THROMBOCYTOPENIA IN INFECTIONS
THESIS

SUBMITTED FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN THE

UNIVERSITY OF ADELAIDE

BY

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ADELAIDE, 1973
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ERRATA

Page numbering errors: Page 55 and 164 omitted, page 78 duplicated. Text is in correct sequence.
DECLARATION

I hereby declare that this thesis is of my own composition and is a true record of original work which has not been submitted for the award of any degree or diploma in any university. To the best of my knowledge and belief the thesis contains no material previously published or written except where due reference to such material is made in the text.

ACKNOWLEDGEMENTS

The studies reported in this thesis were carried out in the Division of Haematology of the Institute of Medical and Veterinary Science, Adelaide. I worked under the supervision of Dr. Richard Kimber and Dr. Harry Lander who gave me considerable encouragement and helpful criticism. I was also greatly assisted by Dr. Donald Handley whose continued interest in the problems involved and technical experience in the area of blood coagulation proved to be of inestimable value.

I gratefully acknowledge the assistance given by members of the Staff of the Institute of Medical and Veterinary Science in performing the routine haematological and coagulation studies. This work would also not have been possible without the kind cooperation of Professor D.J. Deller and Professor J. Ludbrook and members of the Visiting Medical Staff of the Royal Adelaide Hospital, who permitted studies of patients under their care.

I am indebted to Mrs. Iraida Abolins and Miss Christine Holzer for the illustrations and to Miss J. Devaney for making the text legible.

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INTRODUCTION AND AIMS OF THE STUDY
The platelet, a small structure circulating in the blood, has, in the past two decades, become an object which has attracted interest and research activity of impressive proportions. Most of this research has been directed towards answering some of the many complex questions underlying the functions of platelets in coagulation, haemostasis and their role in the pathogenesis of occlusive vascular disease and thrombosis.

Haemorrhagic and thrombotic complications can arise in association with a wide variety of pathological processes of different aetiology. In pestilential fevers, for example, the appearance of purpura and bleeding have, since the time of Hippocrates in the fifth century B.C., been recognized as grave signs usually foreshadowing death. Towards the end of the nineteenth century changes in the numbers of platelets circulating in the blood were noted in acute febrile illnesses. A reduction in the number of circulating platelets was noted in some infections but frequently the platelet numbers remained at normal levels or were even increased. This led to the belief that most of the haemorrhagic manifestations seen in fevers were the result of injury to the vascular endothelium caused directly by the micro-organisms of their toxins.
More recently it has been recognized that when microorganisms or their toxins enter the blood stream they can interact with platelets and other coagulation factors. This may initiate clotting of the circulating blood. Multiple deficiencies of circulating haemostatic components may follow due to the consumption of platelets and other coagulation factors. If this is associated with the deposition of fibrin thrombi in the microvasculature it will cause ischaemic necrosis of the more distal tissues. This consumption of clotting factors can, if severe, result in a generalised bleeding diathesis. "Disseminated intravascular coagulation" or "consumption coagulopathy", terms which are generally accepted to describe this process, is now recognised as a complication of sepsis.

However, in addition to their haemostatic properties, platelets interact both in vitro and in vivo with particulate matter such as carbon particles, thorotrast, lipid emulsions and also with bacteria, endotoxins and viruses. These reactions in many ways resemble those of leucocytes. The coagulation system as a whole may not be significantly involved. This has led some authors to suggest the possibility that platelets may, along with other cells of the body, play a part in resisting infections. Doubt still remains, however,
as to the exact mode and sphere of activity of platelets in this respect.

It is the purpose of this thesis to review the research made in this field to the present time, and to attempt to define more clearly the effect of bacterial infections on platelet number, and the relationship of these changes to the abnormalities in the coagulation system sometimes associated with infectious diseases.

To this aim changes in platelet numbers and some tests of haemostasis are examined in a number of patients admitted to the Royal Adelaide Hospital in whom infection was diagnosed as the primary or predominant disorder. Alterations in platelet numbers and coagulation system are also examined in an experimental animal, the rabbit, in response to inoculations with various organisms and bacterial endotoxin.
CHAPTER I

THE NATURE, STRUCTURE, METABOLISM

AND FUNCTION OF PLATELETS
The Discovery of Platelets

Although Donne is frequently credited with the first observation of platelets in 1842, it appears that there were a number of investigators just before, and about this time, who suspected the presence of small formed elements in the blood apart from erythrocytes and leukocytes. Donne, however, labelled these particles "les globulins du chyle" believing them to be derived from lymph (Donne, 1842). This term, "les globulins", was used by many French workers to describe platelets until as recently as the 1920's.

There can be little doubt that the particles in the blood described by Osler in 1874, who mistook them for bacteria (Osler, 1874) and by Hayem in 1878, who thought they were precursors of red cells and called them "haematoblasts" (Hayem, 1878a, 1878b) were in fact platelets. Both these observers appreciated that these particles were in some way associated with the coagulation of the blood.

It remained however, for Bizzozero in 1881 to lay the foundation of present day concepts concerning platelets which he called "piastrini" (Bizzozero, 1881). As well as describing platelets as they appeared in the mesenteric vessels of rabbits and guinea pigs he recognised, more accurately than
his predecessors, their association with haemostasis and thrombosis. Bizzozero was also the first to recognise the presence of giant cells in the bone marrow (Bizzozero, 1869), later to be called "megakaryocytes" by Howell (1890).

Another twenty years were to elapse before Wright was to present the hypothesis that platelets are derived by fragmentation of the megakaryocytes (Wright, 1910). This hypothesis concerning the origin of platelets is accepted to the present day.

Since 1910 the term "platelet" has been generally accepted, although in French literature the term "globin" persisted as late as 1927. Also in current usage is the term "thrombocyte", which was first used by Dekhuyzen in 1901 to describe the nucleated cells associated with haemostasis seen in the blood of birds (Dekhuysen, 1901).

In a recent review entitled "Why the platelets were discovered", Robb-Smith traces the history of the controversy which surrounded the discovery of platelets for more than eighty years and illustrates how it could be related, in part, to the increasing sophistication of the light microscope and improvement in staining techniques occurring during the latter half of the last century (Robb-Smith, 1967).
The Formation and Structure of Platelets

The small size of platelets (1 - 5 μ in diameter) imposes some limits upon the detail that can be seen with the light microscope. Until the advent of the electron microscope, the appearance of platelets when stained with Wright's stain is no better described than by Wright himself in 1910 (Wright, 1910).

"They consist of a hyaline blue staining substance in which are embedded closely set, minute, red to purple staining granules. The red to purple staining granules may be aggregated in a more or less sharply outlined mass in the central part of the platelet so as to suggest a nucleus surrounded by hyaline cytoplasm. In some platelets a clear, unstained, more or less sharply outlined vacuole-like area may be seen in the midst of the granules."

It was also appreciated that this separation into two zones, the clear hyalomere and the chromomere made up of granules, probably occurred after the platelet left the vessel, whereas in the circulating intact platelet the granular material is distributed finely and evenly through-
Platelets are formed by fragmentation of the megakaryocyte in, for the most part, the bone marrow, although this may also occur in the lungs and to some extent in the spleen (Kaufman, 1965). Each platelet is bounded by a triple layer plasma membrane similar to the "unit membranes" of other cells. This membrane which is composed of phospholipid micelles and sulphated mucopolysaccharides appears to be derived from the membrane of the megakaryocyte by a process of invaginations of this membrane into the cytoplasm of the megakaryocyte (Behnke, 1968; Nakao and Angrist, 1968) a process often referred to as "platelet budding". The platelets, so formed, become detached from the megakaryocyte and are released into the circulating blood.

Within the lumen of normal vessels the platelets are usually randomly distributed amongst the other formed elements of the blood. Circulating platelets have an elliptical profile and their outline is more or less smooth and free of the spikes and projections so frequently seen when platelets are separated from blood and fixed in vitro (French, 1967; Hovig, 1970).
Before describing the visible structures contained within the platelet, mention should be made to the presence of a "plasmatic atmosphere" over its surface which was first recognised by Roskam in 1922 (Roskam, 1922). It consists of plasma proteins, including coagulation factors and gamma globulins, absorbed onto the surface of the platelet. Indeed, because of the concentration of these proteins which can be removed by repeated washings in saline, some authors have likened the platelet to a "sponge" (Adelson et alii, 1961). It does appear that this surface coat of plasma proteins is of great importance in many of the adhesive reactions of platelets.

Mammalian platelets have no nucleus, contain no deoxyribonucleic acid (DNA) and contain only trace amounts of ribonucleic acid (RNA). They do, however, contain a number of organelles. These can, with the electron microscope, be separated into several distinct categories.

1. Dense granules. These are the largest organelles (0.2 - 0.3 μ in diameter) and correspond with the azurophil granules seen with the light microscope. These granules, bounded by a "unit membrane", contain a number of substances which are important to platelet function. They appear similar to the lysosome granules in leukocytes, the contents
of which are lost during platelet aggregation and when platelets phagocytose small particulate matter (Marcus et alii, 1966). Other granules appear to contain an abundance of glycogen (French, 1967).

Highly osmiophilic granules (also known as "dense bodies") have been identified. Although these dense bodies are relatively sparse in human platelets (Davis and White, 1967) they are common in rabbit platelets (Wurzel et alii, 1965). They contain serotonin (5-hydroxytryptamine) which is absorbed with great avidity, even against a concentration gradient, from the medium surrounding the platelet and concentrated in these dense bodies (Maynert and Isaac, 1968). Structural or biochemical disturbance of the platelet by thrombin or bacterial endotoxins causes a rapid disappearance of these bodies and the liberation of serotonin into the surrounding medium (Des Prez, 1961).

2. Mitochondria. These are smaller (0.15 - 0.2 μ in diameter) in platelets than in most mammalian cells, but readily recognised in electron micrographs by their double boundary membrane and christae mitochondriales. This correlates with biochemical evidence of mitochondrial enzyme activity which has been shown to be present (Marcus et alii, 1966).
3. **Vesicular elements.** These are of a variety of shapes and sizes, they are bounded by a smooth surfaced membrane and are distinguishable from other cytoplasmic inclusions by the relative translucency of their contents. When particulate matter, such as thorotrast or ferritin, is injected into the circulation it can, within a few minutes, be seen inside these platelet vesicles (French, 1967). This has been confirmed by a number of observers using a variety of small particles (Movat et alii, 1965; Schulz, 1961).

4. **Microtubules.** Microtubules (200 - 250 A in diameter), arranged in bundles of five to ten, circumscribe the platelet disc in the equatorial plane and lie just below the plasma membrane. These microtubules are similar to those of other animal and plant cells. It has been suggested that the microtubules are rigid and under tension in the intact platelet and, as such, act as a cytoskeleton for the maintenance of its normal discoid shape (Behnke and Zelander, 1967).

Associated with the microtubules are finer microfilaments ("microfibrils"). There is some speculation as to whether there may be a relationship between the microtubules, microfibrils and the contractile protein, thrombasthenin, which platelets have been shown to contain (Zucker-Franklin, 1969).
There appear to be two forms of thrombasthenin; the first is found within the cytoplasm (C-thrombasthenin) and the second is found on the external surface of the platelet (S-thrombasthenin). It is the S-thrombasthenin which appears to be important in forming molecular bridges between adjacent platelets in response to aggregating agents (i.e. thrombin), which in turn can contract drawing the platelets into a tight mass or thrombus (Booyse and Rafelson, 1971).

The platelet population is not homogeneous; it can be separated into two density populations, a large-heavy platelet population with a S.G. > 1.042 and a light-small population with a S.G. < 1.032. Each represent 15-20% of the total population of platelets with the volume of each being 12 and 5 cu.μ respectively. The heavy-large platelets contain more glycogen, adenosinetriphosphate (ATP), adenosinemonophosphate (AMP), glutathione (GSH) and protein than the light-small platelets. It is postulated that the heavy-large platelets are metabolically more active and that they are younger than the light-small platelets (Karpatkin, 1971).

**Regulation of Platelet Numbers**

The number of platelets in the normal human circulation was accurately determined by Hayem in 1878. Since then normal
levels of 140,000 to 350,000 cu mm have been confirmed by many observers using a variety of techniques (Brecher et alii, 1953; Bull et alii, 1965; Wintrobe, 1967).

Under normal circumstances the platelet count remains remarkably constant and, in the past few years, considerable attention has been directed towards elucidating the mechanisms by which blood platelet numbers are regulated. If thrombocytopenia is induced by anti-platelet serum or by exchange transfusion it is followed, after a delay of a few days, by a transient thrombocytosis (Craddock et alii, 1955; Matter et alii, 1960). Thrombocytopenia induced stimulation of thrombopoiesis results in increased entry of precursor cells into the megakaryocyte pool, an increased maturation of megakaryocytes and an increase in megakaryocyte size and numbers in the bone marrow (Levin, 1970). On the other hand transfusion induced thrombocytosis is followed by a transient thrombocytopenia (De Gabriele and Penington, 1967). Studies using $^{75}$Se Methione, which is incorporated into the megakaryocytes and is subsequently present in platelets, show an increased rate of incorporation of $^{75}$Se Methione in the platelets of normal recipients infused with the plasma of thrombocytopenic donors (Levin, 1970). These observations do suggest the presence of some humoral factor controlling the rate of production of
platelets, i.e. thrombopoietin.

Other factors such as destruction and the sequestration of circulating platelets also influence the platelet count. The role of the spleen in this regard is not clear; there is, however, evidence of a "splenic pool" of platelets and, in conditions associated with splenomegaly, the increase in the size of the spleen alone may substantially influence platelet numbers in the circulation (Penny et alii, 1966).

Despite the considerable manipulation involved in the tagging of platelets with Chromium 51, which are then re-infused, this method has yielded much useful information concerning platelet survival and destruction. When platelets of a normal subject are labelled with Chromium 51, the radioactivity disappears in 8 to 11 days, possibly by both random disappearance and senescence. The spleen appears to be the primary site of accumulation of transfused and non-viable platelets (Davey, 1966).

Biochemistry and Metabolism of Platelets

The metabolic activity of platelets was, for a long time, considered to be insignificant despite Aynaud's observations in 1909 which indicated that platelets consumed
oxygen (Aynaud, 1909). It is now realised that platelets contain all the enzymes required for glycolysis and oxidative metabolism necessary for the maintenance of a constant supply of adenosine triphosphate (ATP) needed for their metabolic activity (Doery et alii, 1970b).

As with most other cells of mammalian origin, glucose seems to be the primary source of energy for platelets, although a substantial ability to oxidase long chain fatty acids still exists (Doery et alii, 1970a; Cohen and Wittels, 1970). Glycolysis, the metabolism of glucose via the glycolytic pathway, is more active in the platelet than is oxidative metabolism via the citric acid cycle (Marcus and Zucker, 1965; Karpatkin, 1967; Haslam, 1968). The platelet enzyme phosphofructokinase, which has one of the lowest measurable in vitro activities, is probably the site of regulation of platelet glycolysis. It is likely that, as in other cells, the overall control of the various metabolic cycles in the platelet is mediated by the ADP/ATP ratio acting at this step (Doery et alii, 1970a).

Energy derived from the pentose phosphate or glycolytic pathways can support platelet reactions but both appear to be necessary for normal platelet function. To some extent
either pathway may compensate for a decrease in activity of
the other (Doery et al., 1970a). Oxidative metabolism via
an intact citric acid cycle is required for the synthesis of
platelet phosphatides, amino acids and for the process of de-
granulation associated with the "release reaction" (Steiner
and Kuramoto, 1971) which will be described in the following
section. Interference to either glycolysis or oxidative
metabolism can inhibit platelet functions such as aggregation
(including the changes in shape preceding it) induced by
adenosinediphosphate (ADP), adhesion to glass and clot
retraction (Murer et al., 1967).

Aggregating agents which cause degranulation of the
platelet such as thrombin, epinephrine, collagen and ADP (in
appropriate concentrations) increase glucose and oxygen
consumption and increase the production of lactate. This
increased rate of breakdown of glucose occurs as a result of
stimulation of oxidative metabolism via the citric acid cycle.
Glycolysis is stimulated to a lesser degree while the activity
of pentose phosphate shunt does not appear to be altered
significantly (Steiner and Kuramoto, 1971; Warshaw et al.,
1966). Glycogen, which is stored in abundance in the granules
of fresh platelets, constitutes one of the principal substrates
for this increased metabolism (Karpatick, 1967). Aggregating
agents also cause accelerated incorporation of glucose carbon into glycogen, lipids and proteins but only ADP appears to enhance the rate of oxidation of fatty acids (Steiner and Kuramoto, 1971).

In contrast to erythrocytes and leukocytes, platelets contain enzymes of fatty acid synthesis including acetyl-CoA carboxylase. Studies of the patterns of incorporation of C\textsuperscript{14} acetate into platelet fatty acids indicate both a \textit{de novo} synthetic pathway and a chain elongation pathway (Deykin and Desser, 1968; Majerus et alii, 1969). It is probable that this fatty acid synthesis and metabolism in platelets is specially related to their role in haemostasis since the availability of platelet phospholipid is normally essential in the process of blood coagulation (Majerus et alii, 1969; Davie et alii, 1969; Marcus, 1969).

Platelets have also been shown to synthesize proteins (Warshaw et alii, 1967) and, in addition to the specific platelet proteins (thrombasthenin and platelet factor IV, the anti-heparin factor), they also contain plasma proteins which include albumin, gamma-globulins, plasminogen, fibrinogen and other coagulation factors (Davey and Luscher, 1967; Horowitz and Futimoto, 1965; Kiesselbach and Warner, 1966).
Platelets, however, cannot synthesize adenine nucleotides de novo but do so from some preformed purines, i.e. adenine and adenosine. Platelet adenine nucleotides are compartmentalized, one pool participating in metabolism, and another, metabolically active, is stored in specific granules. During energy-requiring platelet functions, such as the "release reaction", there is a rapid net depletion of metabolically active ATP and the extrusion of stored ADP into the environment surrounding the platelet (Holmsen and Day, 1971).

**Platelet Adhesive, Release and Aggregation Reactions**

Hayem (1878b), Bizzozero (1881) and Eberth and Schimmelbush (1886) recognised that platelets differed from other elements in the blood by their ability to adhere to surfaces and to form clumps, but not until 1958 following Hellem's observations of the biochemical and physical response of platelets to ADP and of those induced by collagen observed by Bounnameaux in 1959, did the complexity of the biochemical and metabolic changes in platelets associated with adhesive, release and aggregation reactions induced by collagen become apparent.

This response of platelets to collagen is central to its primary role in the process of haemostasis. However, many
other chemical and physical agents can induce a similar chain of reactions.

A summary of the biochemical changes associated with these reactions is presented as they are pertinent to some aspects of the response of platelets to particulate matter, micro-organisms and their endotoxins. With reference to the haemostatic process, these reactions can be divided into five steps (Hellem and Stormorken, 1969):

1. Adhesion of platelets to injured vessels, particularly to collagen fibres.
2. Release reaction phase of adhered platelets.
3. Reversible aggregation, an aggregation of platelets which is loose and still permeable to the bloodstream.
4. Irreversible aggregation, the transformation of the permeable haemostatic plug into the impermeable plug ("viscous metamorphosis").
5. Reinforcement of the platelet plug by the formation of fibrin from plasma coagulation factors.

The first reaction of platelets coming into contact with collagen is one of adhesion. The reaction appears to be
highly specific as blood cells other than platelets do not adhere to collagen (Hellem and Stormorken, 1969).

The adhesion of platelets to collagen is independent of calcium ions and of metabolic functions but it can be inhibited by the pre-treatment of the platelets with proteolytic enzymes. This suggests that chemical or physical surface property of the platelet membrane is involved (Hellem, 1958; Hovig, 1965).

The second phase is the "release reaction", a term introduced by Grette in 1962 to describe the explosive release of adenine nucleotides, serotonin, fibrinogen and other proteins induced by thrombin. A year later, Hovig showed that when platelets adhered to collagen they lost their granules and that the aggregation which followed was secondary to the release of ADP from the platelets themselves (Hovig, 1963a, 1963b).

During these early thrombin and collagen induced reactions surprisingly few breaks, as judged by electron microscopy, are apparent in the platelet membrane (Hovig, 1963a; Kjaerheim and Hovig, 1962). This has led to the concept that the release reaction constitutes a rapid discharge of the contents of the
dense bodies and granules from the platelet to the extracellular environment which occurs as a result of a sudden change in membrane permeability. The release process is selective; only granule associated material is specifically released (lysis has to be excluded under experimental conditions by measuring markers for cytoplasm and mitochondria) (Day and Holmsen, 1971).

Components released from platelets in addition to ADP include ATP (Holmsen, 1967), serotonin (Buckingham and Maynert, 1964), potassium (Zieve et alii, 1964), fibrinogen and other proteins (Grette, 1962), platelet factor 3 (Spaet and Cintron, 1965), platelet factor 4 (Farbieszewski et alii, 1968), and a material with a "tissue thromboplastic-like activity" (Biggs et alii, 1968). Other substances include enzymes, one at least is a protease which resembles cathepsin A of tissues and which can digest fibrinogen but is distinct from plasmin (Nachman and Ferris, 1968), and a substance(s) which in some way can alter vascular permeability and possibly muscle tone (Packham et alii, 1968; Donaldson, 1970). Histamine, contained but not concentrated in platelets except those of the rabbit, adrenalin and noradrenalin are also released (Waalkes and Coburn, 1959; Weil-Malherbe and Bone, 1954).
The number of both chemical and physical stimuli, apart from thrombin and collagen, shown to provoke the release reaction has been extended to include snake venoms (Davey and Lüscher, 1967), aggregated gamma-globulins (Ishizaka and Ishizaka, 1962), long chain fatty acids (Haslam, 1964), antigen-antibody complexes (Movat et alii, 1965), latex particles (Glynn et alii, 1965), and bacterial endotoxins (Des Prez, 1961).

Contact with non-enzymic stimuli will cause changes in platelets which bear a close resemblance to those seen in other phagocytic cells. Like phagocytosis these changes, and the release reaction, are energy dependent since they can be inhibited by blocking platelet glycolytic and oxidative metabolic activity (Packham et alii, 1968; Murer et alii, 1967; Kuramoto et alii, 1970).

The release of ADP from the platelet is of primary importance in the formation of the loose and reversible aggregates of platelets which follows the release reaction. ADP, as well as appearing to specifically aggregate platelets, also causes them to become more sticky which gives them the ability to adhere to foreign surfaces such as glass. Several other substances will convert non-sticky platelets to sticky
platelets. It has been suggested that this effect is mediated through ADP released from the platelets themselves (Haslam, 1967); however, this explanation does not satisfy the effects observed with all platelet aggregating substances, i.e. adrenalin (Hellem and Stormorken, 1969; Haslam, 1968).

Low concentrations of ADP from sources external to the platelet will also induce aggregation, but only at relatively high concentrations will it cause the release reaction. The mechanism by which ADP induces this reversible aggregation of platelets has, and continues, to receive considerable attention. No attempt will be made to summarise the work in this field. This has been amply reviewed by a number of investigators (Hellem and Stormorken, 1969; Marcus, 1969; O'Brien, 1966; Mustard, 1968; Brinkhous, 1967).

After the release reaction the platelets then become irreversibly aggregated, a phase which corresponds to the process of "viscous metamorphosis" observed by light microscopy. The presence of thrombin is required for this phase which entails the rupture of the membrane and lysis of the platelets (Hellem and Stormorken, 1969; Marcus, 1969).
And finally, this mass of irreversibly aggregated platelets is reinforced by the laying down of fibrin. The release of platelet factor 3 plays a part in this process. There is also some evidence to suggest that the breakdown of platelets can supply a "tissue thromboplastic-like activity" (Biggs et alii, 1968), although the primary trigger for coagulation in the normal haemostatic process is predominantly "tissue thromboplastin" (Marcus, 1969).

