STUDIES ON

BLOOD PLATELETS IN URAEMIA

by

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VOLUME I.

PREFACE.

The work presented in this thesis was carried out while on leave of absence from U.A.R. government service in the University Department of Medicine, Royal Infirmary, Glasgow, under the guidance of Professor E. M. McGirr, Professor A. S. Douglas and Dr G. P. McNicol.

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ABBREVIATIONS.

ADP	-	Adenosine diphosphate
AMP	-	Adenosine monophosphate
ATP	-	Adenosine triphosphate
PCV	-	Packed cell volume
Platelet count	-	Platelet rich plasma platelet count.

REFERENCES.

Where more than one reference has been used the references are given in alphabetical order.

CHAPTER I.

THE PLATELET

Historical Introduction

Although the credit for the first observation of a blood platelet is usually given to Alfred Donne (1842), the first drawings of platelets were made independently by two English practitioners, George Gulliver (1841) and William Addison (1840, 1841).

In 1842, Donne stated that there are three elements in the blood; red cells, white cells and globules of chyle. Franz Simon in 1842 treated anticoagulated blood with potassium ferrocyanide and saw numerous small bodies, almost certainly platelets, which he thought were the early stages of erythrocytes. Arnold (1845) was the first professional anatomist to recognize and illustrate platelets which he called elementary grains. Zimmermann (1846) repeated and confirmed Simon's work. He observed that if blood is rendered incoagulable it contained numerous small colourless bodies 2 to 4 μ in diameter, which were refractile and had a well defined contour. He called them "elementary bodies".

Wharton Jones (1850) was the first to describe stages of thrombosis, using frogs as experimental animals. He examined the web of a frog's leg and observed that arteries became blocked by a mass composed apparently of colourless corpuscles and fibrin. In 1864, there appeared the first clear drawing of a platelet (Beale). Schultze in 1865 using a warm stage, observed the coalescence of platelets in "granular masses". This work was further extended by Osler (1874) who gave a detailed account of individual platelets, of Schulte's granular masses, and the changes that take place in the peripheral portion of platelets. He observed that these peripheral parts become glassy, throw out pseudo-pods and are associated with fibrin formation. Unfortunately, Osler thought that these bodies were peculiar types of bacteria.

Ranvier, in 1873, gave a paper on the formation of fibrin in which he referred to its relationship to granules which he believed were chemically identical to fibrin. Vulpian (1873) commenting on this paper said that he found these corpuscles in normal blood and that they adhered to glass. Hayem (1877), calling the platelet a haemoblast, thought that it was an early stage in the development of the erythrocyte. He was able to count platelets; he studied their morphology and the part they played in coagulation and clot retraction.

Bizzozero (1881) described the presence of free platelets in omental vessels; he damaged the vessel wall and saw that the first effect was the adhesion of platelets to the point of injury. He also showed that it was platelets, not leucocytes as had previously been believed, that formed the first stage of a thrombus, and demonstrated their relationship to coagulation.

In 1885 Schimmelbusch described the morphological changes that occur when platelets come in contact with a foreign surface or a damaged vessel. Enhanced stickiness of platelets resulted and caused individual platelets to adhere to one another as well as to foreign surfaces. He believed that these changes occurred independent of, and before fibrin formation. Eberth and Schimmelbusch (1886) repeated and extended the work of Bizzozero and introduced the term "viscous metamorphosis" to describe the morphological and physical changes that enabled platelets to stick to foreign surfaces and to one another. Later Wright and Minot (1917) used the term viscous metamorphosis to describe the agglutination and fusion of platelets into "glassy masses" and strands, a phenomenon which they distinguished from simple agglutination without fusion.

The confusion as regards the origin of platelets was settled in

1906 by Wright who found the true origin of platelets from the megakaryocyte.

Platelet Ultrastructure.

The use of the electron microscope and the discovery of a new group of aldehyde fixatives have provided valuable information about the ultrastructure of blood platelets. The electron microscope was first applied to the study of platelets by Wolpers and Ruska (1939). Since then several studies have been made on blood platelets employing a spreading technique which utilizes the ability of the platelet to spread when in contact with a solid surface. The introduction of the ultrathin sectioning technique by which sections with a thickness of about 500Å can be examined, and the use of suitable embedding media, allowed for better use of the electron microscope.

The problem of fixing platelets without the use of anti-coagulants and centrifugation was solved by the introduction of glutaraldehyde (Sabatini et al., 1963) which permits rapid fixation by drawing the blood directly into the fixative. Aldehyde fixation is of special value in the localization of specific chemical components and enzymatic actions. Adenosine triphosphatase and acid phosphatase retain their activity in aldehyde-fixed tissue and may be localized in this way.

Electron microscopy is also used with immunochemical staining where sufficient electron density must be associated with the antibody molecule. Autoradiography is another method adapted for the electron microscope in the study of the platelet ultrastructure in which the position of the radioactive material incorporated into the specimen can be observed. Negative staining of the specimens is

used when very high resolution is required as in studies of isolated subcellular components. The principle of negative staining is to surround the particles with an electron dense material, thus making them visible.

All the above methods have been used in electron microscopic studies of blood platelets or their subcellular components and contributed to better understanding of the platelet morphology and function.

Platelets which have been rapidly fixed in glutaraldehyde and examined in ultrathin sections usually have an elliptical, rod like or disc shape. The longitudinal diameter of the disc form is usually between 1.5 to 4μ and the transversal diameter is within the range of 0.5 to 2μ. The blood platelet is formed of a cytoplasmic matrix which is surrounded by a membrane and contains various organnlles. These include mitochondria, granules, vacuoles, Gogli complex, siderosomes, microtubules, ribosomes, glycogen and lipid inclusions.

Electron microscopic studies have shown that the platelet is surrounded by a well defined membrane similar in structure to other cells. When potassium permanganate is used as a fixative the triple layered structure of the unit membrane can be easily demonstrated (Robertson, 1960). The unit membrane is commonly thought to be formed of a double layer of lipids and phospholipids covered on both sides by layers of proteins (Stoeckenius, 1962). The phospholipid fraction of the platelet membrane (platelet factor 3) becomes exposed on the platelet surface, and thereby capable of reacting with other clotting factors in the plasma (Marcus et al., 1966).

In ultrathin sections a fluffy coat can be seen outside the platelet membrane, which appears as amorphous substance or as short threads extending radially from the membrane. This extracellular

coat is thought by Marcus and Zucker (1965) to be an inherent part of the membrane structure, while Sixma and Molenaar (1967) regard it as adsorbed plasma proteins. It is possible that this surface coat corresponds to what Roskam (1923) described as the "plasmatic atmosphere" of the platelet. This coat is likely to be composed of mucopoly-sacchasides, proteins and muco-proteins and to contain many, if not all, the plasma coagulation factors. It has been stated by Adelson et al (1961) that platelets adsorb and concentrate coagulation and other factors and transport them in the circulation, the ability of the platelet to act as a sponge being one of its basic functions. The plasma coagulation factors stated by various authors to be present in the plasmatic atmosphere include fibrinogen, prothrombin, factors V, VII, VIII, IX, X, surface factors (XI, XII) and factor XIII. Some of these factors are tightly bound to the platelet whilst others can easily be removed by washing. Prothrombin, factors VII, IX and X are loosely bound and may easily be eluted (Bounameaux, 1957a). Fibrinogen, factors V, VIII and XIII as well as surface factors are more tightly bound and resist repeated washing (Iatridis and Ferguson, 1965). Electron microscopic studies have shown that during ADP-induced platelet aggregation, the fluffy coat appears to form bridges in the zones of contact between platelets (Hovig, 1968).

Enzymatic reactions may also take place on the platelet surface. Adenosine triphosphatase activity has been demonstrated by White and Krivit (1965) on the surface membrane; however, this was not confirmed by Behnke (1967) or Vethamany and Lazarus (1967).

Blood platelets contain from 1 to 6 mitochondria, whose function is presumably similar to that in other cells being the principle site of oxidative reactions. These reactions are mediated by adenosine triphosphate (ATP). It is of interest to note that platelets contain more ATP and fewer mitochondria than other cells indicating that the

ATP may be involved in processes besides cell metabolism.

There are between 2 and 20 granules in the human blood platelet. Although the nature of these granules is not quite clear, granules are supposed to contain lipid (platelet factor 3), lysosomal enzymes, fibrinogen and nucleotides.

A variable number of vacuoles and vesicles of different sizes and shapes can be seen in sections of blood platelets. Platelets fixed in glutaraldehyde and post-fixed in osmium tetroxide show cavities surrounded by a membrane, which appears to be continuous with the surface membrane.

Structures similar to that of the Golgi complex can sometimes be found in normal platelets. These structures probably originate from the megakaryocytes and seem to have no important function in platelets.

A bundle of tubular filaments can be observed along the inner circumference of the platelets when glutaraldehyde is used as a fixative. In saggital sections of platelets the marginal band of tubules appears at the poles of the disc-shaped cells as loosely associated groups of small circles. Although the function of the microtubules is still unknown, Behnke (1965) suggests that the tubules may play a role in maintaining the discoid shape of platelets. The microtubules may also have a role in the formation of pseudopod-like projections of the cytoplasm (Silver, 1966). It is possible that these microtubules may represent a retractile element of the platelet cytoplasm.

Glycogen granules occurring singly or in large aggregates have been demonstrated in platelets. Lipid inclusions may occasionally be found in normal human platelets suggesting that platelets phagocytose and transport lipids.

The presence of ribosomes in platelets has not been proved conclusively. Recent evidence suggests that platelets are able to synthesize proteins (Booyse and Rafelson, 1967), an activity known to

take place in ribosomes.

THE FUNCTIONS OF PLATELETS

Platelets and Haemostasis

Recent advances in the fields of microphotography and cinemicrophotography (Kwaan et al., 1967) have been applied to the study of platelet behaviour in small blood vessels in vivo. These techniques have been also applied to the study of the arrest of bleeding from transected small blood vessels and have established that bleeding is stopped by the formation of a platelet mass which occludes the gap in the vessel wall (Zucker, 1947; Jorgansen and Borchgrevink, 1963; Hovig et al., 1967). Platelets interact with the collagen in the connective tissue as one of the first steps in the formation of the haemostatic plug (Hovig et al., 1967). This results in the release of ADP from platelets (Zucker and Borrelli, 1962; Hovig, 1963; Spaet and Zucker, 1964) which causes the platelets to adhere to each other (Gaarder et al., 1961; Born, 1962b).

According to the experiments made by Bounameaux (1961), Zucker and Borrelli (1962); Hugues and Lapiere (1964), Spaet and Erichson (1965) and to the observation in vivo by Hugues (1962), at the site of injury to a blood vessel, platelets adhere in preference if not exclusively to collagen. Platelets in contact with collagen show structural changes which include swelling and loss of platelet constituents (Hovig, 1963; Hovig et al., 1967). Adherence of platelets to collagen does not require the presence of divalent cations (Spaet and Zucker, 1964) and is independent of the blood clotting mechanism. However divalent cations are essential for collagen-induced release of platelet constituents (Hovig, 1964). Together with the release of platelet nucleotides including ADP, collagen also induces the release of serotonin and histamine which influence vessel permeability and cause contraction of

smooth muscle (Packham et al., 1968). It is presumed that lysosomal enzymes are also released at the site of injury since the platelet granules which contain them disappear during this process.

ADP is probably supplied by both the injured cells in the area and by the platelets. It is doubtful whether red cells, which are rich in nucleotides, make a substantial contribution to extracellular ADP; detailed studies of the haemostatic plugs have not shown extensive accumulation of red cells in the platelet mass (Zucker, 1947; Jorgenson and Borchgrevink, 1963; Hovig et al., 1967). Red cells are shown to be trapped in the fibrin around the periphery of haemostatic plugs, but their position suggests that they are not involved in the early events of plug formation.

ADP has been found to be the most potent compound for inducing platelet aggregation but other compounds like uridine diphosphate, cytidine diphosphate, inosine diphosphate and guanosine diphosphate can also cause platelet aggregation (Spaet and Zucker, 1964; Salzman, 1967). Adenosine triphosphate has been found by some workers to cause platelet aggregation (Mustard et al., 1964b; Mitchell and Sharp, 1964). However, O'Brien, (1964) reported that fresh solutions of ATP have little or no activity.

ADP can also cause platelet aggregation in vivo. ADP infusions cause intravascular platelet aggregation which is associated with a fall in the platelet count. In most instances ADP-induced platelet aggregation appears to be reversible both in vitro and in vivo. It has been shown that adenosine inhibits ADP-induced platelet aggregation in vivo (Born and Cross, 1963a) and probably acts by preventing the accumulation of platelets at the site of injury by inhibiting the action of ADP released from damaged tissues or from the platelets.

The platelet mass formed in vivo in experimental situations is usually unstable. Stabilization of the platelet masses in vivo requires

the action of thrombin; it has been demonstrated that when coagulation is inhibited, the platelet masses formed in the microcirculation are unstable (Fulton et al., 1953; Berman, 1961). Thrombin will cause platelets to adhere to each other probably by inducing the release of platelet ADP (Haslam, 1964; Mustard et al., 1967b). Thrombin also catalyses the formation of fibrin which stabilizes the platelet mass by binding the platelets together. It is probably the formation of fibrin which is the main factor in stabilizing the platelet plug because if fibrin is lysed, platelet aggregates break up (Hirsh et al., 1968).

The sequence of steps by which thrombin is generated in vivo has not yet been fully elucidated, but there is evidence to suggest that collagen may activate Factor XII thus activating the intrinsic coagulation pathway (Niewiarowski et al., 1965). Tissue thromboplastin released at the site of injury may be responsible for initiating coagulation through the extrinsic pathway (Biggs and MacFarlane, 1962). The release of platelet factor 3 through platelet aggregation by ADP may also have a relationship to thrombin formation (Mustard et al., 1964b; Castaldi et al., 1965; Hardisty and Hutton, 1966b). Associated with the platelet membrane are some plasma coagulation factors as Factor V and Factor VIII which may be involved in the coagulation process.

Finally, to become an effective haemostatic plug, the fibrin around the platelet mass may undergo retraction; as in a blood clot tightening of the peripheral fibrin by concentration of the contractile protein of platelets may help to stabilize the platelet mass (Bettex-Galland and Luscher, 1959; 1965). Figure 1 summarizes the functions of platelets in haemostasis and figure 2 illustrates the mechanism of haemostasis schematically.

PLATELETS AND THROMBOSIS.

The early work of Bizzozero (1882), Eberth and Schimmelbusch (1885) and Welch (1887) has established much knowledge about the morphology and structure of a thrombus.

A thrombus consists of a head or white thrombus which is attached to the vessel wall, and a tail or red thrombus formed from coagulated blood (figure 3). Histological sections of in vivo thrombi, experimental in vivo thrombi (Rowntree and Shionoya, 1927; Shionoya, 1927; Best et al., 1938; Mason and Harrison, 1949) and experimental in vitro thrombi (Chandler, 1958; Poole, 1959; and 1960) have shown that the white head of a thrombus is formed almost exclusively of tightly packed platelets (figure 4). Flow is critical for the development of such structures. These can develop on arteriosclerotic plaques in arteries, and at sites of injury in patent veins.

Thrombosis may however occur in the absence of flow as in lower limb veins filled with stagnant blood, or in eddying pools of blood in the cardiac chambers. In this situation, the thrombus closely resembles a blood clot, being a homogenous structure of red cells, leucocytes and platelets enmeshed in a fibrin network.

There is considerable evidence to suggest that adherence of platelets to the injured vessel wall and to each other is a fundamental step in the formation of thrombi from flowing blood (Berman and Fulton, 1961; Mustard et al., 1962). The initial adherence of platelets to the subendothelial tissue appears to be independent of blood coagulation (Jorgensen and Borchgrevink, 1964; Mustard et al., 1964a) and may be initiated by the release of ADP from injured tissue (Honour and Mitchell, 1963; Born et al., 1964). In regions of disturbed blood

flow and vortex formation, the formed elements of blood may collide with one another and with the vessel wall. Such collisions may cause cellular injury leading to the release of ADP. On the other hand it seems possible that accumulation of platelets and leucocytes on the vessel surface may induce changes in the underlying endothelium. Both leucocytes and platelets contain lysosomal enzymes which when released during degranulation can cause tissue injury (Mustard et al., 1967a). Moreover focal injury of the vessel wall may cause the accumulation of formed elements with exaggeration of the effect of injury, particularly increased permeability of the endothelium (Mustard et al., 1967a).

ADP cannot maintain the platelet aggregate because it is dephosphorylated to AMP by an enzyme in the plasma (Ireland and Mills, 1966; Packham et al., 1967). The most important factor in stabilizing the platelet aggregate is the formation of fibrin around it. Thrombin converts some platelet ATP to ADP which escapes into the ambient fluid causing further platelet aggregation (Kaiser-Glanzmann and Luscher, 1962; Grette, 1962). It also induces serotonin release (Zucker and Borrelli, 1955; Grette, 1962), and renders platelet phospholipid available for the formation of large amounts of thrombin from the intrinsic coagulation mechanism (Kaiser-Glanzmann and Luscher, 1962; Schmid et al., 1962). When the rate of thrombin formation is greater than the effect of dilution by flowing blood and neutralization by antithrombin, fibrin is formed in the area surrounding the platelet aggregate. Subsequently thrombin on the surface of the thrombus is rapidly depleted through its absorption by fibrin (Seegers et al., 1945), neutralization of blood antithrombin (Monkhouse et al., 1955), and dilution in the blood stream.

If the blood flow is slowed or arrested distal to the initial thrombus, thrombin concentration will not diminish rapidly. This will result in extension of the fibrin formation giving the thrombus the appearance of a white head and a red tail.

CHAPTER 2.

GENERAL INTRODUCTION ON PLATELETS IN URAEMIA.

A generalized haemorrhagic state in uraemia has long been recognized. According to Riesman (1907), Morgagni early in the eighteenth century described a woman in whom "though she already had the odour of urine in her breath, vomiting of blood and bleeding from the nose proved beneficial". A bleeding tendency in chronic renal failure has been described by Riesman (1907), Davidson (1937), Loeper and Lebret (1940), and more recently in acute uraemia by Swann and Merrill (1953), Meroney and Herndon (1954), Donner and Neuwirtova (1960) and Kendall et al., (1961). Although gastro-intestinal bleeding has been most frequently described, other mucosal surfaces and the skin may also be involved. At times, serious or fatal haemorrhage has occurred into vital areas such as the mediastinum (Brown et al., 1953) or pericardial space (Guild et al., 1957). Needle biopsy of the liver and kidney in a uraemic patient has led to massive and fatal bleeding (Zelman, 1954).

Despite the clinical appreciation of the occurrence of a haemorrhagic state, the haemostatic mechanism in uraemia received scant attention until recently. Earlier workers attempted to explain this haemorrhagic tendency in uraemia on the basis of a vascular defect (Gualdi, 1935). Riesman postulated "a circulating substance in uraemic plasma that weakened the capillary wall", wheras Jaffe and Laing (1934) described a "toxic effect on the capillaries". They noted marked dilatation of the capillaries and venules in the walls of the gastrointestinal tract in association with narrowed arterioles.

The Bleeding Time and Capillary Fragility Tests.

The bleeding time and capillary fragility tests were two of the first tests employed in an attempt to detect and possibly to explain the haemostatic defect.

In Fishberg's test (1954) it was reported that the tourniquet test was usually positive and it was suggested that decreased capillary resistance was the immediate mechanism of bleeding. A similar observation was also made by Kuhlbäch (1957) in a study of 30 patients with chronic renal failure; of a number of coagulation tests performed the Rumpel-Leede capillary fragility test was the most consistently abnormal.

Meroney and Herndon (1954) reported purpura in post-traumatic renal insufficiency resulting from battlefield casualties. Bleeding into the skin, nasopharynx and intestine appeared after 12 to 15 days and was related only to the duration, not the severity of the uraemia. Unlike other manifestations, the haemorrhagic tendency was entirely unaffected by haemodialysis or by diuresis; on the contrary, the most severe bleeding occurred several days after the onset of diuresis. A search for the cause of the bleeding revealed increased capillary fragility but no intrinsic clotting defect; the condition was unrelated to abnormalities of plasma electrolytes and was unaffected by the administration of Vitamin K, Vitamin C or fresh blood transfusion.

In a more detailed investigation by Rath et al., (1957) of a mixed group of 45 uraemic patients "capillary fragility was the least commonly seen abnormality". Merrill (1955) reported that the bleeding time and tourniquet test are seldom abnormal in patients with renal failure and purpura.

The bleeding time was prolonged in 2 of 12 patients with chronic uraemia studied by Lewis et al., (1956) and apparently in none of

Kuhlbäch's (1957) 30 chronic cases.

However, the bleeding time was prolonged in all 3 cases of acute renal failure studied by Lazrain and Adelson (1956). Rath et al., (1957) also reported that the bleeding time was prolonged in 4 out of 5 cases of acute renal failure due to acute glomerulonephritis, only 3 out of 21 of the other acute cases and only 2 of 15 chronic uraemic patients showed prolongation of the bleeding time.

In Kendall's (1961) group of acute uraemia the capillary fragility was abnormal in only 6 of 21 cases tested. In these 6 patients there was prolonged bleeding time, and thrombocytopenia; in 5 of these the platelet count was below 30,000/c.mm. Kendall et al., (1961) found that a prolonged bleeding time was a common finding, and was seen at some time in 27 of his 46 cases. Bleeding occurred in 24 of the 27 and it therefore appears that a prolonged bleeding time is a useful indication of likelihood of bleeding. In only 4 of these 27 cases was the platelet count greater than 100,000/c.mm. and 3 of the 4 showed an impaired prothrombin consumption (Kendall et al., 1961).

Donner and Neuwirtova (1960) found the bleeding time to be prolonged in only 4 out of 31 patients with acute uraemia and 3 out of 23 with chronic uraemia.

Willoughby and Crouch (1961) studied the Ivy bleeding time and the rate of blood loss from skin punctures in this test; and found both to be increased in many of the renal patients with a clinically manifested problem of haemostasis. However the capillary fragility test of Hess was seldom abnormal in the same group.

Cheney and Donnin (1962) found the bleeding time to be prolonged in only 4 out of 33 cases of uraemia and only 4 were associated with severe haemorrhagic manifestations. However 11 other patients also showed haemorrhagic signs, sometimes of equal severity, yet had a normal

bleeding time. The results of the tourniquet test did not however correlate well with the clinical manifestations of bleeding nor with the levels of platelet thromboplastic function.

Salzman and Neri (1966) determined the Ivy bleeding time on 29 occasions in 24 patients and found this to be prolonged in 13. Patients with a long bleeding time invariably had deficient platelet adhesiveness.

Castaldi et al., (1966) found abnormal capillary fragility in only 3 patients out of 19 of whom 2 had severe bleeding and one had no bleeding. None of the 3 had thrombocytopenia. A prolonged Duke's bleeding time was found only in one patient who had a severe bleeding tendency. In contrast, all patients with evident bleeding had a significantly prolonged Ivy bleeding time but only one of the non-bleeders had a prolonged bleeding time.

Stewart and Castaldi (1967) considered the Ivy bleeding time to be one of the most sensitive measures of impaired platelet functions and this was found to be abnormal in nearly all predialysis tests and was frequently corrected by one or more dialysis treatments.

The Platelet Count.

Marked thrombocytopenia has been found to be an unusual finding in chronic uraemia although a slight reduction in platelet number is not uncommon (Favre-Gilly and Durand, 1950; Kuhlbäch, 1957; Rath et al., 1957; O'Grady, 1959). In acute renal failure thrombocytopenia may become more striking especially when other toxic factors, as hepatocellular jaundice, septicaemia or severe infection are present. Rath et al., (1957) found platelet counts under 150,000/c.mm. in 17 of 26 acute cases including all 5 of their cases with acute

glomerulonephritis. Larrain and Adelson (1956) noted mild to moderate thrombocytopenia at some time in their 3 acute cases.

Wurzel (1960) found reduced platelet counts in 16 of 43 patients with acute uraemia. Kendall et al., (1961) found thrombocytopenia in many cases of acute uraemia; factors other than the uraemic process, such as septicaemia, most probably contributed to this. Castaldi et al., (1966) found thrombocytopenia to be present in only 2 patients with chronic renal failure both of whom had evident bleeding and prolonged Ivy bleeding time. All other patients however had platelet counts in the normal range even when there was severe bleeding.

Stewart (1967) estimated the platelet count in 225 patients with severe renal failure. Thrombocytopenia occurred in one quarter of all patients with renal tubular necrosis, acute or sub-acute glomerulonephritis or malignant hypertension, but only in one twelfth of those with severe uraemia due to chronic renal failure. Recovery of platelet numbers followed partial relief of uraemia by dialysis or return of renal function in 24 patients.

Whole Blood Clotting Time.

The whole blood clotting time in glass tubes was stated to be prolonged (Allen, 1951). Donner and Neuwirtova (1960) found the clotting time to be prolonged in 10 out of 46 cases of acute uraemia and 4 out of 26 cases of chronic uraemia. Willoughby and Crouch (1961) reported the clotting time to be normal except in 2 cases of acute uraemia. However Larrain and Adelson (1956) showed a prolongation of the clotting time in silicone tubes in their 3 patients, and considered this to be one of the earliest changes in acute experimental canine uraemia. Donner and Neuwirtova (1960) reported the silicone clotting time to be prolonged in 8 of 43 acute uraemic patients and in 4 out of 31 patients with chronic renal failure.

One-Stage Prothrombin Time

The one-stage prothrombin time has shown no consistent prolongation in uraemic patients and when present such prolongation has rarely been sufficient to be a likely cause of bleeding. Lewis et al (1956) reported one-stage prothrombin times ranging from high normal to very low levels; this usually reflected abnormalities in prothrombin, factor V and factor VII. Similar results were found by Donner and Neuwirtova (1960). Factor V was deficient in 16 out of 43 patients with acute uraemia and in 6 out of 18 patients with chronic renal failure. Factor VII was diminished in 29 out of 51 patients with acute uraemia and in 7 out of 21 patients with chronic renal failure. However, Willoughby and Crough (1961) reported normal one-stage prothrombin times in all of their 56 uraemic patients except two; the one-stage prothrombin time in these patients was promptly corrected to normal by vitamin K administration. A slight or moderate prolongation of the one-stage prothrombin time was detected in almost every uraemic patient at or near the peak of azotaemia (Kendall et al., 1961). They found that the abnormalities in the one-stage prothrombin time did not correlate with microscopic hepatic pathology in 29 cases examined at autopsy.

Cheney and Bonnin (1962) found a deficiency in factor VII which resulted in prolongation of the one-stage prothrombin time in 13 out of 33 uraemic patients. Only two of their patients showed marked prolongation which was attributed to factor X deficiency in one and to factor VII deficiency in the other patient.

Other plasma coagulation factors were investigated. Factor VIII levels were reported by Lewis et al (1956) to be normal; only two patients in their series showed reduced factor IX associated with factor VII and protheombin deficiency.

Fibrinogen

Plasma fibrinogen levels have been found by many workers to be elevated (Rath et al., 1957; Donner and Neuwirtova, 1960; Kendall et al., 1961 and McNicol et al., 1965). Castaldi et al., (1966) reported

factor XIII by a qualitative test to be normal.

Plasma Thrombin Clotting Time

The plasma thrombin clotting time was prolonged in 6 of the 12 cases of Lewis et al., (1956) and in 9 of the 14 cases investigated by Kendall et al., (1961). In several patients the addition of calcium with thrombin resulted in normalization of the thrombin time. No increased antithrombin activity could be detected in Kendall's cases.

Clot Retraction

Clot retraction was impaired in all uraemic patients with incidental bleeding and in many of those not bleeding (Castaldi et al., 1966). Clot retraction was restored to normal in the majority of uraemic patients after dialysis treatment (Stewart and Castaldi, 1967).

Prothrombin Consumption Test

The prothrombin consumption test has often been reported to be abnormal in patients with acute and chronic uraemia (Larrain and Adelson, 1956; Lewis et al., 1957; Rath et al., 1957; Cahalane et al., 1958; Geiger et al., 1959; O'Grady, 1959; Donner and Neuwirtova, 1960; Alschuler et al., 1960; Wurzel, 1960; Kendall et al., 1961; Willoughby and Crouch, 1961; Castaldi et al., 1966; Salzman and Neri, 1966; Stewart and Castaldi, 1967).

Cahalane et al., (1958) found abnormal prothrombin consumption in all 8 chronic patients studied by him, while Lewis et al., (1956) noted this defect in 7 out of 12 cases. Rath et al., (1957) found a similar abnormality in 38 out of 45 acute and chronic cases and made the same observation in 2 out of 3 acute cases.

Wurzel (1960) reported abnormal prothrombin consumption in a high percentage of patients with both acute and chronic uraemia.

Of particular interest was the finding of O'Grady (1959) that prothrombin consumption abnormality was present in 6 chronic uraemics with platelet counts greater than 150,000/c.mm.

In Altschuler's (1960) group when thrombocytopenia was present in some patients, prothrombin consumption was normal in some, but not in

others. On the other hand, defective prothrombin consumption was demonstrated in cases with normal platelet count. Willoughby and Crouch (1961) found that prothrombin consumption index was the only test of blood coagulation to be abnormal, i.e. over 15%, in a significant number of patients with a clinical haemorrhagic state.

Kendall et al., (1961) investigating cases of acute renal failure found the prothrombin consumption index to be abnormal in 27 out of 34 cases at the peak of azotemia although the abnormality was slight. Platelet counts carried out at the same time were found to be below 150,000/c.mm. in 22 of these patients, but in several cases where the platelet counts were below 100,000/c.mm. the prothrombin consumption index appeared to be normal.

These results suggest a poor correlation between the platelet count and the prothrombin consumption index.

Salzman and Neri (1966) examined 16 patients using the technique of Merskey found the prothrombin consumption index to be abnormal in 13 patients all of whom had a platelet count above 145,000/c.mm. They also found that patients with abnormal platelet adhesiveness tended to have a poor prothrombin consumption index but the correlation was not perfect. Abnormal prothrombin consumption was found in 2 patients with normal platelet adhesiveness and subnormal platelet adhesiveness was seen in 3 patients with normal prothrombin consumption, and in 4 borderline values were obtained.

Castaldi et al., (1966) found the test to be normal in the great majority of patients with no evidence of bleeding.

Stewart and Castaldi (1967) found the prothrombin consumption index to be more frequently abnormal before dialysis and also more readily corrected by treatment than other abnormalities of platelet function.

Platelet Thromboplastic Factor 3 Activity.

Recently more attention has been focussed on the qualitative changes in the uraemic platelets. Lewis et al., (1956) reported reduced platelet thromboplastic factor 3 activity in 8 and reduced platelet accelerator fector I activity in 7 of 12 uraemics (10 chronic and 2 acute) 9 of whom had normal platelet counts.

Subsequently Cahalane et al., (1958) in their studies of 8 patients with chronic uraemia and normal platelet counts found reduced platelet thromboplastic activity in a modified thromboplastin generation test and this was confirmed by the discovery of marked reduction in their platelet factor 3 assay.

Cheney and Bonnin (1962) using the platelet thromboplastic function test, showed reduced levels of platelet thromboplastic function in the majority of the uraemic patients examined by them. Recently, platelet factor 3 availability has been tested using intact platelet rich plasma. Activation of platelet factor 3 was achieved by addition of kaolin (Hardisty and Hutton, 1965; Spaet and Cintron 1965), or by ADP (Horowitz et al., 1967). Castaldi et al., (1966) found reduced platelet factor 3 availability in 56 per cent of uraemic patients with bleeding while the majority of patients with no bleeding showed normal results. The effect of dialysis on platelet factor 3 availability was shown by Stewart and Castaldi (1967). Using the method of Hardisty and Hutton (1965) platelet factor 3 availability was corrected to normal in 3 out of 6 occasions.

Horowitz et al., (1967) used ADP to activate platelet factor 3 and found that 16 out of 17 uraemic patients showed prolonged "Stypven times".

The most detailed studies of the haemostatic mechanism have been carried out in chronic uraemia. Only recently has acute uraemia come under investigation. This has been possible through the prolongation

of patients' lives by improved medical management and the introduction of extracorporal haemodialysis. Because of the rapidly changing clinical state and the effect of the uraemic process and other factors such as the administration of heparin, adrenal and anabolic steroids and blood transfusion, the acute cases require serial studies. Furthermore, the underlying disease process causing the acute renal failure may have widespread systemic effects which may contribute to the observed abnormalities of haemostatic function tests.

Therefore, although platelet count seems to be more reduced in acute than in chronic uraemia, this is not, however, a constant finding.

Rath et al., (1957) suggested that disturbances of the haemostatic mechanism may differ in acute and chronic renal failure, and indicated that the nature of renal disease may influence the type of the coagulation abnormality found. There seems to be a greater likelihood for bleeding to occur in severe uraemia of acute onset than in chronic. Thus Teschan et al., (1955) reported bleeding in 27 of 55 cases of post-traumatic renal insufficiency, and Rath et al., (1957) in their study of 45 civilian cases noted a higher incidence of clinical bleeding in those with acute renal failure than in chronic.

Wurzel (1960) applied screening procedures of the coagulation mechanism in his study of a considerable number of acute and chronic uraemics; he compared "bleeders" with "non-bleeders". Impaired thromboplastin generation time was noted in a high percentage of the former, but no appreciable difference in the test results were noted between acute and chronic cases. However, no mention was made of the stage of acute renal failure nor on the duration of azotemia at the time the studies were undertaken.

Thromboelastographic Patterns.

Donner and Neuwirtova (1960) described the thromboelastographic

patterns in both acute and chronic uraemia. In several cases, uraemia was suspected from thromboelastographic changes even before being ascertained by other laboratory methods. Although similar changes were found in other diseases such as chronic lymphatic leukaemia, thrombocythaemia, bronchopneumonia and post-splenectomy, these changes were never so marked as in uraemia. The patterns described were either narrowing of a bottle-neck or gradual joining or an onion-like pattern. They suggested that platelets take part in the development of these changes.

Platelet Adhesiveness.

The earliest report on platelet adhesiveness in uraemia was described by Donner and Neuvirtova (1960). Using Bounameaux's method (1957a) of examining platelet adhesiveness to yeast cells and Jurgen's platelet adhesion after rotation in a glass rotator (1959); they found platelet adhesiveness to be diminished in some cases of acute and chronic uraemia.

Hellem et al., (1964) using his own technique, found platelet adhesiveness in platelet-rich plasma induced by ADP to be diminished in uraemia.

Salzman and Neri (1966) found in vitro platelet adhesiveness by his own technique to be diminished in 21 of 24 patients with chronic renal failure; the abnormality was strongly correlated with prolongation of Ivy bleeding time, abnormal prothrombin consumption and with clinical bleeding. In vivo platelet adhesiveness (Borchgrevink method) was found by Castaldi et al., (1966) to be diminished below the lower limit of normal in 5 patients with prolonged Ivy bleeding time but was normal in patients without bleeding tendency. Stewart and Castaldi (1967) studied the effect of dialysis on impaired in vivo platelet adhesiveness; 7 patients were restored to normal and one showed an improvement. O'Brien (1967) found that in a few patients with uraemia the glass bead column method showed abnormally low adhesiveness, while with Wright's method (1941) results

were all within the normal range.

Bayer et al., (1966) investigated 25 patients with azotemia due to primary chronic renal disease. In three patients with severe bleeding, platelet adhesiveness was very low. In the remaining patients with minor or no bleeding about one third showed abnormalities which usually correlated with platelet thromboplastic factor. Abnormal platelet adhesiveness and platelet thromboplastic factor were more frequently found in patients with blood urea elevated above 100 mg. per 100 ml.

