merely reflect the fact that those patients selected for anti-

coagulant therapy differed from those not given anticoagulants, possibly on the basis of more extensive infarction. It is apparent that the effect of oral anticoagulants on platelet aggregation in this *in vitro* system is inconclusive.

Summary

Fifty healthy subjects and 52 patients with myocardial infarction were investigated, the Chandler apparatus being used as an *in vitro* model for the study of thrombosis and platelet aggregation. It was found that thrombus weight and length increased with age in healthy subjects, and that both were markedly elevated in patients with myocardial infarction. Thrombus weight and length were less in patients receiving oral anticoagulant therapy, and both declined progressively with the passage of time after infarction. Plasma fibrinogen levels and thrombus weight showed a statistically significant correlation in both control subjects and in patients with myocardial infarction. No significant correlation between total serum cholesterol levels and thrombus weight was found.

Abnormal platelet aggregation was found to increase in frequency with age in healthy control subjects, and in patients with myocardial infarction it was significantly more common than in control subjects. The overall frequency of abnormal platelet aggregation was slightly less in those patients who had sustained their infarcts more than six weeks before the time of study. Patients receiving oral anticoagulants showed no significant decrease in the frequency of abnormal platelet aggregation, but a trend for the snowstorm phenomenon to occur more often in this group was noted.

This study has shown that the blood of patients with myocardial infarction shows a propensity to abnormal *in vitro* thrombosis and platelet aggregation. The possible significance of the findings is discussed.

We are pleased to record the support of the National Heart Foundation of Australia and the technical assistance given by Miss J. Bansemer, Mr. C. Lowden, and Miss M. Hall. We also wish to thank Dr. A. E. Taylor for the cholesterol and fibrinogen determinations.

References

Chandler, A. B. (1958). *Lab. Invest.*, **7**, 110.
Coles, M., and Roman, W. (1957). *J. clin. Path.*, **10**, 282.
Connor, W. E., Poole, J. C. F. (1961). *Quart. J. exp. Physiol.*, **46**, 1.
Cunningham, G. M., McNicol, G. P., and Douglas, A. S. (1965). *Lancet*, **1**, 729.
McDonald, L., and Edgill, M. (1959). Ibid., **1**, 1115.
Poole, J. C. F. (1959). *Quart. J. exp. Physiol.*, **44**, 377.
Zlatkis, A., Zak, B., and Boyle, A. J. (1953). *J. Lab. clin. Med.*, **41**, 486.

Printed in Great Britain by Fisher, Knight and Co., Ltd.,
Gainsborough Press, St. Albans.

Kinlough, R. L., Davey, M. G., & Lander, H. (1966). An evaluation of anticoagulant solutions used in the preparation of platelet concentrates. *Transfusion, 6*(3), 213-223.