**Functions of Platelets**

Hayem (1878b; 1882) and Bizzozero (1881) first appreciated that platelets were involved in haemostasis and thrombosis. They both noted that platelets stuck to the edges of cut vessels and to each other to form a plug which prevented further bleeding. Eberth and Schimmelbusch extended these observations. They noted that not all the platelets which aggregated at the site of vessel injury remained but that some became detached and passed on apparently intact, while the others which remained lost their individual identity to form a fused mass. This phenomenon they termed "viscous metamorphosis" (Eberth and Schimmelbusch, 1886).

Hayem suggested that a deficiency of platelets may result in a bleeding diathesis (Hayem, 1882). This was soon confirmed
by reports of abnormal bleeding in patients with low platelet counts by Krauss (1883), Denys (1889), and by Hayem himself (1891). Hayem also noted that the blood from patients with low platelet counts showed reduced clot retraction (1896). In 1910 Duke reported the finding of a prolonged bleeding time in patients with purpura and low platelet counts but in whom the whole blood coagulation time was normal. This led him to suggest that platelets may in some way be involved in the maintenance of normal vascular integrity, a suggestion that has since been reaffirmed by several investigators although the mechanism of this action is still unknown (Van Horn and Johnson, 1966, 1968; Robson and Duthie, 1950).

Observations concerning the structure of thrombi reported by Hayem and Bizzozero were confirmed by Welch (1887) and it became clear that white thrombi, the lines of Zahn (1875) and the granular collections within blood clots noted by Ranvier (1873) were platelets.

Renewed interest in the platelet's role in coagulation began in the 1930's (Chargaff et alii, 1936), but only in the last fifteen years has there been a general reawakening of interest in the role the platelet plays in "primary haemo-
stasis", thrombus formation and the complex series of biochemical and physical events occurring within the platelet during these processes.

The Role of the Platelet in the Coagulation of the Blood

Thrombus formation, prevention of bleeding and maintenance of the fluidity of the blood rely for the most part on a complex series of interactions between various factors circulating in the plasma which comprise the coagulation system. This system consists of a number of factors which promote the formation of a fibrin clot from the soluble precursor fibrinogen and others which are involved in the breakdown or lysis of fibrin once it is formed. The first process is known as coagulation and the latter as fibrinolysis.

The theory now generally accepted is that there are "two systems" involved in the clotting of blood, the "extrinsic" and "intrinsic" systems. The extrinsic system is activated by tissue factor which enters the blood from damaged cells, with calcium and the plasma protein clotting factors, Factors VII, X and V thromboplastin is generated which converts prothrombin to thrombin. Thrombin then converts circulating fibrinogen to an insoluble gel, fibrin. The intrinsic system is activated by contact of Hageman Factor
(Factor XII) with a foreign surface or material. This is followed in the presence of calcium by activation of Factor XI, IX (Factors VIII and V appear to act as co-factors) in a cascade progression with the formation of thrombin and the conversion of fibrinogen to fibrin. Once formed the insoluble fibrin is stabilized by activation of Factor XIII. A schematic representation of this process is illustrated in Figure 1.

Activation of fibrinolysis possibly occurs simultaneously with activated Factor XII initiating changes in circulating plasminogen resulting in the formation of plasmin which then primarily breaks down fibrin.

Not until 1936 were the earlier suggestions that platelets may promote blood coagulation confirmed (Chargaff et alii, 1936). Lipoprotein isolated from platelet membranes has been found to be an essential catalyst for the interactions of coagulation factors required for the formation of thrombin (Davie et alii, 1969; Marcus, 1966). Although crude mixtures of phospholipid and even certain individual phosphatides can serve as platelet substitutes in in vitro coagulation tests (Marcus and Spaet, 1958), platelet membranes remain the most potent clot promoting substance tested to date (Marcus et alii, 1966).
**FIGURE 1:** Schematic representation of the two systems involved in the clotting of blood.
This platelet lipoprotein is now designated platelet factor 3 or platelet phospholipid. Platelet factor 3 is probably not a biochemical entity that can be isolated but reflects rather a property of the surface structure of the lipoprotein in platelet membranes which promotes the interaction of clotting factors (Marcus, 1969).

Platelet factor 3 activity can be detected in vitro after platelets have been aggregated with ADP or other agents (Hardisty, 1968). It is known to function at two stages in the intrinsic coagulation system and probably acts as a catalyst surface for the interaction of clotting factors. The interactions involved are those between factor VIII and activated factor IX in the activation of factor X, and the conversion of prothrombin to thrombin by a complex of factor V, activated factor X and platelet factor 3 (Davie et alii, 1969; Marcus, 1969).

There is some evidence to suggest that platelets can also supply a "tissue thromboplastic-like" material for the activation of the extrinsic clotting system (Biggs et alii, 1968).

The Role of Platelets in Haemostasis

Platelets are involved in "primary haemostasis"; this
refers to the events occurring immediately after the severance or injury of a blood vessel. Within seconds platelets adhere to the traumatised area. Electron microscope studies have shown that the initial layer of platelets attach themselves to the exposed collagen fibres of the connective tissue beneath the damaged endothelium (French et alii, 1964; Deykin, 1967). The complex sequence of biochemical and physical events set in motion by this platelet-collagen contact have already been referred to in a previous section.

As more platelets arrive in the circulating blood, these also adhere to those already present. The lumen or vessel defect then becomes rapidly occluded by a mass of aggregated platelets in what is frequently termed the "platelet haemostatic plug". Thrombin, formed as a result of activation of the clotting system, then causes this plug to consolidate. This is brought about by the deposition of fibrin primarily over the periphery of the plug and the action of thrombin in the platelet contractile protein, thrombasthenin (Marcus, 1969; Booyse and Rafelson, 1971). If insufficient fibrin is formed the platelet plug will be unstable and break down (Hovig et alii, 1967).

Much of the recent information obtained with regard to
Platelets has been gleaned from research directed towards answering some of the many complex questions underlying the pathogenesis of occlusive vascular disease and thromboembolism, and has been published in a number of reviews. However, this is not of immediate relevance to the present thesis (Mustard, 1968; Deykin, 1967; Brinkhous, 1967; Marcus, 1969; Packham and Mustard, 1971).

Platelet Interactions apart from those associated with Coagulation and Haemostasis

For many years it has been recognised that following the intravenous injection of bacteria there is a rapid fall in the number of circulating platelets (Levaditti, 1901; Aynaud, 1911). A number of studies have demonstrated that platelets adhere to bacteria in vitro (Govaerts, 1921; Houlihan and Copley, 1946; Aynaud, 1911; Houlihan, 1947). There is even evidence that platelets or an extract of platelets (plakin) is capable of killing some microorganisms (Amano, 1952).

Bacteria in the presence of gamma globulin, viruses and bacterial endotoxin have been shown to cause the release reaction and to aggregate platelets (Packham et alii, 1968; Jerushalmy, 1961). When given intravenously, endotoxin is
rapidly and selectively accumulated in the platelets rather than the other formed elements of the blood (Herring et alii, 1963). A similar response is seen following the intravenous infusion of colloidal carbon (Stehbens and Florey, 1960; Cohen et alii, 1965). Platelets have also been shown to phagocytose small particles in a manner which closely resembles that seen in other phagocytic cells (Movat et alii, 1965; Mustard and Packham, 1968).

These interactions of platelets with particulate matter have led a number of authors to suggest that platelets may well play an intermediary role in the removal of such particles from the blood stream (Stehbens and Florey, 1960; Cohen et alii, 1965; Van Aken et alii, 1968; Taniguchi et alii, 1930). However, studies of the mechanisms involved in the response of platelets to bacteria and other micro-organisms, with respect to this, are incomplete (Packham and Mustard, 1971).

These interactions, it is suspected, may contribute to the genesis of the generalised Shwartzman reaction. This reaction, the Sanarelli-Shwartzman phenomenon described in laboratory animals, is frequently taken as a model to explain similar changes sometimes seen in humans with septicaemic infections (Hjort and Rapaport, 1965; Rodriguez-Erdmann, 1965).
In the animal model, spontaneous bleeding and, in the rabbit, acute haemorrhagic necrosis of the renal cortex occurs following two intravenous injections of microbial products spaced 24 to 48 hours apart. This is accompanied by a reduction in the number of circulating platelets and a fall in the level of other clotting factors. Histological examination of tissues of animals dying as a result of, or killed during, this reaction show the presence of widespread platelet and fibrin thrombi in small vessels (Stetson, 1951; Rodriguez-Erdmann, 1964; Hardaway, 1966; McKay, 1965).

Platelets possess, in addition to private antigens, the same antigens as the leucocytes, i.e. the HLA system or the so-called transplantation antigens. They share both with the leucocytes and the red cells the ABO system of antigens. Platelets may be responsible, at least in part, for the rejection of homographs because they adhere non-specifically to antigen-antibody complexes and this can initiate thrombus formation (Svejgaard, 1969; Stargl et alii, 1970). There is some evidence to indicate that bacterial endotoxin and virus induced platelet injury is also mediated through the effect of an antigen-antibody complex (Des Prez and Bryant, 1966; Myllylä et alii, 1969).
These interactions with micro-organisms, endotoxin and antigen-antibody complexes are reviewed in more detail in the next chapter.

Summary

Blood platelets are small structures derived from bone marrow megakaryocytes. They have a distinct and complex ultrastructure and their biochemistry, metabolism and biological functions are as diverse as any other cell. The principal function of the platelet is the maintenance of haemostasis and vascular integrity. They do, however, appear to have other capabilities which include the ability to phagocytose and clear small particles from the blood stream, which may be an important intermediary mechanism for the removal of micro-organisms and microbial toxins.

The role of the platelet in the genesis of consumption coagulopathy associated with sepsis has been the subject of current interest and research because of its possible bearing on the incidence of septic shock and the accompanying acute renal failure. However, the role of the platelet in resisting infection and the mechanisms of platelet interactions with micro-organisms has received much less attention.
CHAPTER II

A REVIEW OF STUDIES OF PLATELETS IN INFECTIONS

AND PLATELET INTERACTIONS WITH MICRO-

ORGANISMS AND THEIR TOXINS
Introduction

The apparent general unawareness in the early 1960's of the reports of changes in the platelet count during the course of infectious diseases is exemplified in a letter to the Journal of the American Medical Association by Reimann as recently as 1968. In it he cites a number of publications appearing after 1964 describing thrombocytopenia, occasionally with purpura, during viral infections as if it were a new observation. Speculation that thrombocytopenia may be a rather frequent characteristic of, for example, rickettsial infections, he suggested could be resolved by reference to earlier literature on the matter of typhus alone (Reimann, 1968).

Indeed, Hayem was amongst the first to note changes in the number of circulating blood platelets in "acute febrile illnesses". In 1882 he described a rise in platelet numbers with the onset of fever with a fall to normal levels during the period of recovery (Hayem, 1882). Two years later, Afanassiew reported the occurrence of thrombocytopenia in typhus (Afanassiew, 1884). Between 1900 and 1940 the literature concerning platelets contained a number of references to thrombocytopenia, often followed by a transient thrombocytosis in the recovery period, in acute infections in general. This
suggested that this may not be an uncommon, albeit a transient, finding. Then, for twenty years from 1940 to 1960 this phenomenon was largely neglected.

Commenting on this in 1966, Cohen and Gardner suggested that the finding of thrombocytopenia had not received sufficient emphasis as an indicator of sepsis in the then recent textbooks of haematology, infectious diseases and medicine. They went further to suggest that thrombocytopenia probably occurs more frequently in bacteraemic infections, and in Gram-negative bacteraemic infections in particular, than could be suspected from reading the more recent literature of infectious diseases. In a review of seven large series of cases of Gram-negative bacillary sepsis since 1956 they found only two out of a total of 1,114 patients in whom thrombocytopenia was documented. It is noteworthy that there was no mention of platelet counts in most of these reviews (Cohen and Gardner, 1966).

Within the past ten years, however, reports of low platelet counts, with or without associated abnormalities in haemostasis, in patients with bacterial, protozoal, rickettsial and viral infections have been appearing with increasing frequency. This has been prompted in great measure by the
recognition that sepsis may be complicated by the syndrome of consumption coagulopathy, and by the increasing use of laboratory tests in the management of all patients.

Data have now accumulated to indicate that platelets are involved in the body's response to infection. The extent and significance of this involvement remains in doubt; the literature has therefore been reviewed in order to collect the information already available concerning the mode and sphere of activity of the platelet in this respect.

Changes in Platelet Numbers Observed during Infections in Man

The most characteristic feature of the changes in platelet numbers seen during the course of an infection, and one which tends to distinguish it from the behaviour of platelet involvement in many other pathological processes, is the lability, rapidity and variability with which they occur. There can be either a fall or rise in platelet count, changes which may occur within the space of a few hours or develop over a period of days or weeks, or there may be no change in platelet numbers at all. Whether fluctuations occur or not, whether this be a fall or rise in platelet numbers, and the timing of onset and duration of these changes appear to depend on several factors. These include the type of
infecting micro-organism, whether the infection is acute or chronic, localised or disseminated, on the severity of the infection and on the development of complications such as bone marrow depression or consumption coagulopathy. Despite these variations certain patterns have emerged.

The most consistently reported pattern is that of an early and rapid fall in the number of circulating platelets which may persist for several days. This is then followed by a rapid rise in platelet numbers to levels exceeding the normal limit, within six to nine days, when the patient is recovering. The thrombocytosis may be mild, persisting only a few days, or marked with the platelet count sometimes rising in excess of 750,000 cu.mm. between the eighth to fourteenth day after the onset of the infection. The platelet count thereafter falls to normal levels during the convalescent period.

In prolonged severe sepsis and in septicaemic states thrombocytopenia is relatively common. Indeed the degree of thrombocytopenia may reflect the severity of the disease and, in those infections with a fatal termination, the platelets virtually disappear from the peripheral blood (Tocantins, 1938). The persistence of thrombocytopenia in septicaemic
states can also be useful as an indicator of continued bacteraemia and endotoxaemia (Cohen and Gardner, 1966; Hendry, 1955; Mackay, 1931).

On the other hand a persistent thrombocytosis can be a prominent feature in localised infections. An immediate rise in platelet numbers at the onset of infection is associated with localised infections of the kidney, abscess formation, chronic lung infections and localised suppurative lesions (Tocantins, 1938; Brock, 1934; Wright and Kinnicutt, 1911; Webb et alii, 1914; Bannerman, 1924).

Different responses, with respect to the time of onset of infection and thrombocytopenia, have been noted in some infections, in particular those caused by viruses. From the onset the platelet count may fall progressively but slowly with the lowest counts, frequently of the order of 50,000 cu. mm. to 100,000 cu.mm., being recorded at the height of the infection. When the patient recovers the platelet count slowly rises to normal levels usually without a subsequent thrombocytosis (Myllylä et alii, 1969; Oski and Naiman, 1966).

Other reports record an increase in the platelet count in the acute phase of infections followed by a return to
normal levels during the convalescent period (Hayem, 1882; Tocantins, 1938), but when such a response has been observed a preceding transient thrombocytopenia cannot be excluded (Cohen and Gardner, 1966). In some viral infections, the thrombocytopenia has been noted during the incubation period with a sudden rise in platelet numbers occurring with the advent of fever (Stahl, 1923).

Thrombocytopenia, often followed by a thrombocytosis is, however, the most consistently reported finding. It has been documented in pneumonia (Reimann, 1924; Wright and Kinnicutt, 1911; Stahl, 1923, Mackay, 1931), miliary tuberculosis (Brock, 1934; Rahal et alii, 1968; Bannerman, 1924; Hendry, 1955), typhoid fever (Raybaud and Scarpellini, 1933; Wright and Kinnicutt, 1911; Banatvala et alii, 1965), other salmonella and shigella infections (Shimamoto et alii, 1958; Freiman and Super, 1966), meningococcal septicaemia (McGehee et alii, 1967; Hitzig et alii, 1968; Rosner and Ritz, 1966), bacterial endocarditis (Pepper, 1927; Mackay, 1931); scarlet fever (Bonciu, 1925), syphilis (Freiman and Super, 1966), clostridial, staphylococcal, streptococcal and Gram-negative bacillary septicaemic infections (Cohen and Gardner, 1966; Raybaud and Scarpellini, 1933; Rubenberg et alii, 1967; Rosner and Ritz, 1966; Rahal et alii, 1968), malaria
(Degkwitz, 1920; Darling, 1911; Hill et alii, 1964; Dennis et alii, 1967), typhus (Afanassiew, 1884; Reimann et alii, 1926), Rocky Mountain spotted fever (Schaffner et alii, 1965), chicken-pox (Welch, 1956; Clarke, 1961), infectious mononucleosis (Schumacher and Barclay, 1962; Clarke and Davis, 1964; Angle and Alt, 1950), influenza (Stahl, 1923; Gram, 1920; Kinsella and Brown, 1920), measles (Fisher and Kraszewski, 1952; Oski and Naiman, 1966), mumps (Fama et alii, 1964; Kolars and Spink, 1958), rubella (Banatvala et alii, 1965; Bayer, 1965; Ackroyd, 1949; Myllylä et alii, 1969; Morse et alii, 1966), smallpox (Keda, 1926; Nicolau, 1928) and a number of other infections (Tocantins, 1938; McKay and Margaretten, 1967; Rubio et alii, 1968; Mackay, 1931).

The Possible Mechanisms Involved in the Causation of the Variations in the Platelet Count Seen in Man during Infections

The rapidity and complexity of the changes in homeostasis and the clinical course in patients often critically ill with infections are sufficient to explain the sparseness of procedures directed towards elucidating the mechanisms involved in the causation of the accompanying fluctuations in platelet count. Several factors appear to be involved. Depression of production of platelets in the bone marrow, sequestration and destruction of circulating platelets may be
responsible to varying degrees even in the same individual.

A reduction in the number of megakaryocytes in the bone marrow has been noted in some viral infections (Oski and Naiman, 1966; Howie and Crosby, 1961). The replacement of the bone marrow by granulomatous infiltration can occur in tuberculosis (Hendry, 1955). And the anaemia, leucopenia and thrombocytopenia not uncommonly observed in severe or prolonged sepsis also suggests that interference with bone marrow function may be responsible for the fall in platelet count in some cases (Vaughan, 1948).

Studies of the survival time of infused Chromium 51 labelled platelets with infections and thrombocytopenia are few and the results contradictory. It is reported in a viral infection that the survival time is normal (Bhanchet et alii, 1968) which suggests depression of production; others have reported a shortening of the platelet survival time both in viral and bacterial infections (Cohen and Gardner, 1966; Morse et alii, 1966).

Human platelets have been shown to clump about some micro-organisms and to be aggregated, in the presence of factor V, by bacterial endotoxin in vitro (Houlihan, 1947; Copley and Houlihan, 1947; Ream et alii, 1965). And, in
human subjects, a rapid and profound fall in platelet count has been observed following the intravenous injection of T.A.B. vaccine. The reduction in platelet count is maximal 30 minutes after the injection, thereafter it rises to normal levels within a few hours (Mackay, 1931). A temporary sequestration of clumped platelets (rather than complete destruction of the circulating platelets) probably in the capillaries of the lung, spleen and liver is the most plausible explanation for such a response, and is similar to that observed in experimental animals.

The thrombocytopenia seen in acute infections, usually persists for several days and is only then followed by a rapid rise to levels often in excess of normal. This suggests that, in addition to sequestration, there is also destruction of platelets. Indeed, in a single thrombocytopenic subject suffering with a Gram-negative septicemic infection, Cohen and Gardner demonstrated a shortened life span for Chromium 51 labelled platelets infused at the height of the infection. In the recovery period, when the platelet count was rising, the life span of infused Chromium 51 labelled platelets returned to normal (Cohen and Gardner, 1966).

There are a number of factors which could cause this destruction of circulating platelets. The direct interaction
of platelets with the invading micro-organisms and their toxins probably accounts for the early and most immediate fall in platelet count.

Antigen-antibody reactions, where antibodies are formed and directed against a platelet-virus complex, is a possible explanation for the fall in platelet count seen in the later stages of some viral infections. The formation of antibodies to such a platelet-virus complex appears to operate in some cases of post-rubella thrombocytopenic purpura (Myllylä, 1969; Ferguson and Cantab, 1960). Such a mechanism could explain the occurrence of the self-limiting idiopathic thrombocytopenic purpura seen in younger people who often give a history of an infection in the preceding weeks (Hardisty and Ingram, 1965).

"Hypersplenism" or the pooling of platelets in an enlarged spleen has also been suggested as a factor contributing to the thrombocytopenia seen in infectious mononucleosis (Angle and Alt, 1950).

Platelets may also be "consumed" in a process of intra-vascular coagulation. Direct injury to the vascular endothelium or to the platelets themselves and the release of
"tissue factors" into the blood stream may all be involved in initiating coagulation of the circulating blood. The consumption of platelets in this process is only one of the manifestations of this complication; the clinical features of septic "shock" which include hypotension, renal failure and, in some, a generalised bleeding diathesis may also be related in some way to the occurrence of intravascular coagulation which is sometimes seen in severe infections.

A haemorrhagic diathesis, however, can occur without any apparent involvement of the circulating haemostatic components. Conversely, significant alterations in circulating coagulation factors can be observed without the appearance of a haemorrhagic diathesis.

The Relationship of Thrombocytopenia to the Haemorrhagic Manifestations of Infections

Long before the discovery of the platelet, purpura and other bleeding phenomena were accepted as a manifestation of pestilential fevers. In the fifth century B.C. Hippocrates recognised the unfavourable prognosis of patients with fevers who bled, particularly when bleeding occurred from the gastrointestinal tract (Hippocrates, in "A Theory and Practice of Medicine", Philosophical Library, 1964). Centuries later,
Giovanni Boccaccio presented a graphic account of purpura in victims of the "Black Death" which swept through northern Italy and his home city of Florence in 1349. He described the appearance of 'bubo' in the groins and armpits and how the "form of the malady began to change, black spots or livid making their appearance in many cases on the arms and thighs or elsewhere, now few and large, now minute and numerous" (Boccaccio, in "Decameron", Navaare Society, London).

Similar descriptions of victims of the 'Great Plague of London' which occurred in 1665 appear in Daniel Defoe's 'Journal of the Plague Year' (Defoe, 1969, Oxford University Press).

Within the past fifty years it has been appreciated that a reduction in platelets may or may not occur in association with these haemorrhagic manifestations but when it does it is recognised to be a poor prognostic sign (Tocantins, 1938). There is, however, some controversy concerning the relationship of platelet involvement in these manifestations.

1. **Bleeding in the absence of thrombocytopenia.** The rash of scarlet fever, measles, typhoid fever and other infectious diseases not uncommonly becomes haemorrhagic, especially over areas of skin subject to trauma. This is
due to local injury to the small blood vessels by the direct action of micro-organisms and their toxins (Hardisty and Ingram, 1965).

Alternatively, purpura may appear several weeks after the onset of the infection when the patient is recovering. It is not associated with thrombocytopenia or any other defect in circulating coagulation factors. This syndrome, known as Schönlein-Henoch purpura, anaphylactoid or allergic purpura, has the clinical features and the vascular lesions which indicate a hypersensitivity phenomenon (Gairdner, 1948; Hardisty and Ingram, 1965). The bleeding appears to be the result of local injury to the vascular endothelium by antigen-antibody complexes induced by the preceding infection. In animals, antisera against vascular endothelium will produce a similar type of bleeding (Clark and Jacobs, 1950).

2. *Bleeding manifestations associated with thrombocytopenia*. A rare complication sometimes occurring after acute infections, at about the same time as anaphylactoid purpura, is so-called "purpura fulminans". The lesions in this condition are primarily gangrenous rather than haemorrhagic, and are caused by local occlusion of small blood vessels. In about half the cases a more generalised bleeding state supervenes and this is characterised by a fall in the platelet
count and levels of plasma clotting factors. Skin biopsy reveals numerous masses of platelets, leukocytes and fibrin within the small vessels of the dermis over the affected areas. This localised intravascular coagulation is not reflected by a reduction in the levels in circulating clotting factors, including platelets, unless the process is extensive and severe or unless it becomes 'disseminated' (Hjort et alii, 1964).

Similar clinical and laboratory features have been noted in patients with bacillary and viral infections. They differ, however, in that there is usually no latent period; the haemorrhagic manifestations occur at the height of the infection. Again it seems that micro-organisms or their toxins injure the vascular endothelium directly (Martin and Shore, 1928). Either excessive local consumption, or loss of coagulation factors or the supervention of disseminated intravascular coagulation may then cause a fall in the platelet count (Bayer, 1965).