Praga and Cortellaro (1967) found no correlation between serum urea levels and the dimunition in platelet adhesiveness by the rotating bulb method. However they found an inverse relationship between serum creatinine and platelet adhesiveness in patients with chronic uraemia. Pitney et al., (1968) measured the adenine nucleolides in platelets from normal and uraemic subjects before and after passage of blood through glass bead filters. They found uraemic platelets to contain normal amounts of ATP, ADP, AMP and total nucleotides. Normal platelets which had passed through filters contained higher concentration of ATP, ADP and total nucleolides than platelets in control blood sample, indicating that either heterogenity of adenine and nucleolide content in normal platelets or adsorption of nucleotides onto platelets during their passage through the filters. This phenomenon was not observed with platelets from uraemic patients.

ADP-induced Platelet Aggregation.

ADP-induced platelet aggregation in uraemia was recently studied by Castaldi et al., 1966; Salzman and Neri, 1966; Rozenberg and Firkin, 1966; Stewart and Castaldi, 1967; O'Brien, 1967; Gan and Firkin, 1968.

Castaldi et al., (1966) found platelet ADP-induced platelet aggregation to be normal in 7 patients without bleeding manifestations

and impaired in 5 of the same group, but every patient with an established bleeding tendency and long bleeding time had grossly depressed or absent platelet aggregation with ADP, whatever their serum urea concentration. There was also partial correction of impaired platelet aggregation in 2 patients with active bleeding after dialysis treatment.

Salzman and Neri, (1966) on the other hand found ADP platelet aggregation to be normal, but thrombin induced platelet aggregation was diminished in uraemia. They also found a significant diminution in ATP content of uraemic platelets.

Rozenberg and Firkin,(1966) found impaired platelet aggregation in 3 out of 5 cases of uraemia with blood urea about 150 mg. per 100 ml. They found a good correlation between prolonged bleeding times (Duke), impaired aggregation and reduction of platelet masses in the viscometer. The plasma of the 5 uraemic patients did not reduce the rate of aggregation of normal platelets and correlation between the level of urea and platelet function was not present. The ADP-induced platelet aggregation was found by O'Brien (1967) to proceed at a normal rate but the time till disaggregation was rather prolonged.

Stewart and Castaldi (1967) demonstrated the beneficial effect of dialysis on ADP-induced platelet aggregation; 4 patients were restored to normal, one improved and two showed no change.

Gan and Firkin, (1968) showed that ADP-induced platelet aggregation was diminished in six uraemic patients with a bleeding diathesis and a prolonged bleeding time, while it was normal in two patients with no haemostatic defect.

Serotonin.

Platelet serotonin was found by Lewis et al., (1956) and Weiner

and Udenfriend (1957) to be reduced in severe anaemia. Lewis et al., (1956) found markedly reduced serum serotinin levels in 9 out of 12 uraemic patients.

Platelet Life Span

The life span of ^{51}chromium labelled autologous platelets was found by Castaldi et al., (1966) to be normal in 7 uraemic patients. Only two of those patients suffered from haemorrhagic manifestations. When isologous platelets from ABO rhesus compatible donors were used, they found a shortended survival in 4 out of 5 uraemic patients.

Similar results were reported by Stewart (1967). The life span of autologous platelets, or of compatible normal homologous platelets given to subjects who had never received blood transfusion was normal in severe renal failure. There was no significant difference between patients with various types or different severities of renal disease. The survival of homologous platelets was slightly or moderately reduced when given to patients who had received a previous transfusion.

A variety of in vitro studies have been carried out to determine the effect of substances known to be raised in the blood or uraemic patients, on the activity of blood coagulation factors on the functions of platelets.

The studies of platelet factor 3 by Cahelane et al., (1958) suggested that uraemic plasma may induce a defect in normal platelet fragments after an incubation period of 18 hours.

Bonner and Neuwirtova (1960) found that the addition in vitro of urea, phenol, creatinine and guanidine did not definitely influence the activity of the principal coagulation factors.

Hellem et al., (1964) suggested that urea impaired ADP-induced platelet adhesiveness and thus interfered with platelet function.

On the other hand Salzman and Nori (1966) found that the

incubation of platelet-rich plasma with urea (200-400 mg. per 100 ml.) in vitro led to platelet aggregation and the subsequent addition of ADP led to further aggregation which was limited only by the extent to which the platelets had already been clumped by urea. Furthermore the addition of normal plasma to uraemic platelets did not improve the response to thrombin, and the addition of uraemic plasma to normal platelets did not impair the response to thrombin.

Fantl (1966) reported on the effect of urea on ADP-induced platelet aggregation. He found that although at very high urea concentration, above a level ever encountered in vivo (1000 m-osmole), urea inhibited ADP-induced aggregation, no effect on ADP. Reactivity was produced by urea concentration at the upper limit of that encountered in vivo in uraemia (440 m-osmole).

Castaldi et al., (1966) found that acute elevation of serum urea to about 200 mg. per 100 ml. by means of intravenous infusion of urea to two normal subjects, failed to cause any detectable abnormality to platelet function. Stewart and Castaldi (1967) failed to produce a qualitative platelet defect as measured by platelet aggregation, factor 3 availability, and clot retraction by in vitro addition of urea, dextrose, mannitol, creatinine, ureate, phosphate, potassium or magnesium or by changes of pH or osmolality within the range which might be encountered in severely uraemic patients.

Jerushalmy et al., (1966) reported 1-4 diguanidino diphenyl sulphone caused inhibition of ADP-induced platelet aggregation. An analogous compound, guanidinosuccinic acid was found by Horowitz et al., (1967) to be a powerful inhibitor of ADP-induced platelet factor 3 activation.

Praga and Cortellaro (1967) found that creatinine in vitro had a direct inhibitory effect on platelet adhesiveness to glass. Somer et al.

(1968) studied the effect of urea on platelet aggregation and found that urea initially inhibits, and later enhances aggregation induced by ADP, thrombin, adrenaline and non-adrenaline.

The present study was undertaken to determine the presence of a platelet defect in uraemia, evaluate the effect of haemodialysis on this defect, and to demonstrate by in vitro tests the effect of certain substances known to be elevated in uraemia on ADP-induced platelet aggregation.

CHAPTER 3.

MATERIALS AND METHODS.

In this chapter is given an account of the materials and methods used in the work for this thesis. A brief outline of the basis of each method and a discussion of some of the possible sources of error in the basic technique are given with each method. A short outline of the historical background to these techniques is also presented.

Standard methods were used for the assessment of platelet function. These include techniques for the estimation of the ability of platelets to aggregate in the presence of calcium ions and calcium plus ADP. Tests used were a Chandler tube system and the turbidimetric method. ADP-induced platelet aggregation and its reversal was studied by the turbidimetric method. The ability of platelets to adhere to glass surfaces was estimated by a modified Hellem's glass bead column method. Platelet factor 3 availability was determined by the method of Spaet and Cintron.

Experiments on the effect of factors influencing the results of some of the tests are presented.

Details of the method for collection and handling of blood used during the work for this thesis are also described.

Materials.

Adenosine-5-diphosphate was used in the form of the sodium salt as supplied by the Sigma Chemical Company, St Louis. This compound was prepared by dissolving the salt in a barbitone-buffered saline pH 7.2. A stock solution was stored at -20°C in a concentration of 10 µg per ml. in 0.3 ml. amounts. Just before use it was diluted

with the same buffer to give a final concentration of 0.25 µg per ml of plasma for ADP-induced platelet aggregation. In the Chandler tube technique and the turbidimetric modification it was used in a final concentration of 0.125 µg per ml.

Barbitone-buffered saline (pH 7.2) was made as follows:- 0.1M barbitone sodium, 57 ml; 0.1 N. hydrochloric acid, 43 ml; sodium chloride 0.56 gm; 0.9 per cent sodium chloride to 200 ml. It was kept at 4°C and prepared freshly every week.

M/4 calcium chloride and M/40 calcium chloride. A stock solution was prepared and used throughout the study. The concentration was checked by titration (Douglas, 1961).

Creatinine solution. Creatinine supplied by Sigma Chemical Company, St. Louis was dissolved in barbitone buffered saline to give a concentration of 0.2 gm per 100 ml.

Guanidinosuccinic acid solution. Guanidinosuccinic acid supplied by Mann Research Laboratories, New York, N.Y., was made up in saline buffered to pH 7.35 with one tengh volume of 0.05 molar imidazole to give a concentration of 0.386 gm per 100 ml.

Imidazole buffer, pH 7.2 . 1.72 gm. imidazole (Koch-Light Laboratories, Colnbrook, Bucks., England) was dissolved in approximately 90 ml 0.1 N hydrochloric acid; pH was adjusted to 7.2, diluted to 100 ml and stored at -20°C.

Imidazole buffered saline, pH 7.2. 0.85 per cent (W/V) sodium chloride in distilled water was brought to pH 7.2 with one-tenth volume of imidazole buffer.

Imidazole buffered saline, pH 7.35. 0.85 per cent (W/V) sodium chloride in distilled water was buffered to pH 7.35 with one-tenth volume of 0.05 M imidazole (Koch-Light Laboratories, Colnbrook, Bucks.,England).

Kaolin suspension. Kaolin (light) supplied by the British Drug House Ltd., was washed in large volume of imidazole buffered saline pH 7.2 and 5 per cent concentration was prepared in the same buffer.

Cephalin. 1 in 100 Bell and Alton platelet substitute (Diagnostic Reagents Ltd., Thame, Oxon).

Kaolin. 5 mgm per ml in 0.85 per cent (W/V) sodium chloride in distilled water.

Russell's viper venom (Stypven, Burroughs, Wellcome and Company) was used at a concentration of 10 µg per ml in imidazole buffered saline (pH 7.2).

Saline 0.86 per cent (W/V) sodium chloride in distilled water.

Urea stock solutions. Urea supplied by Sigma Chemical Company, St. Louis, was made up in barbitone buffered saline pH 7.2 to give a

concentrationof 20 gm. per 100 ml and 30 gm. per 100 ml.

Uric acid solution 50 mg. uric acid (Sigma Chemical Company, St Louis) were dissolved in 100 ml. barbitone buffered saline (pH 7.2) by gentle heating.

Tubing Transparent vinyl tubing (MF/13 Portland Plastics, Kent) and plastic adaptors (10M/634, Portex) were used in the Chandler tube method. For platelet adhesiveness, transparent vinyl tubing (MF/13 Portland Plastics, Kent) and translucent silicone tubing (Esco Rubber Ltd., London) were used in the preparation of the glass bead columns.

Ballotini glass beads 0.57 mm. diameter.

Collection of Blood.

All blood samples used in this thesis were collected by clean venepuncture using 21 G 1½" disposable needles and plastic disposable syringes. (Plastipack, Becton, Dickinson and Company, Ireland; and Grahams Medical Products Ltd., England). Blood was withdrawn slowly and care was taken to avoid frothing. With the minimum delay, the blood was transferred from the syringe to graduated 10 ml. siliconized centrifuge tubes containing 3.8 per cent sodium citrate, the relative proportions of blood to citrate being 9 to 1. The tubes were then covered with parafilm (Gallenkamp, made by Lindsay and Williams Ltd.) and the blood and citrate were mixed gently by inversion 3 times.

Platelet-rich plasma was prepared from citrated whole blood by centrifugation at 600 g for 5 minutes at room temperature in an MSE major centrifuge (Measuring and Scientific Equipment Ltd., England). The blood was centrifuged within 10 minutes from venepuncture. The plasma was gently pipetted off leaving the lower 1 ml. and transferred to another siliconized centrifuge tube. Experiments were carried out immediately after separation of the plasma.

Platelet poor plasma. Citrated blood was centrifuged at 1200 g. for 10 minutes at 4°C the supernatant two thirds of the plasma was centrifuged again in a superspeed angle head at 10,000 g. for 5 minutes at 4°C. The upper two thirds were separated and warmed to room temperature before use.

Siliconized glass-ware was used for all experiments involving study of platelet function. Two methods were used to siliconize the glass ware. The first method used a water-insoluble commercial silicone preparation Silicone M441 (Imperical Chemical Industries Ltd.) as described by Dacie (1956b). Later in this thesis a water soluble commercial silicone preparation was used (Siliclad; Clay Adams Inc., N.J.).

Methods.

Measurement of Platelet Aggregation in Vitro.

By comparing the adhesiveness of whole blood and platelet-rich plasma, Hellem (1960) demonstrated that a factor present in the erythrocytes, was essential for platelet adhesion to glass. The subsequent identification of this factor as ADP (Gaarder et al., 1961) has led to the development of a method for estimating platelet aggregation in platelet-rich plasma (Born, 1962a; O'Brien, 1962b; Cuthbertson and Mills, 1963; Born and Cross, 1963b; Mitchell and Sharp, 1964; Hardisty et al., 1964; Cook and Symons, 1965 and 1966). The method depends on the observation that platelet aggregation may be induced when ADP is added to platelet-rich plasma which is being stirred (Born, 1962a). The progress of platelet aggregation can be measured quantitatively by recording changes in light transmission through the plasma sample, as the clumping of platelets clumps occur (Born, 1962a). The turbidimetric method was first described by Born (1962a) and Born and Cross (1963b) and O'Brien (1962b). Born (1962a), Born and Cross (1963b) described a turbidimetric method for studying platelet aggregation quantitatively in vitro. The method depends on the decrease in optical density

of plasma which occurred when the platelets aggregated. The initial optical density of platelet-rich plasma is found to be proportional to the number of platelets and vigorous stirring alone causes only slight decrease in optical density in 30 minutes. O'Brien (1962b) estimated platelet aggregation using a simple photo-electric colorimeter. He found that platelet aggregation is more rapid at 37°C, with rapid stirring, with high concentration of ADP and a high platelet count.

Cuthbertson and Mills (1963) modified the turbidimetric method of Born to permit temperature control and continuous recording of the state of aggregation in small samples of platelet suspensions.

A minimum stirring speed is required to obtain aggregation for a given dose of ADP O'Brien (1962b). The rate and extent of aggregation increased when stirring is increased from 100 to 600 r.p.m. but the effect at 600 r.p.m. and 1000 r.p.m. was similar (O'Brien, 1962b; Born and Cross, 1963b). Increasing concentrations of ADP caused greater fall in optical density and the logarithm of ADP concentration is proportional to the rate of fall in optical density during the first 30 seconds (Born and Cross, 1963b; O'Brien, 1964). Aggregation is more rapid for a high platelet count (O'Brien, 1962b; Born and Cross, 1963b). The turbidimetric method was further modified by Cook and Symons (1965) using a prothrombin meter (Evans Electroselenium Ltd.) described by Toohy and Cook (1960) and later by another similar apparatus (Cook and Symons, 1966); these modifications also employ the principle of measurement of the decrease in optical density as a result of platelet aggregation. In these modifications the assessment of platelet aggregation is measured as the time in seconds taken by the optical density to reach a pre-set point on the galvanometer scale. Optical density changes are also recorded graphically.

Simpler methods for estimating platelet aggregation are described

by Hardisty et al. (1964) and by Mitchell and Sharp (1964). In the version described by Hardisty et al. platelet aggregation is studied by adding the aggregating agent to platelet-rich plasma in a tube which is then shaken vigorously either by hand or on an automatic pipette shaker. The sample is then examined macroscopically and microscopically for platelet aggregation. Mitchell and Sharp (1964) estimated platelet clumping in glass tubes at room temperature. ADP is added to platelet-rich citrated plasma and the mixture shaken gently and observed against a dark background under powerful oblique illumination. To determine the minimum effective concentration of an active agent, doublin. dilutions of the agent in saline are prepared and the lowest concentration of the agent which would produce visible clumps within one minute is taken as the end point for that system. They found that the threshold concentration needed to produce visible clumping in 60 seconds is 0.04 µg. ADP per ml. Platelet aggregation in platelet-rich plasma can also be measured using an artificial circulation system (Cunningham et al., 1965). In this technique, recalcified platelet-rich plasma is rotated in a loop where it forms an artificial thrombus with a platelet head and a fibrin tail. The platelets clump together to form macroscopic aggregates before fibrin formation occurs; the time is recorded between recalcification and platelet aggregation. Of these methods the following have been used:

1. The Chandler tube method.
2. The turbidimetric method.

Measuremen and Calcium plus ADP. aced by Calcium

1. Chandler Tube Technique.

1. Chandler Tube Technique.

This method is based on the observation made by Chandler (1958) that if blood is made to flow round a closed loop of plastic tubing, a thrombus is formed which resembles in vivo thrombus histologically.

If whole blood is replaced by platelet-rich plasma in this system, at an interval following recalcification a "snow storm" of platelet aggregates appears. These aggregates eventually consolidate to form a white thrombus and later the fibrin tail of the thrombus becomes visible (Cunningham et al., 1965).

In the present experiments, the time interval between recalcification, or the addition of calcium plus ADP and the appearance of the "snow storm" effect, was used as a measure of platelet aggregation.

The method is a modification of that described by Cunningham et al., (1965). A length of vinyl tubing (71 cm.) having a bore of 5.5 mm. (MP/13 Portland Plastics, Kent) was made into a loop by means of a plastic adaptor (10M/634, Portex). The loop was first washed in distilled water, then rinsed thoroughly in 0.9 per cent sodium chloride.

Two tubes were used in each experiment; to each tube were added 1 ml. platelet-rich plasma and 2.8 ml. 0.9 per cent sodium chloride. Into the first tube were also added 0.1 ml. calcium chloride M/4 and 0.1 ml. saline, and in the second 0.1 ml. ADP (final concentration 0.125 µg/ml) was added instead of the saline. As soon as these reagents were added one stop watch was started for tube 1 and two for tube 2. The two loops were then rotated on the turntable of a blood cell suspension mixer (Matburn Ltd., London), rotating at 28.5 r.p.m. in a glass fronted incubator at 37°C.

The results were assessed as follows:- for the first tube the end point was the first visible appearance of platelet aggregates (snow storm). For the second tube the first appearance of platelet aggregates mediated by ADP was recorded and after disaggregation the time from recalcification to aggregation preceding fibrin formation was also noted.

2. The Turbidimetric Method.

The turbidimetric method as described by O'Brien (1962b) and Born and Cross (1963b) was used to measure the platelet aggregation by recording the changes in optical density occurring as a result of recalcification of diluted platelet-rich plasma with and without added ADP.

Platelet-rich plasma is diluted 1:4 with barbitone buffered saline pH 7.2, then 1.9 ml. of the diluted platelet-rich plasma were transferred into a perspex cuvette containing a magnetic stirring rod and fitted onto an EEL titrator. The platelet-rich plasma was allowed to stir for 30 seconds, the optical density was then adjusted to 0.600, 0.05 ml. M/4 calcium chloride and 0.05 ml. barbitone buffered saline added and a stop watch was started. Optical density readings were then taken every 5 seconds until clotting occurred. A similar experiment was carried out in which ADP (final concentration 0.125 µg/ml.) was substituted for the barbitone buffered saline and a third in which ADP was used without calcium chloride, and the optical density readings were taken every 5 seconds for 10 minutes. These experiments were also carried out on the diluted platelet-rich plasma further diluted 1:2, 1:4, with platelet-poor plasma. Platelet counts were done in duplicate on the diluted platelet-rich plasma.

All experiments were performed on the same EEL titrator and galvanometer but the order in which the three experiments were carried out was varied at random from plasma sample to plasma sample.

The optical density readings were plotted on graph paper. Several phases of optical density changes were regularly found to occur, and the curve of Calcium plus ADP aggregation was divided into five phases (figure 9).

1. Phase 1 is measured from the time of addition of the aggregating agents to the point of maximum aggregation and represents the initial platelet aggregation, probably mainly

due to ADP.

2. Phase 2 is measured from the point of maximum aggregation to the point of maximum disaggregation of platelets following initial aggregation.
3. Phase 3 where the optical density remains stationery.
4. Phase 4 represents a slight increase in optical density which occurs before the second phase of platelet aggregation.
5. Phase 5 represents the second phase of platelet aggregation immediately occurring before clot formation.

In the calcium-platelet aggregation only phases 3, 4 and 5 are present (figure 7).

The results of the turbidimetric method were expressed as follows:-

1. Aggregation time is the time at which the first lowering of optical density in phase 5 is recorded.
2. Optical density fall in phase 5 is measured by subtracting the optical density recorded just before the beginning of phase 5 from the optical density recorded just before clot formation.
3. The duration of phase 5 is measured as the time taken by platelets to aggregate in phase 5.
4. The clotting time is the time at which the first increase in optical density is recorded after maximum aggregation in phase 5.
5. 30-60 platelet aggregation is the fall in optical density between 30 and 60 seconds and was used to determine the effect of calcium on ADP-induced platelet aggregation. This was done by comparing the results of 30-60 platelet aggregation in both ADP and ADP plus calcium platelet aggregation experiments.

Calcium and calcium plus ADP platelet aggregation in a normal control (tables 6 and 7, figures 7 and9) and a uraemic patient (tables 8 and 9, figures 8 and 10), both having nearly the same platelet count,

were selected to represent the optical density changes occurring in these experiments.

Turbidimetric Method for Platelet Aggregation.

The apparatus used in this study consisted of an EEL titrator connected to a galvanometer (EEL, type 20, Evans Electroselenium, Ltd., England) figure 5. A perspex cuvette was fitted onto the titrator above a magnetic stirrer and in the light path from the photo-electric cell. Two ml. distilled water were delivered into the cuvette together with a small stirring rod. Both the stirring motor and the light source were switched on and by means of the zero control knob, the hair line was brought to infinity on the upper galvanometer scale and then brought to zero by the sensitivity knob. An identical cuvette containing 1.9 ml. platelet-rich plasma and a stirring rod was substituted for the blank and a stop watch was started at the time the cuvette was placed in position on the machine. The optical density reading was then adjusted to 0.600 by the sensitivity knob. The plasma was left to stir for 30 seconds and any change in the optical density during this period was corrected by returning it to 0.600. At the end of the 30 seconds, the aggregating agent was then added in 0.1 ml. quantity and a stop watch was started. The aggregating agent was always delivered on the wall of the cuvette near the surface of the plasma and away from the light path, caution was taken not to allow formation of air bubbles in the sample. As platelets aggregated the light transmission through the sample was increased and was recorded as a fall in optical density on the galvanometer scale, and as platelets disaggregated an increase in optical density resulted. Changes in light transmission using a green filter (No. 604 Peak length 5200 Angstrom units) were recorded by taking optical density readings every 30 seconds over a period of ten minutes (or longer), from the time of addition of the aggregating agent.

All experiments unless specified were carried out at room

temperature. In an experiment done at 37°C, the titrator was placed in an incubator at 37°C and left for half an hour before starting the experiment. The platelet-rich plasma was separated in a siliconized tube and incubated together with an empty perspex cuvette at 37°C for ten minutes. The cuvette was then filled with the platelet-rich plasma, placed in the titrator and the experiment was started. All experiments were carried out at a fixed appropriate speed. The stirring speed selected caused only a minimum vortex in the plasma, and in no way interfered with the light path.

Parameters for measuring Platelet Aggregation and Disaggregation.

Platelet aggregation was measured by the following parameters:-

1. 30-60 platelet aggregation: The fall in optical density between 30 and 60 seconds, this being the steepest part of the curve.
2. 30-90 platelet aggregation: The fall in optical density between 30 and 90 seconds to include the main part of the optical density fall due to aggregation.
3. Maximum aggregation: This is measured by subtracting the lowest optical density recorded from the optical density before addition of the aggregating agent.
4. Maximum aggregation time: This is the time taken by platelets to reach the first lowest optical density recorded.

Platelet disaggregation.

1. Percentage disaggregation was considered as -

$$\frac{\text{increase in optical density in five minutes from the point of maximum aggregation}}{\text{fall in optical density due to maximum aggregation}} \times 100$$

2. Disaggregation time: is the time at which the optical density shows its first increase from the lowest optical density recorded.

Calculations when the maximum aggregation or disaggregation times were in excess of 10 minutes utilized the arbitrary figure of 600 seconds.

Factors affecting the results in the Turbidimetric Method.

The method used in the work of this thesis to measure platelet aggregation can be affected by a number of variables which may modify the results obtained. Of these, the effect of variation in haematocrit, plasma white cell count, stirring speed and incubation temperature were studied experimentally and the results are discussed below. The ADP concentration added was fixed at 0.25 µg. per ml. of plasma. An appropriate fixed speed was used and experiments were done at room temperature. The effect of platelet count on the results was studied by varying the platelet number through addition of platelet-poor plasma to platelet-rich plasma (Chapter 5).

The Effect of lowering the Haematocrit on ADP-induced Platelet Aggregation.

Seven experiments were carried out in which the haematocrit was lowered in vitro by adding platelet-poor plasma to whole citrated blood. 3.5 ml. platelet-poor plasma was added to 6.5 ml. citrated blood in a siliconized graduated centrifuge tube. The contents of the tube were mixed by inversion 20 times and centrifuged together with another tube containing undiluted citrated blood (control), at 600 g. for 5 minutes at room temperature. Platelet-rich plasma was separated from each tube and ADP-induced platelet aggregation was carried out as described previously.

The results (table 38) showed that platelet aggregation was diminished as the haematocrit was lowered. There was also a diminution in the platelet count. The 30-60 platelet aggregation although diminished was within the normal range $\pm$ 2 standard deviations in 6 out of 7 experiments. However the results of 30-90 platelet aggregation and maximum aggregation were below the lower limit of normal

in four out of seven experiments. There was no significant change in the maximum aggregation time, disaggregation time and percentage disaggregation, all were within the normal limits in the 7 experiments performed.

From the results it can be concluded that the diminution in platelet count that accompanied the lowering in haematocrit partly explains the diminution in platelet aggregation. Another factor is that the larger platelets tend to sediment with the red cells and white cells (Mitchell, 1968). Stormorken et al., (1965) have shown that there is also selective sedimentation of the more adhesive platelets.

However, the 30-60 platelet aggregation was not significantly affected by lowering the haematocrit, and as it was the parameter which gave significant differences between normal controls and uraemic patients, it can be concluded that the abnormalities in platelet aggregation and disaggregation were not to be wholly attributed to lowering of the haematocrit.

The Effect of White Cells on ADP-induced Platelet Aggregation.

White cell counts were performed on 9 normal controls and 7 uraemic patients, using a Coulter counter (Coulter Electronics, Ltd.) In one patient with chronic renal failure the count was done before and after haemodialysis (table 37). No significant difference was obtained between the mean white cell count in normal controls and the mean value of uraemic patients ($t = 0.334$, $P > 0.1$).

The effect of white cells on ADP-induced platelet aggregation has been studied by Harrison et al., (1966). Platelet-rich plasma from citrated blood is separated into two fractions, the first is the top 2 ml. and the second contains the remainder of the plasma, taking the pipette as close to the buffy coat as possible. The top fraction contained less white cells than the bottom fraction. Although the bottom fractions of plasma showed slightly more active reversal of

ADP-induced aggregates than the top fraction, the difference in reversal between the two is not significantly correlated with the difference in their white cell count.

In the work for this thesis, the platelet-rich plasma was separated leaving the bottom 1 ml; the plasma separated was then mixed by inversion and tested.

The Effect of the Stirring Speed on ADP-induced Platelet Aggregation.

The effect of the stirring speed was studied by increasing or decreasing the speed by one quarter turn of the speed knob in the EEL titrator.

Two experiments were conducted in which ADP-induced platelet aggregation was done using a high, low and the ordinary speed used in all experiments. The results in table 25 showed that increasing the stirring speed caused less platelet aggregation and earlier and more complete disaggregation. Using a low speed, although the effect on aggregation was not constant compared to the controls yet less and rather delayed disaggregation resulted.

O'Brien (1962b) has shown that the degree of agitation of plasma influences the extent to which platelet aggregation occurs. He found that at a low stirring speed no aggregation resulted. Increasing the speed caused platelets to aggregate but has not increased significantly the maximal rate of fall inoptical density.

The Effect of Incubation Temperature on ADP-induced Platelet Aggregation.

An experiment was carried out in which platelet-rich plasma was incubated at 37°C for 10 minutes. ADP-induced platelet aggregation was then performed at 37°C. The results from this experiment showed that platelet aggregation was diminished and early and more complete disaggregation occurred when compared to another experiment done at

room temperature (table 35). These results were similar to the findings of O'Brien (1962b) that platelet disaggregation is greatest at 37°C and does not occur at 0°C.

The turbidimetric method measures changes in optical density, but whether a large number of small platelet aggregates can cause a greater fall in optical density than a small number of large aggregates is not clear. Moreover it is not known whether the number and size of platelet aggregates bear any relationship to the rate of aggregation and disaggregation. Born and Hume (1967) investigated the relationship between the optical density and the numbers and sizes of aggregates. The results showed that the increase in light transmittance is associated with two effects, namely the formation of aggregates and their increasing density. Formation of platelet aggregates begins before there is any change in light transmittance. During this time, the concentration of single platelets decreased steeply as they associated to form small aggregates. The number of these aggregates reached maximum about the time when the light transmittance began to increase. The subsequent increase in transmittance is associated with a progressive diminution in the number of both single platelets and small aggregates and the formation of fewer larger aggregates in which the platelets are no longer countable. During early aggregation, the density of the aggregates diminished, indicating that the platelets were packed loosely with considerable space between individual platelets. Born and Hume(1967) also demonstrated that the initial velocity of increase in light transmittance is a measure not of the rate of formation of small aggregates from single platelets, but of large aggregates from small aggregates. Furthermore, a high initial velocity is caused much more by aggregate contraction than by aggregate formation.

Their findings suggest that any mathematical expression of the relation between the light transmittance and the aggregation process is bound to be complex.

PLATELET ADHESIVENESS.

Previous Methods for the Estimation of Platelet Adhesiveness in Vitro.

Baar and Szekely (1929) estimated the decrease of platelet number in plasma after storage of the plasma in glass containers. Baar (1941) claimed this to be the first test devised for platelet function. In 1930 Moravitz and Jürgens constructed an apparatus they called "Kapillar-thrombometer" consisting of two pumps which drew blood without anticoagulant through a calibrated glass capillary tube and the direction of the blood stream is alternated at intervals. The time taken by the blood stream to stop due to plugging of the capillary tube by platelets is taken as an indirect measure of the adhesiveness of platelets.

Methods described for the study of extracorporeal thrombosis (Rowntree and Shinoya, 1927; Best et al., 1938) were used to estimate platelet adhesiveness in a semiquantitative way. The principle is to shunt the blood stream from arteries to veins through tubes or chambers, in which thrombi are formed after a certain period. The time required for the formation of white thrombi or for plugging of the tubes is taken as a rough estimate of platelet adhesiveness.

The first real attempt to measure platelet adhesiveness quantitatively was made by Wright (1941). The principle of the method is to transfer blood mixed with anticoagulant to a special glass flask whose sides have been ground away to form two windows each about 1.5 cm. in diameter. The windows are covered with uncoated glass, while the internal surface of the bulb is coated with melted vaseline. The tubes are rotated at 3½ revolutions per minute and every twenty minutes platelet counts are made in samples withdrawn from the tube, and the results are expressed as percentages of the initial count.

In 1949, Moolten and Vroman devised the glass wool method, in which the time of contact between the platelets and glass surface is

shortened. After a "delay time" of 10 minutes from the venepuncture 1 ml. of citrated blood is passed through a triple-plait of glass wool braid previously moistened with 1.5 ml. physiological saline solution. The red cells and platelets are counted in the blood sample before and after filtration. The platelet adhesiveness is calculated from the ratio red cells/platelets in both samples. The platelet adhesiveness can also be measured by the method described by Rovatti (1951) using the capillary part of a red cell counting pipette as foreign surface. In the method of Revol (1954) platelet-rich plasma is deposited between a slide and a cover-glass and examined microscopically for spreading and adhesion to glass.

Marx and Derlath (1957) described a method in which citrated blood is run into a capillary tube of a fixed length and bore. The tube is then tilted for a fixed time and the difference in the platelet number before and after the procedure is the number of adhesive platelets.

The first of the glass bead column methods was described by Hellem (1960). Estimation of platelet adhesiveness is carried out in citrated blood. By an electrically-driven mechanical device the blood is pushed from a graduated syringe through a standardized glass bead column at a constant rate. The reduction in platelet count after passage through the glass bead column is taken as a measure of platelet adhesiveness.

A similar method was devised by O'Brien (1961) in which native or heparinized blood is allowed to come in contact with a standardized glass bead column. The contact time is short (15 seconds) compared to Hellem's technique. An initial platelet count is made on the blood before passage through the filter, a second count is made on the first drops of blood coming from the filter, and a third taken 15 seconds later when about 1 ml. of blood has passed through the filter. The difference between the initial and the second count expressed as a percentage of the initial count is considered as a measure of the number of the platelets that stick in the leading front

of the blood passing over a large dry glass surface of known area. The difference between the initial and the third counts, expressed as a percentage of the initial count, is a measure of the number of platelets that stick to glass which had been in contact with blood for 15 seconds.

In Salzman's method (1963) a control venous blood sample is collected in ethylene-diamine tetra acetic acid (disodium salt), a second venepuncture was then performed in the opposite arm with a siliconized needle and blood is made to pass directly through a standardized glass bead filter into a 7 ml. "Vacutainer" tube 32040 (Becton, Dickinson and Company, Rutherford, N.J.) containing ethylene diamine tetra acetic acid (disodium salt) as an anticoagulant. The negative pressure in "Vacutainer" tube is uniform within $\pm$ 2 mm. of mercury. The rate of filling of the collection tube and the time of contact of blood do not vary appreciably from one determination to the next.

The difference between the platelet counts in the control and test blood samples is expressed as percentage of the control count and taken as a value for platelet adhesiveness.

Salzman's method differs from Hellem's technique in that native blood is passed through the glass bead column directly from the vein. The rate of blood flow during passage in the column is rapid (180-360 cm. per minute versus 28 cm. per minute in Hellem's method). The time of contact of blood with the glass beads is also shorter than in Hellem's method (1.7 - 4.5 seconds versus 30 seconds).

Hume (1966) described a simple method in which one ml. of native blood is drained by gravity through a vertical column containing 1 gm. of glass beads. The effluent is collected in a plastic container with dried ethylene diamine tetra acetic acid. The percentage adhesiveness is determined by noting the difference in platelet count between the original sample and the sample which has passed through the column. This method is influenced by the

haematocrit as it will determine the amount of ADP liberated from the red cells and also the rate of flow through the column.

All these methods have advantages and disadvantages. It was decided on an arbitrary basis to select the modification of Hellem's technique as described by Hirsh et al., (1966a).

Method for Platelet Adhesiveness.

The technique used in this thesis was a modification of Hellem's original method (Hirsh et al., 1966a). The glass bead columns were made by filling a length of transparent vinyl plastic tubing ("Portex" code number NT/13, Portland Plastics Limited, Kent) with 2.5 grammes of Ballotini glass beads (0.57 mm. diameter) to give a column 6 cm. in length. The glass beads were held in the column by a filter of fine nylon gauze fitted at each end of the column by end pieces made of "Esco" translucent silicone tubing (3mm. bore, 2 mm. wall). Blood was collected by a clean venepuncture into disposable plastic syringe as described on page 30 and mixed with 3.8 per cent sodium citrate solution (one part citrate to nine parts of blood) by gentle inversion 3 times in siliconized graduated centrifuge tube using parafilm over the end of the tube. The blood was allowed to stand at room temperature for 30 to 45 mimutes. Just before starting the test, the blood was mixed by gentle inversion for 20 times using parafilm over the end of the tube and then delivered into a 5 ml. polystyrene tube. 2 ml. of this blood was drawn into a 2ml. graduated plastic syringe ("Plastipak", Becton, Dickinson and Co., Ireland) which was fitted to one end of the glass bead column. The syringe was then fitted to an electrically-driven mechanical pump which gives a constant speed and the blood was forced to one end of the glass bead column, at a constant rate (figure 6). The time taken for the leading edge of the blood to traverse the whole length of the column was measured. Using the same pump, syringes and glass bead columns, the blood-glass bead contact time was 30 $\pm$ 1 second. Any observations lying outside

this range were discarded. The blood issuing from the column was collected in another 5 ml. polystyrene tube. The two blood samples, before and after passage through the column were mixed 10 times by inversion and duplicate platelet counts were performed on each sample. The difference between the two counts was expressed as a percentage of the initial platelet count, and this value was taken as an index of platelet adhesiveness in the sample. The haematocrit was estimated in the citrated blood sample using a microhaematocrit centrifuge (Hawksley, London).