3. Disseminated intravascular coagulation: Consumption coagulopathy. 'Consumption coagulopathy' or 'disseminated intravascular coagulation' (the term 'defibrination syndrome' is inadequate as the level of fibrinogen may be normal, or
even raised) is an intermediary mechanism of disease. Tissue extracts, products from haemolysis of red cells, proteolytic enzymes, colloidal matter, damaged vascular endothelium, micro-organisms and endotoxin can all initiate coagulation of the flowing blood if they enter the circulation. The changes in the haemostatic mechanism which follow initially show a reduction in the number of platelets and the plasma coagulation factors V and VIII. In the more severe cases depletion of fibrinogen and factors II, VII, IX, X, XI, XII and XIII may also occur (Hardaway, 1966; McKay, 1965; Hjort and Rapaport, 1965).

Almost simultaneously, secondary activation of the fibrinolytic system is reflected by a decrease in the level of circulating plasminogen and the appearance of fibrin split products in the serum. In some patients, in particular those with inflammatory conditions, the coagulation process predominates and activation of fibrinolysis is slow or minimal (McKay, 1965; Corrigan and Jordan, 1970, Fearnley, 1969). Platelet and fibrin thrombi then appear in the arterioles, capillaries and venules of many viscera. If these thrombi persist for any length of time, ischaemic and, frequently, haemorrhagic necrosis of organs will occur.
The whole spectrum of reduced platelet numbers, coagulation factors, haemorrhagic and ischaemic necrosis of organs and a generalised bleeding diathesis is fortunately a relatively rare occurrence but has been documented in Gram-negative bacillary infections (Corrigan et alii, 1968; Goldenfarb et alii, 1970; Rapaport et alii, 1964), meningococcal (Abildgaard et alii, 1967; Winkelstein, 1969; Rosner and Ritz, 1966; Hitzig et alii, 1968), clostridial (Rubenberg et alii, 1967), other bacteraemic (Rosner and Ritz, 1966; Hjort and Rapaport, 1965; Goldenfarb et alii, 1970), protozoal (Dennis et alii, 1967) and viral infections in man (McKay and Margaretten, 1967).

**The Incidence of Thrombocytopenia, Thrombocytosis and other Abnormalities of Haemostasis in Human Infections**

There are now a number of reports describing changes which occur in the haemostatic mechanism in septicaemic states. These suggest that thrombocytopenia is not an uncommon finding, albeit a transient phenomenon, in severe sepsis.

In a survey of 36 children with septicaemia, Corrigan and co-workers report thrombocytopenia to be the most frequent single abnormality of the haemostatic mechanism.
It was noted in 61% of all cases; associated abnormalities of plasma coagulation were less common. Bleeding episodes, notably gastrointestinal bleeding, were recorded in eight patients (22%) (Corrigan et alii, 1968). In another study of 26 children with septicaemic shock, Corrigan and Jordan documented thrombocytopenia in 23 (1970). Thrombocytopenia and other abnormalities indicative of intravascular coagulation have also been reported in eight of ten cases of meningococcaemia observed over a period of six years (Hitzig et alii, 1968). In a group of adults with septicaemia, Goldenfarb et alii recorded thrombocytopenia in 16 of 34 cases. A thrombocytosis at some time during the course of these patients' illness was noted in 17, in 12 the platelet count was in excess of 750,000 cu mm and in three greater than 1,000,000 cu mm (Goldenfarb et alii, 1970).

Despite these reports, the relative frequency of infection as a cause of thrombocytopenia, or the incidence of thrombocytopenia, thrombocytosis and abnormalities in haemostatic mechanism in infections in general is not known.
Studies in Experimental Animals of the Response of Platelets to Micro-organisms and Endotoxin

The injection of bacteria, endotoxin and particulate matter into the circulation of experimental animals almost invariably causes a rapid and sudden fall in the number of circulating platelets (Aynaud, 1911; Cohen et alii, 1965; Taniguchi et alii, 1930; Delrez and Govaerts, 1918; Saluidio and Crosby, 1961; Tait and Elvidge, 1926; Dudgeon and Goaby, 1931). Several interactions between platelets and the injected material are involved; adhesion, aggregation, lysis, phagocytosis and participation in intravascular coagulation may all occur to varying degrees.

1. Adhesion and aggregation. Platelets rapidly adhere and aggregate about injected particles, a phenomenon which in the past was often referred to as 'platelet loading'. It was first described by LeVaditti in 1901. He noted, after an intravenous injection of cholera vibrio into rabbits, that in addition to the phagocytosis of organisms by the leukocytes, some became adherent to platelets (LeVaditti, 1901). In 1911 Aynaud extended this observation by showing that 'platelet loading' could be induced by several different species of bacteria and a variety of other materials such as colloids and peptones (Aynaud, 1911).
A few years later Delrez and Govaerts described clumping and adherence of platelets to bacteria almost immediately after the latter were injected into the bloodstream of a dog or rabbit. The bacteria and platelet aggregates so formed rapidly disappeared from the circulating blood but could then be found in the capillaries of the lung and liver (Delrez and Govaerts, 1918). These observations have since been confirmed by several investigators (Dudgeon and Goaby, 1931; Taniguchi et alii, 1930).

Platelets will also adhere to bacteria in vitro. However, the affinity with which they do so varies considerably from one animal species to the next. Rabbit and dog platelets rapidly adhere and clump about a wide variety of different bacterial species, whereas human and pig platelets show far less tendency to do so (Houlihan and Copley, 1946; Houlihan, 1947; Copley and Houlihan, 1947).

This ability of platelets to adhere to bacteria is greatly facilitated by the presence of plasma or serum. Roskam postulated that this was a property of the 'atmosphere plasmatique' which 'opsonised' the bacteria, for if the plasma proteins are removed from the surface of the platelet by repeated washings in saline this reduces their ability to adhere
to bacteria (Houlihan and Copley, 1946; Govaerts, 1921; Roskam, 1921). Direct evidence as to the nature of the factor responsible for platelet adhesiveness and aggregation was not forthcoming but it was differentiated from bacterial agglutinins, complement and opsonins by various means (Houlihan, 1947).

In the light of more recent research it seems probable that the release of ADP from platelets coming into contact with bacteria or endotoxin may be partly responsible, although it has been shown that the presence of plasma proteins, or the coating of the surface of particulate matter, greatly enhances the release reaction and subsequent phagocytosis of small particles by platelets (Glynn et alii, 1965).

The adhesion and aggregation of platelets about particulate matter is a basic response of the platelet as evidenced by the wide variety of agents which cause this phenomenon. In some instances, however, it appears to be selective. If pneumonococci and staphylococci are injected together intravenously, only the staphylococci form aggregates with platelets (Tocantins, 1938).
This aggregation is, for the most part, reversible but a proportion of platelets are injured and undergo lysis. It has been stated by some investigators that platelet lysis correlates better with the thrombocytopenia seen after the injection of bacteria or endotoxin than aggregation and that it is also associated with a rise in circulating heterophil antibody titre (Tocantins, 1938).

2. Phagocytosis. Platelets can phagocytose small particles, including viruses, although there is no evidence available to indicate that bacteria are ingested (Glynn et alii, 1965; Mustard and Packham, 1968; David-Ferriera, 1961). Despite certain differences, the response of platelets coming in contact with particulate matter is similar in many respects to that shown by leukocytes (Glynn et alii, 1965; Mustard and Packham, 1968).

If colloidal carbon is injected into the blood stream there is an immediate fall in platelet count. Furthermore, the platelets become 'loaded' with carbon particles and aggregate about themselves. Clumps of carbon-laden platelets then appear in the capillaries of possibly all the organs of the body (Stehbens and Florey, 1960; Dudgeon and Goaby, 1931; Roskam, 1921). Leukocytes accumulate round these
clumps and may ingest both platelets and carbon particles, although most of the platelets appear to return to the circulation (Stehbens and Florey, 1960). With the return of the platelets, now free of carbon, the process can be repeated, but with each successive injection of colloidal carbon the number of platelets which return to the circulation gradually diminishes (Cohen et alii, 1965). It has also been demonstrated that the rate of clearance of colloidal carbon injected into thrombocytopenic animals can be increased by the transfusion of platelets (Van Aken et alii, 1968).

A response similar to that induced by carbon occurs after the intravenous injection of endotoxin. The fall in platelet count is, however, more prolonged and the return of platelets to the circulation less complete (Cohen et alii, 1965). Like carbon particles, the observation that chromium 51 labelled endotoxin is rapidly taken up by platelets, and that almost all of a dose remaining in the circulation (within 5 minutes of injection) can be found concentrated in the platelets does suggest that platelets may, in some way, be involved in its clearance from the blood stream (Herring et alii, 1963).
Phagocytosis of endotoxin by platelets appears to be the mechanism by which this is achieved. There are two phases, the first consisting of an interaction between endotoxin and antibody to form immune complexes, and the second is the phagocytosis of these complexes by the platelet (Des Prez and Bryant, 1966; Des Prez, 1964).

3. Intravascular coagulation. The more profound and persistent fall in platelet count in response to the intravenous injection of bacterial endotoxin can, to some extent, be explained on the basis of endotoxin-induced platelet injury and intravascular coagulation.

E. coli endotoxin causes rabbit platelets to aggregate in vitro with the release of platelet phospholipid. It also shortens the coagulation time of recalcified plasma or whole blood in siliconised tubes; this acceleration of coagulation only occurs when platelets are present (Des Prez et alii, 1961).

This transfer of platelet factor 3 activity from platelets to plasma can also be demonstrated in vivo after the intravenous injection of a sublethal dose of endotoxin. At the same time the whole blood clotting time is significantly shortened. The effect reaches a maximum one to five minutes
after the injection and becomes more pronounced with increasing doses of endotoxin. The clotting time then returns to normal within an hour. A fall in the platelet count occurs in all animals but usually there is no reduction in the plasma level of fibrinogen; on the contrary there can be a rise, and no prolongation of the one-stage prothrombin time (Horowitz et alii, 1962; Kliman and McKay, 1958).

With the administration of a larger dose, endotoxin-induced shock in dogs results in an immediate disappearance of platelets followed by a gradual decrease in plasma levels of fibrinogen, factor V and factor VII and activation of an endogenous heparin-like activity. Prior heparinization of the animals prevents the fall in fibrinogen and significantly decreases the rate of mortality (Hardaway and Johnson, 1963).

The enzymic and physiological events caused by the intravenous injection of endotoxin are complex. Endotoxin can, for example, initiate coagulation by its action on factor XII. Activated factor XII may also activate the plasma kallikrein and fibrinolytic systems (Erdös and Miwa, 1968). The formation of platelet aggregates and micro-thrombi also appear to be important in the genesis of the clinical manifestations and abnormalities of septic shock, and the Shwartzman reaction.
This latter phenomenon has been used by many investigators as a model for endotoxin-induced intravascular coagulation.

4. *The Schwartzman reaction.* This can be either a localised or a generalised phenomenon. Classically it is elicited in rabbits by the intravenous injection of two appropriately spaced sub-lethal doses of bacterial endotoxin. The first, or 'preparation', dose is injected intradermally for the localised reaction, and intravenously for the generalised reaction. In the localised reaction the capillaries and small veins in the area of prepared skin become occluded with platelets and neutrophils. In the generalised reaction the first dose of endotoxin causes minor changes in the haemostatic system, referred to in the previous section, with the deposition of a few fibrin thrombi in the liver, lungs and spleen. These thrombi do not persist but undergo rapid dissolution.

The second, or 'provoking' dose has to be administered in general between 24 and 48 hours after the 'preparation' dose, intravenously in both the localised and generalised reactions. In the localised reaction, in the area of prepared skin, haemorrhage and necrosis occurs about the involved vessels (Stetson, 1951). In the generalised reaction there is a rapid
and marked fall in platelets together with other coagulation factors and the deposition of fibrin thrombi in the capillaries of the renal glomeruli with the ultimate development of bilateral haemorrhagic renal cortical necrosis (Stetson, 1951; Rodriguez-Erdmann, 1964a; Hardaway, 1965; McKay, 1965; McKay, 1963).

Other known ways of 'preparing' the animal, be it a rabbit or rat, for the Shwartzman reaction include the induction of pregnancy (Kliman and McKay, 1958; Rodriguez-Erdmann, 1964b), administration of cortisone (Thomas and Good, 1951), reticulo-endothelial blockade (Good and Thomas, 1952; Rodriguez-Erdmann, 1964b), the inhibition of fibrinolysis (Lee, 1962), and the production of obstructive jaundice (Wardle and Wright, 1970). A single intravenous dose of endotoxin will cause death with associated consumption coagulopathy in any of these situations.

Intravascular coagulation and impaired fibrinolysis are both facets of this phenomenon. To lend emphasis to this the pre-treatment of animals with anticoagulants before, but not after the second dose of endotoxin (Good and Thomas, 1953; Shapiro, 1958; Corrigan, 1970) and activation of fibrinolysis with streptokinase after the second dose effectively prevents
the tissue damage attributed to the deposition of microthrombi (Condie, 1957; Rodriguez-Erdmann, 1964b).

It has been suggested that platelets are not essential for the genesis of the Shwartzman reaction since animals rendered neutropenic but not thrombocyticopenic with nitrogen mustard do not develop the characteristic features following the injection of endotoxin (Forman et alii, 1969). Conclusions drawn from such experiments may not be entirely valid since drug induced impairment of platelet function is sufficient to prevent the Shwartzman reaction (Evans and Mustard, 1968). Although experiments in which animals rendered thrombocytopenic with antiplatelet serum are also suspect because antigen-antibody reactions can induce a state of 'preparedness', Margaretten and McKay (1969) have devised models which suggest that platelets are essential for the genesis of the Shwartzman reaction.

Even though there is still considerable speculation as to the mechanisms, it does appear that the platelet is intimately involved in the haemorrhagic and tissue injury manifestation induced by endotoxin.
Bactericidal Properties of Platelets

Substances formed or released during the coagulation of whole blood may impart to the serum bactericidal activities not present in the circulating plasma. The presence of platelets appears to be an important factor in contributing to this enhanced activity as significant differences have been noted between the ability of whole blood serum to kill organisms; this is serum obtained after clotting has occurred in the presence of red cells, leukocytes and platelets, and plasma serum which is obtained from the clotting of plasma in the absence of formed elements of the blood.

Small quantities of rabbit whole blood serum has a lethal effect on several Gram-negative organisms, whereas high concentrations of plasma serum does not kill the organisms. Serum collected from plasma rich in platelets is, however, as lethal as whole blood serum, the effect being proportional to the number of platelets contained in the plasma. Serum collected after clotting has occurred in the presence of red cells or leukocytes does not have this effect (Hirsch, 1960; Des Prez et alii, 1961).

A substance, designated 'plakin', has been extracted from horse platelets which is bactericidal to a variety of
organisms. Within ten minutes of adding plakin to a suspension of organisms, the bacteria have been shown by electron-microscopy to become 'ghost cells'. Plakin appears to attack constituents of the surface membrane of the bacteria. There is loss of the normal staining characteristics and semi-permeability properties of the cell wall. The latter results in an outpouring of cell contents when plakin-treated organisms are exposed to hypertonic saline solutions. Concurrent with these changes, oxygen uptake by the bacteria ceases and there is complete inactivation of the cytochrome C oxidase activity within the cell (Amano et alii, 1953a, 1953b, 1953c, 1954).

Human serum is found to contain low levels of bactericidal activity against Gram-positive organisms when compared with that of the horse or rabbit. Human plasma serum is also capable of killing a number of Gram-positive and Gram-negative organisms as effectively as serum collected from whole blood (Hirsch, 1960).

Although controversy still exists concerning the origin, nature and significance of bactericidal substances, for there are many, found in the plasma and sera of animals and man the release of platelet components during aggregation, lysis and clotting do appear to contribute to an increase in the level
of circulating antibacterial substances in some species.

Summary of Theories Proposed Concerning the Role of Platelets in the Body's Defence Against Infections

Govaerts in 1921 was among the first to suggest that platelets may play a role in the body's reaction to invasion by micro-organisms. He did not observe any evidence of destruction of bacteria in the platelet-bacteria aggregates seen in the blood stream and capillaries of the lung and liver, but postulated that the interaction between bacteria and platelets was an important mechanism for the rapid removal of organisms from the blood stream, and that the accumulation of bacteria and platelet aggregates in the capillaries was the means by which the organisms were anchored thereby preventing their dispersion and continued presence in the circulation (Govaerts, 1921).

Platelets aggregate about bacteria and particular matter in general. Accumulation of these aggregates in close proximity to cells of the reticulo-endothelial system then occurs in the capillaries of possibly all the organs of the body. This may facilitate the phagocytosis of foreign matter by leukocytes and macrophages. This concept has been reiterated and extended by a number of investigators over the past fifty years (Popesco-Combiesco, 1928; Taniguchi et alii,
1930; Dudgeon and Goadby, 1931; Tocantins, 1938; Stehbens and Florey, 1960; Cohen et alii, 1965; Mustard and Packham, 1968; Packham et alii, 1968).

Doubt was cast on the significance of platelet adhesion by Bull and McKee. They found that bacteria were removed more rapidly from the circulation of both immune and non-immune rabbits which had been previously depleted of platelets by an infusion of antiplatelet serum (Bull and McKee, 1922). The observations of later workers that lysis of platelets in vivo increased the level of gamma-globulins in the plasma together with the release of other platelet components such as plakin have been offered as an explanation of this phenomenon.

Popesco-Combiesco, while supporting these early observations, went further to suggest that platelets were capable of destroying adherent micro-organisms by undergoing lysis with the liberation of haemolysins, opsonins or agglutinins condensed about the surface of the platelet (Popesco-Combiesco, 1928, 1930).

Taniguchi ascribed to the platelet a role of even greater significance in the body's defence against invasion
by micro-organisms. He observed, as other investigators had noted, that following an intravenous injection of bacteria the same rapid disappearance and associated fall in number of circulating platelets which was, within five minutes of the infusion, followed by the appearance of aggregates of bacteria and platelets in the capillaries of the lung. He then described a process by which the bacteria were digested by secretions elaborated by the adherent platelets. With the destruction of the bacteria most of the platelets returned to the circulation. Designating this process 'pepticytosis', he uses by way of an illustration the analogy that this has the appearance of 'a swarm of insects devouring a fruit'. He maintained that phagocytosis by leukocytes appeared slower, reaching its maximum activity in about thirty minutes and continuing for more than an hour, whereas pepticytosis was complete within fifteen to twenty minutes.

These observations led Taniguchi to conclude that platelets serve as the first line of defence against foreign particulate matter and that leukocytes form the second, since the numbers of micro-organisms acted on by platelets was so much greater and more rapid than by leukocytes (Taniguchi et alii, 1930).
Little interest was shown in the platelets response to infection over the next twenty years; it could be speculated that the advent of effective antibiotics for the treatment of infections had much to do with this decline. Only recently has there been renewed interest in this field with the apparently overstated views of Taniguchi receiving partial endorsement with the confirmation that platelets are involved in the removal of colloidal particles (Stehbens and Florey, 1960; Cohen et alii, 1965) and endotoxin from the circulation (Cohen et alii, 1965; Herring et alii, 1963; Des Prez, 1964) and the reiteration that the platelet may play a role in the body's reaction to infections (Mustard and Packham, 1968). The mode and significance of the platelets reactions with respect to this have not yet been defined.

Summary

Thrombocytopenia in the acute phase of many infections may not be an infrequent finding, albeit a transient and variable phenomenon. Despite the many reports over the past seventy years to suggest that this is so in both man and animals, infections have only recently been accepted as a significant cause of thrombocytopenia. The frequency with which this occurs in human infections has not been fully established.
It is clear that thrombocytopenia in severe infections can be accompanied by other abnormalities of the haemostatic system. There is much evidence to suggest that damage to many organs, in particular the kidneys, occurs at the same time due to the deposition of micro-thrombi deposited in the capillaries. This process appears to be important as one of the factors in the genesis of the acute renal failure and other manifestations seen in patients with septicaemia. Only occasionally will these changes cause an overt thrombo-haemorrhagic diathesis. The platelet is intimately involved in these processes.

Platelets also interact with micro-organisms and endotoxins both in vivo and in vitro. A number of investigators have suggested that the platelet may act as a 'scavenger', removing particulate matter from the circulation and trapping it in capillaries in the vicinity of cells of the reticulo-endothelial system. The plasma proteins condensed about the platelet surface and the release of bactericidal factors may also facilitate the death and phagocytosis of micro-organisms by macrophages. The platelet appears to be even more intimately involved in the removal of bacterial endotoxins from the circulation. Just how important the role of the platelet is
in respect to this and its significance in the overall defence of the body against infection, however, is not yet known.
CHAPTER III

COLLECTION OF BLOOD SAMPLES AND THE EVALUATION OF METHODS USED FOR THE COUNTING OF PLATELETS
Platelet counts are invaluable in the diagnosis of bleeding disorders and other haematological problems and, in general, the main causes of low platelet counts in man are well recognized. The use of platelet counts, however, has been limited by the errors existing in present day techniques.

The accuracy of standard manual techniques is known not to be good. Assuming perfect technical manipulation with phase contrast microscopy, there is an inherent variation of 11 per cent from the Poisson distribution effect. The method is also time consuming and the resultant eye strain, even in the hands of a skilled technician, can increase the risk of error. Electronic methods for counting platelets are more rapid and precise but are associated with many technical variables. In addition, poor venepuncture technique and partial clotting of the blood due to inadequate mixing with anticoagulant have always to be considered as a cause for a low platelet count.

In this chapter the methods of collection and the technical manipulation of the blood samples carried out routinely for diagnostic purposes in the haematology laboratory of the Institute of Medical and Veterinary Science
are described and evaluated.

The Collection of Blood Samples

Blood samples were collected, in the great majority of cases, by the investigator or by a team of nursing sisters whose sole duty was the routine collection of blood specimens. The remaining samples were collected by the medical officers caring for the patients attending the Royal Adelaide Hospital.

Blood for routine haematological procedures was obtained by standard venepuncture techniques using disposable plastic syringes (Jintan Terumo Co. Tokyo, or Pharma-Plast (Australia) Pty. Sydney) through a 0.80 x 25 mm (21G) Terumo sterile disposable needle. For routine haematological procedures which included estimation of haemoglobin, cell counts, blood smear and platelet count, 5 ml of blood was then immediately delivered into a polypropylene container with 1 mg dry di-potassium ethylenediamine tetraacetate (EDTA) and gently mixed.

PLATELET COUNTS

Platelet counts by a visual method. The method used was slightly modified from that described in 'Practical Haematology' by Dacie and Lewis. Whole blood was diluted 1:100 by pipetting 40 μl into 4 ml of a solution of ammonium oxalate, 1G in 100 ml
distilled water. After mixing, an aliquot of this dilution was transferred to a double sided Neubauer counting chamber by means of a capillary tube. Using a Leitz phase contrast system, all platelets in both sides of the chamber were counted. The mean of these two counts was taken as the whole blood platelet count.

Platelet counts using the Coulter counter, model F. The method described by Bull et alii (1965) was modified slightly. Platelet-rich plasma was obtained by centrifuging the blood in the Institute of Medical and Veterinary Science-designed EDTA container for five minutes in a MSE Wintrobe haematocrit centrifuge at 1,000 rpm.

An initial estimate of platelet numbers was made by inspection of the blood film or from knowledge of previous counts. The following dilutions were made with saline with respect to these estimates:

- 20 μl in 20 ml for counts: 10,000-70,000 cu mm
- 20 μl in 40 ml for counts: 50,000-150,000 cu mm
- 20 μl in 100 ml for counts: 100,000-300,000 cu mm
- 20 μl in 200 ml for counts: 300,000-1,000,000 cu mm
A counter setting of 8 for the lower threshold and 70 for the upper threshold was used. The crude counts were then corrected for coincidence error and the whole blood platelet count derived by correcting for the packed cell volume using factors listed by Bull et alii for platelet-rich plasma obtained by sedimentation.

*Visual estimation of platelet counts in blood films.* All platelet counts were checked, and frequently instituted, by inspection of the blood film. In an evenly smeared blood film a single platelet in a high power field was found to roughly correspond to a count of 10,000 cu mm. Inspection of the film also obviated errors due to gross clumping of the platelets and the presence of fibrin strands indicated in which specimens clotting might have occurred.

To assess the reproducibility and reliability of platelet counts obtained in the routine haematology laboratory, counts were performed in patients and normal subjects by both the investigator and laboratory technicians using the visual and Coulter counter methods.

Blood was collected from 46 normal subjects, all members of the staff of the Institute of Medical and Veterinary Science. Visual counts were performed in duplicate by the
investigator; blood was sampled both prior to centrifuging for counts by the Coulter counter method and after remixing the centrifuged blood subsequent to the sampling of the platelet-rich plasma for the Coulter counter dilutions. A further 50 specimens from patients in the wards of the Royal Adelaide Hospital were treated in the same manner. Platelet counts were also repeated on 27 samples by technicians using the Coulter counter in the I.M.V.S. laboratory, and a further count was performed by a technician at the Queen Elizabeth Hospital, Woodville, South Australia, using the same procedure on a different Coulter counter.

**STATISTICAL METHODS**

The analysis of data was carried out by the methods contained in the Geigy Scientific Tables (J.R. Geigy, S.A., Basle, Switzerland, 6th edition, 1962) with the assistance of the mathematical and statistical tables in the same publication. In reporting results, the form A ± B refers to the arithmetical mean, the standard (root mean square) deviation or, as in the animal experiments, the standard error. Probability values, quoted a p < x, are the two sided limits for deviation from the null hypothesis. Correlations between variables have been evaluated by calculation of the regression equations and correlation.
coefficients, quoted as $r$, and by the student $t$ test.

**RESULTS**

Using the visual method, the mean platelet count in 46 normal subjects is $264,000 \pm 51,000$ cu mm. This is in close agreement with the results obtained by other workers (Brecher et alii, 1953; Fry and Hoak, 1969).

The mean platelet count by the Coulter counter method is $322,000 \pm 72,000$ cu mm. This count is significantly higher ($p < 0.001$) than the value obtained by the visual method.

A comparison of 96 duplicate counts on samples from normal subjects and patients reveals satisfactory correlation between counts made by the same method, i.e. visual against visual (Figure 2, $r 0.707$, $t 6.63$ coefficient of variation 13%) and Coulter counter against Coulter counter (Figure 3, $r 0.959$ and $t 17$ coefficient of variation 10.7%). Correlation between the two methods is less satisfactory. Over a wide range of values the platelet counts by the Coulter counter method are higher than those obtained by the visual method. Shown in Figure 4 are the regression lines for values between $0 - 150,000$ cu mm: line A ($r 0.746$, $t 4.48$) and $150,000 -$
FIGURE 2: Comparison of duplicate platelet counts by the visual method.
FIGURE 3: Comparison of duplicate platelet counts by the Coulter counter method.
FIGURE 4: Comparison of platelet counts by the Coulter counter method against platelet counts by the visual method.

Line A: Regression line for values 0 - 150,000 cu mm.

Line B: Regression line for values 150,000 - 1,000,000 cu mm.
1,000,000 cu mm; line B (r 0.585, t 4.79). There is correlation but it is not linear throughout the whole range of counts.

EVALUATION OF THE METHODS USED AND OF THE ACCURACY AND RELIABILITY OF THE PLATELET COUNTS OBTAINED

The accuracy of platelet counts obtained by visual methods is not good. With phase contrast microscopy there is an inherent variation of 11 per cent from the Poisson distribution effect. Assuming perfect technical manipulation, this will give a coefficient of variation of six per cent. In practice the coefficient of variation for manual counts has been shown to be between eleven and thirteen per cent (Brecher et alii, 1953; Fry and Hoak, 1969). The coefficient of variation of 13 per cent obtained when 96 duplicate counts are compared is therefore within the accepted practical limits of accuracy for visual methods.

The platelets are counted electronically by the Coulter counter. Although this procedure is quicker and more precise than visual methods, platelets cannot be counted in the presence of red cells and preparation of platelet-rich plasma is required to determine the whole blood platelet count.
With centrifugation very high plasma platelet counts can be obtained, especially from the upper layers of the plasma. This observation of a predictable excess concentration of platelets in the supernatant plasma has invited speculation. It has been postulated that there is a fixed amount of platelet-free plasma surrounding each red cell which is carried down with the red cell during centrifugation leaving a relatively higher concentration of platelets in the supernate. The excess concentration of platelets in the supernatant plasma is significantly influenced by the haematocrit of the blood sample. When the haematocrit is high, more red cells and therefore more platelet-free plasma will be carried down during centrifugation. This then leaves less supernatant plasma in which the platelets can disperse and thus lead to a greater excess of platelets.

The mechanism by which the red cells retain a platelet-free zone of plasma round them is not clear. It is related most probably to the static electrical charge on the surface of the red cell as substances which reduce the surface charge, such as aluminium chloride, decrease the amount of platelet-free plasma the red cell can retain during centrifugation. Under these conditions the
platelet concentration in the supernatant plasma is decreased. Conversely, substances which increase the surface charge increase the amount of platelet-free plasma surrounding the red cell and thereby increases the concentration of platelets in the supernate.

There is accentuation also of the negative charge on the surface of platelets when they are suspended in a solution of electrolyte in water. This causes the platelets to repel one another with the smaller lighter platelets tending to rise relative to other larger and heavier particles.

Although it is not possible to obtain an accurate whole blood platelet count by the Coulter counter method, this excess of platelets in the supernatant plasma is, in practice, of little consequence and can be overcome by making a correction from an empirically derived table (Eastham, 1963; Bull et alii, 1965).

As size is used to discriminate between platelets and red cells this can also be a source of error in those conditions in which the platelets are larger than normal; this can result in counts lower than the true value (Fry and Hoak, 1969).
When the Coulter method is compared with other methods some authors claim that the errors associated with electronic counting of platelet-rich plasma as a means for assessing the whole blood platelet count are of the same order as those associated with visual methods (Eggleton and Sharp, 1963).

However, the discrepancy between counts obtained by the Coulter counter and visual method in this study does suggest that there are fundamental differences in the counting by the two methods since the coefficients of variation between duplicate counts by the same method are within the accepted limits of accuracy. An explanation for this could be the result of the modification in the method described by Bull et alii (1965) where centrifugation is substituted for sedimentation and the use of polyvinylpyrrolidone - a substance which decreases the surface charge on the red cells and thereby minimising the tendency towards the excess concentration of platelets in plasma - is omitted.

Other variables also have to be considered; the visual counts were all performed by the investigator whereas the counts by the Coulter counter method were carried out by a
number of the laboratory staff treating the specimens as part of the normal workload of the laboratory. The degree of variation between duplicate counts by the Coulter counter method is more than can be achieved under ideal circumstances but is acceptable when viewed as a procedure performed in the course of the routine work of the laboratory.

**SUMMARY**

The procedures for the collection of specimens in the Royal Adelaide Hospital and the methods used to count platelets in the haematology laboratory of the Institute of Medical and Veterinary Science are described. For routine diagnostic purposes platelets are counted by an electronic method with a Coulter counter, model F, using a modification of a previously described procedure.

The platelet count obtained by this method in normal subjects was 322,000 S.D. ± 72,000 cu mm, a value significantly higher than the count obtained by phase contrast microscopy of 264,000 ± 51,000 cu mm. Although the degree of variation between duplicate counts by the same method are just within the accepted practical limits of accuracy, correlation between the two methods is not good. The
Counts obtained by the Coulter counter method are consistently higher than those obtained by the visual method. The possible reasons for this discrepancy have been discussed.

As the intent of this thesis is to examine thrombocytopenia in infections, a platelet count of less than 150,000 cu mm by the Coulter counter method can therefore be regarded to reflect a significant thrombocytopenia.
CHAPTER IV

A SURVEY OF THE CAUSES OF THROMBOCYTOPENIA IN A HOSPITAL POPULATION WITH SPECIAL REFERENCE TO THE OCCURRENCE OF LOW PLATELET COUNTS IN INFECTIONS
Although the main causes of a low platelet count are generally recognized the difficulties associated with the accurate assessment of platelet numbers on a routine basis probably accounts for the few reports concerned with the incidence of thrombocytopenia and its causes in the community in general. Indeed, the frequency with which thrombocytopenia occurs in many disorders and the degree of severity in each do not appear to be widely known.

It was thought, therefore, that a survey of the frequency of the causes of thrombocytopenia detected by routine laboratory methods would be of value in assessing the significance of a low platelet count.

As the provisional diagnoses attached to patients admitted to hospital were frequently modified during the course of the illnesses, it was decided that the incidence of thrombocytopenia in infections and other conditions could be assessed most accurately if platelet counts were performed on all patients within a day or two of their admission to one medical and surgical unit.

**A Prospective Survey of Platelet Counts in 125 Patients**

Platelet counts, using the Coulter counter in the manner previously described, were performed on blood samples
collected by the investigator from 125 patients within 48 hours of their admission to the wards of the Professorial Medical and Surgical Units in the Royal Adelaide Hospital between 1st July 1969 and 12th August 1969.

Results

The initial platelet counts in the different clinical conditions are shown in Table I. Nine patients, or 7.2%, had low initial counts. Of these, two patients with myocardial infarction died within 48 hours of admission to hospital as did one patient with multiple pulmonary emboli and a haemorrhagic pulmonary infarct. The platelet count returned to normal values in all three patients who had suffered major trauma or had undergone major surgery. One patient of Mediterranean origin had large platelets. The occurrence of thrombocytopenia in these conditions will be discussed more fully in the following section.

An infective process was the final diagnosis in 17 of the 125 patients. The pertinent clinical details of the 17 patients are summarised in Table II.

In only two was there a low platelet count and in both the count rose to the upper limits of normal on recovery. In patients presenting with a history of two days or more,
TABLE I. THE RELATIVE FREQUENCY OF FACTORS WHICH APPEARED TO BE THE PRIMARY CAUSE OF LOW PLATELET COUNTS

<table>
<thead>
<tr>
<th>PRIMARY DISORDER</th>
<th>PLATELET COUNT mean/cu mm</th>
<th>NO. OF PATIENTS</th>
<th>PATIENTS WITH LOW PLATELET COUNTS (less than 150,000/cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of patients</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>266,000 S.D. ± 86,000</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Other thromboembolic and vascular disorders</td>
<td>294,000 S.D. ± 84,000</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Infection</td>
<td>315,000 S.D. ± 125,000</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Surgical cases 'cold'</td>
<td>306,000 S.D. ± 67,000</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>Trauma and surgical emergencies</td>
<td>270,000 S.D. ± 108,000</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>Malignant disease</td>
<td>410,000 S.D. ± 281,000</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>Miscellaneous disorders</td>
<td>322,000 S.D. 129,000</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>125</td>
<td>9</td>
</tr>
</tbody>
</table>
TABLE II. CLINICAL DETAILS OF THE 17 PATIENTS WITH LOW PLATELET COUNTS IN WHOM THE FINAL DIAGNOSIS WAS AN INFECTIVE PROCESS

<table>
<thead>
<tr>
<th>INFLAMMATORY DISORDER</th>
<th>PLATELET COUNT (cu mm)</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Renal tract infection</td>
<td>303,000</td>
<td>Fever, pus cells in urine, Proteus species isolated.</td>
</tr>
<tr>
<td>2. Renal tract infection</td>
<td>410,000</td>
<td>Fever, pus cells in urine, Coliform bacillus isolated.</td>
</tr>
<tr>
<td>3. Renal tract infection</td>
<td>88,000</td>
<td>Fever, pus cells in urine, Coliform bacillus isolated.</td>
</tr>
<tr>
<td>4. Renal tract infection</td>
<td>451,000</td>
<td>Fever, pus cells in urine, no organism isolated.</td>
</tr>
<tr>
<td>5. Renal tract infection</td>
<td>237,000</td>
<td>Fever, pus cells in urine, abdominal pain, duplex kidney.</td>
</tr>
<tr>
<td>6. Renal tract infection</td>
<td>446,000</td>
<td>Fever, pus cells in urine, no organism isolated.</td>
</tr>
<tr>
<td>7. Pneumonia</td>
<td>119,000</td>
<td>Strep.pneumoniae and Haemophilus influenzae.</td>
</tr>
<tr>
<td>8. Pneumonia</td>
<td>282,000</td>
<td>No organism isolated.</td>
</tr>
<tr>
<td>9. Pneumonia</td>
<td>416,000</td>
<td>No organism isolated.</td>
</tr>
<tr>
<td>10. Pneumonia</td>
<td>325,000</td>
<td>No organism isolated.</td>
</tr>
<tr>
<td>11. Chronic bronchitis</td>
<td>316,000</td>
<td>No organism isolated.</td>
</tr>
<tr>
<td>12. Upper respiratory tract infection</td>
<td>310,000</td>
<td>No organism isolated.</td>
</tr>
<tr>
<td>13. Throat infection and mesenteric adenitis</td>
<td>270,000</td>
<td>Streptococcus isolated.</td>
</tr>
<tr>
<td>14. Cholecystitis</td>
<td>277,000</td>
<td>Abdominal pain &amp; tenderness, fever, no organism isolated.</td>
</tr>
<tr>
<td>15. Buttock abscess</td>
<td>586,000</td>
<td>Proteus species isolated.</td>
</tr>
<tr>
<td>16. Erysipelas</td>
<td>217,000</td>
<td>Streptolysin titre 604.</td>
</tr>
<tr>
<td>17. Epididymo-orchitis</td>
<td>402,000</td>
<td>No organism isolated.</td>
</tr>
</tbody>
</table>
the platelet counts tended to be in the upper limits of the normal range. In one patient with a buttock abscess from which a Gram-negative organism (a Proteus species) was isolated there was a distinct thrombocytosis.

Comment

Although the number of patients involved is small, the platelet counts obtained do confirm the variability of counts seen in infections noted by earlier investigators. The platelet count may be normal, or low in the early phase of an infection followed by a transient thrombocytosis in the recovery period, or in localised infections there may be a thrombocytosis.

Speculation as to whether a transient fall in the platelet count may have occurred prior to admission in those patients presenting with high normal or increased platelet counts, or that the changes in platelet numbers could in some way be related to the increase in leukopoietic activity of the bone marrow associated with infections, or that there may be a release of platelets from the spleen or from the megakaryocytes in the lung, would be difficult to confirm. As the incidence of thrombocytopenia was relatively low and because this line of study
appeared to give information of limited value, it was decided to make a study of all patients in whom low platelet counts were recorded in samples routinely sent to the Division of Haematology.

The Relative Frequency of the Causes of Thrombocytopenia in a Hospital Population

As the incidence of thrombocytopenia in infections is relatively low it was decided to undertake a survey of the relative frequency of the causes of thrombocytopenia in a hospital population detected by routine methods over a period of six months.

During the period of the survey from the 1st July 1969 to the 31st December 1969, haemoglobin estimations and leukocyte counts were carried out and blood smears made on all blood samples received in the laboratory from patients attending the Royal Adelaide Hospital. The blood was collected and handled in the manner described in Chapter III. A minority of the specimens (12%) were received from sources other than the Royal Adelaide Hospital.

Laboratory Methods

Haemoglobin, leukocyte and red cell numbers were estimated with a Coulter Counter, Model S (Coulter
Electronics, Hileah, Florida, U.S.A.) and blood films were made and stained with Jenner-Giemsa by standard methods. Where discrepancies were noted between the appearance of the blood film and the Coulter counts, these estimations were repeated and confirmed, if necessary, by manual methods (Dacie and Lewis, 1968).

With the initial haematological investigation the blood smear was, in most instances, examined by a pathologist. If there was any indication that the platelet count was reduced, a platelet count was carried out on the same specimen using the Coulter counter. Samples containing small clots or strands of fibrin were discarded. Platelet counts, with examination of the blood smear, were also performed when requested by the referring physician.

Whenever possible, the platelet count was repeated on another blood sample taken the same day or within the next 48 hours to confirm low counts. In most cases, subsequent changes in platelet numbers were recorded by further counts.

Platelet counts of less than 150,000 cu mm using the Coulter counter were designated as indicating thrombocytopenia. The patient and/or the hospital notes were examined to ascertain the most probable cause of the thrombocytopenia
in each case.

In several instances, more extended laboratory investigations of the patient's haemostatic mechanism were undertaken. For the routine studies of coagulation, blood was collected in the standard manner and 4.5 ml was delivered into 0.5 ml of a solution of 3.8% sodium citrate in distilled water. For studies requiring serum and for routine biochemical tests, 10 ml of blood was delivered into containers with ballotini beads added to initiate and accelerate coagulation.

Routine coagulation screening procedures for detecting abnormalities in plasma coagulation included:

1. Prothrombin activity by the Quick one-stage method, expressed as a percentage based on previously constructed dilution curves to assess the extrinsic system (Dacie and Lewis, 1968).

2. A modified Hicks-Pitney screening thromboplastin generation test, hereafter referred to as the T.S.T., to assess the intrinsic system (MacPherson and Hardisty, 1961).
3. A fibrinogen estimation by a clot density method (Ellis and Strensky, 1961). The normal range for the fibrinogen assay is 180-450 mg/100 ml plasma.

4. Thrombin clotting time (T.C.T.) was done with two dilutions of thrombin in Owren's buffer previously adjusted to give clotting times of 10 seconds and 20 seconds when 0.2 ml of the thrombin dilutions were added to 0.4 ml high spun normal platelet-poor plasma. This test was used to assess interference of fibrin polymerization (due to the presence of fibrin degradation products) and heparin activity in the plasma.

When abnormalities in these screening tests were detected, the haemostatic mechanism was frequently investigated further. This entailed, in some patients, assays of factor II (Owren, 1949), factor V (Hoffman, 1967), plasminogen (Berg et alii, 1966) and, as a measure of the activity of plasminogen activators in the plasma, the euglobulin clot lysis time (Buckell, 1958).

The sera of these patients were also examined for the presence of fibrin degradation products (F.D.P.) using the 'Fi test' (Hyland Laboratories, Los Angeles, U.S.A.). The
'Fi test' was used in the following manner. Blood (10 ml) was specifically collected for this purpose into 0.1 ml thrombin (concentration 1,000 units/ml) to ensure adequate clotting and 0.1 ml trasylol (concentration 500 units/ml) to inhibit fibrinolysis. Serial dilutions of the test sera were made and one drop of the 'Fi test' reagent was then mixed with one drop of serum at each dilution on a slide. After standing for 5 minutes at room temperature the slides were inspected for evidence of agglutination of the latex particles. The result is expressed as the highest dilution at which agglutination was observed. A titre of less than 1:16 was considered to be normal. Caution in interpreting the significance of these titres was required in those patients who had established liver disease in whom there is commonly increased fibrinolytic activity in the plasma and those with paraproteinaemias in whom the abnormal proteins interfere with normal polymerization of fibrinogen. In both conditions abnormally high titres are obtained.

Results

Between the 1st July and 31st December 1969 the blood films of 7,570 patients were examined. A platelet count of less than 150,000 cu mm was recorded in 393 patients.
In only 23 (5.8%) were the blood samples received from sources other than the Royal Adelaide Hospital. A low platelet count was confirmed by repeated counts on samples taken on more than one occasion in 320 (81.4%) patients. Death, discharge from hospital or the outpatients department and specimens received from sources other than the Royal Adelaide Hospital were the reasons which prevented confirmation of the reduced platelet counts in a second sample in 52 (13.3%) patients. And in 21 (5.3%) instances the initial platelet count was found to be in error.

The clinical conditions which appeared to be primarily responsible for the low platelet counts are listed in order of frequency in Tables III and IV. The range and arithmetical mean of the counts obtained are listed for each condition. All percentages, unless otherwise stated, in the text and tables relate to the total number of patients (393) in the survey. The 52 patients in whom the low platelet counts were not confirmed have been included in the differential causes as most had clinical conditions compatible with the platelet counts obtained and it seemed reasonable to assume that the majority of the counts were not erroneously low.
TABLE III. THE RELATIVE FREQUENCY OF FACTORS WHICH APPEARED TO BE THE PRIMARY CAUSE OF LOW PLATELET COUNTS

<table>
<thead>
<tr>
<th>Factor</th>
<th>Platelet count</th>
<th>No. patients</th>
<th>No. unconfirmed platelet counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant disease (including haemo-poietic disorders)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) presenting prior to therapy</td>
<td>10-147 m 44</td>
<td>30 (7.6%)</td>
<td>5</td>
</tr>
<tr>
<td>(b) presenting after therapy (radiotherapy, cytotoxic drugs)</td>
<td>10-148 m 75</td>
<td>93 (24.3%)</td>
<td>7</td>
</tr>
<tr>
<td>Infection (bacterial, protozoal, rickettsial and viral)</td>
<td>11-148 m 80</td>
<td>56 (14.8%)</td>
<td>12</td>
</tr>
<tr>
<td>Massive trauma, surgery and post-blood transfusion</td>
<td>44-141 m 97</td>
<td>48 (11.2%)</td>
<td>7</td>
</tr>
<tr>
<td>Patients of Mediterranean origin</td>
<td>55-144 m 96</td>
<td>38 (9.7%)</td>
<td>10</td>
</tr>
<tr>
<td>Bone marrow hypoplasia, drug or toxic agent-induced</td>
<td>10-145 m 69</td>
<td>27 (6.9%)</td>
<td>4</td>
</tr>
<tr>
<td>Hepatic disease</td>
<td>63-148 m 91</td>
<td>26 (6.6%)</td>
<td>4</td>
</tr>
<tr>
<td>Erroneous counts</td>
<td>10-123 m 40</td>
<td>21 (5.3%)</td>
<td>-</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenia</td>
<td>10-83 m 25</td>
<td>16 (4.1%)</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin Bl2 and folate deficiency</td>
<td>28-142 m 74</td>
<td>9 (2.2%)</td>
<td>-</td>
</tr>
<tr>
<td>Disseminated lupus erythematosus</td>
<td>38-146 m 106</td>
<td>4 (1.0%)</td>
<td>-</td>
</tr>
<tr>
<td>Miscellaneous causes</td>
<td>10-145 m 90</td>
<td>23 (5.8%)</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>393</td>
<td></td>
<td>52</td>
</tr>
<tr>
<td>Primary Condition</td>
<td>Platelet count (cu mm)</td>
<td>No. of Patients</td>
<td>Associated findings and outcome</td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>------------------------</td>
<td>----------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Chronic renal disease</td>
<td>6-145</td>
<td>3</td>
<td>Multiple transfusions in all, with a fall from 145,000 cu mm to 6,000 in 1 following transfusion. Low platelet counts persisted in 2 (1 not confirmed by second count).</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>77-116</td>
<td>3</td>
<td>Features of disseminated intravascular coagulation in 1. 2 died.</td>
</tr>
<tr>
<td>Congestive cardiac failure</td>
<td>124-145</td>
<td>2</td>
<td>2 recovered.</td>
</tr>
<tr>
<td>Cerebral thrombotic episodes</td>
<td>100-120</td>
<td>2</td>
<td>1 died, 1 recovered.</td>
</tr>
<tr>
<td>Congenital thrombocytopenia</td>
<td>10-23</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Apparently normal</td>
<td>115-143</td>
<td>2</td>
<td>1 pregnancy.</td>
</tr>
<tr>
<td>Multiple pulmonary emboli (with haemorrhagic pulmonary infarct)</td>
<td>93</td>
<td>1</td>
<td>Died (count not confirmed)</td>
</tr>
<tr>
<td>Abdominal aortic aneurysm</td>
<td>126</td>
<td>1</td>
<td>Died.</td>
</tr>
<tr>
<td>Eosinophilic leukaemoid reaction (possibly due to Sulthiame)</td>
<td>108</td>
<td>1</td>
<td>Died.</td>
</tr>
<tr>
<td>Overdose of ethchlorvynol</td>
<td>109</td>
<td>1</td>
<td>Recovered.</td>
</tr>
</tbody>
</table>

...continued
TABLE IV (Continued)

<table>
<thead>
<tr>
<th>Primary Condition</th>
<th>Platelet count (cu mm)</th>
<th>No. of Patients</th>
<th>Associated findings and outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxic coma</td>
<td>102</td>
<td>1</td>
<td>Died.</td>
</tr>
<tr>
<td>Sodium chloride intoxication</td>
<td>38</td>
<td>1</td>
<td>Features of disseminated intravascular coagulation. Died.</td>
</tr>
<tr>
<td>Ante-partum haemorrhage</td>
<td>124</td>
<td>1</td>
<td>Recovered (count not confirmed).</td>
</tr>
<tr>
<td>Following i.v. infusion of Cr$_{51}$ labelled red cells</td>
<td>110</td>
<td>1</td>
<td>Polycythaemia, recovered.</td>
</tr>
<tr>
<td>Post tetanus toxoid vaccine</td>
<td>92</td>
<td>1</td>
<td>Minor trauma, recovered.</td>
</tr>
</tbody>
</table>
Details concerning the number of patients known to be thrombocytopenic before the survey period, the number in whom the reduced platelet count was not confirmed on a second blood sample, the number in whom the platelet count subsequently rose to levels in excess of 150,000 cu mm, the number who died and the number in whom the platelet count did not rise above 150,000 cu mm by the end of the survey period in each group of conditions, are set out in Table V. Individual patients are frequently included in the figures of several columns in any one group. For example, a patient who was known to be thrombocytopenic before the start of the survey period and died during survey is included in the figures of both column 2 and column 5. The results have been tabulated in this manner to give some indication of the sequential changes in the number of platelets in each of the conditions listed.