As with all glass bead methods, this technique is dependent on many variables which can influence the accuracy of the results. Variations in the size, shape and surface area of the glass beads and their surface properties as regards adsorption of trace moisture, oils, et., may be of importance. In Hellem's original method (1960) it was found that platelet adhesiveness increased as the glass surface area was increased, and some investigators noted difficulty in obtaining consistent results from one batch of glass beads to the next (Zucker, 1963; O'Brien and Heywood, 1967). The bore and surface characteristics of the plastic tubing is another element that must be standardized. The presence of the personal factor in platelet counting has not been completely solved by the use of electronic counting. Hellem (1960) showed that adhesiveness depended directly upon the haematocrit. This observation was confirmed by other investigators (Hirsh et al., 1966, a,b; McClure et al., 1966). Appropriate corrections were made either by adjusting the packed cell volume by adding red cell concentrates or mathematically from the percentage adhesiveness - packed cell volume curve. Harrison and Mitchell (1966) found that reduction of erythrocyte ADP by enzymatic phosphorylation also reduced the platelet adhesiveness in Wright's roller flask method, but such studies have not yet been carried out with the glass bead column method. The question whether the red cells influence platelet adhesiveness as inert particles by affecting the flow through the

column is still to be answered.

Leucocytes in normal blood were found to stick less than platelets to glass. O'Brien (1961) using his own method found no retention of leucocytes to the glass bead column, and it seems that leucocyte stickiness is not greatly influenced by the factors that cause platelet adhesiveness (Garvin, 1961; Allison and Lancaster, 1967).

The driving force which controls the rate of flow of blood through the glass bead column can be precisely controlled in the Hellem's technique. However, this precision is obtained at the expense of using an anticoagulant (citrate), any minor changes in the concentration of which can markedly affect the adhesiveness of platelets (Hellem, 1960; Hirsh et al., 1966b).

In conclusion it seems that in the method used for platelet adhesiveness some variables such as the platelet-glass bead contact time, the glass beads, the plastic tubing can be standardised. The personal factor in platelet counting can be reasonably controlled, by coding and the use of electronic counting. "Blind" counting was adopted in the present study as electronic counting was not available. Other factors such as the effect of erythrocytes and leucocytes, the use of anticoagulant are variables which present problems inherent in the method.

Platelet Adhesiveness: Correction Curve for Packed Cell Volume.

Eighty ml. citrated blood were collected as described above and 40 ml. were then centrifuged at 1200 g. for 10 minutes at 4°C, the supernatant plasma was centrifuged again at 10,000g for 5 minutes and the upper two thirds of plasma was pipetted off. Eight experiments were carried out in which citrated blood was diluted with platelet-poor plasma, to obtain decreasing packed cell volume values. The blood was diluted with the appropriate amount of platelet-poor plasma (table 45), the tubes inverted 20 times and immediately passed through

the glass bead column.

Platelet adhesiveness was estimated as described above, and was found to be significantly correlated with corresponding packed cell volume values (figure 33). The curve obtained was used to correct the platelet adhesiveness results in both normal subjects and uraemic patients.

Measurement of Platelet Factor 3 Availability.

Platelet factor 3 is probably a lipoprotein (Shinowara, 1957) which the platelet contributes to the interaction of plasma coagulation proteins in the formation of intrinsic prothrombin activation (Horowitz and Marcus, 1964).

Platelet factor 3 was first found by van Creveld and Paulssen (1953) in combination with platelet factor 4, the antiheparin factor, in a water insoluble lipid moiety of platelets. The distinction between the two factors was made possible by ultracentrifugation (Deutsch, 1954; Jürgens, 1952; Deutsch and Kain, 1961). The lipoprotein was extracted from platelets by freezing and thawing and/or sonication. Impurities were removed by adsorption with kaolin and charcoal and platelet factor 3 was then sedimented by high speed ultracentrifugation (Alkjaersig et al., 1955), or was separated by barium sulphate adsorption and elution (Stefanini and Campbell,1954).

Platelet phospholipid has been localised in platelet granules (Johnson et al., 1959; Maupin, 1959, Fanio, 1960), and more recently in both platelet granules and membranes (Marcus et al., 1966).

The observation that the Russel Viper venom or "Stypven time" of intact platelet-rich plasma is barely shorter than that of platelet-poor plasma, and that disruption of platelets greatly accelerates clotting (Fantl and Ward, 1958) has led to the belief that in normal circulating platelets the phospholipid is not available for the coagulation reaction. When platelets are fragmented (Fantl and Ward, 1958; O'Brien, 1958) or washed (Marcus, 1965) they become more active

in accelerating the Stypven time.

Platelet factor 3 was determined quantitatively by Alkjaersig et al., (1955) using a platelet extract prepared by ultrasonic treatment of packed platelets. Addition of this extract to a reaction mixture of purified prothrombin, antihistamine and calcium results in the generation of thrombin, the concentration of which is dependent on the amount of platelet factor 3 added.

The "Stypven time" was used by Fantl and Ward, (1958) to determine the availability of platelet lipids in platelet-rich plasma which has been frozen and thawed. O'Brien (1958) applied a similar method but used mechanically fragmented platelets.

Product I substrate time was used by Des Prez et al., (1961) to determine the availability of platelet factor 3 following the incubation of platelet-rich plasma with endotoxin. Preformed product I (diluted serum, adsorbed plasma and calcium) and further calcium were added to test plasma and the clotting time of this mixture became shorter in the presence of more available phospholipids.

One of the most commonly used methods for measuring platelet factor 3 activity is the thromboplastin generation test (Biggs and Douglas, 1953). Abnormalities in the test have been reported in uraemia (Cahalane et al., 1958). However, this was not the experience of Marcus and Zucker (1965) who found no abnormalities in patients with suspected thrombocytopathy.

There are disadvantages of using this test for detecting platelet disfunction; e.g. a large volume of blood is needed to obtain sufficient platelets especially in thrombocytopaenic patients and children, great accuracy is required and the normal range is wide.

Bonnin and Cheney (1961) described the "platelet thromboplastic function test" which is a modification of the thromboplastin generation test of Biggs and Douglas (1953). Platelets used in the test were

obtained by centrifuging platelet-rich plasma and removing the supernatant. Normal saline is then added without disturbing the platelet button and the tube recentrifuged. The supernatant is removed and the platelet button emulsified in serum. It is then incubated with acetone, dried chloroform extracted brain suspension and recalcified. Aliquots of this mixture are added periodically with calcium to high spun substrate plasma, and the clotting times determined. According to Bonnin and Cheney (1961) the results of this technique are better related to the clinical occurrence of haemorrhage in thrombocytopaenic states than those of the thromboplastin generation test. The test is also found to show consistently reduced platelet thromboplastin function in 18 uraemic subjects. Cheney and Bonnin (1962) later reported the same abnormality in 28 out of 31 uraemic patients.

Egli (1961) and Husom (1961) described a simple one-stage method for the assay of platelet factor 3. Husom's test is based on the determination of the recalcification time of contact activated platelet free plasma enriched with factor V and VII through the addition of adsorbed platelet free plasma. The platelets after washing, freezing and thawing, are tested in their own plasma in high dilution.

The prothrombin consumption has been used by many workders to measure platelet factor 3 activity in various platelet dysfunctions. The test was shown by many authors to be abnormal in uraemia (Barrain and Adelson, 1956; Lewis et al., 1957; Rath et al., 1957; Cahalane et al., 1958; Geiger et al., 1959; O'Grady, 1959; Alschuler et al., 1960; Donner and Nouwirtova, 1960; Wurzel, 1960; Kendall et al., 1961; Willoughby and Crouch, 1961; Salzman and Nexi, 1966; Castaldi et al., 1966; Stewart and Castaldi, 1967).

In the method described, platelet phospholipids are made available by disrupting platelets, either by freezing and thawing or by mechanical fragmentation. Moreover, platelets used are subjected

to washing or sedimentation and the trauma to which the platelets are subjected may thus render the results of little physiological significance.

Hardisty and Hutton (1965) described a method for studying platelet factor 3 availability based on the kaolin clotting time of platelet-rich plasma. Incubation of citrated platelet-rich plasma with kaolin makes about 1/20th of the platelet phospholipid available for coagulation but had no such effect on red cell phospholipid. Spaet and Cintron (1965) devised a similar method for studying platelet factor 3 availability. Russell viper venom was used to activate factor X effectively and rapidly. 'Stypven' times were determined on "intact" platelet-rich plasma after incubation with either kaolin or connective tissue fragments. Platelet factor 3 availability developed in response to kaolin in citrated but not in plasma anticoagulated with EDTA. However connective tissue fragments were effective in both types of plasma.

The most recent test for platelet thromboplastic function was described by Horowits et al., (1967) where ADP was used to activate platelet factor 3 in citrated platelet-rich plasma. The 'Stypven' time was then determined.

The use of 'Stypven' in the method of Spaet and Cintron causes full activation of factor X, with subsequent conversion of prothrombin to thrombin in the presence of phospholipid and factor V. This method was therefore selected for use in the present study.

Platelet Factor 3 Availability.

Method of Spaet and Cintron.

The method used in this study was similar to that described by Spaet and Cintron (1965). To 0.9 ml. of platelet-rich plasma obtained as described earlier, was added 0.1 ml. 5 per cent kaolin in a siliconized centrifuge tube. The two reagents were mixed by gentle shaking 10 times and then left undisturbed in a water bath at 37°C

for 30 minutes. After incubation the platelet-rich plasma and kaolin were mixed by resuspending the kaolin using a siliconized pipette, and 0.1 ml of the mixture was added to 0.1 ml M/40 calcium chloride and 0.1 ml Russel viper venom (10 µg per ml). A stop watch was started and the clotting time determined. Duplicate readings were carried out on each sample. Some of the experiments were carried out using platelet-rich plasma diluted with platelet-poor plasma to determine the "stypven time" at different platelet rich plasma counts.

Kaolin Cephalin Clotting Time

The principle of this test (Biggs and Macfarlane, 1966) is that plasma is recalcified in the presence of a platelet substitute after the plasma has been exposed for a standard preliminary period of two minutes to a suspension of kaolin. Reduced levels of plasma factors I, II, V, VIII, IX, X, XI and XII will result in a prolonged clotting time. Although this test has overcome the disadvantages of the recalcification time and the partial thromboplastin time, it still has its own shortcomings. Elevated levels of one or more individual factors or the presence of active intermediates which would tend to reduce the clotting time may mask a prolongation of the clotting time due to a deficiency of another factor. Minor deficiencies in several factors may on the other hand result in prolongation of the clotting time.

Method

0.1 ml of plasma and 0.1 ml of Bell and Alton platelet substitute are pipetted into a clotting tube and left for 1 minute in a water bath at 37°C. 0.1 ml of kaolin suspension (5 mg per ml in saline) is then added and after 2 minutes 0.1 ml of M/40 calcium chloride is added and a stop watch started. The tube is gently tilted at 5 to 10 second intervals and the clotting time recorded.

Being fully aware of the limitations of this test, it was used

together with platelet factor 3 availability test to detect prolonged clotting times due to reduced plasma clotting factors.

Platelet Count.

Platelet counts were performed using formol-citrate as the diluting fluid (Dacie 1956a) 10 ml. freshly filtered diluting fluid were pipetted into a clean test tube together with 0.1 ml. blood or platelet-rich plasma. The contents of the tube were mixed by inversion 20 times and a sample was run onto an improved Neubauer couting chamber. The chamber was allowed to stand in a damp atmosphere for 20 minutes and platelets were counted with a microscope using a white light source.

CHAPTER 4.

PLATELET AGGREGATION INDUCED BY CALCIUM AND CALCIUM WITH ADP.

The presence of calcium ions seems to be essential for platelets to aggregate in response to added ADP. ADP does not clump platelets in platelet-rich plasma from blood collected with erythylenediaminetetracetate (EDTA), but the addition of calcium chloride restores the responsiveness to ADP (Born and Cross, 1963 a and b; Mitchell and Sharp, 1964; and Hovig, 1964). When unwashed platelets are suspended in Tyrode's solution containing heparin, aggregation occurs when ADP is added; aggregation is not affected when glucose, phosphate, bicarbonate or magnesium chloride are omitted. However, aggregation is reduced when calcium chloride is excluded (Born and Cross, 1964). The same authors also found that when ADP is added to suspension of unwashed platelets in solutions containing sodium chloride, potassium chloride, calcium chloride, heparin and imidiazole buffer, the initial rate of aggregation is proportional to the logarithm of the calcium concentration up to 1.7×10^{-3}M. At higher concentrations, calcium chloride inhibits platelet aggregation. Magnesium is relatively ineffective when substituted for calcium at concentrations up to 1.7×10^{-3}M, while at higher concentrations it inhibits aggregation more effectively than calcium (Born and Cross, 1964). This last observation was also confirmed by Hovig (1964) who found that calcium is essential for the clumping of rabbit platelets by ADP, magnesium being only effective in the presence of otherwise ineffective concentrations of calcium.

To study the ionic requirements of platelets ideally a plasma free system should be employed, but the use of washed platelets creates additional difficulties. For example, although human platelets subjected to centrifugation and subsequent suspension in plasma can clump with ADP (Käser-Glanzmann and Lüscher, 1962; Mitchell and

Sharp, 1964), their ability to do so is diminished (Born and Cross, 1964).

Haslam (1964) was able to prepare a buffered suspension of washed human platelets which did not clump with calcium alone, but did react with added ADP. Aggregation of washed human platelets by ADP showed an absolute requirement for either calcium or magnesium ions and the effect of both is enhanced by potassium ions.

Calcium was found to be necessary for the release of ADP by collagen (Hovig, 1964).

Divalent ions are also essential for the aggregation of platelet by thrombin, the effect of thrombin being blocked by the addition of citrate or EDTA (Bounameaux, 1957b; Zucker and Borrelli, 1958; Bounameaux, 1959; Shermer et al., 1961; Grette, 1962; Hovig, 1964).

It can be concluded that calcium is a necessary factor both for the reaction leading to release of ADP from platelets and for the aggregation of platelets produced by ADP.

Born (1962a) using a turbidimetric method, found that the addition of calcium to heparinized pig plasma caused an immediate increase in optical density, followed by a sharp drop over the succeeding 10 minutes; the addition of further calcium at this time causes a rise in optical density. On the other hand, when calcium is added to platelet free plasma, there is an immediate rise in optical density, smaller than the rise seen with platelet-rich plasma, but with platelet-poor plasma the small rise is sustained. The observation suggests that the rise in optical density after addition of calcium to platelet-rich plasma represents both a direct effect of calcium on the plasma and an effect of calcium on the platelets themselves; this point is treated in more detail in the discussion section of this chapter.

In this chapter are described experiments in which the response of platelets from normal and uraemic subjects to calcium and a

combination of calcium and ADP were studied.

RESULTS.

Normal Controls.

1. Results in the Chandler tube system.

Seven normal controls were studied using calcium alone and calcium plus ADP. The results show that the mean aggregation time (snowstorm) occurring before clot formation when adding calcium alone was 456.14 ± 54 seconds and when adding calcium and ADP was 393 ± 25.2 seconds (table 1). The difference between the two means was significant ($t = 2.782$, $0.02 > P > 0.01$). There was no correlation between the aggregation time and the platelet count of diluted platelet-rich plasma.

2. Results in the turbidimetric system.

The changes in optical density following the addition of calcium and calcium plus ADP to platelet-rich plasma have already been described in terms of a representative experiment (Chapter 3, tables 6, 7, 8, 9, figures 7 and 9).

The aggregation time, duration of phase 5 and the clotting times given below were the mean values from seven normal controls. To study the effect of the platelet count on these times and on the optical density fall in phase 5, another 9 experiments were performed in which the platelet-rich plasma was diluted 1:2 and 1:4 with platelet-poor plasma.

All correlations with the platelet count given below include the results of the diluted and undiluted experiments (a total of 16).

(a) Calcium induced platelet aggregation.

It was observed that when calcium chloride was added to stirred diluted platelet-rich plasma an initial fall in optical density occurred due to dilution of the system. This was followed by lag phase

in which no appreciable change in optical density was observed. A small increase in optical density then occurred which usually lasted a few seconds (phase 4) and preceded the fall in optical density due to platelet aggregation (phase 5), eventually followed by clot formation. The increase in optical density seen in phase 4 was a constant finding in all normal controls examined (figure 7).

The onset of platelet aggregation occurred 490.71 ± 63.8 seconds from the addition of calcium and was correlated with the platelet count of diluted platelet-rich plasma ($r = 0.543$, $0.05 > P > 0.02$) (figure 13). The time taken by platelets to aggregate in phase 5 was found to be 60.71 ± 13.36 seconds, and was also correlated with the platelet count ($r = 0.648$, $0.01 > P > 0.001$) (figure 15). The fall in optical density resulting from platelet aggregation in phase 5 was also correlated with the platelet count ($r = 0.966$, $P < 0.001$) (figure 11). The clotting time of diluted platelet-rich plasma was 551.4 ± 62 seconds; there was a significant positive correlation with the platelet count ($r = 0.543$, $0.05 > P > 0.02$, figure 17).

(b) Calcium plus ADP induced platelet aggregation in the turbidimetric system.

When both ADP and calcium were added to diluted platelet-rich plasma an initial fall in optical density due to dilution was followed by a continuous fall due to platelet aggregation (phase 1). After a time the optical density began to increase as a result of platelet disaggregation (phase 2). Disaggregation became almost complete and the optical density reached a constant level (phase 3). This was followed by a small increase in optical density (phase 4), then a fall resulted, continuing until clot formation eventually occurred (phase 5), when a sudden rise in optical density was recorded (figure 9).

The onset of platelet aggregation in phase 5 occurred at 515.71 ± 87 seconds from the addition of the calcium plus ADP. The duration of phase 5 was 100 ± 15.27 seconds and the clotting time was 623.57 ± 91 seconds. The onset of phase 5, the duration of phase 5 and the clotting time were in correlation significantly with the platelet count of the plasma ($r = 0.687$; $0.01 > P > .001$ (figure 14) $r = 0.904$, $P < 0.001$ (figure 16) and, $r = 0.768$, $P < 0.001$ (figure 18)

respectively).

The fall in optical density as a result of platelet aggregation in phase 5 was also in correlation with platelet count ($r = 0.948$, $P < 0.001$; figure 12).

The results of both calcium and calcium plus ADP experiments indicate that the higher the platelet count the more delayed the onset of platelet aggregation in phase 5, the longer its duration and the more prolonged the clotting time.

Comparison between the results of calcium and calcium ADP Experiments

Although the mean onset times of both the platelet aggregation in phase 5 and the clotting times in the calcium ADP system were longer than those with calcium alone, the differences are insignificant ($t = 0.613$, $P > 0.1$ and $t = 1.734$, $P > 0.1$ respectively).

However, there was a significant difference between the mean time taken by platelets to aggregate in phase 5 in the two sets of experiments, being longer in the calcium ADP system ($t = 5.120$, $P < 0.001$).

The correlation between these times and the platelet count though significant in the calcium experiments, became more so when ADP was also added (tables 2 and 3).

The effect of calcium on ADP-induced platelet aggregation was studied in 7 pairs of experiments and the fall in optical density between 30 and 60 seconds in phase 1 was compared. In one set of experiments, ADP was added to diluted platelet-rich plasma and in the other calcium was also added. Analysis of the results showed no significant difference between the two groups.

Uraemic Patients

The results of calcium and calcium plus ADP induced platelet

aggregation in 10 uraemic patients are presented below. Two of these patients were examined before and after haemodialysis.

Calcium induced platelet aggregation

The onset of platelet aggregation in phase 5 occurred 519 $\pm$ 81.4 seconds after the addition of calcium. There was no correlation between the onset of phase 5 and the platelet count ($r = 0.24$, $P > 0.1$).

The duration of phase 5 was 60 $\pm$ 31.4 seconds and the mean clotting time was 579 $\pm$ 77.63 seconds (table 4).

Calcium plus ADP induced platelet aggregation

The onset of platelet aggregation (i.e. the onset of phase 5) occurred 515.7 $\pm$ 109.7 seconds from the addition of calcium and ADP. The duration of phase 5 was 96.25 $\pm$ 14.50 seconds and the mean clotting time was 575 $\pm$ 137.5 seconds.

The results of the onset of platelet aggregation and the duration of phase 5 given above were the mean values of 8 uraemic patients because two failed to disaggregate in phase 2. The mean value of the clotting time was that of 10 uraemic patients.

In three experiments (table 5, experiments 9, 11 and 12), the addition of calcium and ADP caused platelets to aggregate but disaggregation occurring in phase 2 was either absent or very slight. This made the determination of the onset of the second platelet aggregation (phase 5) very difficult or almost impossible. In one patient (table 5, patient No. 15) who was examined before and after dialysis, the pre-dialysis sample showed inability to disaggregate but this finding was later corrected in the post-dialysis sample.

Comparison between the results of Calcium and Calcium plus ADP Experiments

There was no significant difference between the mean onset of platelet aggregation in calcium and calcium plus ADP experiments ($t = 0.075$, $P > 0.1$). The same was also true with clotting times

($t = 0.08$, $P > 0.1$). However, there was a significant difference between the duration of phase 5 in calcium and calcium plus ADP ($t = 2.967$, $0.01 > P > 0.001$).

Comparison between normal controls and uraemic patients.

Although the onset and duration of phase 5 and clotting times were significantly correlated with the platelet count being 131.920 ± 2403/c.mm. in normal controls, and 71.300 ± 2364/c.mm. in uraemic patients ($t = 5.140$, $P < 0.001$), there was no significant difference in the results between the normal and uraemic patients.

When the results of renal failure patients were compared with normal results the following observations emerged (table 10).

Onset of platelet aggregation in phase 5 in both calcium and calcium plus ADP was found to be normal in all patients except two (Nos. 21 and 38, figures 13 and 14). As regards the fall in optical density in the calcium experiments, all were normal except one patient (No. 22, figure 11) while in the calcium plus ADP experiment (figure 12) all were normal except two (Nos. 18 and 22) in whom the abnormality was possibly a result of failure to disaggregate at the end of phase 2. The duration of platelet aggregation in phase 5 was normal in all patients in the calcium experiments (figure 15). However, in the calcium plus ADP series all except three (Nos. 18, 20 and 21, figure 16) fell outside the 95 per cent confidence limit of normal. The clotting time was normal in both sets of experiments in all patients except two (Nos. 21 and 38, figures 17 and 18).

When the results obtained before and after haemodialysis were compared in two patients, there was shortening of the clotting time in both patients after dialysis (calcium plus ADP experiments). However, only one patient (No. 21, table 4) showed such shortening in the calcium experiment, the other showed an increase.

In summary, all uraemic patients (except patient No. 20), showed an abnormality in one or more of the parameters either in the calcium

or calcium plus ADP experiments or both. It is of interest to note that the abnormalities were more evident in the calcium plus ADP experiments and were most marked in the duration of phase 5 (calcium plus ADP experiments).

DISCUSSION

In the Chandler tube technique, when comparing the time of onset of platelet aggregation (snow storm) it is found that addition of both ADP and calcium significantly shortened the time for the snow storm effect to occur as compared with experiments in which calcium alone was added. A similar finding was also reported by Wilson et al., (1967). However, when this method was used to study uraemic patients it was sometimes impossible to record the snow storm time visually, perhaps because of the low platelet count. It was therefore thought worthwhile to examine the phenomenon in the turbidimetric system.

From the results of the normal controls there appears to be a significant correlation between the platelet count and both the time of the onset and duration of platelet aggregation in phase 5, the fall in optical density due to platelet aggregation in phase 5, and the clotting time. These correlations are present in both calcium and calcium plus ADP experiments. The results indicate that the higher the platelet count, the more delayed is the onset, the bigger the magnitude and the longer the duration of platelet aggregation in phase 5, and the longer the clotting time. The correlation between the platelet count and the clotting time is at variance with the finding of Brinkhous (1947) that the clotting time of recalcified plasma is long if platelets have been virtually removed by high speed centrifugation. In the platelet factor 3 availability test (Hardisty and Hutton, 1965) the clotting times fall as the platelet count increases.

The difference between the results obtained in this chapter and the/

the evidence stated above is not fully understood. A difference in the techniques employed can partly explain the discrepancy between the results. Dilution of the platelet rich plasma in barbitone buffered saline with stirring probably causes delayed clotting, thus "telescoping" the changes that occur before fibrin formation.

A possible explanation for these observations may be that when plasma is recalcified the rate and amount of thrombin released should be similar whether the platelet count is high or low. Thrombin when released acts on platelets causing aggregation and eventually clot formation. With a low platelet count, the proportion of thrombin per platelet would be higher and aggregation and subsequently clot formation will result sooner than where the platelet count is high.

There was slight, but constant increase in optical density/....

density (phase 4) which occurred just before the beginning of platelet aggregation in phase 5. Born and Cross (1963b) have also reported that when calcium chloride is added to plasma, a small increase in optical density occurs just before platelet aggregation. This increase is found to be dependent on the presence of platelets, because when calcium chloride is added to platelet-poor plasma, only a very small increase in optical density is noted. It was suggested by Born and Cross that the increase in optical density may be due to a change in platelets themselves or that the platelets acted as nuclei for the precipitation or adsorption of calcium ions. Changes in platelet morphology do occur as one of the earliest changes after recalcification of plasma. A change in shape, formation of spiky pseudopods, and increase in volume resulting in the so called spiny spheres have been described by Zucker and Borrelli (1954) and White and Krivit (1967). An increase in platelet volume may therefore be the cause for the increase in optical density after recalcification.

There was no significant differences in the turbidimetric system with normal plasma in the mean times for platelet aggregation (onset of phase 5), where calcium alone was added (490 $\pm$ 63 seconds) as compared with the addition of calcium plus ADP (576 $\pm$ 87 seconds). In the Chandler tube system on the other hand ADP plus calcium produced significantly faster aggregation than calcium alone.

This discrepancy can possibly be explained by the difference in speed and axis of rotation and incubation temperature in the two systems. An experiment was conducted (see chapter 3) to study the effect of rotation and temperature on platelet aggregation and disaggregation in the turbidimetric system. It was found that if the speed of the rotator was increased in the turbidimetric system, earlier and more complete disaggregation was obtained, while if the speed was reduced less dissaggregation occurred.

It is possible that the low speed in the Chandler tube causes

platelets to aggregate and stay partially aggregated with the result that the second aggregation occurs earlier.

However in another experiment in the turbidimetric system (described in chapter 3) there was less aggregation and more disaggregation at 37°C than at room temperature. The full explanation of the difference in results obtained with the two systems is not apparent.

Calcium (6.25×10^{-3}M) produced no significant changes in the optical density in phase 1. Born (1962a) showed that when platelets are made to aggregate by calcium in the presence of heparin, the initial rate of ADP-platelet aggregation is proportional to the logarithm of the calcium concentration up to 1.7×10^{-3}M but at a higher concentration calcium chloride inhibited platelet aggregation. The calcium chloride concentration used in the present experiment was 6.25×10^{-3}M, that is, a concentration of calcium above the range at which it might be expected to enhance ADP induced aggregation.

When comparing the results of the calcium and calcium plus ADP experiments in normal controls, it was found that although there was no significant difference between the mean aggregation time (phase 5) and clotting time between the two sets of experiments, there was a significant difference between the duration of platelet aggregation in phase 5 in the two experimental groups, being longer in the calcium plus ADP experiments. A possible explanation is that when platelets aggregate and disaggregate in the calcium plus ADP system some of the added ADP may have been converted to AMP (Spaet and Lejnicks, 1966) which inhibits ADP (Born, 1962b; O'Brien, 1962a; Born and Cross, 1963a; O'Brien, 1963 and O'Brien, 1964).

The difference between the duration of platelet aggregation in calcium and calcium plus ADP experiments was also found in uraemic patients ($t = 2.987$, $0.01 > P > 0.001$). When the duration of phase 5 in the calcium plus ADP experiments in normal and uraemic patients

were compared in a normal dilution curve, taking into account the platelet count, it was found that out of 8 patients, 5 fell outside the 95 per cent confidence limit around the regression line (figure 16), in these patients the platelets took a longer time to aggregate than controls having the same platelet count. No such difference was obtained when calcium was used alone.

A possible explanation of this finding is that when ADP is added uraemic platelets aggregate releasing their own ADP in the process (McMillan, 1966). Thrombin brings about platelet aggregation by promoting the release of endogenous ADP from the platelets, the time taken for platelet aggregation is a function of both the extent of ADP release, and the inhibition of ADP by ADP inhibitors. Salzman and Nori (1966) found that the mean ATP content of uraemic platelets is significantly less than normal and hence presumably a low ADP availability. Uraemic platelets cannot release a normal amount of ADP in response to thrombin.

CHAPTER 5.

ADENOSINE DIPHOSPHATE INDUCED PLATELET AGGREGATION.

One of the major physiological functions of blood platelets is their ability to aggregate. Masses of aggregated platelets are a characteristic component of the thrombus or haemostatic plug formed either in vivo (Kjaerheim and Hovig, 1962; French et al., 1964) or in vitro (Poole, 1959; Dintenfass and Rozenberg, 1965). The most important discovery in the understanding of the role of platelets in haemostasis was that of Hellem (1960) who found a factor in erythrocytes which markedly increased the adhesiveness of platelets. This factor was later identified as adenosine diphosphate by Gaarder et al., (1961).

Earlier, Born (1956a) found that platelets contained a high concentration of ATP which disappeared during clotting (Born 1956b), or when the platelets underwent the changes known as viscous metamorphosis (Zucker and Borrelli, 1961). Since ADP is the first breakdown product of ATP it was suggested by Born (1962b) that the increase in adhesiveness brought about by ADP might underlie the physiological mechanism responsible for the formation of haemostatic plugs and be the pathological mechanism responsible for thrombosis.

When platelets are exposed to a variety of stimuli ranging from contact with foreign surface to proteolytic enzymes, there is a release of serotonin and histamine, a fall in platelet ATP and escape of nucleotides including ADP into the ambient fluid (Movat et al., 1965). Since all these stimuli will in appropriate circumstances clump platelets it seems likely that aggregation is produced by some substances liberated from the platelet itself.

Of the various possible stimuli the most impressive claims can be put forward for ADP (Zucker and Borrelli, 1961; Hovig, 1963; Spaet and Zucker, 1964; and Glynn et al., 1965). ADP was found to be able to produce platelet clumping in vivo (Born and Cross,

1963c; Regoli and Clark, 1963; Davey and Landler, 1964; Nordöy and Chandler, 1964) and in vitro (Ollgaard, 1961; Born, 1962b; O'Brien, 1962a; Zucker and Borrelli, 1962; Hellem et al., 1963; Mitchell and Sharp, 1964).

In addition to ADP, four other closely related nucleotides desoxyadenosine diphosphate, the 1-N-oxide of adenosine diphosphate, adenosine tetraphosphate and cordycepin diphosphate also bring about aggregation, but the potency of these is much less than that of ADP (Gaarder et al., 1961; Gaarder and Laland, 1964). Uridine diphosphate, cytidine diphosphate, inosine diphosphate can also cause platelet aggregation (Spaet and Zucker, 1964; Salzman, 1967).

The effect of ADP on platelets was found to be inhibited by adenosine monophosphate, by adenosine and by high concentrations of ATP (Born and Cross, 1963a; Clayton et al., 1963; Skalhegg et al., 1964; Born, 1964; Born et al., 1965). These substances also inhibit platelet aggregation in vivo (Regoli and Clark, 1963); the inhibitors may act by competing with ADP for sites on the platelet surface. Adrenaline, nor adrenaline and serotonin which are also carried by the platelet (Clayton et al., 1963) have been shown to produce clumping in certain circumstances (O'Brien, 1963; O'Brien, 1964; Mitchell and Sharp, 1964). It seems probable that the clumping action of these compounds is mediated through the release of ADP from the platelets themselves, since clumping by adrenaline and serotonin can be blocked by AMP (O'Brien, 1964).

Several authors (Käser-Glanzmann and Lüscher, 1962; Grette, 1962; Haslam, 1964) observed that thrombin can release ADP from platelets and suggested that this activity may be reponsible for the aggregating property of thrombin. However, Niewiarowski and Thomas (1966) have demonstrated that ADP and thrombin act synergistically and not additively in producing platelet aggregation.

Moreover, adherence of platelets to both collagen and latex particles produces aggregation through release of platelet ADP (Zucker and Borelli, 1962; Hovig, 1963; Glyn et al., 1965). In most of these reactions there is a fall in platelet ATP (Mustard et al., 1966) probably mediated through stimulation of adenosine triphosphatase activity leading to conversion of ATP to ADP. Since ADP is known to stimulate glycolysis and respiration within the cell (Chance and Williams, 1956) metabolic changes would be expected within the platelets under these circumstances. It has been shown that thrombin stimulates oxygen consumption by platelets (Hussain and Newcomb, 1964) and this may also be true for most of the other agents which induce platelet aggregation. The amount of ADP present in the platelet will be increased by conversion of ATP to ADP. The extent of platelet aggregation will presumably correlate with the amount of ADP escaping into the ambient fluid.

Haslam (1964) has shown that in the presence of an enzyme system which catalyses the phosphorylation of ADP to ATP, neither thrombin nor fatty acids were able to induce platelet aggregation.

Platelet aggregation by ADP is dependent on viable platelets, calcium (Born and Cross, 1963b; Hellem and Owren, 1964; Mitchell and Sharp, 1964) and on the presence of a plasma factor (Born and Cross, 1964). It is also temperature dependent (O'Brien, 1962a).

The identity of the plasma factor has been the subject of recent conflicting reports. Hellem and Owren (1964) suggested that the plasma factor is that entity missing in von Willebrand's disease but others (Salzman and Chambers, 1964; Cross, 1964) have found that ADP induced platelet aggregation proceeds normally in plasma from patients with established von Willebrand's disease. McLean et al., (1964) and Cross (1964) have indicated that fibrinogen may be the plasma co-factor and Caen (1965) has recently suggested that both

fibrinogen and the von Willebrand factors participate in ADP-induced platelet aggregation. Deykin et al. (1965) demonstrated that at least two plasma factors, fibrinogen and a heat stable plasma protein distinct from the anti-von Willebrand factor, participate in ADP-induced platelet aggregation in the presence of human plasma.

This platelet aggregation induced by ADP can therefore be inhibited by deficiency or inactivation of a component of the platelet, deficiency or inactivation of a plasma protein, lack or excess of ionic calcium, elimination of ADP by the action of enzymes, and by certain substances related to ADP, specifically its breakdown products as adenosine.

The action of AMP and adenosine is competitive, powerful and highly specific for these substances and few of their analogues . The high specificity of ADP in promoting aggregation, and the specificity of its closely related inhibitors, suggest that there are equally specific receptors for these substances probably on the platelet surface.

Many hypotheses have been put forward to explain the mechanism of action of ADP in inducing platelet aggregation. Born and Cross (1963b) suggested that ADP causes aggregation by becoming associated with aggregating sites on the surface of platelets. Owren (1963) proposed that ADP participates in a binding reaction together with calcium ions and also a plasma protein, probably von Willebrand's factor, resulting in a complex bridge between the platelets. This hypothesis has also been adopted by Skalhegg et al. (1964). Mitchell and Sharp (1964) stated that the clumping agents act at the platelet surface enabling intercellular bonds to be formed, perhaps using the divalent ions, calcium or magnesium, as linking groups.