Discussion of the Causes of Thrombocytopenia Encountered in the Six Month Survey

Malignant disease. Not unexpectedly, thrombocytopenia was encountered most frequently in patients receiving cytotoxic drugs or radiotherapy for the treatment of
<table>
<thead>
<tr>
<th>Condition</th>
<th>Total number</th>
<th>Thrombocytopenic prior to survey</th>
<th>Platelet count no. of supernatant specimens</th>
<th>Platelet count returned to normal</th>
<th>Deaths during survey period</th>
<th>Platelet count remained below normal at end of survey period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant disease</td>
<td>125</td>
<td>56</td>
<td>12</td>
<td>24</td>
<td>36</td>
<td>53</td>
</tr>
<tr>
<td>Haemopoietic disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Chronic lymphatic leukaemia</td>
<td>16</td>
<td>14</td>
<td>-</td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>b. Acute leukaemia</td>
<td>14</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>c. Multiple myeloma</td>
<td>8</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>d. Myeloproliferative disorders</td>
<td>20</td>
<td>14</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Infections</td>
<td>56</td>
<td></td>
<td>12</td>
<td>30</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>Mediterranean subjects</td>
<td>38</td>
<td>1</td>
<td>10</td>
<td>3</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>Pancytopenia</td>
<td>27</td>
<td>11</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Liver disease</td>
<td>26</td>
<td>10</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Erroneous counts</td>
<td>21</td>
<td></td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Massive trauma</td>
<td>19</td>
<td></td>
<td>14</td>
<td>5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>I.T.P.</td>
<td>16</td>
<td>7</td>
<td>-</td>
<td>3</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Post blood transfusion</td>
<td>15</td>
<td></td>
<td>3</td>
<td>11</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Post surgery</td>
<td>14</td>
<td></td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>5</td>
<td></td>
<td>5</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Folate deficiency</td>
<td>4</td>
<td></td>
<td>4</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Disseminated lupus erythematosus</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>23</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>
malignant disease. Of the 125 patients with malignant disease only 32 presented with low platelet counts. Of these 14 had disseminated malignancy with bone and/or bone marrow involvement confirmed by radiology or bone marrow biopsy. Malignant diseases of haemopoietic origin accounted for the remainder. Thrombocytopenia was present in 14 of 16 patients with acute leukaemia at the time of diagnosis. It was not, however, a common finding at the time of the patient's presentation with other malignant disorders of haemopoietic origin. Only 2 of 16 patients presenting with chronic lymphatic leukaemia and 2 of the 8 patients with multiple myeloma had low platelet counts before the commencement of therapy with cytotoxic drugs. A fall in platelet count occurred only after therapy in another 20 patients with chronic myeloid leukaemia, polycythaemia vera or other myeloproliferative disorders.

Thrombocytopenia in persons of Mediterranean descent. In the past two years, the finding of stomatocytes, a mild haemolytic anaemia, an increased incidence of splenomegaly, and mild thrombocytopenia associated with abnormally large platelets in persons of Mediterranean descent has been commented on either singly or in association by several centres in Australia (Ducrou and Kimber, 1969; von Behrens et alii, 1970;
Jackson et alii, 1970).

There were 65 persons (16.6%) of Mediterranean descent included in this survey; 24 (6.3%) had definite clinical and pathological causes for the low platelet count. In Adelaide, persons of Mediterranean (Italian and Greek) descent account for approximately 6% to 7% of the population, and therefore this latter figure of 6.3% is not unexpected. However, in 38 patients (9.7%) of Mediterranean descent, no well-defined clinical or pathological cause could be elicited. Five were symptomless, three being relatives of patients under investigation because of a low platelet count and/or stomatocytosis and other features associated with this group. In six subjects the only complaint was of intermittent abdominal pain; in another five, stomatocytosis of the red cells was the only abnormality. A mild anaemia (the lowest haemoglobin level was 9 gm/100 ml), a slightly raised reticulocyte count, a varying percentage of cells showing the configuration of stomatocytes, splenomegaly and a history of abdominal pain were seen in a variety of combinations in the remaining subjects. The microscopic morphological finding of abnormally large platelets together with a marked degree of anisocytosis of the platelets was, however, a constant feature.
An explanation for these findings is at present not forthcoming. An awareness that persons of Mediterranean descent may have low platelet counts without any other distinct abnormality or in association with a mild haemolytic anaemia and splenomegaly, the mechanism of which is not clear (Ducrou and Kimber, 1969) should be borne in mind, since some of these patients may not necessarily require treatment. Indeed, in only six cases was there any suggestion of increased bleeding; four of these patients were women with menorrhagia, and two were suffering from epistaxes of minor degree. The clinical picture in these cases corresponds very closely with the description of chronic idiopathic thrombocytopenic purpura (Hardisty and Ingram, 1966).

Pancytopenia. No precipitating factor as a cause for bone marrow depression or thrombocytopenia was elicited in 13 of these 27 cases. Only in two did the platelet count at any time rise above 150,000 cu mm.

Exposure to drugs or toxic agents reported to cause bone marrow depression or thrombocytopenia (Wintrobe, 1966) appeared to be responsible in 14 cases. Phenylbutazone was incriminated in four (three of these patients had a
generalised bleeding tendency in the form of purpura and bruising), and in none did the platelet count rise to normal. Other agents which caused a persistent thrombocytopenia were chloramphenicol (one case), antituberculosis therapy combined with tolbutamide and digoxin (one case) and chlorpropamide (one case). The platelet count returned to normal with the withdrawal of the toxic stimulus after carbon monoxide poisoning (one case), gold (one case) and organophosphate poisoning (one case), and after excessive alcohol intake, in two cases in which there was no evidence of liver disease.

Liver disease. Thrombocytopenia is a well-recognised feature of liver disease with portal hypertension and splenomegaly. Of the 26 patients presenting in this group, 14 had alcoholic liver disease, and in three the platelet count returned to normal during the period in hospital. Chronic active hepatitis being treated with azathioprine accounted for another seven cases. Cirrhosis with portal hypertension was diagnosed in the remainder.

A generalised bleeding diathesis in this group was predominantly associated with deficiencies of plasma coagulation factors and increased fibrinolysis rather than with a low platelet count.
Erroneous counts. The total number of platelet
counts below 150,000 cu mm not confirmed by a count on a
second specimen was 73 (18.6%). In 21 instances (5.6%),
the initial low platelet count was shown definitely to be
erroneously low by repeated counts on further specimens;
how many of the remaining counts not confirmed were erron-
eously low is not possible to estimate. However, it seems
reasonable to assume that the majority were correct, since
in most cases the clinical conditions were compatible with
the count.

Faulty technique during venepuncture and inadequate
mixing of the blood with anticoagulant were the primary
reasons for the erroneously low platelet counts. Also, in
many instances in which there were reduced numbers of plate-
lets on the blood smear, the need for a platelet count was
obviated when inspection revealed a clot in the blood
sample received.

Massive trauma. Multiple injuries (usually sustained
in road traffic accidents), massive transfusion, surgery,
fat embolism and the multiplicity of drugs used in these
cases could all contribute to the fall in platelet count.
Disseminated intravascular coagulation is not an uncommon
finding (Hardaway, 1970), and suggestive features of this were seen in five cases. Numerous microscopic fibrin thrombi were demonstrated in the lungs, kidney, liver, spleen and other organs of one patient at autopsy (Pembrey et alii, 1970).

_idiopathic thrombocytopenic purpura:_ In this condition the platelet count is generally very low; the mean in this group was 16,000 cu mm, and spontaneous bleeding was frequently troublesome (10 of the 16 cases). However, this condition accounted for less than 5% of all causes of thrombocytopenia, which does emphasise that idiopathic thrombocytopenic purpura should not be accepted as the primary diagnosis until a number of other causes have been excluded.

_Blood transfusion._ Stored blood is deficient in platelets. The transfusion of more than five or six units of stored blood over a period of several hours almost invariably causes a fall in the platelet count as a result of dilution with platelet-poor blood (Mollison, 1967). The lowest count of 44,000 cu mm was recorded in a woman with post-partum bleeding (without evidence of defibrination), who received 12 units of blood within the space of 8 hours.
The transfusion of only two units of blood to one patient with chronic renal failure, already thrombocytopenic with a platelet count of 145,000 cu mm, caused a further precipitous fall to 6,000 cu mm. The platelet count remained below 20,000 cu mm for several days before slowly rising to pretransfusion levels. An antigen-antibody reaction against the recipient's platelets was suspected in this instance.

*Surgery.* After surgery, the occurrence of a transient mild reduction in the platelet count, frequently followed by a transient thrombocytosis, is well recognised (Ygge, 1970). The comparatively low detection rate of this fall in platelet count after surgery generally results from the few routine haematological investigations requested and carried out in this group of cases.

However, a persistently low platelet count in patients who have undergone major abdominal surgery in which sepsis has also been a factor is a particularly poor prognostic sign. In some cases it may have been an indication of Gram-negative bacteraemia or endotoxaemia, as in a number of these cases the clinical manifestations resembled those seen with proven Gram-negative septicaemia.
Vitamin $B_{12}$ and folate deficiency. Pancytopenia is not an infrequent presentation in cases of vitamin $B_{12}$ and folate deficiency, as these factors are essential for the normal maturation of the myeloid, megakaryocytic and erythrocyte series. Thrombocytopenia, as part of this picture, can be moderately severe. Platelets of 34,000 and 28,000 cu mm respectively were noted in this laboratory. The degree of thrombocytopenia produced by deficiency of either factor was comparable.

Miscellaneous. In this group two patients exhibited features of disseminated intravascular coagulation. A fall in all coagulation factors, followed by an episode of increased spontaneous fibrinolysis, was seen in association with an extensive anterior myocardial infarction in one. In the second, these features were seen after sodium chloride intoxication, which resulted from gastric lavage with normal saline after an overdose of barbiturates.

Infection. An infective process appeared to be responsible for thrombocytopenia in 56 patients. After malignant disease, untreated and treated, this was the second most common cause for a low platelet count in this survey. The thrombocytopenia was confirmed by a further count in 44 of the 56 patients. Only one count was performed in the
remaining 12 patients who presented with clinical histories of an infective process; death as a result of overwhelming infection or discharge from hospital because of the minor nature of the presenting illness were the reasons for not obtaining a second sample.

Details of the types of infection in the 56 cases are summarised in Table VI. These have been divided into three groups; the first represents infections proven by positive blood cultures or by the visualisation of parasites in the blood smears.

Gram-negative bacteraemic infections were those most commonly associated with episodes of thrombocytopenia. Indeed low platelet counts were recorded in 18 of 54 patients from whom Gram-negative organisms were isolated by blood culture in the Division of Microbiology during the same six month period. On the other hand thrombocytopenia was recorded in only 7 of 71 patients from whom Gram-positive organisms were isolated, and two of these had concurrent Gram-negative bacteraemia. One patient, who also had chronic lymphatic leukaemia (untreated), had a normal platelet count before a Gram-negative bacteraemic episode which returned to normal levels after treatment of the
<table>
<thead>
<tr>
<th>Systemic infection proven positive blood cultures, parasitaemia</th>
<th>Platelet count/ cu mm</th>
<th>No. of patients in whom low platelet count confirmed</th>
<th>No. of patients in whom low platelet count not confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative septicaemia</td>
<td>77,000</td>
<td>16 (8 died)</td>
<td>2 (2 died)</td>
</tr>
<tr>
<td>Gram-positive septicaemia</td>
<td>100,000</td>
<td>5 (1 died)</td>
<td></td>
</tr>
<tr>
<td>Septicaemia - mixed organisms</td>
<td>20,000</td>
<td>2 (2 died)</td>
<td></td>
</tr>
<tr>
<td>Malaria</td>
<td>65,000</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

| Other infections where there was evidence of systemic involvement |
|---------------------------------------------------------------|------------------------|
| Q fever                                                       | 23,000                 | 1                                                    |
| Infectious mononucleosis                                     | 140,000                | 1                                                    |
| Viral infections                                              | 101,000                | 1                                                   |
|                                                               |                        | 1                                                   |

| Localised infections: blood cultures negative or not performed |
|---------------------------------------------------------------|------------------------|
| Pneumonia                                                     | 79,000                 | 8 (2 died)                                          | 5 (4 died)                                             |
| Biliary tract infections                                     | 81,000                 | 4                                                    | 3                                                      |
| Renal tract infections                                       | 73,000                 | 1                                                    | 1                                                      |
| Other sites                                                  |                        |                                                     |
| Otogenic abscess                                             | 66,000                 | 1                                                    |
| Infected psoriasis                                           | 101,000                | 1                                                    |
|                                                               |                        |                                                     |
| Total                                                        |                        | 44                                                   | 12                                                     |

TABLE VI. Analysis of the types of infection in the 56 patients in whom infection was believed to be the primary cause of the thrombocytopenia.
infection. Three of four patients with malaria (Plasmodium vivax) encountered in the six months had thrombocytopenia.

In the second group with rickettsial and viral infections there was other evidence to suggest systemic infection, for instance, the patient with Q fever had an endocarditis and hepatic involvement, the latter proven by biopsy. Atypical lymphocytes, a positive Paul-Bunnell test, hepatosplenomegaly and mild abnormalities in the biochemical screening tests which suggested hepatic dysfunction were seen in the case of infectious mononucleosis.

In the third group, systemic infection was not proven, that is blood cultures were negative or were not taken. The most frequent cause of thrombocytopenia in this group was pneumonia; this was usually severe and often had a fatal termination. Indeed a falling platelet count in these patients was a poor prognostic sign.

With the appreciation that abnormalities of haemostasis do occur in septicaemic states, coagulation studies were performed in 30 of the 56 patients. The results obtained together with other clinical and laboratory findings are summarised in Table VII,
### TABLE VII. SUMMARY OF CLINICAL AND LABORATORY FINDINGS IN PATIENTS WITH INFECTIONS ASSOCIATED WITH THROMBOCYTOPENIA

<table>
<thead>
<tr>
<th></th>
<th>No. of patients in whom details were recorded</th>
<th>No. of patients. Initial findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TEMPERATURE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Pyrexia</td>
<td>56</td>
<td>54</td>
</tr>
<tr>
<td>2. Normothermic</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>3. Hypothermic</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>BLOOD PRESSURE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Blood pressure &lt; 110 mg Hg systolic</td>
<td>45</td>
<td>18</td>
</tr>
<tr>
<td><strong>LEUCOCYTE COUNT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Leucocytosis</td>
<td>56</td>
<td>32</td>
</tr>
<tr>
<td>2. Leucocyte count within normal range (4,000-11,000 cu mm)</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>3. Leucopenia</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td><strong>COAGULATION STUDIES</strong></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>1. Prolonged 1-stage pro-thrombin time &gt; 2 secs</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>2. Prolonged TST &gt; 2 secs</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>3. Prolonged TCT &gt; 3 secs</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td><strong>BIOCHEMICAL SCREENING TESTS</strong></td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>1. Bilirubin &gt; 1.5 mg/100 ml</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>2. Alkaline phosphatase &gt; 16 KA units/100 ml</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>3. SGOT (transaminase) &gt; 50 KU/ml</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>4. Blood urea nitrogen &gt; 21 mg/100 ml</td>
<td></td>
<td>21</td>
</tr>
</tbody>
</table>
Abnormalities in coagulation tests were detected in 16 patients. In three, liver dysfunction, and in another, vitamin K deficiency, appeared to be the primary cause for the abnormalities. In the remaining 12, despite evidence of hepatic dysfunction in most cases, the presence of consumption coagulopathy of varying degrees was suspected.

The criteria used for suspecting this complication were the sudden onset of hypotension, oliguria or anuria, and respiratory distress taken in association with the laboratory findings of a falling platelet count, a prolongation of prothrombin time (PT), thromboplastin screening test (TST) and the thrombin clotting time (TCT). Fibrinogen levels were usually raised; only in two patients during the survey period did this fall below 100 mg/100 ml.

The diagnosis of consumption coagulopathy was frequently difficult to make, or to prove by laboratory testing. This was especially so when there was associated evidence of hepatic dysfunction. A rise in the platelet count and improvement in other parameters of coagulation in the face of increasing liver dysfunction following the use of heparin did, however, support this suspicion in six patients. In the following chapter a number of these cases will be described
in detail to illustrate the variability of the clinical and laboratory coagulation status.

SUMMARY

Apart from the finding of a reduced platelet count in a proportionally larger group of patients of Mediterranean descent than would be expected, the conditions causing thrombocytopenia recorded in this laboratory can be found listed in most textbooks of haematology and medicine.

Not unexpectedly malignant disease, including haemopoietic malignant disorders, in patients receiving chemotherapy or radiotherapy, was the most commonly encountered cause of a reduced platelet count. In fact, only 32 of 125 patients with malignant disease actually presented with thrombocytopenia, and the majority of these had either disseminated malignant disease with bone marrow involvement or acute leukaemia.

In this survey of the relative frequency of causes of thrombocytopenia, infection was implicated as a factor in 56 patients. Bacterial sepsis was implicated as the primary cause in 23 patients in whom positive blood cultures were obtained. In 16 Gram-negative organisms, in five Gram-positive organisms and in two both a Gram-negative and a
Gram-positive organism were isolated. During the same period Gram-negative organisms were isolated from blood cultures taken from 54 patients and Gram-positive organisms from 71. Low platelet counts were, therefore, encountered in 37% of patients with proven Gram-negative septicaemia and 10% of those patients with proven Gram-positive septicaemia detected during the period of the survey.

Thrombocytopenia was also noted in three of four patients with malaria, in two with viral infections and one with Q fever. In the remaining 13 patients systemic infection could not be proved; however, marked falls in the platelet count were noted in patients with severe and frequently fatal pulmonary infections and in patients with infections of the biliary and renal tracts.

Abnormal haemostasis was noted in 16 patients with infections; in three this could be ascribed primarily to liver disease, and in another to vitamin K deficiency. The evidence favoured disseminated intravascular coagulation in the remaining 12 cases. A prolongation of the one-stage prothrombin time, the thromboplastin screening test time and the thrombin clotting time, together with the clinical picture of hypotension, oliguria and respiratory distress,
was taken as evidence of the presence of this complication. Patients with Gram-negative bacteriæmic infections were particularly susceptible to these changes.

Although infections were the second most common cause of thrombocytopenia, the 56 patients in whom it was recorded represent less than 1% (0.74%) of the 7,570 patients examined in the larger survey.

In the prospective survey, infection was the primary diagnosis in 17 or 13.6% of the 125 patients studied. Only 2 or 11% of the 17 patients with infections had thrombocytopenia. These data are insufficient to accurately assess the frequency of thrombocytopenia in infections, but if the results of the two surveys are taken in conjunction, it is possible to calculate a very approximate incidence.

If it is assumed that the patients in the prospective survey were representative of all admissions, then 1,020 (i.e. 13.6%) of the 7,570 patients investigated during the six month period could be expected to have an infective process. There were 56 patients with infections and thrombocytopenia during this period; this represents 5.5% of the 1,020 patients which it is calculated could be expected to have infection as a primary diagnosis.
It therefore seems reasonable to speculate that the frequency of thrombocytopenia as a complication of infections in a hospital environment is probably of the order of 5 to 10%.

In practice the detection rate is considerably lower. The reasons for this and the significance of the low platelet count in infections will be elaborated on throughout this thesis.
CHAPTER V

THE CASE HISTORIES OF 18 PATIENTS WITH

THROMBOCYTOPENIA ASSOCIATED WITH INFECTIONS
The extremely variable pattern of response in platelet numbers in infections appeared to be influenced by a number of factors. This variability in the platelet response can best be illustrated by a detailed description of the clinical and laboratory manifestations encountered in a number of patients with thrombocytopenia and infections.

The 18 patients under observation were admitted to the Royal Adelaide Hospital between June 1968 and August 1970. The collection of blood samples and methods of laboratory investigation were performed in the manner described in the previous chapter. Whenever possible, autopsies were performed on patients who died and samples from various organs taken for histological examination. The sections were routinely stained with haematoxylin and eosin (H & E) and, to demonstrate fibrin, with Martius Scarlet Blue (MSB).

CASE REPORTS

Case 1 (Figure 5): A 21 year old known diabetic woman was admitted in severe keto-acidotic coma. On the day of admission the leucocyte count was 9,200/cu mm (78% granulocytes) and the platelets appeared to be within the normal range on the smear. She responded adequately to treatment over the next 48 hours. Blood cultures and urine
cultures were done on day 3 and both were sterile.

On the morning of day 5 the leucocyte count was 4,200/cu mm, the neutrophils (88%) showed marked toxic granulation and some vacuolation, and the platelet count was reduced to 68,000/cu mm. Four hours later the patient had a mild rigor and a fever of 102°F. A further blood picture at this time confirmed the thrombocytopenia, platelet count 66,000/cu mm, but the leucocyte count had fallen to 2,900/cu mm. A coagulation screening study was normal apart from a raised fibrinogen level of 520 mg%. Blood cultures taken at the same time grew a coagulase positive *Staphylococcus aureus*. The source of infection was a cellulitis of the forearm which had developed consequent on intravenous therapy.

The patient was started on penicillin G, but this was changed to Lincomycin 48 hours later. The pyrexia settled and the platelet count rose, reaching a recorded maximum count of 449,000/cu mm on day 14. Subsequent platelet counts up to four weeks after the recorded period were all within the normal range.
FIGURE 5: CASE I. Staphylococcal septicaemia.
BC+ Blood culture positive.
BC- Blood culture negative.
Case 2 (Figure 6): A 53 year old man was admitted with a two day history of headache, diarrhoea and malaise. Clinical examination suggested a diagnosis of acute bacterial endocarditis. A blood picture revealed a leucocyte count of 6,700/cu mm with the neutrophils (78%) showing slight toxic granulation and shift to the left, and a marked thrombocytopenia of 30,000/cu mm. The bacteraemia was confirmed with the growth of a beta haemolytic streptococcus in the blood cultures.

On penicillin therapy the patient made a gradual recovery and the platelet count rose to 587,000/cu mm by day 12. The thrombocytosis continued until day 18 (platelet count 623,000/cu mm), when the patient had a second febrile episode, during which the platelet count fell to 279,000/cu mm, but blood cultures taken on days 19 and 21 were sterile. As this pyrexial episode, which was attributed to several haematomata associated with penicillin therapy, settled the platelet count again rose to 436,000/cu mm. The patient made a complete recovery and just prior to discharge from hospital six weeks later a blood picture showed a normal leucocyte and platelet count (186,000/cu mm).
FIGURE 6: Case 2. Beta-hemolytic streptococcal septicaemia.
Case 3: A 72 year old woman was admitted with a history of dysuria, haematuria, a productive cough and chest pain. She was pyrexic, but normotensive and had a satisfactory renal output. There were chest signs suggesting bronchopneumonia and Haemophilus influenzae was isolated from the sputum. Urine cultures were negative.