Gaarder and Laland (1964) suggested that the adenosine part of the ADP molecule forms hydrogen bond with suitably located -CO- and NH- groups present in proteins located on the surface of platelets. The importance of hydrogen bonding in the ADP-platelet aggregation was suggested by the finding of Hellem et al. (1964) that urea, which splits hydrogen bonds, inhibits platelet aggregation. Of the known inhibitors, 2-oxy-6-amino purine riboside diphosphopyridine nucleotide, AMP, ATP, 6-methyl amino purine riboside, adenosine and flavin adenine dinucleotide (Clayton et al., 1963) all can form hydrogen bonds in an identical manner to that of ADP on the platelet surface and thereby compete with ADP for active sites. The nucleotides known to bring about aggregation have at physiological pH an uneven number of negative charges. Inhibitors on the other hand have either no charge e.g. adenosine, or an even number of charges. Calcium which is required for the aggregation of platelets, is attracted by ionic forces to the negatively charged groups of ADP. Due to the double charge of calcium ions, nucleotides with an uneven number of negative charges would, when attached to the platelets, provide more opportunities for calcium ion bridges between platelets than nucleotides with an even number of charges, thereby promoting aggregation. Another hypothesis (Born, 1967a; Born, 1967b) is that bombardment of ADP molecules produces a configurational change in another component (a protein on platelet surface) and that this change is required for the formation of disulphide bonds between the platelets and molecules of the protein co-factor in the plasma. Evidence in favour of this hypothesis is that in the presence of ADP, platelets swell before they aggregate, that there is minimal or no binding of ADP to platelets, and that aggregation but not swelling is inhibited not only by substances which block sulphydyl groups but also by others such as dimercaprol dithiothreitol which bind to prevent sulphydyl group from reacting.

Salzman et al. (1966) proposed that platelet aggregation might

result from the inhibition by ADP of an enzyme in the platelet membrane whose normal function is the dephosphorylation of ATP to ADP. This enzyme utilizes the energy of hydrolysis of the terminal phosphate bond for maintenance of the unsticky state of the platelet through and active process analogous to the contraction of striated muscle. According to this hypothesis, inhibition of membrane adenosine triphosphatase would allow the platelet to become adhesive perhaps by relaxation of a contractile protein involved in the preservation of platelet size and shape. Exposure of adhesive sites on the platelet surface would permit bridging to adjacent platelets through a metal-mediated intermediate most likely composed of fibrinogen or other plasma proteins and calcium. In support of this hypothesis, Salzman et al. (1966) demonstrated a potent adenosine triphosphatase in the platelet surface membrane capable of splitting ATP on the outer surface of the intact platelet with resultant formation of ADP and inorganic phosphate. This enzyme is found to be distinct from the sodium-pump adenosine triphosphatase which is thought to split ATP on the inner surface of the cell membrane. The activity of the ecto-adenosine triphosphatase is inhibited by specific antisera to thrombosthenin suggesting a possible identity with the ecto-adenosine triphosphatase. Spaet and Lejnicks (1966) proposed an energy yielding reaction involving breakdown of ADP as the cause of platelet aggregation.

It has been found that under appropriate circumstances ADP-induced platelet aggregation in vitro is reversible (Born, 1962b; O'Brien, 1962b; Born and Cross, 1963b). Reversibility is greatest at 37°C and does not occur at 0°C (O'Brien, 1962b). In vivo, intravenous infusion of ADP to animals and man produces a fall in platelet count which rapidly reverses when the infusion is stopped (Born and Cross, 1963a; Nordoy and Chandler, 1964; Davey and Lander, 1964). Such spontaneous disaggregation is probably due to destruction of ADP by enzymes in plasma. In vitro destruction of

ADP by plasma is not observed in plasma which has been heated to 58°C for 15 minutes (Hellem and Owren, 1964).

Salzman et al. (1966) supports the view that disaggregation following aggregation produced by ADP results from degradation of ADP which allows membrane adenosine triphosphatase activity to resume. Increasing temperature favours disaggregation (O'Brien, 1962b) by favouring dephosphorylation of ADP of plasma enzymes and by increasing the membrane adenosine triphosphatase activity.

In this chapter are described the results of ADP-induced platelet aggregation in normal controls and uraemic patients.

RESULTS

Normal Controls

ADP-induced platelet aggregation was examined by the turbidimetric method (Born, 1962b; O'Brien, 1962b). The details of the method and parameters used for measuring platelet aggregation and disaggregation were described in Chapter 3. Platelet-rich plasma from nineteen normal healthy controls (eighteen males and one female) were examined; the age range was from 18 to 50 years. An early objective was to study the effect of differing platelet counts in platelet-rich plasma on ADP-platelet aggregation; uraemic patients may have reduced platelet counts. To allow for comparison between normal controls and uraemic patients, some experiments were performed by diluting normal platelet-rich plasma with platelet-poor plasma. In all experiments the same quantity of ADP was added regardless of the platelet count. A total of thirty-five experiments were performed.

The results of normal controls were grouped in four ranges of platelet counts in platelet-rich plasma.

Group 1 between 350,000 and 450,000/c.mm.

Group 2 between 250,000 and 350,000/c.mm.
Group 3 between 150,000 and 250,000/c.mm.
Group 4 between 50,000 and 150,000/c.mm.

Results of the undiluted normal controls.

The mean platelet-rich plasma platelet count in the 19 normal controls examined was 359.447 ± 50.300/c.mm.

The results of ADP-induced platelet aggregion are shown in table 11 and the optical density readings in table 24.

There was no correlation between the platelet aggregation as measured by the 30-60, 30-90 and maximum aggregation and the platelet count ($r = 0.357$, $P > 0.1$; $r = 0.272$, $P > 0.1$; $r = 0.252$, $P > 0.1$) respectively. However, there was a significant correlation between the platelet count and percentage disaggregation ($r = 0.456$, $0.05 > P > 0.02$).

Results in the diluted and undiluted normal control.

The results were grouped in four ranges of platelet-rich plasma platelet count, as shown in tables 12, 13, 14 and 15. There was a significant correlation of the platelet counts in the platelet-rich plasma with all the parameters used to measure platelet aggregation and disaggregation. The 30-60 platelet aggregation, $r = 0.853$, $P < 0.001$, (figure 23,) 30-90 platelet aggregation $r = 0.805$, $P < 0.001$, maximum aggregation, $r = 0.813$, $P < 0.001$, percentage disaggregation $r = 0.590$, $P < 0.001$ (figure 24).

There was also a significant correlation between 30-60 platelet aggregation and percentage disaggregation ($r = 0.503$, $0.01 > P > 0.001$) i.e. the more aggregation the greater the disaggregation (figure 25).

Results in uraemic patients.

Thirty nine uraemic patients were included in this study. Patients Nos. 3, 6, 7, 14, 15 and 28 were examined for ADP-platelet

aggregation more than once during the course of their illness. Patient No. 29 was also examined after diluting her platelet-rich plasma with platelet-poor plasma. A total of 46 experiments were carried out.

Of these patients, 30 were suffering from chronic renal failure. Thirteen of these patients were examined at a time when the uraemic process was fairly stable, and ten when there was an acute exacerbation resulting either from infection or post renal obstruction. Seven chronic uraemic patients were under treatment of haemodialysis.

Nine patients had acute renal failure, seven of whom were given haemodialysis treatment.

Eleven patients were bleeding at the time of study and these included 5 patients with chronic renal failure, and 6 with acute uraemia.

The results of thirty nine patients with uraemia were grouped in four ranges of platelet-rich plasma platelet count regardless of the aetiology, the rate of development of the uraemic process or the blood urea levels (tables, 16, 17, 18 and 19). The mean results of platelet aggregation and disaggregation were compared with those of normal controls, having the same range of platelet-rich plasma platelet count.

Results showed that platelet aggregation and disaggregation were significantly different from normal controls in all four ranges of platelet count (table 22). The most significant and constant differences were found in the 30-60 platelet aggregation, percentage disaggregation, and the disaggregation time as this occurred in all four ranges of platelet-rich plasma counts. However the rest of the parameters (30-90 platelet aggregation, maximum aggregation and maximum aggregation time) were significantly different in some but not all the ranges.

Uraemic patients were divided into four groups.

1. chronic renal failure
2. acute renal failure
3. chronic renal failure on haemodialysis
4. uraemic patients with clinical haemostatic failure.

The results of platelet aggregation and disaggregation from individual experiments (first experiment if more than one was performed on the same patient) were compared with the normal mean values of the same platelet count $\pm$ 2 standard deviations. Any result falling outside the normal range was considered abnormal. The percentage abnormality in the four groups of uraemic patients are shown in table 40. Analysis of the results have shown that the highest percentage diminution in platelet aggregation occurred in patients with bleeding, and was lowest in chronic uraemia patients on haemodialysis and patients with acute renal failure. The disaggregation time was prolonged in 91 per cent of patients with clinical haemostatic failure. The diminution in percentage disaggregation was similar in all four groups but was slightly lower in chronic renal failure patients on haemodialysis.

Chronic Renal Failure patients on Haemodialysis.

Seven patients suffering from terminal chronic renal failure were examined for ADP-platelet aggregation before and after haemodialysis.

There was no significant difference between the platelet count before and after dialysis ($t = 0.136$, $P > 0.1$), the mean count after dialysis being slightly higher. There was an increase in platelet count in four, and a drop in three patients following the dialysis.

To compare the mean pre-and post-dialysis results with the mean values of normal controls, it was found that both their platelet

counts fell in the platelet-rich plasma range of 250,000 - 350,000/c.mm. The two mean values were not significantly different from the normal mean of this range (before dialysis, $t = 1.557$, $P > 0.1$, after dialysis, $t = 1.988$ $0.10 > P > 0.05$). This allowed comparison to be made between the two mean values (i.e., before and after dialysis): also comparison was possible between them and the normal means of that range. When the mean values of pre-and post-dialysis results were compared, it was found that there was no significant change in aggregation, although an increase in aggregation did occur in six out of seven cases, the only case which showed a drop in platelet aggregation also showed an appreciable drop in platelet-rich plasma platelet count after dialysis. However there was a significant increase in percentage disaggregation after dialysis ($t = 4.489$, $P < 0.001$), and this increase was a constant finding in all seven cases.

The 30-60 platelet aggregation before dialysis was significantly different from the normal mean ($t = 3.145$, $0.01 > P > 0.001$), but became very near the normal mean after dialysis ($t = 0.715$, $P > 0.1$). This was also true for the percentage disaggregation (before dialysis, $t = 3.319$, $0.01 > P > 0.001$, after dialysis, $t = 0.318$, $P > 0.1$).

When the results of the pre- and post-dialysis were correlated with the corresponding blood urea levels in mg. per 100 ml, a significant correlation existed between blood urea and 30-60 platelet aggregation ($t = 0.564$, $0.05 > P > 0.02$) (figure 30) while it was insignificant with other parameters of platelet aggregation and disaggregation.

Acute Renal Failure patients on Haemodialysis.

Seven patients with acute renal failure were examined for ADP-induced platelet aggregation. Platelet aggregation increased in 4 patients and diminished in three following dialysis, while percentage disaggregation increased in 5 and diminished in two (figures 26, 27)

The mean platelet count did not change significantly after dialysis ($t = 0.404$, $P > 0.1$). Being a markedly heterogenous group as regards the platelet count, it was not possible to compare the mean pre- and post-dialysis results with the mean normal values. Therefore the results of each experiment were compared with the means of normal controls of the same platelet count $\pm$ 2 standard deviations. From the results shown in figure 28 no consistent improvement was obtained in aggregation and disaggregation.

No correlation existed between the blood urea levels and the results of platelet aggregation and disaggregation, nor between these and the platelet count.

DISCUSSION.

The degree and reversibility of ADP-induced platelet aggregation in vitro depends on the concentration of ADP present (Born, 1962 a and b; Born and Cross, 1963b; O'Brien, 1964), the number of platelets present in the platelet-rich plasma (O'Brien 1962b, Born and Cross, 1963b), the stirring speed (O'Brien, 1962b; Born and Cross, 1963b) and on the temperature of incubation (O'Brien, 1962b).

The effects of the stirring speed, the temperature of incubation and the ADP-concentrations on the results were eliminated by using a fixed speed, a constant incubation temperature and the same dose of ADP in all experiments performed. The only variable was the platelet-rich plasma platelet count.

From the results presented above in undiluted normal control plasmas there appears to be no correlation between the ADP-induced platelet aggregation, and the platelet count of platelet-rich plasma, but such a correlation existed between percentage disaggregation and the platelet count. In these undiluted normal controls, the mean platelet count was 369,477/c.mm. When samples from normal controls

were diluted with platelet-poor plasma to determine the ADP-platelet aggregation in a wide range of platelet count, platelet aggregation, percentage disaggregation and the disaggregation time were significantly correlated with the platelet-rich plasma platelet count.

It seems when using a fixed dose of ADP to aggregate platelets then the more platelets present in the plasma, the larger clumps will form and the greater the optical density will fall. In the presence of a smaller number of platelets and a relatively higher concentration of ADP, the platelet aggregates will be smaller and the optical density fall will be less. As platelets aggregate under the influence of ADP there is also simultaneous dephosphorylation of ADP by plasma and platelet enzymes (Jörgensen, 1956; Ollgaard, 1961; O'Brien, 1962a; Spaet and Lejniecks, 1966) resulting in the formation of AMP and adenosine which are known inhibitors to ADP aggregating effect. The point of maximum aggregation (maximum aggregation time), can be considered as the time at which there is a balance between ADP and inhibitors in the system. When this balance is offset towards more inhibitor formation or the presence of less ADP, platelets begin to disaggregate (disaggregation time). By using a fixed concentration of ADP, then the less platelets present in the system, the longer the time taken by them to reach maximum aggregation, the longer time they take to begin disaggregation, and their disaggregation will be less complete compared to that of a larger number of platelets. The presence of a correlation between platelet aggregation and disaggregation, and their correlation with the platelet count in the diluted group can be explained by this mechanism.

In the comparison of ADP-platelet aggregation in uraemic patients with normal controls, all were subjected to the same ADP-concentration regardless of their platelet count. The results were then grouped in four ranges of platelet count and the mean values

of normals and uraemics were compared. Other authors (Rozenberg and Firkin, 1966; Castaldi et al., 1966; Stewart and Castaldi, 1967) adjusted the platelet count of platelet-rich plasma of normal controls and uraemic patients to 200,000 - 250,000/c.mm. and thus were unable to establish the results in these patients with lower counts. In the series studied in this thesis, patients with a platelet count below 200,000/c.mm. constituted 28% of the total number of observations and included the majority of patients with thrombocytopenia and bleeding.

Platelet Aggregation.

When comparing the mean results of uraemic patients with those of normal controls, it was found that there was a significant difference between the two means in the 30-60 platelet aggregation, percentage disaggregation and the disaggregation time, in all four ranges of platelet count. This shows that when mean results are considered uraemic platelets aggregate less, take a longer time to disaggregate and fail to disaggregate in a normal way. When each observation was compared to a normal range with confidence limits of 95 per cent ($\pm$ 2 standard deviations of the mean), 22 out of 46 experiments (i.e. 48%) showed diminished 30-60 platelet aggregation. These results are similar to the findings of Rozenberg and Firkin (1966) who found impaired platelet aggregation in 5 out of 12 patients without bleeding tendency, and grossly depressed or absent platelet aggregation in 7 patients with established bleeding: of 19 patients, 12 had diminished platelet aggregation (i.e. 53 per cent). Salzman and Nevi (1966) on the other hand found platelet aggregation with ADP to be normal in all five patients examined by them. This was also the finding of O'Brien (1967) that ADP-induced platelet aggregation proceeded at a normal rate in uraemia.

Traeger et al. (1967) used the method of Reval (1954) to measure platelet aggregation and adhesiveness by the phase contract

microscope. They found improvement in platelet aggregation in 11 out of 15 chronic uraemic patients following haemodialysis.

Gan and Firkin (1968) found a correlation between platelet aggregation and the plasma enzyme'(s) ability to degrade ADP. This plasma activity was shown to be reduced in six patients with uraemia in whom platelet aggregation was impaired, but not in two cases whose platelet aggregation was normal. The incorporation of 14C labelled ADP-8-^{14}C was also reduced in the uraemic patients with abnormal platelet aggregation.

Platelet Disaggregation

Percentage disaggregation was found to be diminished in 28 out of 46 observations (60.9 per cent) and disaggregation time to be prolonged in 28 out of 46 (60.9 per cent). This is in accordance with the observation of O'Brien (1967) that the time till disaggregation was probably prolonged.

Comparison between acute and chronic renal failure patient

To make a comparison between the results of acute and chronic renal failure, the first experiments carried out in each patient were grouped according to rate of development of the uraemic process. Of the 39 patients, 30 were suffering from chronic renal failure and 9 from acute uraemia. Three cases had post-renal uraemia. 30-60 platelet aggregation was diminished in 53 per cent of patients in the chronic group and in 33 per cent in acute. However, disaggregation was diminished or absent in 67 per cent of acute uraemic patients in 63 per cent of these with chronic renal failure. These results show that although there was no difference between the acute and chronic uraemics as regards platelet disaggregation, there were less cases with diminished platelet aggregation in the acute group of patients. (Table 40).

Effect of Haemodialysis

The effect of treatment by haemodialysis on patients with acute and chronic renal failure was assessed. The two groups of patients

were studied separately because of the difference in aetiology, the duration of the uraemic process, the type of artificial kidney and the duration of haemodialysis. There was a fixed time at which the post-dialysis sample was collected in each group. Stewart and Castaldi (1967) grouped all patients with acute and chronic renal failure together regardless of whether they received peritoneal or haemodialysis treatment, and there was no fixed time at which the post-dialysis sample was taken, this varied from one to six days.

In the chronic renal failure group, although an increase in platelet aggregation was obtained in six out of seven patients after dialysis, this increase was not statistically significant. However, a significant improvement did occur in percentage disaggregation following dialysis ($t = 4.489$, $P < 0.001$). When compared with normal controls of the same range of platelet count, there was a definite improvement in both aggregation and disaggregation after dialysis.

No significant change occurred in the platelet count or in the whole blood platelet count after dialysis ($t = 0.136$, $P > 0.1$; $t = 0.199$, $P > 0.1$ respectively). This was not the finding of Stewart and Castaldi (1967) who reported a significant increase in platelet count following dialysis in the patients studied by them whether treated by peritoneal or haemodialysis, and for the patient treated by haemodialysis only. These findings may be explained by the fact that in this group of patients, the post-dialysis sample was not taken at a fixed time after the end of dialysis but varied from one to six days and it is probable that the more time allowed between the end of dialysis and the collection of the post-dialysis sample the greater the possibility of quantitative as well as qualitative improvement in platelets.

In the acute uraemic group of patients, there was a great variability in platelet count in different patients and also between the pre- and post-dialysis samples in individual patients. Therefore

it became impossible to consider these cases as a uniform group as in the chronic uraemic patients. Thus the results of each experiment were compared separately with the mean results of normal controls. From the results, there appears to be no consistent change in ADP-platelet aggregation in cases of acute renal failure as a consequence of treatment by haemodialysis. This is probably due to the presence of different added aetiological factors other than the uraemic process, the rate of development and duration of the uraemic process, and the type of artifical kidney used. The platelet count did not change significantly after dialysis ($t = 0.393$, $P > 0.1$); the mean was slightly lower after the dialysis. No significant change occurred also in the citrated blood count ($t = 0.455$, $P > 0.1$).

Uraemic Patients with Clinical Haemostatic Failure.

Eleven patients with bleeding due to haemostatic failure were included in this study (the bleeding group). Six patients were suffering from acute renal failure and five had chronic uraemia, two of whom were having an acute exacerbation of their uraemic process. Thrombocytopenia (below 100,000/c.mm.) was present in 4 patients. Seven patients showed diminished or absent 30-60 platelet aggregation and seven diminished or absent disaggregation. The bleeding group showed the highest abnormality in platelet aggregation and disaggregation time (table 40). Castaldi et al. (1966) found that every patient with an established bleeding tendency and a long bleeding time had grossly depressed or absent platelet aggregation with ADP whatever their serum urea concentration.

Although a significant correlation existed between the blood urea level and the 30-60" platelet aggregation in the chronic haemodialysis patients ($t = 0.574$, $0.05 > P > 0.02$), such a correlation did not exist with percentage disaggregation or with any parameter of aggregation and disaggregation in the acute renal failure groups before and after dialysis.

In summary, there seems to be a tendency for platelets to aggregate and disaggregate, less than normal in cases of uraemia regardless of the progress and duration of the uraemic process. This qualitative defect was corrected by haemodialysis in the chronic uraemic group but not in the acute uraemic patients. Platelet aggregation was lowest in the bleeding group, and also the disaggregation times were longest (table 40).

Mechanism of disordered platelet aggregation and disaggregation

The defective aggregation and disaggregation in cases of renal failure may be explained on the basis of the mechanism and hypotheses of ADP-platelet aggregation mentioned above and disorders of these from the biochemical changes that occur in uraemia. The high blood urea level may have a role in causing this defect. According to Gaarder and Laland (1964) the adenosine part of ADP-molecule forms hydrogen bonds with similarly located-CO-and NH-groups present in proteins located on the surface of platelets. Urea is known to break hydrogen bonds (Hellem et al., 1964) and this might interfere with platelet aggregation in this way.

The osmotic effect of urea on platelets in combination with the electrolyte changes and the excessive endogenous protein catabolism known to occur in uraemia, may result in shrinkage in platelet size thus probably causing less aggregating sites to be available for ADP. Urea in vitro was shown to cause diminished ADP-aggregation and disaggregation (Chapter 8) and this has also been reported by Somer et al. (1968). These in vitro experiments may support the view that urea could be responsible, at least in part, for the changes that occur in uraemic patients. Lack or excess of calcium ions is one of the factors responsible for inhibition of ADP-platelet aggregation. In uraemia, although hypocalcaemia may be present (Fanconi and Rose, 1958) the uraemic patient is protected from tetany by the presence of acidosis, since higher proportion of total calcium is ionized at low plasma pH. Plasma levels of citrate have been reported to be

elevated in renal failure (Black, 1967), leading to an increase in the complexed fraction of plasma calcium and lowering of the amount ionized. Nordmann, et al. (1959) has questioned this, since they found that the methods used were not specific to citrate but subject to interference by ketoacids, and the plasma citrate concentration in their patients was normal.

The ATP content of platelets from patients with uraemia has been reported by Salzman and Neri (1966) to be lower than normal. However, Pitney et al. (1968) found that the adenine nucleotide content of platelets from uraemic subjects did not differ from normal. The filtered uraemic samples did not show the increase in nucleotide content recorded for the normal sample, indicating either lack of heterogenesity of adenine nucleotide content of platelet from cases of uraemic or inability of such platelets to adsorb nucleotides liberated during the passage of blood through glass filters. From this evidence it is probable that uraemic platelets are unable to adsorb or react with the ADP added in a normal way. The intracellular enzymes concerned with the formation or inactivation of ADP may be inhibited as a result of intracellular electrolyte changes. As regards inability to disaggregate this may be due to membrane changes or intracellular enzyme change causing less production of inhibitors. According to Salzman et al. (1967) disaggregation following ADP aggregation results from degradation of ADP which allows membrane adenosine triphosphatase to resume its action in maintaining platelets discrete.

CHAPTER 6.

PLATELET ADHESIVENESS TO GLASS SURFACES.

In addition to their contribution to the coagulation mechanism, platelets also participate in haemostasis by adhering to injured vascular endothelium and to one another, forming a haemostatic plug (Roskam et al., 1959; Sharp, 1961; Käser-Glanzmann and Lüscher, 1962). The formation of a haemostatic plug seems to occur in stages, an early phase of aggregation not requiring thrombin which can be reversed by decalcification, (Sharp, 1961), and a subsequent fusion of the aggregated platelets to form an irreversible amorphous mass (viscous metamorphosis). The latter stage may be mediated by the coagulation mechanism perhaps by the action of thrombin (Zucker and Borrelli, 1959; Schmid et al., 1962; Spaet and Zucker, 1964).

Attempts have been made to measure the in vivo property of platelet adhesion by the use of in vitro tests of platelet adhesiveness to foreign surfaces especially to glass, although there is lack of agreement that in vitro tests of adhesiveness accurately measure in vivo platelet behaviour.

Platelet adhesiveness measured as the adhesion of platelets to glass, is expressed as either the absolute number, or more commonly the percentage of platelets, which stick to glass surfaces by comparing the platelet counts before and after exposure of the specimen to glass surfaces. Although many methods have been described in the past (Chapter 3), the most important and more accurate methods were those which determined the per cent of platelet sticking to the wall of a rotating flask (Wright, 1941) to a glass wool column (Moolten and Vroman, 1949), and to a column of glass beads (Hellem, 1960; O'Brien, 1961; Salzman, 1963). These tests depend not only on platelet-glass adhesion, but also on platelet-platelet aggregation, since it appears that the bulk of platelets retained in the filter are in contact with other platelets rather than with glass (Wright, 1941; Salzman, 1963).

In 1960, Borchgrevink described an in vivo method for platelet adhesiveness, based on comparison of venous blood platelet counts with those in a capillary blood sample from a modified bleeding time incision. The difference between the two counts represents the platelets adherent to the wound surface.

The mechanism of platelet adhesiveness was first explained by Hellem (1960, 1964) and Gaarder et al. (1961). Hellem observed that although about one half of the platelets in citrated blood adhered to a column of glass beads, no platelet adhesion occurred when platelet-rich plasma was used instead of whole blood. This observation was also reported by Honorato et al. (1961). Adhesiveness was found to vary according to haematocrit (Hellem 1960; Hirsh et al., 1966a, McClure et al., 1966). Hellem isolated a "factor R" from boiled red cells which was found to cause platelet aggregation. This factor was identified by Gaarder et al. as ADP and was thought to be released from the erythrocytes during passage of blood through the column.

Of the three glass-bead column methods, the Hellem's technique as modified by Hirsh et al. (1966a) was used to study the platelet adhesiveness in normal controls and uraemic patients. The details of the method used were described in Chapter 3.

RESULTS

Results in Normal Controls

Results without correction for packed red cell volume

Twenty-seven normal healthy controls, 4 of whom were females, were examined for platelet adhesiveness to glass by Hellem's technique as modified by Hirsh et al. (1966a). The mean normal percentage platelet adhesiveness was 50.2 $\pm$ 7.81 ($\pm$ 2 standard deviations: 34.5 - 65.9), and the mean percentage packed cell volume was 40.6 $\pm$ 3.1 (table 41). The mean platelet count in citrated blood was 194,379 $\pm$ 45,000/c.mm. ($\pm$ 2 standard deviations 102,379 - 286,379/c.mm.)

A significant correlation existed between the packed cell volume and platelet adhesiveness ($r = 0.651$, $P < 0.001$), (figure 32). No correlation was found between the percentage adhesiveness and the platelet count in citrated blood ($r = 0.231$, $P > 0.1$).

Relationship between platelet adhesiveness and ADP platelet aggregation and disaggregation.

Platelet adhesiveness was assessed in 17 normal controls together with ADP platelet aggregation in an attempt to find a relationship between the two tests. No correlation existed between platelet aggregation as measured by 30-60, 30-90, maximum aggregation and the percentage adhesiveness ($r = 0.0317$, $P > 0.1$, $r = -0.014$, $P > 0.1$, $r = 0.056$, $P > 0.1$ respectively). However, there was a significant correlation between percentage disaggregation and percentage adhesiveness ($r = 0.549$, $0.05 > P > 0.02$) (table 42, figure 35). The mean platelet count was 365,970 ± 62,690/c.mm.

Results in Uraemic Patients.

Thirty-nine patients suffering from renal failure were examined for platelet adhesiveness, 30 of these were suffering from chronic renal failure, 9 had acute renal failure (table 43). The mean value for platelet adhesiveness was 30.1 ± 12.2 per cent the mean packed cell volume was 26.64 ± 7.75 per cent, and the mean platelet count in citrated blood was 159,936 ± 72,211/c.mm. No correlation existed between the percentage adhesiveness and the percentage packed cell volume ($r = 0.310$, $0.1 > P > 0.05$), nor between the adhesiveness and the platelet count in citrated blood ($r = 0.161$, P 0.1).

The mean values of platelet adhesiveness in acute and chronic renal failure were 31.5 ± 15.6 per cent and 29.7 ± 11.3 per cent respectively, showing no significant difference ($t = 0.382$, $P > 0.1$). Eleven uraemic patients had a clinical haemostatic failure at the time of the study. Their mean percentage platelet adhesiveness was 25.9 ± 16.4 and was not significantly different from the mean value

of the rest of the uraemic patients ($t = 1.33$, $P > 0.1$).

Chronic Renal Failure patients on Haemodialysis.

Seven patients suffering from terminal chronic uraemia were examined before and after dialysis treatment (table 46). The mean platelet adhesiveness before dialysis was 30.2 per cent and after dialysis 42.1 per cent, showing a significant increase ($t = 2.831$, $0.02 > P > 0.01$). This increase in platelet adhesiveness after dialysis was a consistent finding in all 7 patients examined (figure 37). The packed cell volume did not significantly change after the end of dialysis ($t = 1.104$, $P > 0.1$). The results of platelet adhesiveness before and after dialysis were significantly correlated with their corresponding blood urea levels ($r = -0.636$, $0.02 > P > 0.01$) (table 46, figure 38).

Acute Renal Failure patients on Haemodialysis.

Seven patients with acute renal failure were examined for platelet adhesiveness (table 47).

The mean percentage platelet adhesiveness before dialysis was 32.9 ± 16.3 per cent and after dialysis 22.9 ± 19.9 per cent. There was an increased adhesiveness in 3 and a fall in 4 patients following dialysis (figure 37). No significant change occurred in adhesiveness after dialysis ($t = 0.994$, $P > 0.1$) and in this respect these patients differed from those with chronic renal failure. The mean packed cell volume did not change significantly, being 29.5 per cent before and 30 per cent after dialysis ($t = 0.102$, $P > 0.1$). No correlation existed between the blood urea levels and percentage adhesiveness ($r = 0.055$, $P > 0.1$).

Comparison between normal controls and uraemic patients.

Of the 39 patients examined, 26 showed diminished platelet adhesiveness (67 per cent) when compared to the normal range ± 2 standard deviations. When the mean percentage platelet adhesiveness

of normal controls and uraemic patients were compared, the mean value of uraemic patients was significantly lower than the mean of normal controls ($t = 7.483$, $P < 0.001$, figure 31).

The mean values for platelet adhesiveness in acute and chronic uraemic patients were also significantly lower than the normal value ($t = 4.766$, $P < 0.001$; $t = 7.739$, $P < 0.001$ respectively).

Of the 11 uraemic patients with bleeding, 8 showed diminished platelet adhesiveness (73 per cent) when compared to the normal mean value $\pm$ 2 standard deviations. When the mean percentage platelet adhesiveness of the uraemic patients with bleeding and normal controls, were compared the mean value of the patients with bleeding was significantly lower than that of normal controls ($t = 6.214$, $P < 0.001$).

The mean values for platelet adhesiveness of pre- and post-dialysis in chronic renal failure patients were significantly lower than the mean normal value ($t = 5.725$, $P < 0.001$; $t = 2.552$, $0.02 > P > 0.01$ respectively). The same was true for acute renal failure patients treated by haemodialysis (pre-dialysis $t = 4.154$, $P < 0.001$, post-dialysis, $t = 5.767$, $P < 0.001$).

Relationship between Platelet Adhesiveness and Percentage Disaggregation in Uraemic Patients.

Percentage platelet disaggregation was found to be dependent on the platelet count (Chapter 5). The mean platelet count of normal controls used to demonstrate the relationship between percentage platelet disaggregation and percentage platelet adhesiveness was 365,970 $\pm$ 62,690/c.mm. To show such a relationship in uraemic patients, the results having a platelet count within the normal range $\pm$ 2 standard deviations were chosen. Fourteen out of 18 patients (78 per cent) showed an abnormal platelet adhesiveness-disaggregation relationship i.e. a decrease in either adhesiveness or disaggregation or both (table 44, figure 35). The four patients who showed normal relationship included two suffering from chronic renal failure but with a relatively low blood urea level (in fact the two patients in the group

of chronic uraemics with the lowest urea levels, 106 mg per 100 ml., and 85 mg per 100 ml). The other two patients were suffering from renal failure resulting from post-renal obstruction.

Results after correction for Packed Red Cell Volume

By changing the packed cell volume using various concentrations of whole citrated blood and platelet-poor plasma, a curve was constructed in which a significant correlation existed between the percentage packed cell volume and the percentage platelet adhesiveness (figure 33). A correction factor of 1:1.8 was found between the two variables, i.e. for every 1 per cent reduction in packed cell, the platelet adhesiveness was increased by 1.8 per cent. Normal results in the citrated blood showed a mean value of 40.6 per cent for the packed cell volume. Results of platelet adhesiveness obtained from normal controls and uraemic patients were corrected to this level either by increasing or decreasing the percentage adhesiveness.

The mean normal value for platelet adhesiveness after correcting for the packed cell volume was 50.2 $\pm$ 6.95 per cent ($\pm$ 2 standard deviations 38.2 - 62.1), while that of the whole group of uraemic patients was 55.3 $\pm$ 15.9 per cent, the difference between the 2 groups was insignificant ($t = 1.630$, $P > 0.1$, figure 34). The same was also true for acute and chronic uraemic patients ($t = 1.509$, $P > 0.1$; $t = 1.528$, $P > 0.1$ respectively). The negative correlation that existed between percentage platelet adhesiveness and percentage platelet disaggregation became more significant after correcting the results of platelet adhesiveness for PCV ($r = -0.611$, $0.01 > P > 0.001$). This relationship is shown in table 42, figure 36.

When the results of individual uraemic patients were compared to the normal range of platelet adhesiveness (mean $\pm$ 2 standard deviations), 19 patients showed normal results, 14 were increased and only in 6 the platelet adhesiveness was diminished. Uraemic patients

with bleeding had a mean platelet adhesiveness of 52.9 per cent, and showed no significant difference from the mean normal platelet adhesiveness ($t = 0.711$, $P > 0.1$), or from the rest of the uraemic patients ($t = 0.431$, $P > 0.1$). Of the 11 patients with bleeding 7 showed normal results, 2 were diminished and 2 had increased platelet adhesiveness. Chronic renal failure patients under haemodialysis treatment had a mean corrected platelet adhesiveness of 65.6 ± 9.2 per cent before dialysis, and 70.7 ± 7.6 per cent after dialysis ($t = 1.151$, $P > 0.1$); both were significantly higher than the mean of normal controls (before dialysis: $t = 5.399$, $P < 0.001$; after dialysis: $t = 7.660$, $P < 0.001$).

The mean values of pre-and post-dialysis in chronic uraemic patients were compared to the rest of the uraemic group excluding the chronic dialysis patients mean (53.34 ± 16.15 per cent). The mean pre-dialysis results showed no difference from the rest of the uraemic group ($t = 1.908$; $0.1 > P > 0.05$), while the post-dialysis mean showed a significant increase ($t = 2.749$, $0.01 > P > 0.001$). The significant negative correlation that existed between percentage platelet adhesiveness and the blood urea levels before and after haemodialysis became insignificant after correction for PCV ($t = 0.0419$, $P > 0.1$). The mean values of pre- and post-dialysis on the acute uraemic patients undergoing haemodialysis treatment was 51.9 per cent $\pm$ 14.61 before dialysis and 47.5 per cent $\pm$ 15.8 after dialysis thus showing no significant change ($t = 0.951$, $P > 0.1$); furthermore no difference was found between the mean normal and the acute uraemic patient either before or after dialysis (pre-dialysis value $t = 0.505$, $P > 0.1$ and post-dialysis value $t = 0.687$, $P > 0.1$).

DISCUSSION

Platelet adhesiveness to glass appears to be mediated by the release of ADP from red cells during their passage through the glass bead column (Hellem 1960, 1964; Gaarder et al., 1961), a process which

is followed by aggregation between the platelets themselves (Wright 1941; Salzman 1963).

An important factor influencing platelet adhesiveness in the glass bead column method is the packed red cell volume (PCV). A positive correlation between platelet adhesiveness and the PCV was found both in normal controls ($t = 0.651$, $P < 0.001$) (figure 32) and also where the PCV was modified in vitro using various concentrations of citrated blood and platelet-poor plasma ($t = 0.989$, $P < 0.001$) (figure 33). This correlation has previously been reported by Hellem, 1960; Hirsh et al., 1966; McClure et al., 1966. Platelet adhesiveness was also found to be negatively correlated with the ability of platelets to disaggregate following the addition of ADP in the turbidimetric system ($r = 0.549$, $0.05 > P > 0.02$) i.e. the more disaggregation, the less the adhesiveness. As ADP released from the red cells is the factor responsible for platelet adhesiveness, and as platelet-to-platelet aggregation is part of the process of adhesion, it is possible that some platelets aggregate primarily then disaggregate as ADP released from the red cells is converted to AMP; platelets after disaggregation may then pass through the column without aggregating again.

The results of studies of platelet adhesiveness in uraemic patients, when no correction was made for variation in the packed cell volume, have shown that the mean platelet adhesiveness in the whole group was 30.2 ± 12.3 per cent. This was significantly lower than the mean of either normal controls ($t = 7.4831$, $P < 0.001$), chronic uraemic patients undergoing dialysis (pre-dialysis: $t = 5.725$, $P < 0.001$; post-dialysis: $t = 2.552$, $0.02 > P > 0.01$), and acute uraemic patients (pre-dialysis: $t = 4.154$, $P < 0.001$; post-dialysis: $t = 5.767$, $P < 0.001$). Without correction for haematocrit, platelet adhesiveness was diminished in 26 out of 39 cases of uraemic patients examined (66 per cent), there was no significant difference between the acute and chronic uraemic patients under dialysis and the whole group of uraemic patients. It was of interest to note that the mean platelet adhesiveness in the 11 patients with a haemorrhagic tendency was

25.9 per cent which was not significantly lower than the mean of the other uraemic patients ($t = 0.431$ $P > 0.10$).

The effect of haemodialysis on platelet adhesiveness in the chronic group was to cause an increase in platelet adhesiveness. This was statistically significant ($t = 2.831$, $0.02 > P > 0.01$) and occurred in all 7 patients. On the other hand, such an increase did not occur in the acute uraemic patients following dialysis ($t = 0.994$, $P > 0.1$). There was a diminution of platelet adhesiveness in 4 patients and an increase in 3.

Figure 35 shows the relationship between platelet adhesiveness and disaggregation for the normal controls and the uraemic patients. It will be seen that 14 (78 per cent) of the 18 uraemic patients show a relationship outside the 95 per cent confidence limit of the normal controls. Furthermore it is obvious from the figure that in the uraemic group there is no correlation between adhesiveness and disaggregation.

In four uraemic patients, the relationship between adhesiveness and disaggregation was within the 95 per cent confidence limits of normal. Of these four, two were patients with post renal uraemia one of whom was bleeding at the time of study, and the other two were patients with chronic renal failure due to presumed renal tuberculosis and chronic pyelonephritis respectively. When the results of platelet adhesiveness in both normal controls and uraemic patients were corrected for PCV only six out of 18 patients showed an abnormal platelet adhesiveness-disaggregation relationship (figure 36).

In summary, the present studies show that platelet adhesiveness is diminished in the majority of patients with uraemia when no correction is made for variations in haematocrit. The results obtained in the uraemic patients with a bleeding tendency were not significantly different from those of the rest of the uraemic group. An increase in platelet adhesiveness occurred after haemodialysis in

in the chronic but not in the acute uraemic patients. The majority of patients showed an abnormal relationship between platelet adhesiveness and disaggregation.

The low platelet adhesiveness found has been reported by others using different assay system. Donner and Neuwirtova (1960) found a reduced platelet adhesiveness to yeast by the method of Bounameaux (1957a). In cases of uraemia where a functional deficiency of platelets resulted in disturbance of the thromboplastin formation, reduced adhesiveness was found after rotation in a glass rotator according to Jürgen's procedure (1959).

Hellem et al. (1964) found low ADP-induced platelet adhesiveness in citrated platelet-rich plasma in cases of uraemia with a prolonged bleeding time. Salzman and Neri (1966) reported in vitro platelet adhesiveness to be diminished in 21 out of 24 patients with chronic renal failure, the abnormality being correlated with prolongation of Ivy bleeding time, abnormal prothrombin consumption and with clinical bleeding. The in vivo platelet adhesiveness method (Borchgrevink 1960) showed low calues in 5 patients with prolonged Ive bleeding time but was normal in patients without bleeding (Castaldi et al. 1966). The effect of dialysis on platelet adhesiveness has been investigated by Castaldi et al. (1966), Anderson et al. (1966) and Stewart and Castaldi (1967). A return of platelet adhesiveness to normal was observed in two patients following dialysis (Castaldi et al. 1966). Anderson et al. (1966) compared the arterial and venous platelet adhesiveness and found an increase in adhesiveness as blood flowed through the dialyzer. Stewart and Castaldi (1967) showed by Borchgrevink's method a beneficial effect of dialysis in 7 uraemic patients out of 8. O'Brien (1967) reported low platelet adhesiveness in few patients with renal failure using his own glass bead column method, but normal results were found with Wright's technique.

In all the glass bead column methods described in the preceding

paragraph, no correction for packed cell volume was performed either because platelet-rich plasma and ADP were used (Hellem et al., 1964) or because the method was not dependent on haematocrit (Salzman and Neri, 1966) or not reported to be so (O'Brien's method).

Correction for PCV.

The relationship between the PCV and platelet adhesiveness might appear to justify applying a correction factor to the results obtained from controls and from uraemic patients where the PCV differs from the mean results obtained in normal controls (40.6 per cent). A standard curve (figure 33) in which the PCV was varied in vitro by using various concentrations of whole citrated blood and platelet-poor plasma, was employed to determine the correction factor, rather than a standard curve prepared from normal controls (figure 32), in which there were no low PCV values.

The mean corrected platelet adhesiveness in uraemic patients was 53.3 ± 15.9 per cent, and was not found to be significantly different from the value from the normal subjects ($t = 1.63$, $P > 0.1$). Only 6 out of 39 uraemic patients showed low platelet adhesiveness, 14 were increased but the majority (19 cases) showed normal results. The group of patients with bleeding showed no difference in platelet adhesiveness from the normal mean value ($t = 0.711$, $P > 0.1$) and was not significantly lower than the remainder of the uraemic patients ($t = 0.431$, $P > 0.1$).

Before dialysis, patients with chronic renal failure showed a significantly higher mean platelet adhesiveness of 65.5 ± 9.3 per cent than in normal controls ($t = 5.399$, $P < 0.001$). This became even higher after dialysis 70.7 ± 7.6 per cent ($t = 7.660$, $P < 0.001$). However in patients with acute uraemia, the mean platelet adhesiveness before and after dialysis was not different from that of normal controls (before dialysis: $t = 0.505$, $P > 0.1$; after dialysis: $t = 0.687$, $P > 0.1$). The mean of pre-dialysis was not significantly

different from the rest of the uraemic group ($t = 1.908$, $P > 0.1$) but was significantly high after dialysis ($t = 2.749$, $0.01 > P > 0.001$). No significant changes occurred in platelet adhesiveness in either chronic uraemic patients following dialysis ($t = 1.51$, $P > 0.1$) or in the acute group ($t = 0.591$, $P > 0.1$).

When platelet adhesiveness was corrected for PCV adhesiveness and disaggregation became more significant ($t = 0.611$ $0.01 > P > 0.001$) than before correction. Only 8 patients out of 18 (as compared to 14 before correction) fell outside the 95 per cent confidence limit of normal (figure 36).

In summary, after correcting for the PCV, the mean platelet adhesiveness in uraemic patients was within normal limits, as was the mean value of acute uraemic patients before and after dialysis. However, in the chronic uraemic group, the mean platelet adhesiveness was significantly higher than normal and became even more so after dialysis. After correction for PCV less than half of the uraemic patients showed an abnormal platelet adhesiveness-platelet disaggregation relationship.

The results obtained before correction for PCV probably reflect an overall picture in uraemia where platelet adhesiveness is diminished as a result of the low packed cell volume frequently encountered in these cases. However, after mathematical correction for packed cell volume, normal results were obtained in 49 per cent of cases, increased results in 36 per cent and diminished adhesiveness in only 15.4 per cent. The high values obtained in chronic uraemic patients before and the even higher results seen after dialysis may be explained by the fact that these patients' platelets were exposed twice weekly for 10 hours to the dialysis membrane in the kidney machine, such exposure to a foreign surface perhaps being responsible for this increased adhesiveness. However, in acute uraemic patients under dialysis the results were normal before dialysis and did not significantly change after the end of dialysis, perhaps

because these patients have not been dialysed as frequently as the chronic patients, and that the dialysis time was also shorter.

CHAPTER 7

PLATELET FACTOR 3 AVAILABILITY

Platelets are the main source of phospholipid for the intrinsic coagulation pathway. This is evident from the fact that the clotting time of recalcified plasma is long if platelets have been virtually removed by high speed centrifugation (Brinkhous, 1947). It can be restored to normal by addition of platelets or appropriate phospholipid. Conley et al. (1949) found that prothrombin consumption showed a significant decrease when platelets were reduced. Merskey (1950) confirmed the effect of platelets on prothrombin consumption and found it to be almost absent in thrombocytopenia and in normal plasma when platelets were removed by centrifugation.

The observation that the "Stypven" times of intact platelet-rich plasma differs little from that of platelet-poor plasma (Marcus and Zucker, 1965) has led to the conclusion that platelet phospholipid is not available in normal circulating platelets. Disruption of the platelets by freezing and thawing (Fantl and Ward, 1958), by mechanical fragmentation (O'Brien, 1958), sonication (Axelrod, 1956), or even by centrifugation and washing (Marcus and Zucker, 1965) renders their phospholipids available thus shortening the "Stypven" time.

Phospholipid is thought to be necessary for the interaction between factors VIII and IX (Lundblad and Davie, 1964) and also between factors X and V (Davie and Ratnoff, 1964). In vivo, this phospholipid may well be provided by platelet factor 3, which is probably a lipoprotein complex. In vitro, agents such as total platelet lipid extracts or isolated platelet ethanolamine and serine phosphoglyceride provide an effective substitute (Troup et al., 1960; Barkhan et al., 1961; Marcus et al., 1962; Ferguson et al., 1963; Woodside et al., 1964).

The subcellular localization of platelet factor 3 activity was

made difficult by the fact that complete separation of platelet components could not be obtained. So called granule preparations were always found to be mixed with membrane fragments when examined by the electron microscope (Marcus and Zucker-Franklin, 1964). Recently Marcus et al. (1966) using an improved method of platelet homogenization, and ultracentrifugation in a continuous sucrose density gradient, were able to achieve complete separation of platelet granules and membranes. When tested in vitro, platelet membranes were more efficient as clot-promoting agents than were the granules. However, when the lipids were extracted from each fraction, the clot-promoting activity of both granules and membrane lipid was the same. This led to the conclusion that the lipoprotein of platelet membranes is more available for interaction with coagulation proteins than is the lipoprotein from the granules.

Electron microscopy studies showing intact platelet membranes throughout the major part of the clotting process is in favour of the proposition that the platelet contribution to prothrombin activation may be a function of their external or plasma membrane (Castaldi et al., 1962; Rodman et al., 1962; Rodman et al., 1963). The mechanism by which platelet factor 3 is activated from intact platelets is not clear. There is now evidence to support the proposition that ADP may be responsible for both platelet factor 3 activation as well as platelet aggregation (Hardisty and Hutton, 1966b; Horowitz and Papayaunou, 1968). In the process of haemostasis, the ADP released by platelets which have adhered to collagen may be the responsible factor for inducing platelet aggregation and factor 3 activation. Eventually a platelet plug will develop which is the first step in the formation of a haemostatic plug.

Platelet factor 3 availability has been studied by a technique using Russel Viper Venom (Stypven) as described by Spaet and Cintron (1965) in both normal controls and uraemic patients and the results are presented in this chapter.

RESULTS

Results in Normal Controls

Twenty-three normal healthy subjects were examined for platelet factor 3 availability by the method of Spaet and Gintron (1965). The mean 'Stypven' time of normal controls was 19.89 ± 2.8 seconds, and the mean platelet count was 374,217 ± 74,420/c.mm. (table 49, figure 39). The normal Kaolin cephalin clotting time was 50.9 ± 5.3 seconds (table 36).

There was no significant correlation between the platelet count and the 'Stypven' time in 23 normal control subjects tested ($r = 0.131$, $P > 0.1$). There was a tendency for the 'Stypven' time to be prolonged when the platelet count was lowered by dilution of the platelet rich plasma with platelet poor plasma (experiments 24-31, table 49). However, when these latter results were included with the results for control subjects a significant correlation between the platelet count and the 'Stypven' time was not obtained ($r = 0.298$, $0.1 > P > 0.05$; figure 40). It should be noted that in all 5 results with a platelet count less than 177,000/c.mm., the 'Stypven' time exceeded 23 seconds, whereas in only 2 of the 26 in which the count was higher than this figure did the time exceed 23 seconds.

Relationship between platelet factor 3 availability and ADP-induced platelet aggregation.

Ten experiments were carried out in which platelet factor 3 availability test was done together with ADP-induced platelet aggregation from the same blood sample. No correlation existed between platelet aggregation (as measured by 30-60, 30-90, maximum aggregation) and the 'Stypven' time. However, a significant correlation was obtained between percentage disaggregation and the 'Stypven' time ($r = 0.645$, $0.05 > P > 0.02$; table 52, figure 42).

Results in Uraemic Patients

Thirty-one patients with uraemia were examined; of these 25 were suffering from chronic renal failure and 6 from acute uraemia. The mean 'Stypven' time of the whole group was 30.2 ± 10.4 seconds, and

the mean platelet count was 227,750 ± 107,000/c.mm. (table 50, figure 39). In all experiments the kaolin cephalin clotting time was also performed. In studying the results, 3 patients had prolonged kaolin cephalin clotting time.

To determine the relationship between the platelet count and the 'Stypven' time, the results from uraemic patients with prolonged kaolin cephalin clotting time were excluded. This is because any prolongation in the kaolin cephalin clotting time would result in prolonged 'Stypven'time ($r = 0.462$, $0.02 > P > 0.01$; figure 41).

In studying the results, platelet counts below and kaolin cephalin clotting times above ± 2 standard deviations from the normal were excluded, the mean 'Stypven' time from 13 uraemic patients was 24.8 ± 3.9 seconds.

Effect of Haemodialysis

Five chronic renal failure patients were examined before and after haemodialysis (table 53). The mean platelet count was 251,900/c.mm. before dialysis, and 286,300/c.mm. after dialysis, showing no significant change ($t = 0.676$; $P > 0.1$). The mean 'Stypven' time before dialysis was 29 ± 8.8 seconds and after dialysis 26.5 ± 5.8 seconds. The difference between the two was insignificant ($t = 0.531$, $P > 0.1$). Four patients with acute uraemia were tested before and after dialysis (table 54); one patient (No. 23) who had a markedly prolonged 'Stypven' time and kaolin clotting time was excluded from the calculation of the mean. The mean pre-dialysis 'Stypven' time of 3 patients was 30 seconds and post-dialysis 25.13 seconds. All 3 patients had a platelet count below the normal range. There was no significant difference between the two means ($t = 0.587$, $P > 0.1$).

Relationship between platelet factor 3 availability and percentage disaggregation

Of the 13 uraemic patients who showed normal kaolin cephalin clotting time and had a platelet count within the normal range, 12 had

also been examined for ADP-induced platelet aggregation (table 51). The significant correlation found in normal controls between the 'Stypven' time and percentage disaggregation was absent in uraemic patients ($r = 0.389$, $P > 0.1$). The results of 8 uraemic patients fell outside the 95 per cent confidence limit of normal (67 per cent) i.e. prolonged 'Stypven' time or diminished disaggregation, and only 4 showed normal results (figure 42). These were two patients with chronic renal failure under haemodialysis and one case of post-renal uraemia, the fourth patient was suffering from chronic renal failure under treatment in the ward. Her blood urea fell from 310 mg. per 100 ml. 5 days before platelet studies were done to 131 mg. per 100 ml.

Comparison with Normal Controls.

The mean 'Stypven' time of the whole group of uraemic patients was significantly different from the normal mean ($t = 4.613$, $P < 0.001$).

The mean of 13 patients with a platelet count above the low mean normal controls and having a normal kaolin cephalin clotting time was compared with the mean normal. The mean of uraemic patients was significantly longer than the normal ($t = 4.256$, $P < 0.001$).

Comparison was made between the mean 'Stypven' time in 10 normal controls and 12 uraemic patients having a platelet count in the range of 200,000 - 300,000/c.mm. (table 51). In some experiments the platelet count was adjusted to this range by appropriate dilution of platelet-rich plasma with platelet-poor plasma. The mean 'Stypven' time in normal controls was 20.5 ± 2.7 seconds, and in uraemic patients 26.6 ± 4.5 seconds. The mean 'Stypven' time in uraemic patients was significantly longer than in normal controls ($t = 3.764$, $0.01 > P > 0.001$).

Both pre- and post-dialysis mean 'Stypven' times of chronic uraemic patients were significantly longer than the normal mean (pre-dialysis: $t = 4.227$, $P < 0.001$; post-dialysis: $t = 3.878$, $P < 0.001$). The same was true for the acute uraemic patients (pre-dialysis: $t = 3.581$, 0.01

$> P > 0.001$; Post-dialysis: $t = 2.544$, $0.02 > P > 0.01$).

DISCUSSION.

It has been shown by Hardisty and Hutton (1965, 1966a) that kaolin makes platelet factor 3 available in platelet-rich plasma by a reaction which involves platelet aggregation, and which can be inhibited by antagonists of ADP such as adenosine and related compounds. Hardisty and Hutton (1966b) confirmed the observations of Mustard et al. (1964b), and Castaldi et al. (1965) on the role of ADP in the clotting activity of platelets. They measured the changes in optical density and 'Stypven' time of platelet-rich plasma aggregated by ADP. Their results showed that aggregation by ADP, adrenaline, noradrenaline, 5-hydroxytryptamine or collagen is closely followed by a reduction in the 'Stypven' time which reaches a maximum after about 20 minutes. The effect of the 'Stypven' depends on the rate, degree and duration of platelet aggregation. They suggested that the membrane changes which lead to platelet aggregation may also provide an active catalytic surface for the interaction of coagulation factors.

The work of Horowitz and Papayoanou (1968) also confirms that ADP activates platelet factor 3 of intact platelet-rich plasma. Activation is dependent on the concentration of ADP and it occurs with sufficient speed to have a potential physiological role in blood coagulation.

Inhibition of aggregation by adenosine is associated with a proportionate inhibition of the fall of 'Stypven' time; and phenothiazine derivatives, which also inhibit ADP, halt the effect of ADP on the 'Stypven' time but do not reverse it (Hardisty and Hutton, 1966). The relationship presented in this chapter between platelet disaggregation and the 'Stypven' time in normal controls ($r = 0.645$; $0.05 > P > 0.02$) may be explained from the above findings. Disaggregation following platelet aggregation induced by ADP results from degradation of ADP by plasma enzymes (Hellem and Owren, 1964) and by platelet enzymes (Salzman et al., 1966); hence in platelets with a

high capacity for destruction of ADP it may be that the more disaggregation occurs, the earlier and greater is the degradation of ADP so the greater the amount of ADP inhibitors formed. As these inhibitors can halt the fall of the 'Stypven' time (Hardisty and Hutton, 1966; Horowitz and Papayoanou, 1968) then it should be anticipated that the more inhibitor formation, the more disaggregation and the more prolonged the 'Stypven' time will be. Platelet factor 3 availability as tested by the method of Spaet and Cintron (1965) was found to have a mean of 19.98 $\pm$ 2.8 seconds in normal controls. There was no correlation between the 'Stypven' times and corresponding platelet counts. The correlation was still insignificant when experiments were included where the platelet-rich plasma was diluted with platelet-poor plasma. The results with normal controls show that 'Stypven' times within the normal $\pm$ 2 standard deviations were always obtained with platelet counts down to 177,000/c.mm.; below this level either normal or prolonged 'Stypven' times may be found.

Tests of platelet factor 3 availability in uraemic patients showed prolonged clotting times when compared with normal controls. This difference was obtained with the whole group ($t = 4.613$, $P < 0.001$), and also when samples showing a prolonged kaolin cephalin clotting time and/or a platelet count two standard deviations below the normal mean were excluded ($t = 4.256$, $P < 0.0001$). The mean 'Stypven' time in uraemic patients was also significantly longer than normal controls when the platelet count in both groups was adjusted to 200,000 - 300,000/c.mm. ($t = 3.764$, $0.01 > P > 0.001$).

Results with normal subjects have shown that with a platelet count above 177,000/c.mm., the 'Stypven' time will be within $\pm$ 2 standard deviations of normal (figure 40). When these criteria were applied to uraemic patients the following 4 groups were observed. Patients with prolonged kaolin cephalin clotting times were excluded and 28 were analysed (figure 41).

Results from normal subjects have shown that with a platelet count above 177,000/c.mm. the 'Stypven' time will be normal (figure 40). The results from 3 patients with prolonged kaolin cephalin clotting time were excluded and the remaining 28 patients were divided into four groups based on their platelet counts and 'Stypven' time. For the purpose of this analysis a normal platelet count is defined as being greater than 177,000/c.mm.

(1) Low platelet count and prolonged 'Stypven' time

Six patients had a platelet count less than 177,000/c.mm. and prolonged 'Stypven' time. Of these, three showed a bleeding disorder; two had acute renal failure (Nos. 27, 28, table 50,55); and one had chronic renal failure (No. 13). Of the remaining three, two had chronic renal failure (Nos. 21, 26) and one had acute or chronic renal failure (No. 19).

(2) Low platelet count and normal 'Stypven' time

Three patients with acute renal failure (Nos. 25, 37 and 39), two of whom were bleeding (Nos. 25, 37), showed normal 'Stypven' time despite low platelet count. All three patients were jaundiced.

(3) Normal platelet count and prolonged 'Stypven' time

Six patients with a platelet count above 177,000/c.mm. showed prolonged 'Stypven' time. All were suffering from chronic uraemia (Nos. 14,15,16, 22, 30,33). Three patients were bleeding at the time of study (nos. 14, 15, 30).

(4) Normal platelet count and normal 'Stypven' time

Thirteen patients (46%) showed normal 'Stypven' times and platelet counts above 177,000/c.mm. One patient had acute renal failure and bleeding.

Platelet factor 3 availability was tested in 9 of the 11 patients with clinical haemostatic defect (table 55). Four of these 9 patients had a platelet count above 177,000/c.mm.; in 3 the 'Stypven' time was prolonged and in one the result was normal. Five of the 9 patients had a platelet count below 177,000/c.mm.; in two the 'Stypven' time was normal, and in three it was prolonged.

A significant negative correlation between the 'Stypven' time and the platelet count was obtained in uraemic patients, but was insignificant in normal controls. This may suggest that in normal controls, platelet factor 3 can be made available for the intrinsic coagulation mechanism from relatively low platelet count and is not highly dependent on the number of platelets.

However, in uraemic patients, lowering of the platelet count can significantly affect the 'Stypven' time.

A significant correlation existed between the 'Stypven' time and percentage disaggregation in normal controls, i.e. the less disaggregation, the shorter the 'Stypven' time. In uraemic patients such a correlation was absent and most patients showed diminished disaggregation or prolonged 'Stypven' time or both.

ADP is found to activate platelet factor 3 (Hardisty and Hutton, 1966b; Horowitz and Papayoanou, 1968). Kaolin makes platelet factor 3 available in platelet-rich plasma by a reaction which involves platelet aggregation and which is inhibited by ADP antagonists, such as adenosine and AMP (Hardisty and Hutton, 1965, 1966a). It is possible that kaolin acts by releasing ADP from platelets resulting in platelet aggregation and availability of platelet factor 3.

Analyses of the results in uraemic patients (table 52, figure 42) have shown that the 'Stypven' time was normal in 80 per cent of patients with low disaggregation, as compared to 67 per cent when the disaggregation was normal. This shows that diminished platelet disaggregation in uraemic patients may be responsible for correcting the 'Stypven' time but did not cause the expected increase in platelet factor 3 availability.

The effect of haemodialysis on platelet factor 3 availability in chronic uraemia was studied (Table 53). Three patients who showed normal 'Stypven' times before dialysis remained unchanged after dialysis. In two others the 'Stypven' time was prolonged before dialysis, becoming normal in one, and improving in the others, after dialysis. In patients with acute uraemia, one patient showed normal results before and after dialysis, one showed improvement after dialysis and one showed a prolongation in the clotting time after dialysis (table 54).

These results are similar to those reported by Castaldi et al (1966) who tested platelet coagulant activity by the method of Hardisty and Hutton (1965) and the method of Spaet and Gintron (1965) in two cases of chronic renal failure before and after dialysis. They reported a return to normal in both cases following dialysis. Stewart and Castaldi (1967) also reported similar findings using the prothrombin consumption index and platelet factor 3 availability test.

The prothrombin consumption index, abnormal before dialysis on 10 occasions, was restored to normal 7 times and improved 3 times. The test of platelet factor 3 availability was abnormal on 6 occasions, became normal in 3 but showed no improvement after the other 3 haemodialyses.

Platelet thromboplastic function in uraemia has been investigated by several authors using different techniques. A poor prothrombin consumption is a common finding (Larrain and Adelson, 1956; Lewis et al., 1957; Alschuler et al., 1960; Donner and Neuwirtova, 1960; Wurzel, 1960; Willoughby and Crouch, 1961; Kendall et al., 1961; Salzman and Neri, 1966; Castaldi et al., 1966; Stewart and Castaldi, 1967). Serum prothrombin times were found to be longer in chronic than in acute uraemia (Donner and Neuwirtova, 1960). Defective consumption has been attributed by some to an abnormality in platelet thromboplastin function (Cahalane et al., 1958), while others found poor prothrombin consumption in patients who had no measurable platelet abnormality (Altschuler et al., 1960). Cahalane et al. (1958) showed a poor platelet function in the thromboplastin generation test using a diluted system but could not bring the results back to normal by sonicating the platelets, contrary to the findings in thrombocytopathy. Cheney and Bonnin (1962) studied 33 cases of uraemia using the platelet thromboplastic function test. Twenty-eight patients showed reduced levels of platelet thromboplastic function and there was a positive relationship between the levels of blood urea nitrogen and the results of the test.

Investigation of platelet factor 3 availability in uraemia has been recently carried out using intact platelet-rich plasma. Activation of platelet factor 3 was achieved either by addition of kaolin (Hardisty and Hutton, 1965; Spaet and Cintron, 1965) or by ADP (Horowitz et al., 1967). Castaldi et al. (1966) reported that 56 per cent of uraemic patients with bleeding had abnormal platelet

factor 3 availability while the majority of the patients with no bleeding had normal tests.

When ADP was used to activate the platelet factor 3, 16 of 17 uraemic patients showed prolonged 'Stypven' times (Horowitz et al., 1967).

In summary, the results show a reduced platelet factor 3 availability in 15 out of 31 uraemic patients examined (50 per cent). However, when the results from uraemic patients with low platelet counts and prolonged kaolin cephalin clotting times are excluded, only 3 out of 13 patients showed a prolonged 'Stypven' time (23 per cent). The majority of the patients with bleeding were thrombocytopenic and/or had prolonged 'Stypven' times. Dialysis had no effect where platelet factor 3 availability was normal, but tended to restore normality in patients with reduced platelet factor 3 availability.

CHAPTER 8.

THE IN VITRO EFFECT OF UREA, CREATININE, URIC ACID GUANIDINO SUCCINIC ACID ON ADP-INDUCED PLATELET AGGREGATION.

For many years, investigators have been trying to identify the substance or substances responsible for the toxic changes that occur in uraemia, and in particular for the haemostatic abnormalities encountered in some uraemic patients.

Although abnormalities of blood coagulation have been reported in some patients with uraemia (Donner and Neuwirtova, 1960), and the prolonged bleeding time frequently found has been attributed to a vascular defect (Gualdi, 1935), recent evidence points to a functional platelet disorder.

Attempts to determine the agent responsible for thedefect and the nature of the platelet dysfunction took many lines of approach. One was to determine the deleterious effect of uraemic plasma on normal platelets and the correcting effect of normal plasma on uraemic platelets. Cahalane et al. (1958) demonstrated an inhibitory effect of uraemic plasma on platelet factor 3 extracted from normal human platelets. Although Salzman and Neri (1966) detected abnormal findings in thrombin-induced platelet aggregation in uraemic patients, they were unable to reproduce these results in vitro, either by adding normal plasma to uraemic platelets or vice versa. Stewart and Castaldi (1967) found that neither in vitro dialysis, nor mixing normal plasma with abnormal platelet and vice versa, for 2 hours, had any effect on aggregation, coagulation activity and clot retraction in either uraemic or normal platelet-rich plasma. However, Horowitz et al.(1967) using ADP to activate platelet factor 3 found that the presence of uraemic platelet-poor plasma prevented ADP-induced platelet factor 3 activation in normal plasma with rough dose-response relationship. Fully activated factor 3, obtained from frozen and thawed platelets was not inactivated during 60 minutes of

incubation in uraemic plasma.

The high blood urea found in uraemic patients had led many investigators to believe that urea may have a role in causing platelet dysfunction.

Hellem et al (1964) infused 150 g. urea intravenously to a subject with normal haemostasis. This resulted in lowering of platelet adhesiveness and prolongation of the bleeding time. They also obtained a prolonged bleeding time when the skin wound is flushed with a solution of urea in buffered saline. However, this is not the finding of Castaldi et al (1966) who failed to detect any abnormality in platelet function when the blood urea is raised to about 200 mg per 100 ml in normal controls by means of intravenous infusion of urea. Urea itself is found to cause platelet aggregation at a concentration of 200-400 mg per 100 ml but failed to inhibit ADP-induced platelet aggregation (Salzman and Neri, 1966).

Addition of urea in a concentration of 50 to 1000 mg per 100 ml of normal platelet-rich plasma did not inhibit factor 3 activation with ADP (Horowitz et al. 1967).

Fantl (1966) found that urea at a concentration of 820 mg per cent (400 m-osmole) did not inhibit ADP-induced platelet aggregation.

Recently Somer et al. (1968) studied the effect of urea on platelet aggregation and found that urea in concentrations frequently encountered in the uraemic state initially inhibits and later enhances aggregation induced by ADP.

Substances other than urea, known to be raised in uraemia, have also been introduced in in-vitro systems to determine their effect on platelet functions. Hellem et al (1964) found that high

concentrations of creatinine and inorganic phosphate had no effect on ADP-induced platelet adhesiveness of platelet-rich plasma in vitro. However, Praga and Cortellaro (1967) reported a direct inhibitory effect of creatinine on platelet adhesiveness in vitro. The in vitro addition of creatinine, ureate, phosphate, potassium and magnesium in concentrations that might occur in uraemia, or changes in pH to 5.2 and 8.1 had no deleterious effect on platelet aggregation, coagulant activity of platelets or clot retraction (Stewart and Castaldi, 1967). These authors also found that dextrose and mannitol impaired platelet coagulant function at concentrations of 167 m Mol. per litre, clot retraction at 250 m Mol. per litre and aggregation at 333 m Mol. per litre.

Phase contrast microscopy showed that impairment of platelet function resulting from addition of urea, dextrose and mannitol is associated with shrinkage and distortion of platelets.

The finding that 1-4 diguanidino diphenyl sulphone causes inhibition of ADP-induced platelet aggregation (Jerushalmy et al., 1966) has led Horowitz et al. (1967) to investigate the effect of analogous compounds which might be present in uraemia.

Of these, guanidino succinic acid was found to be a powerful inhibitor of ADP-induced platelet factor 3 activation, while arginine and guanidino acetic acid had no such effect.

In this chapter are presented an account of the in vitro study of the effects of urea, creatinine, uric acid and guanidino succinic acid on ADP-induced platelet aggregation.

MEASUREMENT OF THE IN VITRO EFFECT OF UREA, CREATININE, URIC ACID AND GUANIDINO SUCCINIC ACID ON ADP-INDUCED PLATELET AGGREGATION

1. Urea

Urea was added to citrated platelet rich plasma to give a final concentration of 800, 750, 500, 400, 250, and 200 mg. per 100 ml. This was done in the following manner:

1. 800 mg. per 100 ml:- 0.1 ml. of 20 gm per 100 ml. stock urea solution was added to 2.4 ml. platelet-rich plasma.
2. 750 mg. per 100 ml:- 0.1 ml. of 30 gm per 100 ml. stock urea solution diluted in 1:2 was added to 1.9 ml. platelet-rich plasma.
3. 500 mg. per 100 ml:- 0.1 ml. of 20 gm per 100 ml. stock urea solution diluted in 1:2 was added to 1.9 ml. platelet-rich plasma.
4. 400 mg. per 100 ml.:- 0.05 ml. of 20 gm per 100 ml. stock urea solution was added to 2.45 ml. platelet-rich plasma.
5. 375 mg. per 100 ml. :- 0.1 ml. of 30 gm per 100 ml. stock urea solution diluted 1:4 was added to 1.9 ml. platelet-rich plasma .
6. 250 mg. per 100 ml:- 0.1 ml. of 20 gm per 100 ml. stock urea solution diluted 1:4 was added to 1.9 ml. platelet-rich plasma.
7. 200 mg. per 100 ml:- 0.05 ml. of 20 gm per 100 ml. stock urea solution diluted 1:2 was added to 2.45 ml. platelet-rich plasma.

All dilutions were prepared in barbitone buffered saline pH 7.2. For the controls the appropriate amounts of barbitone buffered saline was added to platelet-rich plasma.

After the platelet-rich plasma had been incubated with the urea

for 5 minutes at room temperature the plasma was mixed by inversion for 5 times; a 1.9 ml. aliquot was transferred to a cuvette and the ADP-induced platelet aggregation assessed by the turbidimetric method described above.

Three experiments were carried out for each urea concentration. A test followed by a normal then another test were carried out in that order.

The mean results obtained from the 2 test experiments were subtracted from the value obtained with the normal control. The result obtained was considered a measure of the degree of inhibition by urea of platelet aggregation and disaggregation. A negative value would mean that the test is greater than the control.

2. Creatinine

0.1 ml. of 0.2 gm per 100 ml. creatinine in barbitone buffered saline was added to 1.9 ml. platelet-rich plasma and incubated for 5 minutes at room temperature. Final concentration of creatinine was 10 mg. per 100 ml. ADP-platelet aggregation was assessed as described above.

3. Uric Acid

0.2 ml. of 50 mg per 100 ml. uric acid in barbitone buffered saline was added to 1.8 ml. platelet-rich plasma and incubated for 5 minutes at room temperature. Final concentration of uric acid was 5 mg. per 100 ml. ADP-induced platelet aggregation was assessed as described above.

4. Guanidino succicinic Acid

0.2 ml of an o.386 gm per 100 ml. solution of guanidino succinic acid in imidazole buffered saline were added to 1.8 ml. platelet-rich plasma in a siliconized centrifuge tube and incubated at room temperature for 10 minutes. The final concentration of the guanidino succinic acid was 2×10^{-3}M.

1.9 ml. of the plasma was transferred to a cuvette and placed in position in an EEL titrator. 0.1 ml. ADP were added and ADP platelet aggregation was assessed as described. A control experiment was

performed in which 0.2 ml. imidiazole buffered saline were added, then another test experiment was performed. The degree of inhibition was calculated as described before.

RESULTS.

The Effect of Urea on ADP-induced Platelet Aggregation.

Eight experiments were carried out to determine the effect of urea in vitro on ADP-induced platelet aggregation and its reversal using a wide range of concentrations (from 200-800 mg. per 100 ml. of plasma). The details of the method used on the calculation of the results were given previously in this chapter.

Urea caused an inhibition of platelet aggregation in all 8 experiments performed. However, the degree of inhibition was not significantly correlated with the corresponding urea concentrations.