The blood picture showed a leucocyte count of 8,300/cu mm (79% granulocytes) and a platelet count of 42,000/cu mm. The prothrombin activity was 100% and the TST normal. The fibrinogen was raised (704 mg %) and the TCT slightly prolonged (patient 20 seconds, normal 15 seconds) and the 'Fi-test' was positive to a titre of 1:64.

She was treated with penicillin. On day 3 the platelet count was 53,000/cu mm; thereafter it rapidly rose to 161,000/cu mm by day 8 during which time she made a complete recovery.

Case 4 (Figure 7): A 70 year old man was admitted with a history of severe upper abdominal pain for one day. Serum chemistry (bilirubin 4.0 mg/100 ml, raised transaminase and normal amylase) and the blood picture suggested a biliary tract infection. Twelve hours after admission the
leucocyte count was 48,000/cu mm (90% granulocytes) and a platelet count of 64,000/cu mm. Treatment with penicillin and streptomycin was started without taking blood cultures.

Although the patient was clinically improved the following day the platelet count had fallen to 34,000/cu mm. Coagulation studies revealed no abnormality apart from a raised level of fibrinogen (680 mg/100 ml). The platelet count then rose steadily despite the patient having a rigor on day 6. Blood cultures taken on days 4 and 8 were sterile.

At operation on day 14 the gall bladder, which showed signs of chronic cholecystitis, was removed and a culture taken which grew a coliform bacillus. During the recovery period the platelet and leucocyte counts remained normal.

Case 5: A 78 year old woman with a one week history of dizziness, failing vision and weakness, and fever for one day was admitted pyrexic (101°F) and had the signs of a right lower lobe pneumonia. She was normotensive and renal output was satisfactory.

A blood picture showed a leucocyte count of 7,100/cu mm (85% granulocytes) and a platelet count of 88,000/cu mm. She was treated with penicillin.
FIGURE 7: Case 4. Gram-negative bacillary infection of the biliary tract.
On day 5 bruising and a morbilliform rash was noted over the abdomen. The platelet count, however, had risen to 123,000/cu mm and, although the prothrombin activity and the TST were normal, the fibrinogen was reduced to 64 mg %. By day 7 the prothrombin activity had fallen to 55%, the TST was prolonged (patient 12 seconds, normal 8 seconds) and the TCT (patient 17 seconds, normal 10 seconds) and the fibrinogen remained low (94 mg %). There was evidence of increased fibrinolysis, the euglobulin lysis time was slightly shortened (110 minutes) and the 'Fi-test' was positive to a titre of 1:64.

There was no evidence to suggest hepatic dysfunction and at this time the patient appeared to be making a satisfactory recovery. This episode of increased fibrinolysis resolved over the next two days with the coagulation parameters returning to normal, the fibrinogen rose to 439 mg % by day 18 and the platelet count to 344,000/cu mm.

The patient was then discharged to an old people's home after investigations for an occult malignancy had proved negative.

Case 6 (Figure 8): A 62 year old bedridden elderly diabetic woman who had a bed sore over the buttocks and
sacrum was admitted with suspected bronchopneumonia. She was pyrexic and her renal output was 'poor'. She was normotensive.

The blood picture showed a leucocyte count of 14,200/cu mm (95% granulocytes) and a platelet count of 56,000/cu mm. Treatment with ampicillin was commenced but when a Coliform bacillus was isolated from blood cultures on day 2, this was changed to kanamycin.

On day 2, the prothrombin activity was 55 per cent, the TST was normal, the TCT prolonged (patient 16 seconds, normal 10 seconds), the fibrinogen raised to 972 mg % and the 'Fi-test' positive to a titre of 1:64. Serum biochemistry indicated no abnormality in hepatic function (bili-rubin 0.9 mg/100 ml); however, the blood urea nitrogen was 73 mg/100 ml.

The patient made an uneventful recovery with a rise in platelet count to 423,000/cu mm on day 13, the coagulation test returned to normal and the blood urea nitrogen fell to 27 mg/100 ml. The bed sore was skin grafted on the 34th day.

Case 7: A 63 year old man with a past history of chronic bronchitis was admitted with upper abdominal pain.
FIGURE 8: Case 6. Gram-negative bacillary septicaemia.
A laparotomy revealed a pseudocyst of the pancreas and evidence of previous pancreatitis. No further action was taken.

Blood pictures on the third and eighth post-operative days showed a normal leucocyte count and platelets appeared normal on the smear. On day 14 a leakage of sero-sanguinous fluid from the abdominal wound was noted. On the following day there was a platelet count of 64,000/cu mm and a leucocyte count of 6,900/cu mm (94% granulocytes).

Twenty-four hours later the platelet count had fallen to 47,000 cu mm. The same day the patient suffered a burst abdomen, and during resuturing it was noted that his blood did not clot. Coagulation studies showed the features of an acute defibrination syndrome, prothrombin activity 10%, TST 43 seconds (normal 8 seconds) and fibrinogen 30 mg/100 ml. The patient received an infusion of 15 g of fibrinogen and epsilon-aminocaproic acid in addition to blood transfusion with no effect on the bleeding. Heparin was then given in a single intravenous dose of 10,000 units and this was followed by the administration of factor II, VII, IX and X concentrate and cryoprecipitate (factor VIII). The patient by this time was markedly hypotensive, anuric and comatose. He died two hours later.
Autopsy revealed massive haemorrhage into both pleural cavities and into the peritoneal cavity. A ruptured pseudo-cyst of the pancreas connected with a large abscess lying under the left lobe of the liver from which a Coliform bacillus was isolated. There was massive antemortem thrombosis of both intra- and extrahepatic portal vessels. The intestines were congested with blood but not infarcted. Thrombi occluded several medium sized and peripheral pulmonary vessels in both lungs. Microscopically there were numerous small thrombi, especially in the lung, with fewer seen in the liver, kidneys and pancreas.

Case 8 (Figure 9): A 63 year old man was admitted with a three day history of a febrile illness. On admission he was hypotensive and there was marked peripheral vascular constriction. A blood picture showed platelets 83,000/cu mm and a leucocyte count of 24,600/cu mm (92% granulocytes). A diagnosis of a Gram-negative bacteraemia infection was confirmed by blood cultures.

Coagulation studies revealed a slight decrease in prothrombin activity (57%), borderline prolongation of the TST (10 seconds, normal 8 seconds), a fibrinogen level of 938 mg/100 ml and a fibrinogen breakdown product titre of 1:128.
There was hypotension, anuria (the blood urea nitrogen was 88 mg/100 ml) and a further fall in platelet count to 44,000/cu mm, and it was felt that these findings indicated the presence of disseminated intravascular coagulation. Treatment with heparin, in a dose of 1,500 units hourly, was started.

As there was no improvement in renal output, the blood urea nitrogen continued to rise, he was transferred to a renal unit for dialysis on day 4. The platelet count had risen slightly (75,000/cu mm), the fibrinogen had fallen to 470 mg/100 ml and fibrinogen breakdown products were present in the serum to a titre of 1:128 ('Fi-test'). Dialysis was continued into day 6 but it was not until day 7 that the patient entered a diuretic phase. The subsequent decrease in blood urea nitrogen is indicated in Figure 9.

The rise in platelet count was associated with a fall in the serum fibrinogen breakdown products to 1:8 on day 19. Heparin therapy was stopped on day 20, although a slight rise in fibrinogen breakdown products was recorded the following day. This rapidly fell to 1:8 by day 25.

Antibiotic therapy during this patient's illness was an initial dose of chloramphenicol, followed by kanamycin. This
was changed to methicillin and gentamycin on day 7.

Case 9 (Figure 10): Four days after an appendicectomy, a man of 56 years developed symptoms and signs of pulmonary embolism. He was treated with heparin which was given intravenously as a dose of 1,500 units every hour. The next 13 days were uneventful, then on the 17th postoperative day the patient manifested a swinging pyrexia. Blood cultures were taken and the patient was immediately started on a course of kanamycin and tetracycline. A Coliform bacillus was isolated from the blood cultures.

The patient's pyrexia persisted for the next few days. On the morning of day 23 it was decided to stop the heparin therapy which had continued since the fourth post-operative day. Within 4 hours of cessation of the heparin the patient developed the features of 'septic shock'. He was shortly afterwards transferred to the intensive care unit of the Royal Adelaide Hospital.

On admission he was hypotensive (systolic pressure 65 mm Hg) and oliguric. The platelet count had fallen from 57,000/cu mm in the specimen from the private hospital at 9 a.m. to 25,000/cu mm in the one taken at 1 p.m. shortly after admission. The leucocyte count was 9,000/cu mm and
the neutrophils showed toxic changes. The prothrombin activity was 19% (29 seconds) and the TST prolonged to 13.5 seconds (normal 8 seconds). There was slight prolongation of the TCT to 13 seconds (normal 10 seconds), fibrin degradation products in the serum were increased to a titre of 1:64 ('Fi test') and the fibrinogen level was 635 mg %.

A single dose of methedrine (10 mg) was given intravenously and heparin at a dose of 2,000 units every hour was restarted. The antibiotic therapy was changed to chloramphenicol 250 mg 6 hourly.

Within the next 3 hours the patient’s blood pressure rose to 130/80 and he began to pass urine to a total of 450 ml in the first 12 hours. The following day the urinary output returned to normal (2,385 ml/24 hours), he remained normotensive, the platelet count rose to 47,000 cu mm and the prothrombin activity increased to 59% even with the presence of heparin. There was only a slight rise in bilirubin to 1.4 mg/100 ml which fell to normal limits within 48 hours.

The following day (day 25) there was a continued increase in the platelet count and prothrombin activity and,
after an initial rise, the blood urea nitrogen began to
decrease. Over the next 5 days there was a steady improve-
ment, with a continued rise in platelet count and a fall in
the blood urea nitrogen.

Although the dose of heparin remained unchanged from
day 24 to day 32, a progressive increase in heparin activity,
reflected by increased prolongation of the TCT, was seen.
This was associated with a fall in the PT after an initial
rise to 88%. When a haematoma developed at the site of
the sphygmomanometer cuff on day 32 the heparin was stopped;
this was followed immediately by a shortening of the pro-
thrombin time. The patient then proceeded to a complete
recovery. The sequence of events, treatment and changes
in some coagulation test parameters are summarised in Table
VIII.

Case 10 (Figure 11): A 73 year old man who had four
days previously visited a doctor complaining of a sore
throat and fever was found in a delerious state in a hotel
room. There was also a history of cirrhosis of the liver.
On admission he was semi-comatose, pyrexic and exhibited
the signs of a left lower lobe pneumonia. He was normo-
tensive.
FIGURE 10: Case 9. Gram-negative bacillary septicaemia occurring in a man being treated with heparin and the onset of disseminated intravascular coagulation with interruption of therapy.
### TABLE VIII.  A SUMMARY OF THE CLINICAL AND LABORATORY FINDINGS IN CASE 9.

<table>
<thead>
<tr>
<th>Day</th>
<th>Progress</th>
<th>Treatment</th>
<th>Platelets cu mm</th>
<th>PT %</th>
<th>B.U.N. mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Appendicectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pulmonary embolus</td>
<td>Heparin 1,500 units i.v./hrly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Blood cultures grew a coliform bacillus</td>
<td>Kanamycin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Heparin ceased</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BP 70/-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>'Septic shock'</td>
<td>Heparin 2,000 units i.v./hrly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heparin recommenced</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>BP 130/80</td>
<td>Chloramphenicol 250 mg q.i.d.</td>
<td>25,000</td>
<td>19</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Urinary output 450 ml</td>
<td>Vitamin K 10 mg</td>
<td>47,000</td>
<td>59</td>
<td>80</td>
</tr>
<tr>
<td>25</td>
<td>Urinary output 2,385 ml</td>
<td></td>
<td>71,000</td>
<td>88</td>
<td>70</td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td>98,000</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td>152,000</td>
<td>48</td>
<td>22</td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td>180,000</td>
<td>59</td>
<td>17</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td>148,000</td>
<td>51</td>
<td>19</td>
</tr>
<tr>
<td>32</td>
<td>Bleed into arm</td>
<td>Heparin stopped</td>
<td>158,000</td>
<td>55</td>
<td>16</td>
</tr>
<tr>
<td>33</td>
<td></td>
<td></td>
<td>175,000</td>
<td>79</td>
<td>17</td>
</tr>
</tbody>
</table>
A blood picture showed a leucocyte count of 5,700/cu mm (90% granulocytes) and a platelet count of 42,000/cu mm. The prothrombin activity was 38%, the TST showed a borderline prolongation (patient 11 seconds, normal 8 seconds), the fibrinogen was raised (500 mg %) and the euglobulin clot lysis time greater than 5½ hours, indicating there was no increase in fibrinolytic activity. The bilirubin was slightly raised (2.4 mg/100 ml) and the transaminase markedly elevated (in excess of 1250 K units/ml). The blood urea nitrogen was 26 mg/100 ml. No alcohol was detected in the blood. No organisms were isolated from blood cultures.

He was treated with kanamycin and his condition improved. The platelet count returned to normal on day 8, as did the blood urea nitrogen and bilirubin, and the transaminase reduced to 80 K units/ml.

Case 11 (Figure 12): A 29 year old man presented with fever and in a state of mild confusion. There was a history of coryza and a sore throat one week previously, and of abdominal pain, diarrhoea, vomiting and headache two days prior to admission.

A diagnosis of meningitis or encephalitis was made. Although there were 18 polymorphs/cu mm in the cerebral
FIGURE II: Case 10. Bronchopneumonia.
spinal fluid, no organism was isolated. A Staph. pyogenes was, however, isolated from a throat swab and from five consecutive blood cultures.

On day 2 the blood picture showed a mild leucocytosis (white blood count 12,300/cu mm with 89% granulocytes) and a platelet count of 82,000/cu mm. There was a mild decrease in prothrombin activity (55%) and the fibrinogen was raised to 658 mg %. The TST and TCT were normal. The bilirubin was mildly elevated at 2.4 mg/100 ml. The patient was treated with parenteral lincomycin and given vitamin K1.

On day 4 the platelet count was 54,000/cu mm but the prothrombin activity had risen to 88%.

There was a rise in temperature on day 8; this was associated with a decrease in prothrombin activity (55%) and prolongation of the TCT (patient 31 seconds, normal 22 seconds; there was no difference between the patient and the normal at the higher concentration of thrombin which gave a clotting time of 13 seconds). There was no hypotension or oliguria. Blood cultures were positive for Staph. pyogenes on one occasion only at this time.
FIGURE 12: Case II. Staphylococcal septicaemia.
Within two days the coagulation studies returned to normal values, and thereafter the patient made a complete recovery. A mild compensatory thrombocytosis was noted in the convalescent period.

**Case 12 (Figure 13):** A 62 year old woman was admitted with a history suggestive of acute cholecystitis. A laparotomy on day 1 revealed no positive findings apart from a few gall stones. The gall bladder was not removed, because of continued hypotension.

Blood cultures taken post-operatively were sterile, but there was a thrombocytopenia (90,000/cu mm) and a leucocytosis (16,000/cu mm). The patient remained hypotensive and became oliguric with the blood urea nitrogen rising to 115 mg/100 ml on day 5 when she entered a diuretic phase and then appeared to improve.

On day 9 she suffered a burst abdomen and following re-suturing she had bouts of rigors and hypotension. Blood cultures were sterile. Biochemical evidence of liver damage and renal insufficiency became increasingly apparent by day 13 when a platelet count of 22,000/cu mm was recorded. Purpura was first noted on day 14. From day 15 to day 19 there was some clinical improvement and the platelet count
rose to 68,000/cu mm.

On day 19 she had further bouts of rigors and became hypotensive, and the following day the platelet count had fallen to 19,000/cu mm. Coagulation studies, which were repeated until the patient's death, are summarised in Table IX. A bone marrow biopsy on day 23 showed megakaryocytes to be present in normal numbers. Blood cultures taken 2 days prior to the patient's death grew Staphylococcus aureus and a coliform bacillus. A Staphylococcus aureus with a similar antibiotic sensitivity pattern had been isolated from bronchial secretions on day 14.

Autopsy revealed a suppurative cholangitis and multiple liver abscesses. In addition there was also a subphrenic abscess. A haemorrhagic pulmonary infarct was noted in the right lung and the myocardium and kidneys were described as toxic. Swabs taken from the liver, subphrenic abscess and the gall bladder all grew a coliform bacillus.

Case 13 (Figure 14): A 33 year old man was admitted in a state of severe shock (blood pressure 60/40), jaundice and oliguria. A diagnosis of Gram-negative septicaemia was suspected. Blood cultures were taken which grew a coliform bacillus. Treatment with kanamycin and ampicillin was
TABLE IX. CHANGES IN THE TESTS OF COAGULATION RECORDED
IN THE TERMINAL STAGES OF THE ILLNESS OF CASE 12

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Prothrombin activity %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TST screen (sec)</td>
<td>10.5</td>
<td>9</td>
</tr>
<tr>
<td>Fibrinogen mg/100 ml</td>
<td>435</td>
<td>350</td>
</tr>
<tr>
<td>Factor II %</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>Factor V %</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>Plasminogen μ/ml</td>
<td>0.25</td>
<td>0.44</td>
</tr>
<tr>
<td>I(^{131}) fibrinogen T(_{1/2}) (hours)</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen breakdown products (index)</td>
<td></td>
<td>6.7</td>
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started.

The blood picture showed a leucocytosis (white blood count 35,200/cu mm, 89% granulocytes) and a platelet count of 70,000/cu mm. Eighteen hours later the platelet count had fallen to less than 10,000/cu mm. Although the TCT was slightly prolonged, the prothrombin activity was not significantly reduced (65%). The fibrinogen was 524 mg %.

During the first two days he passed only 570 ml of urine, and the blood urea nitrogen rose from 56 mg/100 ml to 105 mg/100 ml. In view of the fall in the platelet count and the acute renal failure, heparin 100 units/hour was commenced.

Despite the evidence of severe hepatic dysfunction (bilirubin 9.5 mg/100 ml, alkaline phosphatase 80 KA units/100 ml and SGOT of 305 K units/ml) and the presence of marked heparin activity (TCT - patient in excess of 120 seconds, normal 9 seconds), the prothrombin activity was not markedly reduced (55%).

It was assumed, because of continued fever and episodes of hypotension, that the septicaemia was due to a cholangitis consequent to biliary obstruction. By day 5 the patient had shown sufficient improvement to allow a
laparotomy to be performed. A stenosed choledocho-duodenal anastomosis and an enlarged fibroed knobbly liver were found. The anastomosis was refashioned.

Following the operation, the heparin therapy was continued and within 24 hours there was a diuresis (3,705 ml in 24 hours) and the blood pressure was sustained at about 110/60. The platelet count rose to 38,000/cu mm and the prothrombin activity was 79%.

The patient passed melaena stools on day 7, and the heparin was therefore stopped. At the same time there was a fall in blood pressure, renal output, prothrombin activity (51%), prolongation of the TCT (patient 17 seconds, normal 11 seconds) and a fall in the platelet count to 18,000/cu mm on day 9, 24 hours before he died.

Case 14: A 78 year old man was admitted with a history of painless jaundice. A laparotomy revealed cholelithiasis and a chronically fibroed gall bladder, which was removed. There was transient postoperative hypotension.

On day 2 the leucocyte count was 3,500/cu mm (61% granulocytes) and the platelets were noted to be clumped. On day 3 there developed cellulitis and gangrene of the anterior abdominal wall. Blood cultures grew a coliform
bacillus and Streptococcus faecalis. A platelet count of 61,000/cu mm was recorded on day 4. Coagulation studies showed a prothrombin activity of 64%, a prolonged TST (patient 12 seconds, normal 8 seconds) with a decrease in factor V (9%) and factor II (50%). The fibrinogen, however, was 635 mg/100 ml. The platelet count rose slightly to 81,000/cu mm and there was a leucocytosis (13,400/cu mm) on day 6. The patient at this stage was severely jaundiced (bilirubin 12 mg/100 ml) and oliguric. Although a leucocytosis was maintained until death a few days later, no further platelet counts were recorded. Autopsy revealed a large abscess under the right lobe of the liver. No histological details were available.

Case 15: A 64 year old man was admitted with a 7 month history of intermittent upper abdominal pains and mild jaundice for one week. At operation the gall bladder showed signs of chronic cholecystitis. A chronically fibrosed gall bladder was removed and the common bile duct explored from which several calculi were removed. The immediate postoperative recovery was uneventful. On day 6 he had several rigors and became hypotensive. There was a leucocytosis (12,800/cu mm, 97% granulocytes) and platelets were clumped on the smear. Blood cultures grew a coliform
bacillus and treatment with kanamycin was started.

A platelet count of 22,000/cu mm was recorded on days 8 and 9, but the prothrombin activity and TST were normal. By day 10 there was a marked leucocytosis (48,000/cu mm, 93% granulocytes) but the platelet count remained low at 18,000/cu mm. Over the next 36 hours the patient rapidly deteriorated, becoming deeply jaundiced (bilirubin 14 mg/100 ml) and anuric just before death. Autopsy was refused.

Case 16 (Figure 15): An 80 year old woman with a 6 week history of painless jaundice was admitted following the onset of rigors and upper abdominal pain. On admission she was deeply jaundiced (bilirubin 9.4 mg/100 ml), hypotensive and showed marked peripheral vascular constriction. The initial blood picture showed a leucocyte count of 41,000/cu mm (91% granulocytes) and a platelet count of 156,000/cu mm. Coagulation studies showed a raised fibrinogen (650 mg/100 ml), with a prothrombin activity (67%) at the lower limits of normal. The TST was normal. Blood cultures grew a coliform bacillus and the patient was treated with kanamycin.

The following day there was a slight fall in the plate-
let count to 106,000/cu mm and a decrease in prothrombin activity (38%), borderline prolongation of the TST (10 seconds, normal 8 seconds) and reduction in fibrinogen to 500 mg/100 ml.

Coagulation studies on day 4 showed a return to normal in the prothrombin activity and TST and the fibrinogen had risen to 610 mg/100 ml, but the platelet count had fallen further to 84,000/cu mm. On day 6 she again became hypotensive. Following a bone marrow biopsy, which showed megakaryocytes to be present in normal numbers, there was a further fall in platelet count to 60,000/cu mm and it was decided to give a trial of heparin. After a single dose of 4,000 units of heparin intravenously, she developed a haematoma at the site of the sternal puncture and this therapy was discontinued.

The following day the blood picture showed a leucocyte count of 23,600/cu mm (96% granulocytes) and a platelet count of 72,000/cu mm. The patient died 12 hours later. Autopsy revealed a chronically fibrosed and inflamed gall bladder which contained pus and one large calculus. There was a suppurative cholangitis and the liver was studded with numerous small abscesses. Small subcapsular haemorr-
FIGURE 15: Case 16: Gram-negative bacillary septicaemia and associated liver dysfunction.
hages were evident in both kidneys, which also showed
cortical necrosis. A few microemboli were detected in
the capillaries of the lungs, kidney and liver. There
was also evidence of a recent small subepicardial myo-
cardial infarct.

Case 17 (Figure 16): A 34 year old woman, 22
weeks pregnant, was admitted to a maternity hospital with
abdominal pain. Laparotomy confirmed a clinically
suspected abdominal pregnancy. There was some difficulty
in controlling bleeding postoperatively so the patient was
transferred to the Royal Adelaide Hospital. On day 3 the
platelet count appeared to be normal on inspection of the
blood film and a further laparotomy was performed to
remove the remains of the placenta. Histology of the
tissue showed 'non-specific inflammatory changes'.

Postoperatively she improved but remained febrile.
Swabs taken from the abdominal wound grew a Staph.
pyogenes and treatment with ampicillin and lincomycin was
duly commenced.