30-60 platelet aggregation: $r = 0.668$, $0.1 > P > 0.05$
30-90 platelet aggregation: $r = 0.261$, $P > 0.1$
Maximum platelet aggregation: $r = 0.403$, $P > 0.1$

Urea also resulted in a diminution of percentage disaggregation in all experiments, and was in correlation with the respective urea concentration ($r = 0.882$, $0.01 > P > 0.001$). This relationship is shown in figure 43.

A representative experiment (No. 4, table 56 and 59) is shown in figure 44.

The Effect of Creatinine on ADP-induced Platelet Aggregation.

Creatinine added in a concentration of 10 mg. per 100 ml. of plasma caused a negligible increase in platelet aggregation and disaggregation when compared to a saline control (table 57).

The Effect of Uric Acid on ADP-induced Platelet Aggregation.

Uric acid added in a concentration of 5 mg. per 100 ml. of plasma

caused a negligible diminution in platelet aggregation and a slight increase in percentage disaggregation as compared to a saline control (table 57).

The Effect of Guanidino Succinic Acid on ADP-induced Platelet Aggregation.

No effect was obtained when guanidino succinic acid was added in a final concentration of 2×10^{-4} molar which is the concentration used by Horowitz et al. (1967) to demonstrate its effect on ADP-induced factor 3 availability. However when the concentration was increased to 2×10^{-3} molar a constant diminution of platelet aggregation and an increase in percentage disaggregation was obtained in all 7 experiments performed. This effect was not significantly different from the saline controls (table 58, figure 45).

30-60 platelet aggregation: $t = 1.813$, $0.1 > P > 0.05$
30-90 platelet aggregation: $t = 2.146$, $0.1 > P > 0.05$
Maximum platelet aggregation: $t = 1.309$, $P > 0.1$
Percentage disaggregation: $t = 1.172$, $P > 0.1$

DISCUSSION.

The platelet dysfunction in uraemia has been described by several authors to be reversed by correction of the uraemic state (Castaldi et al., 1966; Traegar et al., 1967; Stewart and Castaldi, 1967). The corrective effect of haemodialysis and peritoneal dialysis on the abnormalities in platelet function suggest that the cause may be due to dialysable metabolites.

An attempt was made to reproduce a platelet abnormality by incubating urea, creatinine, uric acid and guanidino succinic acid with platelet-rich plasma. After a short incubation, ADP-induced platelet aggregation was assessed.

Urea caused a constant diminution in platelet aggregation and disaggregation when added in concentrations varying from 200 to 800 mg per 100 ml. of plasma. The degree of inhibition of platelet aggregation was not significantly correlated with the corresponding urea levels. However the inhibitory effect on platelet disaggregation was significantly correlated with the respective urea concentrations (figure 43).

There are conflicting reports in the literature as regards the in vitro effect of urea on platelets. Fanti (1966) showed that urea can inhibit ADP-platelet aggregation only in a concentration of 1000 m-osmole or more. Stewart and Castaldi (1967) observed that urea alone has no effect on ADP-induced platelet aggregation within the range that may be encountered in uraemia. Studies performed by Somer et al. (1968) have shown that over a wide range of concentrations, urea initially inhibits and later enhances platelet aggregation induced by ADP. This biphasic effect is explained by the authors as resulting from the formation of stable platelet aggregates probably due to damage by urea. Salzman and Neri (1966) and Somer et al. (1968) have shown that urea can cause platelet aggregation. This effect is independent of cations and differs from aggregation by ADP in that the inhibitory effect of adenosine and AMP is markedly delayed.

The mechanism by which urea acts on platelets is not clear. Hellem et al. (1964) postulated that urea breaks hydrogen bonds thus interfering with the binding of ADP to proteins located on the platelet surface. Somer et al. (1968) explained the urea effect on platelets as being due to alterations in the cohesive property of platelets, leading to stable platelet aggregation.

The different results obtained by various authors regarding the effect of urea on ADP-induced platelet aggregation may be due to

individual variations and different methods of study.

The findings in this thesis of the in vitro effect of urea on ADP-platelet aggregation were similar to the results obtained from patients with uraemia, i.e. diminished aggregation and disaggregation (Chapter 5).

Other metabolites like creatinine and uric acid added in concentrations which might be found in uraemia had negligible effect on ADP-induced platelet aggregation. This is in agreement with the results of Stewart and Castaldi (1967) who reported that urcate and creatinine had no effect on platelet aggregation induced by ADP.

Guanidinosuccinic acid, an intermediate or urea metabolism, has been reported by Cohen et al (1966) to be increased in the urine of uraemic patients and is thought by Horowitz et al. (1967) to be the uraemic inhibitor. When guanidinosuccinic acid and ADP are incubated with platelet-rich plasma for 1 hour at 37°C ADP induced platelet factor 3 availability is markedly diminished (Horowitz et al, 1967).

The effect of guanidinosuccinic acid on ADP-induced platelet aggregation was tested in the turbidimetric system. No effect was obtained when the substance was added in a concentration of 2×10^{-4} Molar. However, when a concentration of 2×10^{-3} Molar was used a diminution in platelet aggregation and an increase in platelet disaggregation was found in all 7 experiments performed (table 58). This effect was found to be insignificant when compared to controls. Failure to obtain significance may be due to the shorter incubation time (10 minutes as opposed to 60 minutes) and the incubation temperature (room temperature as compared with 37 °C) as used in the present experiments as compared with Horowitz et al (1967).

The evidence presented in this chapter and the results of in vitro and in vivo experiments by various authors on the effect of urea and its intermediate, guanidinosuccinic acid, on platelet function

support the view that these may have a role in causing the platelet abnormality in uraemia.

CHAPTER 9.

SUMMARY AND CONCLUSIONS.

The haemorrhagic tendency in uraemia has been recognized for many years, but its specific nature has not been established. It has been attributed to vascular fragility, thrombocytopenia and abnormalities in the coagulation mechanism. The clinical pattern of the bleeding in uraemia suggests that the haemostatic failure is likely to be in the platelet-vascular group, i.e. bleeding from mucous membranes rather than deep tissue haematoma; the latter clinical feature is characteristic of defects of the blood coagulation mechanism. Recent evidence suggests a qualitative platelet defect (Farrain and Adelson, 1956; Lewis et al., 1956; Rath et al., 1957; Cahalane et al., 1958; O'Grady, 1959; Donner and Neuwirtova, 1960; Wurzel, 1960; Willoughby and Crouch, 1961; Cheney and Bonnin, 1962; Hellem et al., 1964; Castaldi et al., 1966; Bayer et al., 1966; Salzman and Nexi, 1966; Rozenberg and Firkin, 1966; Stewart and Castaldi, 1967; O'Brien, 1967; Praga and Cortellaro, 1967; Horowitz et al., 1967; Gan and Firkin, 1968). In this thesis an attempt has been made to investigate some aspects of platelet function in uraemia, to evaluate the effect of haemodialysis on platelet function in acute and chronic renal failure and to study the effect on ADP-induced platelet aggregation in vitro of some biochemical abnormalities known to be present in uraemia.

1. Platelet morphology and function.

Chapter 1 contains a short account of platelet morphology and the role of platelets in haemostasis and thrombosis.

2. General introduction on platelets in uraemia.

In Chapter 2 an historical account is given of the progress of knowledge on the bleeding tendency in uraemia and the evolution of

ideas on etiology, together with some account of the methods used in these studies.

3. Materials and methods

Chapter 3 contains a description of the materials and methods used in this thesis. The methods outlined are those used to study some of the properties of platelets. These include platelet aggregation, platelet adhesiveness to glass and the availability of platelet factor 3.

The ability of platelets to aggregate with calcium and with calcium plus ADP was tested by a Chandler tube method and by the turbidimetric method. In the Chandler tube system the time interval between recalcification, or the addition of calcium plus ADP and the appearance of the "snow storm" effect, was used as a measure of platelet aggregation. In the turbidimetric modification, optical density readings were taken to measure platelet aggregation and the clotting time.

ADP-induced platelet aggregation and its reversal was determined by a turbidimetric method (O'Brien, 1962b; Born and Cross, 1963b). Optical density readings were taken every 30 seconds for 10 minutes or longer.

Platelet adhesiveness to glass was measured by a modified Hellem's glass bead column method as described by Hirsh et al. (1966a). In this method citrated blood is forced through a glass bead column by an electrically driven mechanical pump which gives a constant speed.

Platelet factor 3 availability was estimated by determining the "Stypven" time of kaolin-activated platelet rich plasma (Spaet and Cintron, 1965).

Experiments on the effect of factors influencing the results of some of the tests were described e.g. the effect of haematocrit on platelet aggregation and platelet adhesiveness, and the effect of

the stirring speed, incubation temperature and plasma white cell count on platelet aggregation.

In Chapter 3, the kaolin cephalin clotting time is also described, this was sused as a simple screening test to detect any plasma coagulation abnormalities in uraemic patients.

4. Platelet aggregation induced by calcium and calcium plus ADP

As shown in Chapter 4, the addition of ADP to recalcified platelet-rich plasma in the Chandler tube system resulted in significant shortening of the platelet aggregation time. However, a comparable effect on platelet aggregation was not obtained in the turbidimetric system. The cause for this discrepancy is unknown.

A significant positive correlation was obtained in normal controls between the platelet count and platelet aggregation time, duration of platelet aggregation and the fall in optical density occurring just before clot formation. A similar correlation was found with the clotting time and the platelet count i.e. the higher the platelet count, the more delayed the onset of platelet aggregation occurring before clot formation, the longer its duration and the more prolonged the clotting time. It is possible that thrombin released as a result of recalcification will act on platelets causing aggregation and eventually clot formation. With a low platelet count, the proportion of thrombin per platelet would be higher and consequently aggregation and clot formation will occur sooner.

Comparison between the results of calcium and calcium plus ADP experiments have shown that the addition of ADP as well as calcium significantly prolonged the duration of phase 5 (i.e. the time taken by platelets to aggregate prior to clot formation). This effect of ADP was also obtained in uraemic patients. In normal controls no difference was obtained in the mean platelet aggregation time and the clotting

times in the experiments with ADP and calcium as compared with calcium alone. There was no difference between uraemic patients and normal controls as regards the aggregation time and the clotting time, although the uraemic patients had a significantly lower platelet count than normal controls. However, the duration of phase 5 in the calcium plus ADP experiment was found to be prolonged in 5 out of 8 uraemic patients examined. This may be a reflection of a qualitative platelet defect in uraemia resulting in inability of the platelets to release a normal amount of ADP in response to thrombin.

5. ADP-induced platelet aggregation and disaggregation

Chapter 5 deals with the platelet response to ADP in both normal control subjects and uraemic patients as measured by the turbidimetric method. Uraemic patients have a wide range of platelet counts, and, to study the patients with low counts as compared to normal controls, the ADP induced platelet aggregation was studied after diluting the platelet-rich plasma with platelet-poor plasma.

In normal plasma a positive correlation was found between platelet disaggregation and the platelet count but not between the count and platelet aggregation. However, when the results of experiments in which the platelet count was diluted with platelet-poor plasma were included, both aggregation and disaggregation were significantly correlated with the platelet count. A positive correlation also existed between aggregation and disaggregation. Because of the correlation with the platelet count, the results obtained from normal subjects and uraemic patients were grouped into four ranges of platelet count and the mean values compared. In all four ranges the 30-60 platelet aggregation and the percentage disaggregation were significantly lower in uraemic patients than in control subjects and the disaggregation time was more prolonged in uraemic patients.

The effect of haemodialysis on ADP-induced platelet aggregation was assessed. In chronic renal failure, although platelet aggregation

increased in the majority of patients examined, The difference before and after dialysis did not reach levels of technical significance. Platelet aggregation results were significantly correlated with their corresponding blood urea level. Platelet disaggregation showed a constant and significant increase after dialysis, usually reaching normal levels.

It was not possible to consider the effect of haemodialysis in acute uraemic patients as a group because of the marked variations in the platelet count; this variation was much greater than in the chronic uraemic patients. For this reason the results of each experiment were compared with the result of normal controls diluted when required with platelet free plasma to produce the same range of platelet count. The effect of haemodialysis on the platelet behaviour in acute uraemic patients was not constant. This could be due to the varying aetiological factors producing acute uraemia, the rate of development and duration of the uraemic process and the type of dialysis.

In uraemic patients with a haemostatic defect, the time taken by their platelets to disaggregate was prolonged in the majority of cases, their ability to aggregate was more diminished than the rest of the uraemic group.

6. Platelet adhesiveness to glass.

In Chapter 6 the results are described of platelet adhesiveness to glass in control subjects and uraemic patients by a modified Mollem's glass bead column method. In the controls, the percentage platelet adhesiveness was found to be correlated with the haematocrit value, but not with the platelet count of the citrated blood. When the haematocrit was varied in vitro by addition of platelet-poor plasma to citrated blod, a significant correlation was obtained between the percentage adhesiveness and their corresponding haematocrit values. A significant negative correlation existed between the percentage disaggregation and percentage platelet adhesiveness, but

not between the latter and platelet aggregation.

When no correction was made for the haematocrit value, the majority of uraemic patients showed low platelet adhesiveness, and the mean value was significantly lower than the mean of controls. The mean value of platelet adhesiveness of uraemic patients with clinical bleeding was not significantly different from the mean of the rest of the uraemic group.

Haemodialysis increased platelet adhesiveness consistently in chronic uraemic patients, and the results before and after haemodialysis were correlated with the corresponding blood urea levels. However platelet adhesiveness did not change significantly in the acute uraemic group following haemodialysis.

Most uraemic patients (70 per cent) had an abnormal relationship between the ability of platelets to adhere to glass and their ability to disaggregate after aggregation with ADP.

When the results of platelet adhesiveness were corrected for haematocrit, the mean value of uraemic patients was within normal limits. Uraemic patients with bleeding also had a normal mean value. However patients with chronic renal failure under haemodialysis showed significantly high results before dialysis (i.e. the platelets were more adhesive), becoming more so after haemodialysis.

The relationship between percentage platelet adhesiveness and percentage platelet disaggregation in uraemic patients was still insignificant when the results of platelet adhesiveness were corrected for PCV. However this relationship became normal in the majority of patients after correction.

The results obtained before correction for PCV probably reflect an overall picture in uraemia where platelet adhesiveness is diminished at least partly as a result of low PCV. The increased adhesiveness in patients with chronic renal failure on dialysis is

unknown; one might speculate that this is the result of frequent exposure to the dialysing membrane.

7. Platelet factor-3 availability

In Chapter 7 the results are described of platelet factor-3 availability in control subjects and uraemic patients using the method described by Spaet and Cintron (1965). In normal subjects there was no significant correlation between the platelet count and the 'Stypven' time, although there was a tendency for 'Stypven' time to be prolonged in the presence of a low platelet count. When this test was done in conjunction with ADP-induced platelet aggregation, the 'Stypven' times were significantly correlated with percentage disaggregation but not with platelet aggregation.

The mean value of the 'Stypven' times in uraemic patients was significantly longer than the mean value from normal controls. A significant correlation existed between the 'Stypven' times in uraemic patients and the platelet count.

The normal relationship between the 'Stypven' time and percentage disaggregation was absent in uraemic patients. The majority of patients showed either prolonged 'Stypven' time or diminished disaggregation or both. Haemodialysis had no effect where platelet factor-3 availability was normal, but tended to restore normality in patients with reduced platelet factor-3 availability.

8. The effect of urea, uric acid, creatinine and guanidinosuccinic acid on ADP-induced platelet aggregation

In Chapter 8 the results are described of the above mentioned chemical substances on platelet aggregation in vitro.

Urea caused inhibition of ADP-induced platelet aggregation in a final concentration ranging from 200-800 mg per 100 ml platelet-rich plasma, but the inhibition was not correlated with the urea concentration. However, the degrees of inhibition of percentage disaggregation by added urea were correlated with the corresponding urea concentration. The effect of urea on ADP-induced platelet aggregation was similar to the findings obtained from patients with uraemia i.e. diminished aggregation and disaggregation.

Uric acid in a concentration of 5 mg. per 100 ml. platelet-rich plasma caused slight diminution in platelet aggregation and slight increase in disaggregation. Creatinine when added in a final concentration of 10 mg. per 100 ml. had no effect on platelet aggregation but slightly increased disaggregation. Guanidino succinic acid inhibited platelet aggregation but increased platelet disaggregation.

In conclusion, the evidence suggests that the haemostatic defect in uraemia may be due in part at least to a qualitative platelet abnormality. This abnormality could arise from inability of the uraemic platelets to react with or release ADP possibly as a result of intracellular enzymatic inhibition. In this way an explanation can be found for the low platelet aggregation, disaggregation and low factor 3 availability. The normal results obtained in assessment of platelet adhesiveness after correction for haematocrit could be a balance between diminished reactivity to ADP and diminished platelet disaggregation.

The defect in platelet function can be corrected by haemodialysis in most patients with chronic renal failure but not in acute uraemia.

APPENDIX.

CLINICAL DETAILS OF URAEMIC PATIENTS IN WHOM PLATELET STUDIES WERE MADE.

Forty patients with uraemia were studied in this thesis; the blood urea levels ranged from 85 to 920 mg. per 100 ml. Of these 31 patients were suffering from chronic renal failure and 9 from acute uraemia. There were 20 males and 20 females and their ages ranged from 13 to 86 years. Some of these patients were studied more than once during the course of their illness. The patients included in this study were either attending the renal clinic, treated in the wards or under treatment by haemodialysis.

Of these patients, eleven were bleeding at the time of the study.

Seven patients with chronic renal failure were examined before and after haemodialysis. In all patients the pre-dialysis sample was taken in the afternoon from the arterio-venous shunt, before starting the dialysis and before heparin was administered. The post-dialysis sample was collected by a clean venepuncture the next morning and between six to seven hours after the end of dialysis. None of the patients were under oral anticoagulant therapy at the time of the study.

Six patients were dialysed for 10 hours by a "chron-a-coil" twin coil (Baxter) in a Travenol twin coil artificial kidney. The seventh patient (No. 40) was dialysed for the same period using a "chron-a-coil" twin coil (Baxter) in a recirculating single pass artificial kidney machine.

Seven patients suffering from acute uraemia were also examined before and after dialysis. The pre-dialysis samples were taken at different times of the day, and the post-dialysis on the day after.

All seven patients were dialysed by a rotating-drum artificial kidney (made by Usifroid of France, a modification of their Necker hospital model, incorporating suggestions by Dr. E.H. Parsons of Leeds). The patients are described in the chronological order in which they were studied.

Case No. 1: Male, age 20 years: This patient presented two years prior to investigation with a 3 month history of tiredness and exertional dyspnoea. At that time his haemoglobin was 7.3 gm. per cent and his blood urea was 200 mg per 100 ml. Retrograde pyelogram showed very small kidneys and was diagnosed provisionally as chronic renal failure due to congenital hypoplasia of the kidney. In August 1966 haemodialysis was started and the patient has been maintained on this form of treatment ever since. His platelet studies were performed before and after single treatments by haemodialysis.

Case No. 2: Male, age 33 years: For 7 years this patient has been known to have heavy albuminuria, high blood urea and radiological findings suggestive of chronic nephritis. Microscopy of urine showed granular casts and occasional red blood cells but the urine was sterile on culture. This patient has been treated by dialysis since the beginning of 1967 and was examined for platelet function on two occasions before and after haemodialysis. In the results section the figures quoted were on an occasion when the blood urea was 150 mg per 100 ml before haemodialysis and 55 mg per 100 ml at the end of haemodialysis.

Case No. 3: Male, age 31 years: This male patient had been known to have albuminuria since 1948 and was later diagnosed as chronic glomerulonephritis. In 1965 he was placed on haemodialysis treatment. He was examined 3 times for platelet function before and after haemodialysis. In the results section the figures quoted were on an occasion when the blood urea was 168 mg per 100 ml before haemodialysis and 52 mg per 100 ml after haemodialysis.

Case No. 4: Female, age 30 years: This patient had a history of nephritis at the age of 15 years. She had persisting renal impairment and had superimposed toxaemia of pregnancy during her only pregnancy in 1950. In the past few months she developed headaches, general

malaise and blurring of vision and on admission to hospital she was diagnosed as suffering from chronic glomerulonephritis plus malignant hypertension. She was given treatment for her hypertension and decision taken to give her regular dialysis treatment. Her platelet studies were performed before starting dialysis when the blood urea was 277 mg per 100 ml.

Case No. 5: Female, age 24 years: In the year prior to admission to hospital in 1967 this young woman had suffered from bouts of "indigestion" with vomiting. For several months she had become increasingly dyspnoeic, had intermittent ankle swelling and occasional transient epistaxis. On admission she had anaemia and hypertension and was found to have chronic renal failure possibly due to congenital renal anomaly. She was started on haemodialysis treatment and was examined for platelet function before and after haemodialysis. There was great difficulty in maintaining the patient on regular haemodialysis because of trouble with her arteriovenous shunt and she was maintained by peritoneal dialysis. The patient's general condition gradually deteriorated and following convulsions she died. Her blood urea levels before and after haemodialysis were 155 mg per 100 ml and 22 mg per 100 ml respectively. Autopsy showed hypoplasia of the kidneys.

Case No. 6: Male, age 59 years: This patient was admitted to the artificial kidney unit with a severe degree of acute renal failure (blood urea 540 mg per 100 ml). The primary cause for uraemia was not clear but was possibly due to septicaemia secondary to pneumonia. The patient was haemodialysed on six occasions over a period of two weeks. His renal function improved to a great extent and he was discharged with a blood urea of 82 mg per 100 ml. Platelet studies were carried out before and after the third haemodialysis.

Case No. 7: Female, age 62 years : This patient was admitted to the artificial kidney unit having a severe degree of acute renal failure which had followed perforation of a peptic ulcer six days before.

Haemodialysis was carried out on the following day. Blood urea before haemodialysis was 340 mg per 100 ml and after haemodialysis 154 mg per 100 ml. An intraperitoneal drain was inserted with the aspiration of a copious amount of dark fluid containing some altered blood. Bacteriological examination of this fluid showed a profuse growth of monilia. A further haemodialysis was carried out three days later. On both occasions platelet studies were done before and after dialysis. Following the first haemodialysis there was a fall in the platelet count and the patient developed ecchymosis at venepuncture site. After the second haemodialysis there was a further drop in platelet count with profuse upper alimentary and peritoneal bleeding as judged by the aspiration from these sites. Her blood urea before the second haemodialysis was 345 mg per 100 ml; this fell to 190 mg per 100 ml at the end of haemodialysis. The patient's general condition deteriorated and she died 9 days after admission.

Case No. 8: Female, age 53 years: There was a history of profuse diarrhoea for two weeks before admission followed by right-sided abdominal pain. On admission to the renal unit the patient was dehydrated, pyroxial and subsequently noted to be oliguric. Marked haematuria was also present. Haemodialysis was carried out on three occasions. A percutaneous kidney biopsy showed changes compatible with acute tubular necrosis. The patient was discharged 39 days later with a blood urea of 48 mg per 100 ml.

Investigations at a later date suggested that the patient was suffering from cholangitis or cholecystitis. The platelet function of this patient was investigated before and after her second haemodialysis. Blood urea was 310 mg per 100 ml before haemodialysis and 130 mg per 100 ml after haemodialysis.

Case No. 9: Male, age 13 years: This patient had a life-long history of enuresis, polydypsia and urinary frequency. He also had spina bifida. Proteus urinary infection was demonstrated at the age of 5 weeks. Blood urea was not markedly increased, ranging from 41 to 85 mg per 100 ml in the previous 5 months. The urine was sterile on several occasions before and on the day of the platelet studies.

Case No. 10: Female, age 25 years: In 1965 this patient had been suffering from a left perinephric abcess. She was also found to have a right staghorn calculus, impaired renal function with a non-functioning left kidney. She was admitted to hospital because of progressive weakness, tiredness, vomiting and oliguria. Her blood urea on admission was 310 mg. per 100 ml. Treatment was given for dehydration and pyelonephritis. Her platelet studies were done five days after admission when the blood urea was 131 mg. per 100 ml.

Case No. 11: Female, age 57 years: This patient was suffering from chronic pyelonephritis with chronic renal failure, hypertension and myxoedema. This has been associated with frequent episodes of epistaxis. Platelet studies were carried out 6 months after the last epistaxis. At that time her blood urea was 122 mg. per 100 ml.

Case No. 12: Female, age 58 years: This female patient had long standing rheumatoid arthritis with chronic renal failure, possibly on the basis of renal amyloid. She was admitted to hospital because of increasing tiredness, breathlessness and ankle swelling. Purpuric spots on the face were seen on the day of the platelet studies. She had a low white cell count (1,300/c.mm.) and later developed a gum infection possibly as a consequence of the agranulocytosis (900/c.mm). Her blood urea was 87 mg. per 100 ml. Two months later the patient died.

Case No. 13: Female, age 35 years: At the age of seven years this patient had acute glomerulonephritis and thereafter recurrent episodes of acute pyelonephritis. The last episode was 4 months before she was examined for platelet function. She had spontaneous bruising on the limbs particularly the lower limbs. Her blood urea was 180 mg. per 100 ml. on the day her platelet studies were performed.

Case No. 14: Male, age 58 years: For 44 years this patient had been known to have chronic renal insufficiency due to hydronephrosis

resulting from stenosed urethra following a fractured pelvis. He was admitted as an emergency because he bled profusely from the lower gastrointestinal tract. The source of bleeding was sought and may have occurred from colon diverticuli demonstrated by barium enema. The platelet studies were done 17 days after the onset of bleeding. After admission his blood urea rose slightly from 188 to 203 mg. per 100 ml.

Case No. 15: Female, age 42 years: This patient was suffering from chronic renal failure and pyelonephritis due to B.coli and B. proteus. She bruised easily but had no history of other bleeding and was never jaundiced. This patient was examined for platelet function when she was on out-patient surveillance (blood urea 169 mg. per 100 ml.) and again when she was put on regular haemodialysis treatment.

Case No. 16: Female, age 37 years: This patient was investigated in 1961 for renal tuberculosis and although no certain diagnosis was made at that time, she received antituberculous treatment for 18 months. A past history of tuberculosis of the hip was also obtained. She had intermittent frequency of micturition, loin pain and headache. She had a creatinine clearance of 30 ml. per minute and the urine culture was positive for B.coli. The platelet studies were done while the patient was on out-patient attendance at the renal clinic. Her blood urea was usually maintained around 100 mg. per 100 ml.

Case No. 17: Male, age 41 years: This patient had been known to be suffering from chronic renal failure secondary to polycystic kidney disease since the age of 9 years. Urine culture was repeatedly sterile. His blood urea was 248 mg. per 100 ml. at the time of study.

Case No. 18: Female, age 50 years: This patient was suffering from chronic renal insufficiency due to chronic pyelonephritis. She had a history of renal calculi in 1965 and nephrectomy in 1966. Thereafter she developed chronic pyelonephritis due to B. coli infection. Her blood urea was stable around 100 mg per 100 ml and was 116 mg per 100 ml when her platelet studies were done.

Case No. 19: Female, age 37 years: This woman's history started with a left pyelolithotomy for calculi in 1950. She has had bilateral renal calculi since that time. In the two pregnancies (1958 and 1959) she had toxaemia of pregnancy and recurrent pyelonephritis. In the six months prior to study she developed exertional dyspnoea and was found to be hypertensive with cardiovascular changes and albuminuria. Intravenous pyelography showed poor right renal function and aortogram revealed right renal artery stenosis. Her condition rapidly deteriorated with uncontrollable hypertension, left ventricular failure and hypertensive encephalopathy. Her skin showed a yellowish hue. Her blood urea rose steadily to 180 mg per 100 ml and she rapidly deteriorated and died. Her platelet studies were done a few hours before death.

Case No. 20: Male, age 32 years: This patient had been suffering from chronic renal failure due to polycystic kidney disease recognised since the age of 26. He also had chronic pyelonephritis with urine culture positive for B. coli. He was studied for platelet function while under in-patient treatment for acute pyelonephritis. His blood urea was 120 mg per 100 ml at the time of study.

Case No. 21: Female, age 34 years: This patient had been known to have chronic renal failure and malignant hypertension for two years before admission. She was treated for the hypertension and was put on regular haemodialysis treatment since one year. Platelet studies were done before and after haemodialysis. Her blood urea was 262 mg per 100 ml before haemodialysis and 112 mg per 100 ml after haemodialysis.

Case No. 22: Female, age 13 years: This patient was admitted to hospital in 1965 with acute glomerulonephritis. Her condition deteriorated in the five months prior to admission with vomiting, weakness and oedema with chronic renal failure, the blood urea increasing from 247 to 605 mg per 100 ml. The latter was the blood urea value on the day her platelet studies were done.

Case No. 23: Male, age 43 years: This patient developed acute renal failure following post-operative peritonitis and septicaemia. The platelet studies were done before and after haemodialysis at one week after operation. The predialysis blood urea was 205 mg per 100 ml and the postdialysis blood urea was 140 mg per 100 ml.

Case No. 24: Female, age 53 years: This female patient had chronic renal failure resulting from chronic pyelonephritis in a solitary left kidney. Urine culture was positive for Klebsèilla organism. She had no history of abnormal bleeding. The blood urea was 162 mg per 100 ml at the time of examination.

Case No. 25: Female, age 46 years: This patient was admitted as an emergency with acute renal failure following delivery. Other features include haematemesis, melaena and jaundice. Her serum bilirubin was 4.6 mg per 100 ml., direct bilirubin 3.1 mg per 100 ml; alkaline phosphatase 15 K.A. units per 100 ml. Her blood urea was 121 mg per 100 ml at the time the platelet studies were carried out.

Case No. 26: Male, age 28 years: Eight months before study this patient developed frontal headache and was found to have hypertension. It was not possible to control his hypertension and he developed chronic renal failure and left ventricular failure. His blood urea was 188 mg per 100 ml on the day of the study.

Case No. 27: Male age 59 years: Five months before study this patient had a respiratory infection followed by an acute proliferative glomerulonephritis; this passed in to the nephrotic phase. There was evidence of marked renal failure as shown by proteinurea and haematuria, creatinine clearance and estimation of blood urea.

Three days before the platelet studies were done, the patient was admitted as an emergency with rigors, pyrexia, delirium and abdominal pain. Bacteraemic shock and gram-negative septicaemia were diagnosed and the patient was treated accordingly. However, his condition deteriorated steadily, he became anuric, began to bleed from his nose and mucous membranes and died 4 days after admission. Blood culture immediately before death showed the presence of haemolytic streptococcus. His blood urea was 220 mg. per 100 ml; he had thrombocytopaenia on the day the platelet studies were performed.

Case No. 28: Female, aged 32 years: This patient developed acute renal failure following septic abortion. Bleeding per vagina continued and later a purpuric rash appeared on the abdomen. Following the first dialysis the vaginal bleeding and purpura disappeared but the patient remained anuric. Her platelet count was low (61,500/c.mm.) before the first dialysis but improved steadily afterwards. Her platelet studies were carried out on several occasions before and after dialysis.

Case No. 29: Female, age 53 years: This patient was admitted as an emergency bleeding per urethra and with anuria. She was found to have an extensive tumour of the vault of vagina and base of the bladder. Although urethrostomy was performed, the patient's general condition deteriorated with little drainage of urine and

gradually rising blood urea. Her platelet studies were done on the 4th post-operative day and the patient died 10 days later.

Case No. 30: Male, age 53 years: This patient was admitted as an emergency. He was in coma and had epistaxis; acute renal failure resulting from prostatic enlargement with obstruction was diagnosed. The patient had a blood urea of 650 mg. per 100 ml., haematuria but no casts. Platelet studies were done just after admission and repeated one week later, that is 7 days after the obstruction had been relieved. The blood urea fell to 45 mg. per 100 ml. before discharge.

Case No. 31: Male, age 86 years: This patient developed acute renal failure supervening on top of chronic failure as a consequence of urinary retention due to phimosis. He had a history of myocardial infarction. His blood urea was the highest in the group (920 mg. per 100 ml.) The kaolin cephalin clotting time was 72.5 seconds, one-stage prothrombin time was 37.9 seconds (control 22 seconds). Prothrombin assay was 40.8 per cent and factor X assay was 40 per cent.

Case No. 32: Male, age 49 years: This male patient was admitted as an emergency one day before his platelet studies were done. Three months before he had experienced increasing fatigue, anorexia, nausea, vomiting and loss of weight. The diagnosis of chronic renal failure due to polycystic disease of kidneys was established. The patient also had pyelonephritis due to a mixed growth of B. coli, B. proteus and streptococcus faecalis. Following admission his condition deteriorated, he became comatosed and died five days later. His blood urea at the time of the study was 350 mg. per 100 ml.

Case No. 33: Female, age 28 years: This patient gave a four year history of recurrent attacks of right loin pain associated with dysuria. Six weeks before admission she had noticed deterioration in her vision, became breathless anddeveloped polyuria with nocturia. She had a past history of pulmonary tuberculosis and had received

therapy for this. She was in left ventricular failure with bilateral pleural effusion at the time of admission. She was also found to be hypertensive with Grade IV retinopathy. Her blood urea on admission was 154 mg. per 100 ml. A diagnosis of chronic renal failure secondary to malignant hypertension was made. She was not bleeding at the time her platelet studies were made but ecchymosis on the legs was noted 10 days later.

Case No. 34: Male, age 55 years: This male patient was admitted as an emergency with bronchopneumonia, bronchial carcinoma and acute chronic renal failure. His blood urea was 435 mg. per 100 ml. on admission, this became 234 mg. per 100 ml. when his platelet studies were done and fell to 52 mg. per 100 ml. on discharge.

Case No. 35: Male, age 31 years: This patient was suffering from chronic renal failure of unknown aetiology. The blood urea on the day of the study was 250 mg. per 100 ml.

Case No. 36: Male, age 51 years: This male patient had congenital bilateral duplex ureter and had developed chronic renal failure with chronic pyelonephritis since 1962. He had no history of bleeding. Urine culture was positive for B.coli. He was admitted as an emergency and was found to have hypokalaemic alkalosis and a blood urea of 370 mg. per 100 ml. at the time of the platelet studies.

Case No. 37: Female, age 38 years: She was admitted with acute renal failure following incomplete abortion and Clostridium Welchii septicaemia. She was jaundiced, pyrexic and oliguric. Her serum bilirubin was 14 mg per 100 ml. and her urine contained methaemoglobin and bilirubin. She had a positive direct antihuman globulin (Coombs) test to a titre of 1/320. Her platelet count was 50,000/c.mm. on admission and she had ecchymosis on the anterior tibial area, before dialysis and bruising at venepuncture sites after dialysis. Haematuria and haemoptysis were also features. The blood urea fell from 347 to 161 mg. per 100 ml. after dialysis.

Case No. 38: Male, age 42 years: This patient was suffering from epidermolysis bullosa and acute renal failure. He was investigated because he had epistaxis and haematemesis. The blood urea on the day of the platelet studies was 190 mg per 100 ml.

Case No. 39: Male, age 32 years: Two months before investigation this patient developed acute renal failure with thrombotic microangiopathy and jaundice. He also had uncontrollable malignant hypertension. There was a history of ingestion of analgesics. The patient was treated by haemodialysis on several occasions; platelet function was examined before and after haemodialysis. The blood urea before haemodialysis was 300 mg per 100 ml and 219 mg per 100 ml after haemodialysis.

Case No. 40: Male, age 23 years: This patient had acute nephritis at the age of four and frequent attacks of sore throat which culminated in tonsillectomy at the age of twelve. On admission he was found to be anaemic, hypertensive and he had a blood urea of 145 mg per 100 ml. Urine cultures were repeatedly negative. He was treated by regular haemodialysis and his platelet studies were done before and after dialysis.

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STUDIES ON BLOOD PLATELETS IN URAEMIA

Summary of thesis submitted for degree of Ph.D.

by

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The bleeding tendency in uraemia has been under investigation in recent years. It has been attributed to vascular fragility, thrombocytopenia and abnormalities in plasma coagulation factors. A qualitative platelet defect has also been suggested as being partly responsible for the haemostatic failure in uraemia.

In this thesis an attempt has been made to define the defect in platelet function in uraemia, to assess the effect of haemodialysis on the defect and to demonstrate by in vitro tests the effect on the reactivity of platelets to adenosine diphosphate of certain substances known to be present in increased amounts in uraemic plasma.

The discovery of platelets, their morphology and the role they play in haemostasis and thrombosis is described.

An historical account is given of the progress of knowledge on the bleeding tendency in uraemia and the evolution of ideas on aetiology and methods used in these studies.

An account is given on the materials and methods used to study some of the properties of platelets in normal controls and uraemic patients. These include a method for estimation of platelet aggregation by addition of calcium and calcium plus adenosine diphosphate (ADP) in an artificial circulation system (the Chandler tube technique), and by a turbidimetric method. Platelet aggregation with ADP and its reversal was studied by a turbidimetric system. The ability/

ability of platelets to adhere to glass surfaces was measured by a modified Hellem's glass bead column method. Platelet factor 3 availability was estimated by determining the 'Stypven' time of kaolin activated platelet-rich plasma (the method of Spaet and Cintron). A brief outline of the basis of each method and a discussion of some of the possible sources of error in the basic techniques are given with each method. Experiments on the effect of factors influencing the results of some of the tests were also described.

The addition of ADP to platelet-rich plasma in the Chandler tube system resulted in significant shortening of the platelet aggregation time.

When calcium and calcium plus ADP were added to platelet-rich plasma in turbidimetric system, a significant positive correlation was obtained between the platelet-rich plasma platelet count and the platelet aggregation time, duration of platelet aggregation and the fall in optical density occurring just before clot formation. A similar correlation was found with the clotting time and the platelet count.

The addition of ADP as well as calcium significantly prolonged the duration of platelet aggregation occurring just before clot formation.

When the results from normal controls and uraemic patients were compared, no difference was obtained in the mean aggregation time and clotting time, although uraemic patients had a significantly lower platelet count. However, the duration of platelet aggregation occurring before clot formation in the calcium plus ADP experiment was found to be prolonged in most of the uraemic patients examined. This may be a reflection of a qualitative platelet defect in uraemia resulting in inability of the platelets to release a normal amount of ADP in response to thrombin.

ADP/

ADP-induced platelet aggregation in normal control and uraemic patients was also investigated by the turbidimetric system. In normal controls a positive correlation was found between platelet disaggregation and the platelet count, but not between the count and platelet aggregation. However, when the results of experiments in which the platelet count was diluted with platelet-poor plasma were included, both aggregation and disaggregation were significantly correlated with the platelet count. A positive correlation also existed between platelet aggregation and disaggregation. Because of these correlations with the platelet count, the results from normal subjects and uraemic patients were grouped into four ranges of platelet count and the mean values compared. Platelet aggregation and disaggregation in uraemic patients were significantly lower than in normal controls.

The effect of haemodialysis on ADP-induced platelet aggregation was assessed. In chronic renal failure there was an insignificant increase in platelet aggregation following dialysis but the increase in platelet disaggregation was both constant and significant, usually reaching normal levels.

In acute uraemia, the effect of dialysis on platelet aggregation and disaggregation was not constant; in this respect these patients differed from those with chronic uraemia. In uraemic patients with a haemostatic failure, the time taken by their platelets to disaggregate was prolonged in the majority of cases, their ability to aggregate being more diminished than the rest of the uraemic group.

Platelet adhesiveness to glass was studied in normal subjects and uraemic patients. A significant correlation existed between platelet adhesiveness and the haematocrit value, both in normal controls and when the packed cell volume was varied in vitro. A negative correlation was obtained between the percentage disaggregation and percentage platelet adhesiveness.

When/

When no correction was made for the haematocrit value, the majority of uraemic patients showed low adhesiveness, while after correction their mean value was not significantly different from the normal mean.

Haemodialysis increased platelet adhesiveness consistently in chronic uraemic patients but not in the patients with acute renal failure.

Most uraemic patients had an abnormal relationship between disaggregation and adhesiveness. However, this relationship became normal in the majority of patients when the results of platelet adhesiveness were corrected for haematocrit.

Platelet factor 3 availability was studied in normal controls and uraemic patients. The 'Stypven' times of normal subjects were not correlated with the platelet count but were significantly correlated with the ability of platelets to disaggregate following the addition of ADP in the turbidimetric system. The mean value of the 'Stypven' times in uraemic patients was significantly lower than the mean value from normal controls and was also correlated with the platelet count. The normal relationship between the 'Stypven' time and percentage disaggregation was absent in uraemic patients.

Haemodialysis had no effect where platelet factor 3 availability was normal, but tended to restore normality in patients with reduced platelet factor 3 availability.

The in vitro effect of urea, creatinine, uric acid and guanidino succinic acid on ADP aggregation was assessed. Urea caused a diminution in both aggregation and disaggregation, an effect similar to the findings obtained from uraemic patients. Percentage disaggregation was significantly correlated with the corresponding urea concentration added to the plasma.

Creatinine, uric acid had a negligible effect on aggregation and disaggregation while guanidino succinic acid caused an insignificant/

insignificant diminution in aggregation and an increase in disaggregation.

The thesis concludes with a summary of the work.

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CONTENTS.

Table 1. The effect of adding ADP on the "snow storm" time in the Chandler tube method.

Subject No.	Control (calcium)	Test (Calcium + ADP)		Platelet count/c.mm.
	Snow storm	First snow storm	Second snow storm	
1	484	60	379	450,000
2	381	46	404	412,000
3	542	50	370	423,000
4	475	54	399	445,000
5	446	44	440	444,000
6	465	60	366	423,000
7	400	62	396	363,000
Mean	456.14	53.5	393.4	422,857
S.D.	$\pm$54	$\pm$7.43	$\pm$25.2	$\pm$29,930

Table 1 shows the effect of ADP on the "snow storm" time of recalcified platelet-rich plasma in the Chandler tube technique. The mean "snow storm" time when ADP was added with the calcium chloride was significantly shorter than when calcium chloride was added alone ($t = 2.782$, $.02 > P > 0.01$).

Table 2. Calcium-platelet aggregation in normal controls
Turbidimetric method.

Experiment No.	Subject No.	Optical density fall in phase 5	Duration of phase 5 (seconds)	Aggregation time (seconds)	Clotting time (seconds)	Platelet count/c.mm.
1	1	0.290	65	425	490	143,000
2	2	0.140	45	490	535	110,000
3	3	0.125	50	595	645	150,000
4		0.095	45	490	535	95,000
5		0.025	30	505	535	57,000
6	4	0.290	75	540	615	153,000
7		0.080	75	560	635	91,500
8		0.025	30	335	385	45,000
9	5	0.135	80	435	515	89,000
10		0.035	40	290	330	62,000
11		0.010	30	310	340	37,000
12	6	0.205	50	435	485	131,000
13		0.060	45	445	490	76,500
14		0.035	25	535	560	57,000
15	7	0.250	60	515	575	142,500
16		0.095	50	455	505	74,500

Table 2 shows the aggregating effect of calcium on platelets in plasma diluted 1:4 with barbitone buffered saline in seven normal controls. Some experiments (Nos. 4, 5, 7, 8, 10, 11, 13, 14 and 16) were carried out after diluting the platelet-rich plasma with platelet-poor plasma. A significant correlation existed between the diluted plasma platelet count and the fall in optical density in phase 5 ($r = 0.966$, $P < 0.001$), the duration of phase 5 ($r = 0.648$, $0.01 > P > 0.001$), the aggregation time ($r = 0.543$, $0.05 > P > 0.02$), and the clotting time ($r = 0.703$, $0.01 > P > 0.001$).

Table 3. Calcium plus ADP-platelet aggregation in normal controls
Turbidimetric method.

Experiment No.	Subject No.	Optical density fall in phase 5	Duration of phase 5 (seconds)	Aggregation time (seconds)	Clotting time (seconds)	Platelet count/c.mm.
1	1	0.210	110	445	555	148,000
2	2	0.155	95	450	600	110,000
3	3	0.270	105	485	590	150,000
4		0.085	70	430	500	95,000
5		0.020	50	505	555	57,000
6	4	0.265	100	630	730	153,000
7		0.105	75	505	580	91,000
8		0.045	55	335	390	45,000
9	5	0.170	80	435	515	89,000
10		0.060	65	330	395	62,000
11		0.030	55	285	340	37,000
12	6	0.240	85	525	610	131,000
13		0.090	75	550	625	76,500
14		0.050	75	425	500	57,000
15	7	0.280	125	640	765	142,500
16		0.110	70	475	545	74,500

Table 3 shows the results of platelet aggregation when calcium and ADP were added to diluted platelet-rich plasma. There was a significant correlation between the platelet count and the fall in optical density in phase 5 ($r = 0.948$, $P < 0.001$), the duration of phase 5 ($r = 0.904$, $P < 0.001$), the aggregation time ($r = 0.687$, $0.01 > P > 0.001$), and the clotting time ($r = 0.768$, $P < 0.001$).

Table 4. Calcium platelet aggregation in uraemic patients
Turbidimetric method.

Experiment No.	Subject No.	Optical density fall in phase 5	Duration of phase 5 (seconds)	Aggregation time (seconds)	Clotting time (seconds)	Platelet count/c.mm.	Blood urea (mg. per 100 ml.)	
1	12	0.010	45	480	525	62,000	87	
2	15	0.025	35	550	585	55,000	169	
3	15	0.045	55	525	580	66,000	160	Pre-dialysis
4	15	0.045	50	610	660	49,500	81	Post-dialysis
5	17	0.065	65	455	520	54,000	258	
6	18	0.170	75	430	505	120,000	116	
7	20	0.025	35	530	565	74,000	120	
8	21	0.025	35	605	640	56,000	262	Pre-dialysis
9	21	0.030	50	245	295	92,000	112	Post-dialysis
10	22	0.075	65	515	580	104,000	605	
11	35	0.110	140	485	625	75,000	280	
12	36	0.025	50	445	495	45,000	370	
13	38	0.035	55	695	750	68,000	190	

Table 4 shows the results of calcium-platelet aggregation in 10 uraemic patients. Patient No. 15 was repeated before and after haemodialysis. Patient No. 21 was also examined before and after haemodialysis.

Table 5. Calcium plus ADP-platelet aggregation in uraemic patients. Turbidimetric method.

Experiment No.	Subject No.	Optical density fall in phase 5	Duration of phase 5 (seconds)	Aggregation time (seconds)	Clotting time (seconds)	Platelet count/c.mm.	Blood urea (mg. per 100 ml.)	
1	12	0.030	95	395	490	62,000	87	
2	15	0.040	85	465	550	55,000	169	
3	15	-	-	-	595	66,000	150	Pre-dialysis
4	15	0.040	110	365	475	49,500	80	Post-dialysis
5	17	0.055	100	485	585	54,000	258	
6	18	0.025	100	545	645	120,000	116	
7	20	0.045	120	425	545	74,000	120	
8	21	0.035	70	655	725	56,000	262	Pre-dialysis
9	21	0.050	100	365	465	92,000	112	Post-dialysis
10	22	0.045	105	455	550	104,000	605	
11	35	-	-	-	585	75,000	280	
12	36	-	-	-	285	45,000	370	
13	38	0.015	95	700	795	68,000	190	

Table 5 shows the aggregating effect of calcium plus ADP in uraemic patients. Two patients (No. 15 and 21) were examined before and after dialysis. In three experiments (Nos. 3, 11 and 12) it was impossible to determine the optical density fall in phase 5, the duration of phase 5 and the aggregation time because of the inability of platelets to disaggregate in phase 2.

Table 6. Optical density readings of calcium-platelet aggregation in a normal control. Turbidimetric method.

Time (seconds)	Optical density	Time (seconds)	Optical density	Time (seconds)	Optical density	Time (seconds)	Optical density
Start	0.600	155	0.565	310	0.560	465	0.545
5	0.565	160	0.565	315	0.560	470	0.540
10	0.560	165	0.565	320	0.560	475	0.530
15	0.565	170	0.565	325	0.560	480	0.520
20	0.565	175	0.565	330	0.565	485	0.510
25	0.565	180	0.565	335	0.565	490	0.495
30	0.565	185	0.565	340	0.565	495	0.480
35	0.565	190	0.565	345	0.565	500	0.470
40	0.565	195	0.565	350	0.565	505	CLOT
45	0.565	200	0.565	355	0.565		
50	0.565	205	0.565	360	0.565		
55	0.565	210	0.565	365	0.565		
60	0.565	215	0.565	370	0.565		
65	0.565	220	0.565	375	0.565		
70	0.565	225	0.565	380	0.565		
75	0.565	230	0.565	385	0.565		
80	0.565	235	0.565	390	0.565		
85	0.565	240	0.565	395	0.565		
90	0.565	245	0.565	400	0.565		
95	0.565	250	0.565	405	0.565		
100	0.565	255	0.565	410	0.565		
105	0.565	260	0.565	415	0.565		
110	0.565	265	0.565	420	0.565		
115	0.565	270	0.565	425	0.565		
120	0.565	275	0.565	430	0.565		
125	0.565	280	0.565	435	0.565		
130	0.565	285	0.565	440	0.565		
135	0.565	290	0.565	445	0.565		
140	0.565	295	0.565	450	0.565		
145	0.565	300	0.565	455	0.565		
150	0.565	305	0.565	460	0.565		

Table 6 shows the optical density readings in the turbidimetric method after recalcification of diluted platelet-rich plasma. This is a representative experiment in a normal control (experiment No. 16, table 2, figure 7).

Table 7. Optical density reading of calcium plus ADP platelet aggregation in a normal control. Turbidimetric method

Time (seconds)	Optical density	Time (seconds)	Optical density	Time (seconds)	Optical density	Time (seconds)	Optical density
Start	0.600	155	0.460	310	0.535	465	0.565
5	0.580	160	0.460	315	0.535	470	0.565
10	0.580	165	0.460	320	0.540	475	0.555
15	0.580	170	0.460	325	0.540	480	0.555
20	0.580	175	0.455	330	0.540	485	0.550
25	0.575	180	0.460	335	0.545	490	0.545
30	0.575	185	0.455	340	0.545	495	0.540
35	0.565	190	0.460	345	0.550	500	0.535
40	0.560	195	0.460	350	0.550	505	0.525
45	0.540	200	0.460	355	0.550	500	0.520
50	0.540	205	0.460	360	0.555	515	0.510
55	0.540	210	0.465	365	0.560	520	0.500
60	0.540	215	0.470	370	0.560	525	0.490
65	0.535	220	0.470	375	0.560	530	0.475
70	0.530	225	0.475	380	0.560	535	0.470
75	0.520	230	0.480	385	0.560	540	0.455
80	0.515	235	0.480	390	0.560	545	CLOT
85	0.510	240	0.480	395	0.560		
90	0.505	245	0.485	400	0.560		
95	0.500	250	0.490	405	0.560		
100	0.495	255	0.490	410	0.560		
105	0.490	260	0.495	415	0.560		
110	0.485	265	0.500	420	0.560		
115	0.480	270	0.500	425	0.560		
120	0.480	275	0.510	430	0.565		
125	0.470	280	0.510	435	0.565		
130	0.470	285	0.515	440	0.565		
135	0.470	290	0.520	445	0.565		
140	0.470	295	0.520	450	0.565		
145	0.465	300	0.525	455	0.565		
150	0.465	305	0.530	460	0.565		

Table 7 shows the optical density readings in the turbidimetric method after addition of calcium plus ADP to diluted platelet-rich plasma. This is a representative experiment in a normal control (experiment No. 16, table 3, figure 9).

Table 8. Optical density readings of calcium-platelet aggregation in a uraemic patient. Turbidimetric method.

Time (seconds)	Optical density	Time (seconds)	Optical density	Time (seconds)	Optical density	Time (seconds)	Optical density
Start	0.600	155	0.590	310	0.590	465	0.600
5	0.585	160	0.590	315	0.590	470	0.600
10	0.585	165	0.590	320	0.590	475	0.600
15	0.585	170	0.590	325	0.590	480	0.600
20	0.585	175	0.590	330	0.590	485	0.600
25	0.590	180	0.590	335	0.590	490	0.600
30	0.590	185	0.590	340	0.590	495	0.600
35	0.590	190	0.590	345	0.590	500	0.600
40	0.590	195	0.590	350	0.590	505	0.600
45	0.590	200	0.590	355	0.590	510	0.600
50	0.590	205	0.590	360	0.590	515	0.600
55	0.590	210	0.590	365	0.590	520	0.600
60	0.590	215	0.590	370	0.590	525	0.600
65	0.590	220	0.590	375	0.595	530	0.595
70	0.590	225	0.590	380	0.595	535	0.595
75	0.590	230	0.590	385	0.595	540	0.595
80	0.590	235	0.590	390	0.595	545	0.585
85	0.590	240	0.590	395	0.595	550	0.580
90	0.590	245	0.590	400	0.595	555	0.575
95	0.590	250	0.590	405	0.595	560	0.575
100	0.590	255	0.590	410	0.595	565	CLOT
105.	0.590	260	0.590	415	0.595		
110	0.590	265	0.590	420	0.595		
115	0.590	270	0.590	425	0.595		
120	0.590	275	0.590	430	0.595		
125	0.590	280	0.590	435	0.595		
130	0.590	285	0.590	440	0.595		
135	0.590	290	0.590	445	0.600		
140	0.590	295	0.590	450	0.600		
145	0.590	300	0.590	455	0.600		
150	0.590	305	0.590	460	0.600		

Table 8 shows the optical density reading in the turbidimetric method after recalcification of diluted platelet-rich plasma. This is a representative experiment in a uraemic patient (patient No. 20, table 4, figure 8).

Table 9. Optical density readings of calcium plus ADP-platelet aggregation in a uraemic patient
Turbidimetric method

Time (seconds)	Optical density	Time (seconds)	Optical density	Time (seconds)	Optical density	Time (seconds)	Optical density
Start	0.600	155	0.585	310	0.595	465	0.585
5	0.600	160	0.585	315	0.595	470	0.585
10	0.600	165	0.585	320	0.595	475	0.580
15	0.600	170	0.585	325	0.595	480	0.580
20	0.600	175	0.580	330	0.595	485	0.580
25	0.610	180	0.580	335	0.595	490	0.580
30	0.610	185	0.580	340	0.595	495	0.575
35	0.610	190	0.580	345	0.595	500	0.570
40	0.610	195	0.580	350	0.600	505	0.570
45	0.610	200	0.580	355	0.600	510	0.570
50	0.605	205	0.580	360	0.600	515	0.565
55	0.605	210	0.580	365	0.600	520	0.565
60	0.600	215	0.580	370	0.600	525	0.560
65	0.600	220	0.580	375	0.600	530	0.555
70	0.600	225	0.580	280	0.600	535	0.555
75	0.600	230	0.580	385	0.600	540	0.555
80	0.600	235	0.580	390	0.600	545	CLOT
85	0.600	240	0.580	395	0.600		
90	0.600	245	0.580	400	0.600		
95	0.595	250	0.585	405	0.600		
100	0.595	255	0.585	410	0.600		
105	0.595	260	0.585	415	0.600		
110	0.590	265	0.585	420	0.600		
115	0.590	270	0.585	425	0.595		
120	0.590	275	0.585	430	0.595		
125	0.590	280	0.585	435	0.595		
130	0.585	285	0.590	440	0.595		
135	0.585	290	0.590	445	0.590		
140	0.585	295	0.590	450	0.590		
145	0.585	300	0.590	455	0.590		
150	0.585	305	0.590	460	0.585		

Table 9. shows the opticaldensity readings in the turbidimetric method after addition of Calcium plus ADP to diluted platelet-rich plasma. This is a representative experiment in a uraemic patient (patient No. 20, table 5, figure 10).

Table 10. Results in uraemic patients in calcium and calcium plus ADP-platelet aggregation
Turbidimetric method

Subject No.	Calcium-platelet aggregation				Calcium-ADP platelet aggregation				
	Optical density fall in phase 5	Duration of phase 5 (seconds)	Aggregation time (seconds)	Clotting time (seconds)	Optical density fall in phase 5	Duration of phase 5 (seconds)	Aggregation time (seconds)	Clotting time (seconds)	Blood urea (mg. per 100 ml.)
12	N	N	N	N	N	P	N	N	87
15	N	N	N	N	N	P	N	N	160
17	N	N	N	N	N	P	N	N	258
18	N	N	N	N	D	N	N	N	116
20	N	N	N	N	N	N	N	N	120
21	N	N	P	P	N	N	P	P	262
22	D	N	N	N	D	P	N	N	605
35	N	P	N	N	-	-	-	N	280
36	N	N	N	N	-	-	-	N	370
38	N	N	P	P	N	P	P	P	190
Abnormality (per cent)	10	10	20	20	25	62	25	20	

Table 10 shows the results in uraemic patients as compared to normal values within the 95 per cent confidence limit (figures 11 to 18).
N = Normal: P = prolonged: D = diminished.

Table 11. ADP-induced platelet aggregation in 19 normal controls
Turbidimetric method

Subject No.	Platelet aggregation				Disaggregation		
	30-60	30-90	Maximum	M.A.T. (seconds)	Time (seconds)	Percentage	Platelet count/c.mm.
1	0.120	0.180	0.380	120	150	51.3	301,000
2	0.120	0.180	0.365	180	210	46.5	345,000
3	0.180	0.280	0.480	180	210	41.6	360,000
4	0.130	0.170	0.130	150	180	72.6	375,000
5	0.170	0.230	0.425	150	180	87	340,000
6	0.190	0.300	0.485	150	180	81.4	400,500
7	0.160	0.240	0.415	150	180	74.7	369,000
8	0.125	0.185	0.300	150	180	76.6	432,000
9	0.115	0.145	0.290	90	120	86.2	442,000
10	0.065	0.115	0.200	180	210	62.5	380,000
11	0.185	0.275	0.450	180	210	57.8	435,000
12	0.085	0.150	0.245	180	240	57.1	240,500
13	0.120	0.195	0.330	180	210	65.1	442,500
14	0.165	0.240	0.480	270	300	47.9	390,000
15	0.115	0.190	0.315	180	240	50.8	255,000
16	0.165	0.240	0.380	90	120	86.8	425,000
17	0.090	0.130	0.230	100	150	76	350,000
18	0.145	0.215	0.410	150	210	65.8	422,000
19	0.130	0.190	0.380	180	210	44.7	315,000
Mean	0.1355	0.2026	0.3642	159.47	194.21	64.86	369,447
S.D.	±0.0352	±0.0514	±0.0857	±40	±42.8	±15.26	±60,300

Table 11 shows the results of ADP-induced platelet aggregation in 19 normal controls. There was no correlation between the platelet count and the parameter used to measure platelet aggregation. However, there was a significant correlation between the platelet count and percentage disaggregation ($r = 0.466$, $0.02 > P > 0.05$).

M.A.T. = Maximum aggregation time.

Table 12. ADP-induced platelet aggregation in normal controls
Group 1 (Platelet count between 350,000 - 450,000/c.mm.)
Turbidimetric method.

Subject No.	Platelet aggregation				Disaggregation		Platelet count/c.mm.	
	30-60	30-90	Maximum	M.A.T. (seconds)	Time (seconds)	Percentage		
1	0.180	0.280	0.480	180	210	41.6	360,000	undiluted
2	0.130	0.170	0.310	150	180	72.6	375,000	undiluted
3	0.190	0.300	0.485	150	180	81.4	400,500	undiluted
4	0.160	0.240	0.415	150	180	74.7	369,000	undiluted
5	0.125	0.185	0.300	150	180	76.6	432,000	undiluted
6	0.115	0.145	0.290	90	120	86.2	442,000	undiluted
7	0.065	0.115	0.200	180	210	62.5	380,000	undiluted
8	0.185	0.275	0.450	180	210	57.8	435,000	undiluted
9	0.120	0.195	0.380	180	210	65.1	442,500	undiluted
10	0.165	0.240	0.480	270	300	47.9	390,000	undiluted
11	0.165	0.240	0.380	90	120	86.8	425,000	undiluted
12	0.145	0.215	0.410	150	210	65.8	422,0000	undiluted
Mean	0.145	0.216	0.3816	160	192.5	68.25	406,083	
S.D.	±0.0362	±0.0565	±0.0904	±46.7	± 46.9	± 14.31	±30,400	

Table 12 shows the results of ADP-induced platelet aggregation in 12 normal controls with a platelet count ranging from 350,000 - 450,000/c.mm.

M.A.T. = maximum aggregation time.

Table 13. ADP-induced platelet aggregation in normal controls
Group 2 (Platelet count between 250,000 - 350,000/c.mm.)
Turbidimetric method.

Subject No.	Platelet aggregation				Disaggregation		Platelet count/c.mm.	
	30-60	30-90	Maximum	M.A.T. (seconds)	Time (seconds)	Percentage		
1	0.120	0.180	0.380	120	150	51.3	301,000	undiluted
2	0.120	0.180	0.365	180	210	46.5	345,000	undiluted
3	0.170	0.230	0.425	150	180	87	340,000	undiluted
4	0.115	0.190	0.315	180	240	50.8	255,000	undiluted
5	0.115	0.195	0.320	150	210	46.8	268,000	diluted
6	0.110	0.170	0.320	150	210	43.7	340,000	diluted
7	0.105	0.170	0.290	120	210	72.4	279,000	diluted
8	0.090	0.130	0.230	120	150	76	350,000	undiluted
9	0.130	0.190	0.380	180	210	44.7	315,000	undiluted
Mean	0.119	0.181	0.336	150	196.6	57.68	310,333	
S.D.	±0.022	±0.0264	±0.058	±25.98	±30.41	±16.23	±36,200	

Table 13 shows the results of ADP-induced platelet aggregation in 9 normal controls with a platelet count ranging between 250,000 - 350,000/c.mm. Experiments (5, 6, 7) were performed after diluting the platelet-rich plasma with platelet-poor plasma.

M.A.T. = Maximum aggregation time.

Table 14. ADP-induced platelet aggregation in normal controls
Group 3 (Platelet count between 150,000 - 250,000/c.mm.)
Turbidimetric method.

Subject No.	Platelet aggregation				Disaggregation		Platelet count/c.mm.	
	30-60	30-90	Maximum	M.A.T (seconds)	Time (seconds)	Percentage		
1	0.100	0.150	0.320	150	240	39.1	236,000	diluted
2	0.050	0.180	0.280	120	150	28.6	177,000	diluted
3	0.090	0.140	0.240	120	150	70.8	194,000	diluted
4	0.085	0.150	0.245	180	240	57.1	240,000	undiluted
5	0.065	0.115	0.200	180	210	62.5	212,000	diluted
6	0.105	0.150	0.275	150	240	67.3	223,000	diluted
7	0.080	0.120	0.210	150	180	59.5	185,000	diluted
Mean	0.082	0.1436	0.2528	150	201.4	54.9	209,571	
S.D.	0.0193	0.0217	0.042	24.49	41.4	15.45	24,890	

Table 14 shows the results of ADP-induced platelet aggregation in 7 normal controls having a platelet count in the range of 150,000 - 250,000/c.mm. Experiments 1, 2, 3, 5, 6, 7 were performed after diluting the platelet-rich plasma with platelet-poor plasma.

M.A.T. = maximum aggregation time.

Table 15. ADP-induced platelet aggregation in normal controls
Group 4 (platelet count between 50,000 - 150,000/c.mm)
Turbidimetric method.

Subject No.	Platelet aggregation				Disaggregation		Platelet count/c.mm.	
	30-60	30-90	Maximum	M.A.T. (seconds)	Time (seconds)	Percentage		
1	0.025	0.050	0.110	150	210	45.4	100,000	diluted
2	0.010	0.025	0.050	180	210	30	72,000	diluted
3	0.020	0.040	0.105	150	180	47.6	126,000	diluted
4	0.020	0.050	0.110	210	270	50	90,500	diluted
5	0.030	0.050	0.100	180	210	45	90,000	diluted
6	0.030	0.065	0.150	210	300	40	93,000	diluted
7	0.045	0.065	0.170	270	300	50	114,000	diluted
Mean	0.0257	0.049	0.1137	192.8	240	44	97,978	
S.D.	0.0109	0.0139	0.038	41.9	48.9	7.07	18,000	

Table 15 shows the results of ADP-induced platelet aggregation in 7 normal controls having a platelet count ranging between 50,000 - 150,000/c.mm. All experiments were performed after diluting the platelet-rich plasma with platelet-poor plasma.

M.A.T. = maximum aggregation.

Table 16. ADP-induced platelet aggregation in uraemic patients
Group 1 (platelet count between 350,000 - 450,000/c.mm)
Turbidimetric method

Subject No.	Platelet aggregation				Disaggregation		Platelet count/c.mm.	Blood urea mg. per 100 ml.	
	30-60	30-90	Maximum	M.A.T. (seconds)	time (seconds)	Percentage			
3	0.085	0.145	0.230	150	210	60.8	375,000	142	undiluted
5	0.070	0.135	0.270	210	270	33.3	427,000	155	undiluted
6	0.040	0.095	0.240	270	300	37.5	384,000	290	undiluted
8	0.140	0.210	0.430	210	240	33.7	362,000	310	undiluted
29	0.130	0.195	0.345	180	240	56.5	440,000	300	undiluted
Mean	0.093	0.156	0.303	204	252	44.4	397,600		
S.D.	0.0069	0.0088	0.084	44.5	34.2	13.2	34,000		

Table 16 shows the results of ADP-induced platelet aggregation in 5 uraemic patients having a platelet count ranging between 350,000 - 450,000/c.mm.

M.A.T. = maximum aggregation time .

Table 17. ADP-induced platelet aggregation in uraemic patients
Group 2 (platelet count between 250,000 - 350,000/c.mm.)

Subject No.	Platelet aggregation				Disaggregation		Platelet count/c.mm.	Blood urea mg. per 100 ml.
	30-60	30-90	Maximum	M.A.T. (seconds)	Time (seconds)	Percentage		
1	0.085	0.160	0.410	240	360	19.5	254,000	153
3	0.090	0.160	0.280	150	180	42.8	250,250	168
6	0.105	0.185	0.290	180	210	34.4	340,000	264
10	0.105	0.170	0.315	210	270	38.1	320,000	131
11	0.080	0.120	0.270	330	360	25.9	300,000	120
14	0.060	0.140	0.360	600	600	0	305,000	203
16	0.170	0.240	0.420	120	150	72.6	325,000	106
15	0.070	0.125	0.310	360	450	3.2	253,000	160
28	0.080	0.450	0.235	150	210	61.7	341,000	390
29	0.070	0.125	0.215	180	240	41.8	250,000	300
30	0.105	0.165	0.280	150	240	64.3	260,000	650
31	0.030	0.075	0.185	210	270	48.6	320,000	920
32	0.030	0.075	0.165	210	240	60.6	325,000	360
35	0.025	0.070	0.245	300	420	10.2	252,500	280
38	0.040	0.080	0.175	240	330	31.4	270,500	190
39	0.035	0.080	0.220	300	390	15.9	266,000	270
40	0.075	0.115	0.190	120	150	50	270,000	154
Mean	0.0738	0.131	0.268	238.2	298.23	36.53	287,779	
S.D.	0.0368	0.0468	0.0772	117.84	120.2	21.9	37,400	

Table 17 shows the results of ADP-induced platelet aggregation in 17 uraemic patients having a platelet count ranging between 250,000 - 350,000/c.mm.

N.B. Subject No. 28: This experiment was carried out after diluting the patient's platelet-rich plasma with platelet-poor plasma.

M.A.T. = Maximum aggregation time.

Table 18. ADP-induced platelet aggregation in uraemic patients
Group 3 (platelet count between 150,000 - 250,000/c.mm.)
Turbidimetric method.

Subject No.	Platelet aggregation				Disaggregation		Platelet count/c.mm.	Blood urea mg. per 100 ml.
	30-60	30-90	Maximum	M.A.T. (seconds)	Time (seconds)	Percentage		
2	0.130	0.230	0.425	240	300	20	231,000	150
4	0.025	0.060	0.130	600	600	0	207,000	277
7	0.060	0.115	0.280	270	300	14.2	183,000	345
9	0.020	0.060	0.160	180	210	50	245,000	85
12	0.010	0.030	0.135	600	600	0	222,000	87
14	0.030	0.080	0.205	300	420	14.6	198,000	302
15	0.050	0.110	0.280	600	600	0	205,000	169
17	0.040	0.105	0.330	600	600	0	204,000	252
20	0.090	0.145	0.305	600	600	0	229,000	120
21	0.035	0.110	0.200	180	240	42.5	160,000	262
22	0.050	0.120	0.235	240	360	21.3	211,000	605
24	0.055	0.120	0.285	240	330	19.3	204,000	162
25	0.030	0.070	0.185	360	600	0	152,000	121
26	0.015	0.050	0.145	210	330	34.5	151,500	272
33	0.025	0.095	0.175	180	270	45.7	212,000	165
36	0.080	0.130	0.280	300	330	17.8	190,000	370
Mean	0.0466	0.1018	0.237	356.2	418.1	17.5	200,281	
S.D.	0.0315	0.047	0.0823	176.2	152,8	17.5	27,490	

Table 18 shows the ADP-induced platelet aggregation in 16 uraemic patients having a platelet count between 150,000 - 250,000/c.mm.

M.A.T. = maximum aggregation time

Table 19. ADP-induced platelet aggregation in uraemic patients
Group 4 (platelet count between 50,000 - 150,000/c.mm.

Subject No.	Platelet aggregation				Disaggregation		Platelet count/c.mm.	Blood urea mg. per 100 ml.
	30-60	30-90	Maximum	M.A.T. (seconds)	Time (seconds)	Percentage		
7	0	0.010	0.050	210	350	30	74,000	345
13	0.015	0.040	0.110	120	180	50	145,000	180
19	0	0	0	600	600	0	52,000	110
23	0.020	0.035	0.095	180	300	36.8	96,000	205
27	0	0	0.020	150	600	0	52,000	200
28	0.010	0.015	0.037	150	600	0	81,000	365
37	0	0.005	0.042	330	540	5.9	75,0000	347
39	0.015	0.030	0.140	330	480	7.1	103,000	300
Mean	0.0075	0.0168	0.0617	258.9	457.5	16.35	84,750	
S.D.	0.008	0.0160	0.048	130	160	19.6	30.370	

Table 19 shows the results of ADP-induced platelet aggregation in 8 uraemic patients having a platelet count ranging between 50,000 - 150,000/c.mm.

M.A.T. = maximum aggregation time.

Table 20. The effect of haemodialysis on ADP-induced platelet aggregation in chronic renal failure patients
Turbidimetric method

Subject No.		Platelet aggregation				Disaggregation		Platelet count/c.mm.	Blood urea mg. per 100 ml.
		39-60	30-90	Maximum	M.A.T. (seconds)	Time (seconds)	Percentage		
1	Pre-dialysis	0.085	0.160	0.410	240	360	19.5	254.000	153
	Post-dialysis	0.160	0.250	0.475	240	270	55.8	289,000	54
2	Pre-dialysis	0.130	0.230	0.425	240	300	20	231,000	150
	Post-dialysis	0.160	0.255	0.470	210	270	54.2	357,000	55
3	Pre-dialysis	0.090	0.160	0.280	150	180	42.8	250,250	168
	Post-dialysis	0.120	0.200	0.370	150	180	62.1	289,750	52
5	Pre-dialysis	0.070	0.135	0.270	210	270	33.3	427,000	155
	Post-dialysis	0.065	0.130	0.240	180	300	35.4	225,000	22
21	Pre-dialysis	0.035	0.110	0.200	180	240	42.5	160,000	262
	Post-dialysis	0.070	0.135	0.240	180	270	45.8	217,000	112
15	Pre-dialysis	0.070	0.125	0.315	360	540	3.2	253,000	160
	Post-dialysis	0.095	0.160	0.315	240	300	44.4	249,000	80
40	Pre-dialysis	0.075	0.115	0.190	120	180	50	270,000	154
	Post-dialysis	0.085	0.135	0.200	150	180	87.5	252,000	80

Table 20 shows the effect of haemodialysis on 7 chronic uraemic patients. An insignificant increase in platelet aggregation occurred in 6 out of 7 patients following haemodialysis. However a constant and significant increase in percentage disaggregation was obtained ($t = 4.489$, $P < 0.001$).