On day 8 she vomited fresh blood. The platelet count
count was 316,000/cu mm. There was no evidence of hepatic
dysfunction at this time; however the prothrombin activity
was reduced (16%), the TST prolonged (patient 13 seconds, normal 8 seconds) and the TCT (patient 20 seconds, normal 10 seconds). The fibrinogen was 270 mg/100 ml.

The prothrombin activity rose to 48% within 24 hours of the parenteral administration of vitamin K₁. As there was no evidence of increased fibrinolysis (ECL > 2 hours) it was thought that the abnormality of coagulation was vitamin K deficiency consequent to intravenous and antibiotic therapy. Blood cultures on four occasions at that time were negative.

Over the next 10 days she developed the signs of a lung abscess which subsequently gradually resolved. The abdominal wound, however, failed to heal adequately and on day 19 pseudomonas was isolated from it. Treatment with Gentamycin was commenced.

On day 21 she became hypotensive, the platelet count fell to 78,000/cu mm, the prothrombin activity was 30%, the TCT prolonged (patient 27 seconds, normal 12 seconds) and the fibrinogen 130 mg/100 ml. Blood cultures were negative.

The following day there was a further reduction in prothrombin activity (21 %) and fibrinogen (55 mg/100 ml). There was also at this stage increasing evidence of hepatic
dysfunction; the bilirubin had risen from 2.7 mg/100 ml on day 20 to 9.4 mg/100 ml on day 25. The blood urea nitrogen was also raised (101 mg/100 ml).

The patient then became desperately ill on day 25; she was hypotensive and virtually anuric. Heparin 1,000 units hourly was given. There was some improvement in renal output and a temporary rise in prothrombin activity to 40%, but this subsequently fell to 26% two days later. The level of fibrinogen remained below 100 mg/100 ml and the 'F1 test' was positive to a titre of 1:64. Despite the evidence of hepatic dysfunction, the euglobulin clot lysis time did not become shortened, remaining greater than 5 hours on day 26 and 2 hours on day 28.

Because fresh blood was aspirated from the stomach and there was some bleeding per rectum, heparin therapy was stopped on day 28. Blood cultures taken at this time grew a coliform bacillus.

Over the next 3 days the patient's condition deteriorated further and she died on the 31st day of her admission.

Autopsy revealed miliary tuberculosis involving lungs, pleura, peritoneum, bowel, liver and uterus. There was
FIGURE 16: Case 17. Disseminated tuberculosis and terminal Gram-negative bacillary septicaemia.
also a huge mass of fibrin deposited in the peritoneal
cavity. There was not, however, any evidence of fibrin
deposited within the vessels of the kidneys or lungs.

Case 18 (Figure 17): A 46 year old male meat
inspector with a four day history of sweats, fever, rigors
and passing dark urine was admitted with dyspnoea, jaundice,
pyrexia and dehydration. He was normotensive.

The blood pictures showed a leucocyte count of 10,200/
cu mm (92% granulocytes) and a platelet count of 26,000/cu
mm. The prothrombin activity was 67% and the TST showed
slight prolongation (patient 12 seconds, normal 8 seconds).

The initial diagnosis was that of septicaemia, and
the patient was treated with kanamycin. Blood cultures,
however, were negative. Because of the low platelet count
consumption coagulopathy was suspected and the patient was
given heparin 1,000 units hourly.

The following day the platelet count was 36,000/cu mm,
the prothrombin activity was 79% and TST normal despite the
presence of heparin, a bilirubin of 11.5 mg/100 ml, an
alkaline phosphatase of 27 KA units/100 ml, and a SGOT of
85 K units/ml. Fibrinogen was 280 mg/100 ml and the 'Fi
test positive to a titre of 1:64.

On day 5 the heparin was stopped. At this time a diagnosis of Q fever was entertained. This was confirmed by a titre for Q fever rising from 1:5 to 1:350 by day 7. The patient was then started on a course of tetracycline.

Although the patient gradually improved, the fever persisted until day 9 and it was not until day 12 that the platelet count began to rise significantly. Throughout this period the prothrombin activity remained slightly reduced at between 50 and 60% but the other parameters of coagulation remained normal even though the serum chemistry did not suggest improvement in hepatic function until day 11 when the bilirubin, alkaline phosphatase and SGOT began to fall.

**DISCUSSION**

Thrombocytopenia was recorded in 16 of 36 patients with septicaemia by Goldenfarb and co-workers in 1970 (Goldenfarb et alii, 1970). Primarily concerned with the abnormalities in haemostasis occurring in septicaemia they noted that these ranged from isolated thrombocytopenia to involvement of all the parameters of coagulation studied. These
FIGURE 17: Case 18. Q fever.
observations are amply confirmed by the findings in the 18 cases described in this thesis.

Goldenfarb and co-workers, although subjecting the patients studied to intensive investigation of the coagulation system, make only a passing reference to evidence of liver disease which was noted in a number of their cases. Abnormalities of hepatic function were, however, a prominent feature in the cases studied here. Indeed, the patients described can be divided into three main groups - those with thrombocytopenia with little evidence of involvement of the other parameters of coagulation, those with evidence of consumption coagulopathy, and those with abnormalities of haemostasis and hepatic dysfunction which were by far the largest group.

In cases 1, 2, 3 and 4 only a transient fall in the platelet count was recorded in the acute phase of the infection; nevertheless, it did appear to be a good index for the presence of bacteremia and endotoxaemia. This was particularly striking in case 1, for the reduction in platelet count noted on a routine blood film examination actually preceded the clinical manifestations of bacteremia by several hours. Blood cultures taken at the onset of
fever confirmed the presence of a staphylococcal bacter-
aemia.

Thrombocytopenia together with toxic changes seen in
the neutrophils were the earliest confirmatory laboratory
findings of a clinically suspected septicaemia in case 2.
Case 3 illustrates the pattern frequently seen in patients
with acute severe pulmonary infections with the thrombo-
cytopenia rapidly resolving with treatment of the infection.
In case 4 the association of a low platelet count and a
marked leucocytosis suggested the presence of a subsequently
proven Gram-negative bacterial infection of the biliary
tract.

In these patients the rapid and profound fall in the
platelet count in the acute phase of the infection followed
by a rapid rise in platelet count in the period of clinical
recovery does suggest a destruction or sequestration of
circulating platelets. The appearance of a thrombocytosis
as seen in cases 1, 2 and 4, is almost certainly a reflection
of the earlier thrombocytopenia (Matter et alii, 1960).

A direct interaction between platelets and bacteria
with no, or minimal, involvement of the whole coagulation
system is proposed as the mechanism for the thrombocytopenia
in these cases. Reference has already been made to the clumping of platelets about bacteria which occurs when bacteria are injected intravenously into experimental animals, and to the transient thrombocytopenia induced by small doses of bacterial endotoxin.

In bacteraemic infections a transient thrombocytopenia can therefore be expected, and indeed it is seen, and is not necessarily associated with a depletion of other coagulation factors. The rapid fall in the number of circulating platelets in the acute phase of infections may be a reflection of their function as one of the mechanisms for the removal of the bacteria from the blood stream. The platelet count, once the bacteria are cleared, and in the absence of further invasion, then rapidly rises. The compensatory thrombocytosis seen in the convalescent period is due, most likely, to an increase in production of platelets by the bone marrow. Bone marrow biopsies in two patients (case 12 and case 16) did show an abundance of megakaryocytes.

Minor degrees of consumption of coagulation factors occurring during the reaction between platelets, bacteria and endotoxins in these cases cannot be excluded.
Activation of the coagulation system is probably of no consequence, however, since the reticulo-endothelial system is able to remove toxins, clotting intermediaries and fibrin degradation products (McKay, 1965). Clinically insignificant activation of the coagulation system, on the other hand, may be the stimulus for an initial rise in the level of clotting factors during infections and, therefore, account for the raised levels of fibrinogen so frequently noted in these cases.

A simple interaction between platelets, bacteria and endotoxin with minimal involvement of the coagulation mechanism is an insufficient explanation for the thrombocytopenia, abnormalities and other manifestations seen in cases 6, 7, 8 and 9.

The aggregation of platelets and the subsequent trapping of these aggregates in the vascular capillaries appears to be a salient feature in the genesis of the generalised Shwartzman reaction. The similarities of the coagulation abnormalities and the resulting histological features seen in such organs as the kidney in rabbits to clinical cases with bacteraemias have been the subject of detailed discussion elsewhere. The clinical importance of
recognising the presence of consumption coagulopathy is that it can be blocked with anticoagulants.

Thrombocytopenia was the earliest laboratory finding to suggest what may have been the human counterpart of the generalised Shwartzman reaction in three patients.

In case 7, despite the absence of pyrexia, thrombocytopenia did indicate the presence of sepsis. The full significance of this reduction in platelet count was not realised until 24 hours later when it was noted that the patient's blood did not clot. A markedly decreased prothrombin active (39 seconds, 10% activity), prolonged TST (patient 43 seconds, normal 8 seconds) and a fibrinogen assay of 30 mg/100 ml were consistent with acute defibrin-ation due to consumption coagulopathy, and at autopsy there was both macroscopic and microscopic evidence of extensive intravascular coagulation. It is only possible to speculate whether recognition of this condition 24 hours earlier, followed by the administration of heparin, could have altered the prognosis.

Case 8 was admitted with a Gram-negative septicaemia and a low platelet count, a decrease in prothrombin activity, a slight prolongation of the TST together with the onset of
moderate hypotension and oliguria did suggest the presence of consumption coagulopathy despite a fibrinogen assay of 980 mg/100 ml. Heparin therapy was begun. This was accompanied by a rapid rise in platelet count and a steady decrease in the titre of fibrin degradation products in the patient's serum which in turn, with dialysis, was followed by the restoration of normal renal function.

The efficacy of anticoagulant therapy in the possible prevention of the morbid sequelae of consumption coagulopathy is further reinforced by the sequence of events seen in case 9. This patient acquired a Gram-negative septic caemia while receiving heparin but did not exhibit the features of 'septic shock' until after the heparin was stopped 72 hours after confirmation of the bacteraemia. Hypotension and oliguria occurred within 2 hours of the cessation of heparin therapy. Associated with this was a rapid and profound fall in the platelet count and levels of other coagulation factors. The prompt improvement in clinical state when heparin was restarted and the subsequent rise in platelet count and other coagulation factors does suggest that the heparin was effective first in preventing then controlling this complication.
Patients with bacteraemic infections with coagulation abnormalities occurring in the absence of either vitamin K deficiency or hepatic dysfunction were, however, uncommon. In many cases the Gram-negative bacteraemic infections appeared to originate from the biliary tract, renal or other sources of abdominal sepsis. Because of the varying degrees of hepatic dysfunction in these patients it was not possible to attribute the changes in haemostasis entirely to consumption coagulopathy since the pattern of abnormalities in the coagulation studies induced by both conditions are very similar.

This is particularly well illustrated in case 12 where it was difficult to assess the relative importance of hepatic dysfunction and consumption coagulopathy even in the face of more extensive investigation of the coagulation system. In the remaining cases all showed varying degrees of hepatic dysfunction and abnormalities in the coagulation screening test at some stage during their illness, although the severity of the changes in coagulation did not usually coincide with the stage at which the patient showed the greatest degree of impairment of hepatic function. In general the changes in coagulation preceded the evidence of hepatic impairment and it may be that they were secondary
to the changes in coagulation. There is no doubt that a combination of the two processes can rapidly lead to a measurable lowering of the clotting factors concerned (Rapaport et alii, 1966). In case 18, one explanation for the decrease in coagulation factors is that it could have been due to the laying down of the mass of fibrin found in the peritoneal cavity - indeed, due to 'extravascular coagulation' as there was little evidence for intravascular coagulation at autopsy.

Despite the uncertainty of the mechanism for the fall in platelet count and the abnormalities in coagulation seen in these patients, thrombocytopenia was still a useful index of continued sepsis. In cases 12 and 18, thrombocytopenia closely reflected the clinically suspected presence of sepsis even though blood cultures were negative until a few days prior to death. Endotoxaemia associated with the Gram-negative bacillary infection could have accounted for this (Porter et alii, 1964).
SUMMARY

In the survey of the causes of a low platelet count in a hospital population it appeared that the probable incidence of thrombocytopenia in infections was of the order of 5 to 10%. When a number of patients were studied in more detail the thrombocytopenia was found usually to be a transient phenomenon, persisting only for a day or two, and which was frequently followed by a transient thrombocytosis in the convalescent period. A reduced platelet count was sometimes, but not always, associated with abnormalities in the screening tests of plasma coagulation.

Thrombocytopenia was much more frequently encountered in infections by Gram-negative bacilli than those due to Gram-positive organisms. In patients with Gram-negative bacillary infections associated abnormalities in coagulation were common. Hepatic dysfunction could have contributed, and probably did, to the changes in the haemostatic mechanism in many of these patients. The finding at autopsy of multiple thrombi in many vessels would seem conclusive evidence, however, that massive intravascular clotting was a significant factor in the causation of these changes in one patient. This same process was possibly
also a significant factor in contributing to the genesis of renal dysfunction in another two patients in whom the use of heparin appeared to be of some benefit in improving the outcome of the septicaemic illness. In the remaining patients the abnormalities in haemostasis were difficult to define.

Disseminated intravascular coagulation is, however, only one of the many facets of associated abnormalities in body homeostasis encountered in the septicaemic patient. As an intermediary mechanism of disease its effect on the course of the illness and its significance in the pathogenesis of complications including the haemorrhagic and renal manifestations in these patients remains unclear. The abnormalities in haemostasis do, however, appear to be a reflection of the severity of the disease process.

Nevertheless, this study does emphasize the usefulness of thrombocytopenia as a relatively simple laboratory sign which can alert the physician to the possible presence of bacteraemia and continued sepsis, in particular when this is caused by a Gram-negative organism, and to the presence of disseminated intravascular coagulation.
CHAPTER VI

STUDIES OF THE RESPONSE OF PLATELETS AND THE COAGULATION SYSTEM TO EXPERIMENTALLY INDUCED BACTERAEMIAS AND ENDOTOXAEMIA IN RABBITS
Intensive investigation into the mechanism of thrombocytopenia in very ill patients with sepsis was felt not to be justified. The relative infrequency and the rapidity of change in the clinical status of these patients also made such a study impracticable in the hands of the observer who was not directly involved in the clinical management.

The patterns of the response in platelet numbers to bacteraemia and endotoxaemia were therefore studied in an experimental animal, the rabbit. Experiments were designed to study:

1. The changes in platelet numbers in response to artificially induced bacteraemia and endotoxaemia in the intact animal.

2. The relationship of these changes to alterations in the coagulation system.

MATERIALS AND METHODS

As the majority of previous investigators have used the rabbit for the study of changes in the coagulation system induced by endotoxin, this animal was chosen for this study of the response of the platelet to artificially induced bacteraemias and endotoxaemia.
All the animals were of the Institute of Medical and Veterinary Science 'group 0' strain, a closed colony inbred for the past 20 years. Young animals on attaining a weight of 2 to 3 kilograms were used for the experiments. They were housed in a separate room and fed on M and V pellets manufactured by W. Charlick Ltd. Adelaide to specifications produced by the Veterinary Division of the Institute of Medical and Veterinary Science.

Blood for platelet counts, packed cell volume and leucocyte counts was obtained in the following manner. The ear vein was first shaved. A 10 SWG needle was then scored across the shaft with a file and the hub of the needle snapped off. The remaining section of the shaft of the needle, measuring 1 to 2 cm long, was used to insert into the ear vein of the rabbit. A tourniquet applied lightly to the base of the ear to facilitate entry into the vein was removed immediately the needle was inserted. With a rate of flow of at least one drop a second, blood was collected directly into the standard I.M.V.S. sequestrene (EDTA) container. Fifteen drops of blood were found to be approximately equivalent to 0.5 ml. This procedure is illustrated in Plate I.
It is possible by this method to obtain a clean sample and to minimise blood loss, both factors which can themselves substantially alter the platelet count. There is also very little trauma associated with this procedure, bleeding can be easily controlled when the needle is removed and it permits the use of the same ear vein on a number of occasions.

Platelet counts were performed, in most cases, within an hour of collection using the same visual method described for the counting of human platelets. The packed cell volume and leucocyte counts were performed by routine manual laboratory methods.

Normal Values in Rabbits

The following results were obtained in 100 untreated animals:

Platelet count: mean 426,000 S.D. ± 106,000 cu mm
PCV: mean 39 S.D. ± 3
Leucocyte count: mean 7,900 S.D. ± 2,500 cu mm

The results obtained for the packed cell volume and the leucocyte count are in close agreement with those obtained by other investigators. There does not appear to be the same general agreement with regard to the platelet
Mean values of 251,000, 552,000 and 743,000 cu mm with ranges of 200,000 to 1,000,000 cu mm have been reported (Schalm, 1965). The values obtained in the I.M.V.S. strain of rabbits falls in the centre of these estimates and the range of counts is not as wide as reported by some authors.

Changes in Platelet Numbers in Response to Intravenous Infusions of Micro-organisms and Endotoxin

The purpose of these experiments was to observe the changes in the platelet count in response to the intravenous infusion of suspensions of freshly washed organisms in saline and to 'endotoxin'. Escherichia coli, Staphylococcus aureus and, to assess the effect of a non-pathogen, Sarcina lutea were chosen.

Materials and Methods

Micro-organisms:

Escherichia coli: 075 (0 somatic serotype)
Staphylococcus aureus: National collection typed cultures
Sarcina lutea: N.C.T.C. 8340
Preparation of the bacterial suspensions: As suspensions of organisms obtained from colonies grown on agar plates are contaminated with agar, which itself induces aggregation of platelets, a broth culture was used. Wright-Munday broth causes no alteration in the platelet or leucocyte counts when infused intravenously into rabbits. The composition of this broth includes acid icase, magnesium sulphate, potassium dihydrogen phosphate, disodium hydrogen phosphate, nicotinamide and thiamine in 1% dextrose.

An eight hour (overnight) culture was prepared by taking a single colony and incubating it in a flat bottomed glass bottle containing 30 ml of the broth. After incubation, the culture was centrifuged for 30 minutes at 12,000 rpm to produce a compact deposit. The supernatant was removed and the deposit resuspended in normal saline. This procedure was repeated three times to wash the organisms. The density of the final suspension was determined against Brown opacity tubes. A count for viable organisms was performed on the same suspension according to the method of Miles and Misra using 0.02 ml drops of ten-fold dilutions on to blood agar and reading the viable count at 24 hours (Miles and Misra, 1938).
The supernatant (containing endotoxin) was filtered through a Seitz EK filter. The filtrate was then steamed for 15 minutes to destroy any heat-labile toxins that may have been present in addition to the heat stable '0' fraction.

Procedure

Following the collection of 0.5 ml blood from an ear vein, 0.5 ml of freshly prepared suspension of bacteria, supernatant fluid, broth mixture or saline per kilogram body weight was infused intravenously into the rabbit over a period of about one minute.

Further specimens of blood were collected from the ear vein at 10 minutes after the infusion then again at 24 hours and 2, 4 and 7 days later. Packed cell volume, platelet and leucocyte counts were estimated within two hours of collection of the samples in each case.

In the event of the death of the animal, an autopsy was performed and sections of the kidneys, lung, liver and spleen were made and stained with haematoxylin and eosin (H & E) and to demonstrate fibrin, with Martius scarlet blue (MSB).
Experiment 1 (A)

Sarcina lutea, NCTC 8340, is a non-pathogenic organism. It was chosen in order to assess the response in platelet numbers to the infusion of varying concentrations of bacteria into the circulation.

The animals were divided into the following groups:

(a) Controls. Four animals were infused with the filtrate of the supernatant fluid from the broth cultures of the organism. Two animals were infused with fresh uncontaminated Wright-Munday broth and two with 0.9% saline. The quantity of material injected in each case was 0.5 ml per kilogram body weight.

(b) Test animals.

1. Four animals received infusions of a saline suspension of Sarcina lutea with a final organism density of $5.4 \times 10^6$/ml.

2. Four animals received an infusion of a suspension with an organism density of $3.1 \times 10^{12}$/ml.

3. Four animals received an infusion of a suspension with an organism density of approximately $1 \times 10^{18}$/ml determined by a turbidometric method.
Results

The results of the counts obtained in the control animal are shown in Figure 18. These results are expressed in this and subsequent experiments as the percentage change in the platelet count from a baseline value, designated 100%, obtained at zero time before the intravenous infusions. This mode of expression has been adopted because of the very wide range of platelet counts found in normal animals. For example, a fall in the platelet count from an initial level of 600,000/cu mm to 300,000/cu mm represents a fall of 50% in that animal; even so, this will not bring the count below the lower limit of the normal range. The standard error is shown for the platelet counts but only the arithmetical mean values for the packed cell volume and leucocyte counts.

It is apparent from this experiment that the infusion of supernatant culture fluid, broth and saline and even the procedure of repeated small venesections has no effect on the parameters measured.

The response to the infusion of varying concentrations of organisms into the circulation is illustrated in sequence in Figures 19, 20 and 21. The degree of fall in platelet
FIGURE 18: Changes in the platelet count, packed cell volume and leucocyte count in four animals infused with the filtrate of the supernatant fluid obtained from a culture of Sarcina lutea, two animals infused with fresh, uncontaminated broth and two animals infused with 'normal' saline.
count correlated well with the quantity of organisms injected: the greater the number of organisms infused, the greater is the fall in the platelet count.

The platelet count remains low for 24 hours in every case before rapidly rising to levels in excess of the initial count on days 4 and 7. The magnitude of this thrombocytosis appears to be dependent on the degree of thrombocytopenia induced. Platelet counts in excess of 1,000,000/cu mm were encountered in those animals in which the reduction in platelet count had been most marked.

The changes in the leucocyte count are variable. An immediate rise in the leucocyte count is seen following the injection of a few organisms but with the infusion of greater numbers of bacteria the changes in leucocyte count are similar to those of the platelet count, although the time sequence of these changes is more rapid.

Throughout this series of infusions the rabbits showed no evidence of constitutional upset.

Experiment 1 (B): Shown in Figure 22 are the changes in packed cell volume, platelet and leucocyte counts in:

1. Four animals infused with a saline suspension of
FIGURE 19: Changes in the platelet count, packed cell volume and leucocyte count in four animals infused with a suspension of Sarcina lutea in 'normal' saline (organism density 5.4 x 10^6/ml).
FIGURE 20: Changes in the platelet count, packed cell volume and leucocyte count in four animals infused with a suspension of Sarcina lutea in 'normal' saline (organism density $3.1 \times 10^{12}/\text{ml}$).
FIGURE 21: Changes in the platelet count, packed cell volume and leucocyte count in four animals infused with a suspension of Sarcina lutea in 'normal' saline (organism density approximately $1 \times 10^{18}$/ml).
freshly washed E. coli with a final organism density of 
$8.8 \times 10^8$ ml.

2. Three animals infused with the supernatant endotoxin derived from the same culture.

Results

There is a significant fall in the platelet count at 10 minutes after the infusion of both the suspension of bacteria and of the endotoxin. The reduction in platelet numbers is maximal on day 1 (26% of the baseline value) in the animals infused with bacteria. Two of the animals died within the first 24 hours. The greatest reduction in platelet numbers in the endotoxin infused animals occurred on day 2. Two of these animals died between day 3 and 4.

The rise in the packed cell volume almost certainly reflects haemoconcentration as all the animals were febrile, weak and not taking usual quantities of water and food. The sickest animals also had diarrhoea.

No macroscopic evidence of haemorrhage was discernible at autopsy in any of these animals. There was, however, an abundance of fibrin in the small vessels of the lungs and kidneys (see Plate II).
FIGURE 22: Changes in the platelet count, packed cell volume and leucocyte count in:

1. Four animals infused with a suspension of E. coli in 'normal' saline (organism density $8.8 \times 10^8$/ml).

2. Four animals infused with E.coli endotoxin.
Fibrin deposited in the small vessels of the glomerulus (MSB stain).

Fibrin deposited in the small vessels of the lungs (MSB stain).

PLATE II
The remaining animals recovered quickly and this was accompanied by a rapid rise in the platelet count to levels well in excess of the initial values. Not until 14 to 21 days later (not shown on the graphs) did the platelet count return to normal levels. There appears to be no significant difference in the response to bacteria and endotoxin infused in these amounts.

The changes in the leucocyte count are similar to the changes seen in the platelet count, although the fall is less marked and during recovery the return to normal levels more rapid. In the one surviving animal infused with endotoxin the leucocyte count remained low throughout the period of the experiment.

The same experiment was repeated using weaker suspensions of bacteria (organism density $6.4 \times 10^4$ /ml) and a correspondingly weaker endotoxin.

The changes are shown in Figure 23. The fall in platelet count is less marked. None of the animals died and during recovery the thrombocytosis is particularly pronounced, reaching 255% of the baseline value on day 7.
FIGURE 23: Changes in the platelet count, packed cell volume and leucocyte count in:

1. Four animals infused with a suspension of E. coli in 'normal' saline (organism density 6.4 x 10^6/ml).

2. Four animals infused with E. coli endotoxin.
Experiment 1 (C): In Figure 24 are shown the changes occurring in the packed cell volume, platelet and leucocyte counts in:

1. Four animals infused with a saline suspension of Staphylococcus aureus (NCTC 4163, organism density $9.4 \times 10^7$/ml).

2. Four animals infused with the supernatant of this culture.

Results

The suspension of organisms causes a fall in the platelet count; the supernatant fluid of the culture did not, although there is a wider variation in the platelet count about the baseline level in these animals than in the control group (Figure 18).

One of the animals given the bacteria died before there was a rise in the platelet count and the remaining three died between day 5 and 6. In the latter animals death was preceded by a moderate thrombocytosis and an extreme leucocytosis.

Autopsy revealed extensive pneumonia, enlarged, congested livers and abscesses in the kidneys of all
FIGURE 24: Changes in the platelet count, packed cell volume and leucocyte count in:

1. Four animals infused with a suspension of staphylococcus aureus in 'normal' saline (organism density 9.4 x 10^7/ml).

2. Four animals infused with the supernatant fluid (enterotoxin).
animals. There was no evidence of fibrin deposition in any of these organs.

CONCLUSIONS

This series of experiments confirms the rapid fall in the number of circulating platelets within 10 minutes of the intravenous infusion of bacteria noted by previous authors. It is also possible to demonstrate that the degree of fall in the platelet count following the infusion of non-pathogenic organisms (Sarcina lutea) is proportional to the number of bacteria injected; the more organisms injected the greater is the fall in the platelet count. This response is very similar to that reported following the intravenous infusion of colloidal carbon (Cohen et alii, 1965).

Unlike the response to colloidal carbon where reports indicate that the platelet count rises within a period of several hours of the infusion, the platelet count did not rise until at least 24 hours after the infusion of the bacteria. The thesis presented by Taniguchi that the platelets rapidly return to the circulation in such circumstances does not appear to be substantiated. On the contrary, the present findings suggest that the platelets are destroyed.
The appearance of a compensatory thrombocytosis seen from day 4 adds weight to this suggestion.

Not unexpectedly, changes in the platelet count were more profound when pathogenic organisms are injected. There is a major difference between the two organisms used. Staph. aureus does not appear to elaborate a toxin capable of inducing thrombocytopenia under the conditions of the experiment, whereas E. coli endotoxin has an effect as profound as the organisms themselves. The thrombocytopenia in Gram-negative infections therefore appears to be caused by a combination of the effects of bacteraemia and endotoxaemia. This could be an explanation as to why thrombocytopenia is seen more commonly in Gram-negative bacteraemia than in Gram-positive bacteraemic infections.

The response to the E. coli endotoxin in these experiments is more profound and persistent than that reported by other investigators; this is almost certainly due to the quantity infused. A more rapid recovery in the platelet count in response to endotoxin is demonstrated in later experiments.

Although the haemostatic system in general was not
examined, the presence of fibrin in the capillaries of the E. coli treated animals does suggest that intravascular clotting was a factor in those which died, whereas no such evidence was apparent in the Staph. aureus treated animals.

The Consumption of Clotting Factors in Response to Infusions of Small Amounts of Bacterial Endotoxin

Factors VII, IX and X persist in the serum of blood clotted in glass tubes; platelets, fibrinogen, factors II (prothrombin), V and VIII are consumed. Extensive clotting in vivo also causes thrombocytopenia and reduced levels of factors II, V and VIII, and in patients with massive intravascular clotting there may be a reduction of all the coagulation factors (Rapaport et alii, 1964).

To examine more accurately and sensitively the effect of an infusion of dilute tissue thromboplastin on the coagulation system of the rabbit, Rapaport et alii inhibited the synthesis of factors II, VII, IX and X in the liver by administering large doses of sodium warfarin intravenously. Four hours after the initial dose the animals were infused with either saline or dilute tissue thromboplastin. In both groups of animals there was a fall in the levels of the clotting factors but in those infused with thromboplastin
they were able to clearly demonstrate that consumption and clearance of these factors was increased during episodes of clotting (Rapaport et alii, 1966).

Using this experimental model, small doses of endotoxin capable of causing a transient thrombocytopenia but no significant alteration in the levels of factors II and X in the intact animal, were infused instead of dilute thromboplastin.

MATERIALS AND METHODS

Bacterial endotoxin: This was prepared from a culture of E. coli in broth in the manner previously described. It was stored at -20°C and thawed just prior to infusion on each occasion.

Citrated diluting fluid: This contained one part citrate anticoagulant (a solution of 0.06 M sodium citrate and 0.04 M citric acid) to five parts isotonic saline solution.

Methods of assay of factors II and X: Factor II was measured by a modification of method described by Owren (1949) using normal serum and Simplastin A (Warner-Chilcott Laboratories, Morris Plains, N.J., U.S.A.). The latter
reagent supplies factors V, fibrinogen, tissue factor and calcium.

Factor X was measured by a one-stage method where dilutions of the test plasma were mixed with Seitz filtered bovine plasma and Russell viper venom (Stypven, Burroughs Wellcome Co. London) diluted in 0.02% inosithin. The test was performed in the same manner as the one-stage prothrombin time with an incubation time from the addition of the Stypven to the addition of the calcium chloride of 3 minutes (using 0.1 ml of each reagent).

The clotting times were converted to per cent activity from dilution curves prepared from plasma samples taken at zero time in each animal. Alterations in the levels of the clotting factors therefore represent the per cent change in activity from the baseline value (100%) at zero time in each animal.

Procedure: The animals were handled in batches of three; each was given 50 mg sodium warfarin (Endo Laboratories Inc. U.S.A.) intravenously. Three infusions of warfarin at intervals of six hours were required to induce a steady fall in factors II and X. Four hours after the first intravenous injection of warfarin blood (0.5 ml) was
collected into a plastic syringe through a 21 SWG needle from an ear vein. The blood was then immediately expelled on to a sheet of 'Parafilm' (American Can Co. Wisconsin, U.S.A.) and dilutions made by pipetting 0.05 ml of blood into 0.45 ml citrated diluting fluid. This procedure had always to be completed within 30 seconds.

One animal was then infused with 0.2 ml normal saline, and two animals with 0.2 ml bacterial endotoxin. Further samples were collected at 5, 30 and 60 minutes and at 4, 6, 10, 18 and 24 hours after the infusion of saline or endotoxin.

The samples were frozen immediately after collection and stored at -20°C for 24 to 48 hours before being thawed prior to assay of factors II and X.

**EXPERIMENT II (A)**

Eight animals were infused with 0.2 ml bacterial endotoxin at a concentration adjusted to cause a significant fall in the platelet count. Apart from a transient pyrexia no apparent systemic manifestations were noted in any of the rabbits.
Results: The changes in platelet count are shown in Figure 27A. The fall in the platelet count is transient, a response which is in close agreement with that observed by other investigators.

EXPERIMENT II (B)

Serial estimations of factors II and X were made in six animals not treated with sodium warfarin; two received an infusion of 0.2 ml normal saline and four 0.2 ml endotoxin. The mean per cent change in the levels of the two clotting factors from a baseline value of 100% in the saline and endotoxin treated animals are shown in Figure 25.

Results: There does appear to be some fall in factor II immediately after the infusion of the endotoxin but this is found not to be significant on statistical grounds. The mean value of both factors in the endotoxin treated animals does, however, remain consistently lower than those of the saline treated animals throughout the period of the experiment. A gradual rise in the levels of the clotting factors, in particular of factor II in the saline treated animals, occurs during the course of the experiment. This represents, almost certainly, a response to blood loss.
FIGURE 25: Changes in the levels of factors II and X in:
1. Two animals infused with 0.2 ml 'normal' saline.
2. Four animals infused with 0.2 ml endotoxin.
A rise in the level of fibrinogen in both groups increasing from a mean of 348 mg/100 ml prior to the infusion of endotoxin to 603 mg/100 ml at 24 hours also occurs.

**EXPERIMENT II (C)**

Nine animals were given three intravenous doses of 50 mg sodium warfarin at intervals of six hours. Three animals were infused with 0.2 ml normal saline and six with 0.2 ml endotoxin four hours after the initial dose of sodium warfarin. The mean values of the serial estimations of factors II and X are shown in Figure 26.

**Results:** In the endotoxin treated animals the mean rate of fall in factor II is significantly greater (58% t 8.3 p < 0.001) in the first four hours following infusion than in the saline treated animals. During the remaining 28 hours the rate of fall in the level of this factor in the endotoxin and saline treated animals is similar.

A different pattern in the rate of fall is seen in the levels of factor X. In the first six hours the rate of fall of factor X is the same after the infusion of endotoxin or saline. There is, however, an increase in the rate of decline in the level of this factor in the endotoxin
FIGURE 26: Changes in the levels of factors II and X in:

1. Three animals administered 50 mg sodium warfarin intravenously at intervals of six hours.

2. Six animals infused additionally with 0.2 ml endotoxin four hours after the initial dose of sodium warfarin.
treated animals eight to sixteen hours later (30% t 8.2 p < 0.001).

CONCLUSIONS

There is a steady exponential decline in the levels of factor II, half life 42 hours, and factor X, half life 18 hours, when synthesis of these clotting factors by the liver is inhibited by the administration of sodium warfarin. The rate of decline of factor II is significantly increased in the first four hours after the infusion of endotoxin. This is very similar to findings in warfarin treated rabbits following the infusion of tissue thromboplastin observed by Rapaport.

It is known that factor II is consumed in the process of coagulation; the finding that there is an increased rate of consumption of this factor does, therefore, suggest that clotting does occur after the infusion of endotoxin. In the intact animal this increased rate of consumption is, however, insufficient to cause a significant fall in the level of factor II where production of clotting factors by the liver, possibly at an increased rate, compensates for its loss.
Factor X is not consumed in the clotting process; as expected, no increase in the rate of decline of this factor is noted in the initial hours after the infusion of endotoxin. The removal of activated factor X by the reticuloendothelial system could explain why there is a more rapid fall of this factor eight to sixteen hours later in the endotoxin treated animals.

It is concluded that in the rabbit small intravenous doses of bacterial endotoxin which are capable of causing a transient fall in the platelet count will also induce an episode of intravascular clotting.

The Response in Platelet Count in Rabbits Treated with Heparin Prior to the Infusion of Endotoxin and Organisms

As consumption of clotting factors does occur after the intravenous infusion of even small quantities of bacterial endotoxin, rabbits were anticoagulated with heparin prior to the infusion of endotoxin in order to assess whether or not inhibition of clotting would prevent the fall in the platelet count.

EXPERIMENT III (A)

Six animals were given 1 ml of heparin in a concen-
tration of 1,000 units per ml (Weddel Pharmaceutical Ltd. London) intravenously. This was sufficient to render the circulating blood incoagualable. Five animals were then infused with 0.2 ml endotoxin.

Results: The changes in the platelet count are shown in Figure 27B. There is no difference in the degree of fall in the platelet counts in the heparinised animals compared to that occurring in the non-anticoagulated animals (cf experiment II(A), Figure 27A).

EXPERIMENT III (B)

A similar study was made with respect to the changes in the platelet count after the infusion of a suspension of micro-organisms (Sarcina lutea, organism density 8.9 x 10^8/ml) into the circulation. The changes in platelet numbers are shown in Figure 28. The response in the heparinized animals is no different to that seen in the untreated animals (cf Figures 19 and 20).

CONCLUSIONS

Despite inhibition of clotting with large doses of heparin the changes in platelet numbers following the intravenous infusion of endotoxin and of bacteria are not significantly different to those seen in the intact animal.
FIGURE 27A (ABOVE): Changes in the platelet count in eight animals infused with 0.2 ml endotoxin.

FIGURE 27B (BELOW): Changes in the platelet count in five animals infused with 1,000 units heparin followed by 0.2 ml endotoxin, and one animal infused with 1,000 units heparin alone.
FIGURE 28: Changes in the platelet count in:

1. Three animals infused with 1,000 units heparin.

2. Six animals infused with 1,000 units heparin followed by a suspension of Sarcina lutea in saline (organism density $8.9 \times 10^8$/ml).
It does appear that the fall in platelet count induced by these agents is independent of coagulation.

**SUMMARY**

Changes in the platelet count in response to the intravenous infusion of bacteria and endotoxin were studied in the rabbit.

The observation of previous workers who reported a rapid fall in the number of circulating platelets following the intravenous infusion of bacteria is confirmed. It is further demonstrated that the degree of thrombocytopenia induced is proportional to the number of organisms infused when a non-pathogenic strain is used. The greater the number of organisms infused, the more profound is the thrombocytopenia.

The rapid return of the platelet count to nearly normal levels within a few hours with the injection of small doses of endotoxin reported by other investigators is also confirmed. That this also occurs with bacterial suspensions is not substantiated. The platelet count in the latter group remained low for 24 hours before rising to levels in excess of the baseline value four to seven days later.
This suggests that there is destruction of platelets following the infusion of bacteria.

The changes in the platelet count are more profound if the pathogenic organisms E. coli and Staph. aureus are used. The animals also showed severe constitutional symptoms and several died. Fibrin demonstrated in the capillaries of the lungs and kidneys of those animals which received the products of Gram-negative bacteria does suggest that intravascular clotting had occurred. These features are not seen in the sections of those animals which received the products of the Gram-positive organism. Staph. aureus also does not appear to produce, under the experimental conditions described, a toxin capable of inducing changes in the platelet count whereas E. coli endotoxin produces changes as profound as the bacteria itself.

When some facets of the coagulation system were studied, it was not possible to demonstrate a significant change in the levels of the clotting factors II and X after the infusion of small doses of bacterial endotoxin in the intact animal. When synthesis of these factors by the liver is inhibited by sodium warfarin, a highly significant
increase in the rate of consumption of factor II occurs within a few hours of the infusion of endotoxin. Factor II is consumed in the process of clotting; this finding does, therefore, suggest that endotoxin causes an episode of intravascular clotting. A corresponding increase in the rate of fall of factor X occurs several hours later. The clearance of activated factor X, which is not consumed in the clotting process by the reticulo-endothelial system, is the most probable explanation for this phenomenon.

Consumption of platelets during intravascular clotting is one mechanism by which endotoxin and bacteria may cause a fall in the platelet count but this is not, however, the complete explanation, because thrombocytopenia cannot be prevented by the inhibition of clotting with heparin.
In 1968, when this study of thrombocytopenia in infectious illnesses was begun, the literature and textbooks of Medicine and Haematology gave little emphasis to this association; indeed, there appeared to be a general lack of awareness that thrombocytopenia was a significant manifestation of sepsis. This prompted several authors to suggest that thrombocytopenia in bacteraemic illnesses may be a more frequent occurrence than was then generally supposed.

That this general unawareness of the association of thrombocytopenia in infectious illnesses should have been so does not appear to have been entirely warranted since there is ample literature, especially from France and Germany, available from the early decades of this century to indicate that thrombocytopenia is a significant manifestation in severe sepsis. Most of the changes in platelet count observed during the course of illnesses due to infections reported in more recent literature were well documented forty to fifty years ago.

In addition to documenting these changes in platelet numbers, a number of investigators prior to 1930 did turn
their attention to the possibility that platelets may also in some way be involved in the body's defence against infections. It was appreciated that when organisms entered the blood stream platelets rapidly clumped about the bacteria and disappeared from the circulation. Aggregates of platelets and bacteria then could be found in the capillaries of many organs, in particular the lungs, liver and spleen. Only recently has there been a resurgence of interest in this behaviour of platelets in relation to foreign material entering the circulation. There is now evidence to suggest that platelets are involved in the removal of small particles and bacterial endotoxin.

Haemorrhagic manifestations and the occasional reference to the finding that the blood of a patient with sepsis did not clot are also well documented by many of the earlier observers. The concept that other facets of the coagulation system may also be involved were however not expressed until the late 1950's. This is not surprising as a fuller understanding of the coagulation system and tests of its integrity were not devised until the 1940's. More recently the concept that consumption of coagulation factors, including platelets, in a process termed disseminated intravascular
coagulation, has been promulgated as a mechanism for the thrombocytopenia, haemorrhagic and other manifestations sometimes seen in infectious diseases. Beginning in the late 1950's and continuing up to the present time there has been a considerable and increasing flow of reports concerned with the abnormalities of haemostasis in bacterial, protozoal, rickettsial and viral illnesses. Many of the findings reported in this thesis reflect and confirm much that has already appeared in this recent and current literature.

The frequency with which infections in general are associated with thrombocytopenia is still not known. To answer this question, a limited prospective study of all patients admitted to a medical and surgical unit and a larger survey of all cases of thrombocytopenia detected in the laboratory by routine haematological examination was undertaken. From these surveys it was possible to estimate that the incidence of thrombocytopenia in infections in a hospital population to be probably in the order of five to ten per cent.

In a more detailed study of patients with thrombocyto-
penia and infections it became apparent that a low platelet count is frequently a transient phenomenon, with the platelet count rapidly rising with resolution of the illness to levels in excess of normal in the convalescent period.

Bacteraemia was demonstrated in about half the patients. It is highly probable that if an intensive search by means of repeated blood cultures were made in all patients with bacterial infections and thrombocytopenia that most would be found to have a bacteraemia. Gram-negative bacteria were much more frequently isolated from these patients with thrombocytopenia than were Gram-positive bacteria.

Thrombocytopenia also proved to be a useful laboratory sign suggesting bacteraemia, while a persistent or falling platelet count was highly suggestive of continuing bacteraemia or endoxaemia and usually indicated the presence of the complication of disseminated intravascular coagulation. Patients with biliary tract infection and jaundice appeared to be particularly susceptible to this complication. The association of liver disease often made it difficult to decide whether the changes in haemostasis were predominantly due to the liver disease or to disseminated intravascular coagulation as both produce similar patterns.
In three patients there was unequivocal evidence of disseminated intravascular coagulation. Two appeared to have a satisfactory response to the use of heparin, the third was given an antifibrinolytic agent (Epsilon amino caproic acid) and at autopsy there was evidence of massive intravascular thrombus formation. This latter case does add confirmation to the view expressed by most authors that the fibrinolysis seen in this syndrome is secondary to the clotting process and represents a compensatory response. In the remaining patients treated with heparin there was an improvement in the laboratory coagulation tests but a fatal outcome to the illness was not averted in most cases.

It was not practicable to investigate in more detail the changes induced by septicaemia in man in relation to the platelets, so further studies of the platelet response to artificially induced bacteraemia were made in rabbits.

The rapid fall in platelet count within five minutes of an intravenous infusion of bacteria noted by previous investigators is confirmed. It was also possible to show that the degree of fall in the platelet count is related to the number of organisms injected when a non-pathogenic bacteria, Sarcina lutea, was used. The more organisms
injected the greater was the fall in the platelet count. The persistence of a low platelet count in these animals for more than twentyfour hours does suggest that the platelets were destroyed. The rapid rise to levels in excess of the baseline values seven days later with the highest counts being inversely proportional to the preceding fall in platelet count tends to confirm this suspicion. A similar sequence of events in platelet count were seen in a few uncomplicated cases of infections in man.

The fall in platelet count was more pronounced when the pathogenic organism Escherichia coli and Staphylococcus aureus were injected intravenously. Several of these animals died. Those infused with the Gram-negative organisms showed evidence of fibrin in the small vessels of the lungs and kidney, whereas this was not demonstrated in those animals infused with the Gram-positive organisms.

Escherichia coli elaborates an endotoxin which is itself capable of inducing as profound a fall in the platelet count as the bacteria themselves; this does not occur with Staphylococcus aureus under the conditions used for these experiments. This finding again reflects and is in keeping with the observation that human infections in which Gram-negative
organisms were isolated were more commonly associated with profound falls in platelets than in the cases where Gram-positive organisms were isolated.

A series of experiments were then devised to assess whether the infusion of small doses of E. coli endotoxin capable of causing a transient thrombocytopenia lasting only a few hours is also associated with activation of the coagulation system. In the intact animal no significant change in the levels of the coagulation factors II and X could be shown following the intravenous injection of small quantities of endotoxin. If the production of these factors by the liver is inhibited with large doses of sodium warfarin, it was possible to demonstrate that there is an increase in the rate of fall in level of factor II immediately after the injection of endotoxin compared to animals which were treated with warfarin alone. This strongly suggests that there is consumption of factor II in response to the infusion of endotoxin. An increase in the rate of fall in the level of factor X was not apparent until twelve to eighteen hours after the infusion of endotoxin. This factor, unlike factor II, is not consumed in the clotting process and this late change almost certainly reflects removal of activated factor X.
by the reticuloendothelial system.

Although these findings indicate that there is activation of the coagulation system in response to very small doses of endotoxin, the fall in platelet count could not be prevented by prior treatment of the animals with large doses of heparin sufficient to render the blood incoagulable. The fall in platelet count was identical in both the heparinized and unheparinized animals. A similar finding was also noted with the intravenous injection of Sarcina lutea. This does confirm the in vitro findings of other investigators that platelet adhesion and aggregation can occur independently of the coagulation system as a whole. Although it was not demonstrated, this interaction between platelets, bacteria and endotoxin most probably depends on the release of ADP consequent to the "platelet release" reaction.

It would be of interest to devise experiments whereby the role of platelets in clearing endotoxin and bacteria from the circulation could be more clearly defined. Attempts were made to render animals thrombocytopenic and neutropenic with cytotoxic drugs and radioactive phosphorus-32 with the intention of comparing the clearance of bacteria from the circulation of rabbits which had received a platelet trans-
fusion and those which had not. The results obtained have not been reported in this thesis since the degree of thrombocytopenia and neutropenia induced in individual animals was extremely variable. Once the animals had been reduced to this state their physical condition then did not allow for the manipulation required for such experiments.

There are many cellular and humoral factors involved in the body's reaction to invasion by micro-organisms. At the present time it appears difficult to devise a means by which the role of the platelet in this scheme could be more adequately investigated. It is probable that the role of the platelet is not great since patients with congenital amegakaryocytic thrombocytopenia (two such cases were encountered in the survey period) do not appear to be more susceptible to infections that the general population.

In conclusion, it is now firmly established that thrombocytopenia is a significant manifestation of sepsis. Indeed the more recent textbooks of Medicine and Haematology have given increased prominence to this finding. There are without doubt a number of factors and mechanisms involved in causing the fall in platelet count. Consumption of platelets in a process of disseminated intravascular coagulation is the
mechanism that has received most attention in the past five years as this complication also appears to be important in the genesis of other features such as acute renal failure seen in association with septicaemic shock. It has been possible to demonstrate that even very small doses of endotoxin will activate and cause consumption of clotting factors. It is therefore very probable that there is an increase in turnover of clotting factors in all infectious illnesses, but only in a few will these changes be so marked as to cause signs and abnormalities in the laboratory tests of coagulation.

Other mechanisms are also involved. It has been demonstrated that the changes in the platelet count in response to endotoxin and bacteria cannot be influenced by the inhibition of the clotting system with heparin. This strongly suggests that the platelet interaction with these agents can be independent of the coagulation system as a whole.

It is also appreciated that many other factors not studied in this thesis could be involved in the genesis of the thrombocytopenia. There appears every indication that further research in this field will continue at an
increasing rate as it has done in the many other fields of the behaviour of the complex interactions in which the platelet is involved.


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