Table 21. The effect of haemodialysis on ADP-induced platelet aggregation in acute renal failure patients
Turbidimetric method.

Subject No.		Platelet aggregation				Disaggregation		Platelet count/c.mm.	Blood urea mg. per 100 ml.
		30-60	30-90	Maximum	M.A.T. (seconds)	Time (seconds)	Percentage		
6	Pre-dialysis	0.040	0.090	0.240	270	300	37.5	384,000	
	Post-dialysis	0.140	0.200	0.345	120	180	39.1	396,000	
7	Pre-dialysis	0.060	0.115	0.280	270	300	14.2	183,000	
	Post-dialysis	0	0	0	600	600	0	40,000	
8	Pre-dialysis	0.140	0.230	0.430	210	240	33.7	362,000	
	Post-dialysis	0.045	0.120	0.300	270	300	28.3	259,000	
23	Pre-dialysis	0.020	0.035	0.095	180	300	36.8	96,000	
	Post-dialysis	0.005	0.015	0.065	180	300	38.4	67,000	
28	Pre-dialysis	0.005	0.010	0.0375	180	600	0	81,000	
	Post-dialysis	0.010	0.025	0.060	180	240	33.3	92,000	
37	Pre-dialysis	0	0.005	0.0425	300	540	5.8	75,000	
	Post-dialysis	0.015	0.030	0.080	210	480	6.2	66,000	
39	Pre-dialysis	0.015	0.030	0.140	330	480	7.1	112,750	
	Post-dialysis	0.030	0.060	0.180	270	330	16.6	174,000	

Table 21 shows the effect of haemodialysis on ADP-induced platelet aggregation in 7 patients with acute renal failure. Platelet aggregation increased in 4 patients and diminished in 3 following dialysis, while percentage disaggregation increased in 5 and diminished in two (figure 26, 27). The results of each experiment were compared to the mean normal values $\pm$ 2 standard deviation in the 4 rantes of platelet count (figure 28).

M.A.T. = Maximum aggregation time.

Table 22. Comparison between the mean results of normal controls and the mean results of uraemic patients in the ADP-induced platelet aggregation.
Turbidimetric method.

		Number of Controls	Mean	S.D.	Range ± 2 S.D.	Number of experiments in uraemic patients	Mean	S.D.	t-test	P value
GROUP 1	30-60 aggregation	12	0.145	0.0362	0.0726 - 0.2174	5	0.093	0.0069	2.593	0.05 > P > 0.02
	30-90 aggregation		0.216	0.0565	0.103 - 0.329		0.156	0.0088	2.085	0.1 > P > 0.05
	Maximum		0.3816	0.0904	0.2008 - 0.5624		0.303	0.084	0.526	P > 0.1
	M.A.T.		160	46.7	66.6 - 253.4		204	44.5	1.794	0.1 > P > 0.05
	D.T.		192.5	46.9	98.7 - 286.3		252	34.2	2.548	0.05 > P > 0.02
	Percentage disaggregation		68.25	14.31	39.63 - 96.87		44.36	13.23	3.201	0.02 > P > 0.01
	Platelet count		406.083	30.400	345.283 - 466.883		397.600	34.000	0.507	P > 0.1
GROUP 2	30-60 aggregation	9	0.119	0.022	0.075 - 0.163	17	0.0738	0.0368	3.362	0.01 > P > 0.001
	30-90 aggregation		0.181	0.264	0.1282 - 0.2338		0.131	0.0468	2.950	0.01 > P > 0.001
	Maximum		0.336	0.058	0.22 - 0.452		0.268	0.0772	2.309	0.05 > P > 0.02
	M.A.T.		150	25.98	98.04 - 201.96		238.2	117.84	2.196	0.05 > P > 0.02
	D.T.		196.6	30.41	135.78 - 257.42		298.23	120.2	2.472	0.05 > P > 0.02
	Percentage disaggregation		57.68	16.23	25.24 - 90.12		36.53	21.9	2.540	0.02 > P > 0.01
	Platelet count		310.333	36.200	237.933 - 382.733		287.779	37.400	1.482	P > 0.01
GROUP 3	30-60 aggregation	7	0.082	0.0193	0.043 - 0.12	16	0.0466	0.0315	2.739	0.02 > P > 0.01
	30-90 aggregation		0.1436	0.0217	0.1 - 0.187		0.1018	0.047	2.165	0.05 > P > 0.02
	Maximum		0.2528	0.042	0.169 - 0.337		0.237	0.0823	0.477	P > 0.1
	M.A.T.		150	24.49	101 - 199		356.2	176.2	3.044	0.01 > P > 0.001
	D.T.		201.4	41.4	118.6 - 284.2		418.1	152.8	3.651	0.01 > P > 0.001
	Percentage disaggregation		54.9	15.45	24 - 85.8		17.5	17.5	4.852	P < 0.001
	Platelet count		209.571	24.890	159.791 - 259.351		200.281	27.490	0.765	P > 0.1
GROUP 4	30-60 aggregation	7	0.0257	0.0109	0.004 - 0.0475	8	0.0075	0.008	3.628	0.01 > P > 0.001
	30-90 aggregation		0.049	0.0139	0.021 - 0.0768		0.0168	0.016	4.151	0.01 > P > 0.001
	Maximum		0.1137	0.038	0.0377 - 0.189		0.0617	0.048	2.283	0.05 > P > 0.02
	M.A.T.		192.8	41.9	109 - 276.6		258.9	130	1.543	P > 0.1
	D.T.		240	48.9	142.2 - 337.8		457.5	160	3.438	0.01 > P > 0.001
	Percentage disaggregation		44	7.07	29.86 - 58.14		16.35	19.6	3.653	0.01 > P > 0.001
	Platelet count		97.978	18.000	61.978 - 103.978		84.750	30.370	1.005	P > 0.1

M.A.T. = Maximum aggregation time (in seconds)
D.T. = Disaggregation time (in seconds)
Platelet count = platelet-rich plasma platelet count/c.mm.
Total of 35 experiments in normal controls and 46 experiments in uraemic patients.
Group 1 = platelet count between 350,000 - 450,000/c.mm.
Group 2 = platelet count between 250,000 - 350,000/c.mm.
Group 3 = platelet count between 150,000 - 250,000/c.mm.
Group 4 = platelet count between 50,000 - 150,000/c.mm.

Table 23. Results of ADP-induced platelet aggregation and the corresponding platelet count in normal controls.

Experiment No.	Platelet aggregation			Percentage disaggregation	Platelet count/c.mm.
	30-60	30-90	Maximum		
1	0.025	0.050	0.110	45.4	100,000
2	0.010	0.025	0.050	30	72,000
3	0.020	0.040	0.105	47.6	126,000
4	0.020	0.050	0.110	50	90,500
5	0.030	0.050	0.100	45	90,000
6	0.030	0.065	0.150	40	93,000
7	0.045	0.065	0.170	50	114,000
8	0.100	0.150	0.320	39.1	236,000
9	0.050	0.180	0.280	28.6	177,000
10	0.090	0.140	0.240	70.8	194,000
11	0.085	0.150	0.245	59.1	240,000
12	0.065	0.115	0.200	62.5	212,000
13	0.105	0.150	0.275	67.3	223,000
14	0.080	0.120	0.210	59.5	185,000
15	0.120	0.180	0.380	51.3	301,000
16	0.120	0.180	0.365	46.5	345,000
17	0.170	0.230	0.425	87	340,000
18	0.115	0.190	0.315	50.8	255,000
19	0.115	0.195	0.320	46.8	268,000
20	0.110	0.170	0.320	43.7	340,000
21	0.105	0.170	0.290	72.4	279,000
22	0.090	0.130	0.230	76	350,000
23	0.180	0.280	0.480	41.6	360,000
24	0.130	0.170	0.310	72.6	375,000
25	0.190	0.300	0.485	81.4	400,500
26	0.160	0.240	0.415	74.7	369,000
27	0.125	0.185	0.300	76.6	432,000
28	0.115	0.145	0.290	86.2	442,000
29	0.065	0.115	0.200	62.5	380,000
30	0.185	0.275	0.450	57.8	435,000
31	0.120	0.195	0.380	65.1	442,500
32	0.165	0.240	0.480	47.9	390,000
33	0.165	0.240	0.380	86.8	425,000
34	0.145	0.215	0.410	65.8	422,000
35	0.130	0.190	0.380	44.7	315,000

Table 23 shows the results of ADP-induced platelet aggregation in 35 normal experiments. Fifteen experiments were carried out after diluting the platelet-rich plasma with platelet-poor plasma. All parameters of platelet aggregation and disaggregation were significantly correlated with the plateletcount (Chapter 5).

Table 24. ADP-induced platelet aggregation in 19 normal controls.
Optical density readings.

	1	2	3	4	5	6	7	8	9	10	11
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.430	0.480	0.470	0.510	0.440	0.480	0.460	0.525	0.455	0.560	0.480
1	0.310	0.360	0.290	0.380	0.270	0.290	0.300	0.400	0.340	0.495	0.295
1.30	0.250	0.340	0.190	0.340	0.210	0.180	0.220	0.340	0.310	0.445	0.205
2	0.220	0.270	0.160	0.300	0.180	0.140	0.190	0.305	0.345	0.420	0.170
2.30	0.235	0.250	0.130	0.290	0.175	0.115	0.185	0.300	0.425	0.405	0.155
3	0.275	0.235	0.120	0.295	0.230	0.120	0.200	0.305	0.495	0.400	0.150
3.30	0.310	0.245	0.125	0.315	0.295	0.160	0.230	0.320	0.530	0.405	0.195
4	0.350	0.250	0.130	0.345	0.360	0.225	0.270	0.350	0.550	0.410	0.210
4.30	0.375	0.260	0.145	0.380	0.445	0.290	0.310	0.385	0.560	0.425	0.250
5	0.395	0.280	0.180	0.415	0.490	0.350	0.360	0.420	0.560	0.445	0.295
5.30	0.400	0.300	0.195	0.445	0.520	0.410	0.405	0.450	0.560	0.465	0.335
6	0.410	0.330	0.230	0.470	0.530	0.450	0.440	0.480	0.560	0.480	0.510
6.30	0.410	0.360	0.250	0.495	0.540	0.480	0.465	0.505	0.560	0.500	0.390
7	0.415	0.375	0.280	0.505	0.540	0.495	0.480	0.520	0.560	0.510	0.400
7.30	0.415	0.390	0.300	0.515	0.545	0.510	0.495	0.530	0.560	0.520	0.405
8	0.420	0.405	0.320	0.520	0.545	0.520	0.500	0.540	0.560	0.525	0.410
8.30	0.425	0.415	0.340	0.525	0.550	0.520	0.505	0.540	0.560	0.530	0.415
9	0.435	0.420	0.360	0.525	0.550	0.520	0.505	0.545	0.560	0.530	0.425
9.30	0.440	0.430	0.375	0.525	0.550	0.520	0.510	0.550	0.560	0.530	0.440
10	0.440	0.440	0.385	0.525	0.550	0.520	0.510	0.550	0.560	0.530	0.460

Table 24 (continued).

	12	13	14	15	16	17	18	19
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.560	0.525	0.465	0.540	0.460	0.510	0.465	0.450
1	0.475	0.405	0.300	0.425	0.295	0.420	0.320	0.320
1.30	0.410	0.330	0.225	0.350	0.220	0.380	0.250	0.260
2	0.375	0.290	0.185	0.315	0.225	0.370	0.215	0.235
2.30	0.360	0.275	0.160	0.295	0.295	0.390	0.190	0.225
3	0.355	0.270	0.140	0.285	0.395	0.420	0.190	0.220
3.30	0.355	0.275	0.130	0.285	0.480	0.460	0.205	0.230
4	0.370	0.300	0.125	0.290	0.530	0.490	0.230	0.255
4.30	0.390	0.330	0.120	0.295	0.550	0.515	0.265	0.275
5	0.410	0.360	0.130	0.315	0.555	0.520	0.295	0.295
5.30	0.430	0.395	0.140	0.340	0.560	0.535	0.335	0.315
6	0.450	0.425	0.160	0.360	0.560	0.545	0.380	0.325
6.30	0.465	0.450	0.180	0.385	0.550	0.545	0.415	0.350
7	0.475	0.465	0.210	0.405	0.545	0.545	0.440	0.365
7.30	0.485	0.475	0.230	0.425	0.545	0.545	0.460	0.375
8	0.495	0.495	0.260	0.445	0.545	0.545	0.470	0.390
8.30	0.500	0.490	0.295	0.455	0.545	0.545	0.480	0.400
9	0.500	0.500	0.320	0.465	0.545	0.545	0.490	0.410
9.30	0.505	0.510	0.350	0.475	0.545	0.545	0.500	0.415
10	0.510	0.500	0.370	0.480	0.545	0.545	0.500	0.420

Table 25. ADP-induced platelet aggregation in normal controls
Optical density readings in Group I
Turbidimetric method

Time (minutes)	1	2	3	4	5	6	7	8	9	10	11	12
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.470	0.510	0.480	0.460	0.525	0.455	0.560	0.480	0.525	0.465	0.460	0.465
1	0.290	0.380	0.290	0.300	0.400	0.340	0.495	0.295	0.405	0.300	0.295	0.320
1.30	0.190	0.340	0.180	0.220	0.340	0.310	0.445	0.205	0.330	0.225	0.220	0.250
2	0.160	0.300	0.140	0.190	0.305	0.345	0.420	0.170	0.290	0.185	0.225	0.215
2.30	0.130	0.290	0.115	0.185	0.300	0.425	0.405	0.155	0.275	0.160	0.295	0.190
3	0.120	0.295	0.120	0.200	0.305	0.495	0.400	0.150	0.270	0.140	0.395	0.190
3.30	0.125	0.315	0.160	0.230	0.320	0.530	0.405	0.175	0.275	0.130	0.480	0.205
4	0.130	0.345	0.225	0.270	0.350	0.550	0.410	0.210	0.300	0.125	0.530	0.230
4.30	0.145	0.380	0.290	0.310	0.385	0.560	0.425	0.250	0.330	0.120	0.550	0.265
5	0.180	0.415	0.350	0.360	0.420	0.560	0.445	0.295	0.360	0.130	0.555	0.295
5.30	0.195	0.445	0.410	0.405	0.450	0.560	0.465	0.335	0.395	0.140	0.560	0.335
6	0.230	0.470	0.450	0.440	0.480	0.560	0.480	0.370	0.425	0.160	0.560	0.380
6.30	0.250	0.495	0.480	0.465	0.505	0.560	0.500	0.390	0.450	0.180	0.550	0.415
7	0.280	0.505	0.495	0.480	0.520	0.560	0.510	0.400	0.465	0.210	0.545	0.440
7.30	0.300	0.515	0.510	0.495	0.530	0.560	0.520	0.405	0.475	0.230	0.545	0.460
8	0.370	0.520	0.520	0.500	0.540	0.560	0.525	0.410	0.485	0.260	0.545	0.470
8.30	0.340	0.525	0.520	0.505	0.540	0.560	0.530	0.415	0.490	0.295	0.545	0.480
9	0.360	0.525	0.520	0.505	0.545	0.560	0.530	0.425	0.500	0.320	0.545	0.490
9.30	0.375	0.525	0.520	0.510	0.550	0.560	0.530	0.440	0.500	0.350	0.545	0.500
10	0.385	0.525	0.520	0.510	0.550	0.560	0.530	0.460	0.500	0.370	0.545	0.500

Table 26. ADP-induced platelet aggregation in normal controls optical density readings in Group 2.

Time Minutes	1	2	3	4	5	6	7	8	9
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.430	0.480	0.440	0.540	0.520	0.490	0.505	0.510	0.450
1	0.310	0.360	0.270	0.425	0.405	0.380	0.400	0.420	0.320
1.30	0.250	0.300	0.210	0.350	0.325	0.320	0.335	0.380	0.260
2	0.220	0.270	0.180	0.315	0.290	0.295	0.310	0.370	0.235
2.30	0.235	0.250	0.175	0.295	0.280	0.280	0.310	0.390	0.225
3	0.275	0.235	0.23	0.285	0.280	0.280	0.310	0.420	0.220
3.30	0.310	0.245	0.295	0.285	0.310	0.290	0.330	0.460	0.230
4	0.350	0.250	0.360	0.290	0.350	0.305	0.365	0.490	0.255
4.30	0.375	0.260	0.445	0.295	0.375	0.330	0.405	0.515	0.275
5	0.395	0.280	0.490	0.315	0.395	0.355	0.440	0.520	0.295
5.30	0.400	0.300	0.520	0.340	0.410	0.375	0.475	0.535	0.315
6	0.410	0.330	0.530	0.360	0.420	0.395	0.500	0.545	0.325
6.30	0.410	0.360	0.540	0.385	0.420	0.405	0.515	0.545	0.350
7	0.415	0.375	0.540	0.405	0.430	0.415	0.520	0.545	0.365
7.30	0.415	0.390	0.545	0.425	0.430	0.420	0.530	0.545	0.375
8	0.420	0.405	0.545	0.445	0.440	0.425	0.535	0.545	0.390
8.30	0.425	0.415	0.550	0.455	0.450	0.425	0.540	0.545	0.400
9	0.435	0.420	0.550	0.465	0.450	0.430	0.540	0.545	0.410
9.30	0.440	0.430	0.550	0.475	0.455	0.430	0.540	0.545	0.415
10	0.440	0.440	0.550	0.480	0.460	0.435	0.540	0.545	0.420

Table 27. ADP-induced platelet aggregation in normal control optical density readings in Group 3.

	1	2	3	4	5	6	7
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.480	0.510	0.510	0.560	0.560	0.510	0.530
1	0.380	0.460	0.420	0.475	0.495	0.405	0.450
1.30	0.330	0.330	0.370	0.410	0.445	0.360	0.410
2	0.295	0.320	0.360	0.375	0.420	0.330	0.395
2.30	0.280	0.335	0.270	0.360	0.405	0.325	0.390
3	0.280	0.360	0.410	0.355	0.400	0.335	0.395
3.30	0.280	0.380	0.450	0.355	0.405	0.355	0.410
4	0.290	0.390	0.485	0.370	0.410	0.380	0.435
4.30	0.300	0.390	0.510	0.390	0.425	0.410	0.460
5	0.320	0.390	0.520	0.410	0.445	0.440	0.480
5.30	0.340	0.395	0.525	0.430	0.465	0.465	0.495
6	0.360	0.395	0.530	0.450	0.480	0.480	0.500
6.30	0.375	0.400	0.530	0.465	0.500	0.495	0.505
7	0.390	0.400	0.530	0.475	0.510	0.500	0.510
7.30	0.405	0.405	0.530	0.485	0.520	0.510	0.515
8	0.410	0.420	0.530	0.495	0.525	0.510	0.520
8.30	0.420	0.420	0.530	0.500	0.530	0.515	0.520
9	0.420	0.430	0.530	0.500	0.530	0.520	0.525
9.30	0.425	0.430	0.530	0.505	0.530	0.520	0.525
10	0.430	0.440	0.530	0.510	0.530	0.520	0.530

Table 28. ADP-induced platelet aggregation in normal control. Optical density readings in Group 4. Turbidimetric method.

	1	2	3	4	5	6	7
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.510	0.580	0.565	0.510	0.565	0.565	0.570
1	0.545	0.570	0.545	0.550	0.535	0.535	0.525
1.30	0.520	0.555	0.525	0.520	0.515	0.500	0.485
2	0.500	0.560	0.510	0.500	0.505	0.480	0.460
2.30	0.490	0.555	0.500	0.495	0.500	0.465	0.450
3	0.490	0.550	0.495	0.495	0.510	0.455	0.470
2.30	0.495	0.555	0.500	0.490	0.520	0.455	0.440
4	0.500	0.557	0.515	0.490	0.525	0.450	0.435
4.30	0.510	0.560	0.520	0.495	0.525	0.450	0.430
5	0.520	0.560	0.530	0.500	0.540	0.455	0.435
5.30	0.530	0.560	0.540	0.505	0.540	0.455	0.440
6	0.535	0.560	0.540	0.515	0.540	0.460	0.445
6.30	0.540	0.565	0.545	0.520	0.545	0.470	0.455
7	0.540	0.565	0.545	0.530	0.545	0.480	0.470
7.30	0.545	0.565	0.545	0.530	0.545	0.485	0.480
8	0.545	0.570	0.545	0.540	0.550	0.495	0.495
8.30	0.545	0.570	0.545	0.545	0.550	0.505	0.500
9	0.545	0.565	0.550	0.550	0.550	0.510	0.505
9.30	0.545	0.565	0.550	0.560	0.550	0.515	0.515
10	0.545	0.565	0.550	0.560	0.550	0.520	0.520

Table 29. ADP-induced platelet aggregation in uraemic patients Optical density readings Group 1 (platelet count 350,000 - 450,000/c.mm).

Time (minutes)	Subject number				
	3	5	6	8	29
Start	0.600	0.600	0.600	0.600	0.600
30	0.550	0.540	0.560	0.520	0.525
1	0.465	0.470	0.520	0.380	0.395
1.30	0.405	0.405	0.465	0.290	0.330
2	0.375	0.365	0.420	0.240	0.285
2.30	0.370	0.350	0.395	0.190	0.270
3	0.370	0.340	0.375	0.180	0.255
3.30	0.385	0.330	0.370	0.170	0.255
4	0.410	0.330	0.365	0.175	0.260
4.30	0.435	0.335	0.360	0.175	0.280
5	0.465	0.340	0.365	0.185	0.295
5.30	0.480	0.350	0.370	0.210	0.320
6	0.495	0.355	0.375	0.230	0.350
6.30	0.500	0.370	0.385	0.250	0.385
7	0.510	0.390	0.400	0.280	0.410
7.30	0.510	0.400	0.410	0.290	0.430
8	0.520	0.410	0.420	0.305	0.450
8.30	0.520	0.420	0.430	0.315	0.455
9	0.520	0.430	0.440	0.325	0.470
9.30	0.520	0.440	0.450	0.330	0.475
10	0.520	0.450	0.455	0.340	0.480

Table 30. ADP-induced platelet aggregation in uraemic patients.
Optical density readings Group 2 (platelet count 250,000 - 350,000/c.mm.).

	Subject number								
Time (minutes)	1	3	6	10	11	14	16	15	28
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.510	0.510	0.530	0.530	0.530	0.570	0.460	0.545	0.550
1	0.425	0.420	0.415	0.425	0.450	0.510	0.290	0.475	0.470
1.30	0.350	0.350	0.345	0.360	0.410	0.430	0.220	0.420	0.405
2	0.250	0.325	0.315	0.315	0.380	0.380	0.180	0.365	0.375
2.30	0.215	0.320	0.305	0.300	0.370	0.340	0.200	0.340	0.365
3	0.205	0.330	0.310	0.290	0.350	0.320	0.260	0.325	0.365
3.30	0.195	0.350	0.330	0.285	0.345	0.310	0.330	0.310	0.400
4	0.190	0.370	0.360	0.285	0.340	0.290	0.400	0.300	0.430
4.30	0.190	0.395	0.375	0.290	0.340	0.270	0.450	0.295	0.460
5	0.190	0.405	0.385	0.295	0.345	0.270	0.470	0.290	0.480
5.30	0.190	0.415	0.390	0.310	0.330	0.265	0.480	0.290	0.490
6	0.195	0.420	0.390	0.315	0.340	0.260	0.480	0.285	0.500
6.30	0.205	0.425	0.395	0.340	0.345	0.260	0.485	0.285	0.505
7	0.215	0.435	0.395	0.360	0.350	0.250	0.490	0.285	0.510
7.30	0.225	0.440	0.400	0.385	0.355	0.250	0.490	0.285	0.510
8	0.240	0.445	0.410	0.400	0.360	0.250	0.495	0.285	0.515
8.30	0.260	0.450	0.415	0.405	0.370	0.250	0.495	0.285	0.515
9	0.270	0.460	0.420	0.435	0.380	0.245	0.495	0.290	0.520
9.30	0.275	0.470	0.425	0.450	0.390	0.240	0.500	0.290	0.520
10	0.285	0.470	0.430	0.450	0.400	0.240	0.500	0.295	0.520
10.30	-	-	-	-	0.400	-	-	0.295	-
11	-	-	-	-	-	-	-	0.295	-

Table 30 (continued).

Time (minutes)	Subject number							
	29	30	31	32	35	38	34	40
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.555	0.525	0.570	0.570	0.555	0.560	0.575	0.540
1	0.485	0.420	0.540	0.540	0.530	0.520	0.540	0.465
1.30	0.430	0.360	0.495	0.495	0.485	0.480	0.495	0.425
2	0.405	0.330	0.460	0.465	0.450	0.455	0.460	0.410
2.30	0.390	0.320	0.435	0.450	0.415	0.440	0.415	0.410
3	0.385	0.320	0.425	0.440	0.400	0.430	0.400	0.420
3.30	0.385	0.320	0.415	0.435	0.380	0.430	0.395	0.445
4	0.395	0.335	0.415	0.440	0.370	0.425	0.385	0.475
4.30	0.405	0.355	0.420	0.455	0.365	0.425	0.385	0.505
5	0.415	0.390	0.435	0.485	0.355	0.425	0.380	0.525
5.30	0.435	0.420	0.450	0.480	0.355	0.430	0.380	0.540
6	0.450	0.450	0.460	0.495	0.355	0.435	0.380	0.540
6.30	0.455	0.475	0.470	0.505	0.355	0.440	0.385	0.540
7	0.465	0.490	0.480	0.515	0.365	0.445	0.385	0.540
7.30	0.470	0.500	0.490	0.525	0.360	0.445	0.390	0.540
8	0.475	0.505	0.495	0.530	0.360	0.460	0.395	0.545
8.30	0.475	0.510	0.505	0.535	0.365	0.470	0.400	0.545
9	0.480	0.515	0.505	0.535	0.370	0.480	0.405	0.545
9.30	0.480	0.520	0.510	0.535	0.375	0.485	0.410	0.545
10	0.480	0.520	0.510	0.540	0.380	0.490	0.415	0.550
10.30	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-

Table 31. ADP-induced platelet aggregation in uraemic patients
Optical density readings Group 3 (platelet count 150,000 - 250,000/c.mm)

	Subject number							
Time (minutes)	2	4	7	9	12	14	15	17
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.500	0.580	0.540	0.555	0.580	0.580	0.570	0.540
1	0.370	0.555	0.480	0.465	0.570	0.550	0.520	0.500
1.30	0.270	0.520	0.425	0.410	0.550	0.500	0.460	0.435
2	0.230	0.500	0.390	0.375	0.530	0.465	0.420	0.380
2.30	0.205	0.490	0.360	0.360	0.515	0.440	0.390	0.355
3	0.190	0.490	0.340	0.350	0.500	0.425	0.380	0.330
3.30	0.185	0.485	0.335	0.335	0.490	0.415	0.360	0.315
4	0.175	0.480	0.325	0.330	0.485	0.405	0.350	0.305
4.30	0.175	0.480	0.320	0.325	0.480	0.400	0.345	0.295
5	0.180	0.475	0.325	0.320	0.470	0.395	0.340	0.295
5.30	0.180	0.475	0.330	0.315	0.470	0.395	0.330	0.285
6	0.195	0.470	0.335	0.315	0.465	0.395	0.325	0.280
6.30	0.200	0.470	0.340	0.310	0.465	0.395	0.320	0.280
7	0.215	0.470	0.345	0.305	0.465	0.400	0.320	0.280
7.30	0.230	0.470	0.350	0.305	0.465	0.400	0.320	0.280
8	0.240	0.470	0.355	0.300	0.465	0.405	0.320	0.280
8.30	0.250	0.470	0.355	0.300	0.465	0.410	0.320	0.275
9	0.260	0.490	0.360	0.300	0.465	0.415	0.320	0.270
9.30	0.275	0.470	0.360	0.295	0.465	0.420	0.320	0.270
10	0.285	0.470	0.365	0.295	0.465	0.425	0.320	0.270

Table 31 (continued).

Time (minutes)	Subject number							
	20	21	22	24	25	26	33	35
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.555	0.560	0.570	0.545	0.570	0.585	0.575	0.530
1	0.465	0.505	0.520	0.490	0.540	0.570	0.520	0.450
1.30	0.410	0.450	0.450	0.425	0.500	0.535	0.480	0.400
2	0.375	0.420	0.415	0.385	0.465	0.500	0.455	0.370
2.30	0.360	0.405	0.395	0.355	0.455	0.480	0.440	0.355
3	0.350	0.400	0.380	0.330	0.435	0.465	0.430	0.340
3.30	0.335	0.400	0.370	0.325	0.430	0.455	0.425	0.330
4	0.330	0.405	0.365	0.315	0.425	0.455	0.425	0.325
4.30	0.325	0.420	0.365	0.315	0.425	0.455	0.430	0.325
5	0.320	0.435	0.365	0.315	0.420	0.455	0.440	0.320
5.30	0.315	0.445	0.365	0.320	0.420	0.465	0.455	0.325
6	0.315	0.460	0.370	0.325	0.415	0.475	0.465	0.330
6.30	0.310	0.470	0.375	0.325	0.420	0.480	0.480	0.335
7	0.305	0.475	0.385	0.330	0.420	0.485	0.490	0.335
7.30	0.300	0.480	0.390	0.340	0.420	0.495	0.495	0.340
8	0.300	0.485	0.400	0.350	0.420	0.500	0.500	0.345
8.30	0.300	0.490	0.405	0.360	0.420	0.505	0.505	0.355
9	0.295	0.495	0.415	0.370	0.420	0.510	0.505	0.355
9.30	0.295	0.495	0.420	0.370	0.420	0.515	0.505	0.365
10	0.295	0.495	0.430	0.380	0.420	0.515	0.505	0.370

Table 32. ADP-induced platelet aggregation in uraemic patients
Optical density readings of group 4 (platelet count 50,000 - 150,000/c.mm)

Time (minutes)	Subject number							
	7	13	19	23	27	28	37	39
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.580	0.540	0.590	0.560	0.585	0.585	0.570	0.575
1	0.580	0.525	0.590	0.540	0.585	0.575	0.570	0.560
1.30	0.570	0.500	0.590	0.525	0.585	0.570	0.565	0.545
2	0.560	0.490	0.590	0.515	0.585	0.565	0.565	0.525
2.30	0.560	0.490	0.590	0.510	0.580	0.565	0.562	0.510
3	0.560	0.500	0.590	0.505	0.580	0.565	0.560	0.500
3.30	0.550	0.505	0.590	0.505	0.580	0.565	0.560	0.485
4	0.550	0.510	0.590	0.505	0.580	0.565	0.560	0.475
4.30	0.550	0.520	0.590	0.505	0.580	0.565	0.560	0.470
5	0.550	0.525	0.590	0.510	0.580	0.565	0.560	0.465
5.30	0.550	0.530	0.590	0.515	0.580	0.565	0.557	0.460
6	0.555	0.535	0.590	0.520	0.580	0.565	0.557	0.460
6.30	0.560	0.540	0.590	0.525	0.580	0.565	0.557	0.460
7	0.560	0.545	0.590	0.530	0.580	0.565	0.557	0.460
7.30	0.560	0.545	0.590	0.535	0.580	0.565	0.557	0.460
8	0.560	0.550	0.590	0.540	0.575	0.565	0.557	0.465
8.30	0.565	0.555	0.590	0.540	0.580	0.565	0.557	0.465
9	0.565	0.560	0.590	0.540	0.580	0.565	0.560	0.465
9.30	0.565	0.560	0.590	0.540	0.580	0.565	0.560	0.465
10	0.565	0.560	0.590	0.540	0.580	0.565	0.560	0.470
10.30	-	-	-	-	-	-	0.560	0.470

Table 33. ADP-induced platelet aggregation
Optical density readings before and after haemodialysis in chronic renal failure patients.
Turbidimetric method.

	Before haemodialysis							After haemodialysis						
	Subject number							Subject number						
Time (minutes)	1	2	3	5	15	21	40	1	2	3	5	15	21	40
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.510	0.500	0.510	0.540	0.545	0.560	0.540	0.510	0.490	0.450	0.550	0.520	0.550	0.555
1	0.425	0.370	0.420	0.470	0.475	0.505	0.465	0.350	0.330	0.330	0.485	0.425	0.480	0.470
1.30	0.350	0.270	0.350	0.405	0.420	0.450	0.425	0.260	0.235	0.250	0.430	0.360	0.415	0.420
2	0.250	0.230	0.325	0.365	0.365	0.420	0.410	0.225	0.180	0.230	0.395	0.320	0.385	0.405
2.30	0.215	0.205	0.370	0.350	0.340	0.405	0.410	0.200	0.155	0.250	0.370	0.310	0.370	0.400
3	0.205	0.190	0.330	0.340	0.325	0.400	0.420	0.160	0.140	0.300	0.360	0.295	0.360	0.410
3.30	0.195	0.185	0.350	0.330	0.310	0.400	0.445	0.140	0.130	0.360	0.360	0.290	0.360	0.430
4	0.190	0.175	0.370	0.330	0.300	0.405	0.475	0.125	0.130	0.400	0.360	0.285	0.360	0.470
4.30	0.190	0.175	0.395	0.335	0.295	0.420	0.505	0.130	0.135	0.430	0.360	0.285	0.375	0.510
5	0.190	0.180	0.405	0.340	0.290	0.435	0.525	0.135	0.170	0.440	0.365	0.290	0.380	0.540
5.30	0.190	0.180	0.415	0.350	0.290	0.445	0.540	0.150	0.200	0.450	0.375	0.300	0.415	0.560
6	0.195	0.195	0.420	0.355	0.285	0.460	0.540	0.175	0.230	0.455	0.385	0.310	0.425	0.565
6.30	0.205	0.200	0.425	0.370	0.285	0.470	0.540	0.210	0.280	0.460	0.400	0.325	0.440	0.570
7	0.215	0.215	0.435	0.390	0.285	0.475	0.540	0.275	0.320	0.460	0.415	0.345	0.460	0.575
7.30	0.225	0.230	0.440	0.400	0.285	0.480	0.545	0.320	0.350	0.465	0.435	0.365	0.465	0.575
8	0.240	0.240	0.445	0.410	0.285	0.485	0.545	0.360	0.370	0.465	0.445	0.385	0.470	0.575
8.30	0.260	0.250	0.450	0.420	0.285	0.490	0.545	0.375	0.385	0.465	0.455	0.400	0.480	0.575
9	0.270	0.260	0.460	0.430	0.29	0.495	0.545	0.390	0.385	0.470	0.465	0.415	0.485	0.575
9.30	0.275	0.275	0.470	0.440	0.29	0.495	0.545	0.405	0.390	0.470	0.470	0.425	0.490	0.575
10	0.285	0.285	0.470	0.450	0.295	0.495	0.55	0.410	0.390	0.470	0.475	0.435	0.500	0.575
10.30					0.295									
11					0.295									

Table 34. ADP-induced platelet aggregation
Optical density readings before and after haemodialysis in acute renal failure patients
Turbidimetric method

Time (minutes)	Before haemodialysis							After haemodialysis						
	Subject number							Subject number						
	6	7	8	23	28	37	39	6	7	8	23	28	37	39
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.560	0.540	0.520	0.560	0.580	0.570	0.575	0.500	0.580	0.560	0.585	0.585	0.565	0.555
1	0.520	0.480	0.380	0.540	0.575	0.570	0.560	0.360	0.580	0.515	0.580	0.525	0.550	0.525
1.30	0.470	0.425	0.290	0.525	0.570	0.565	0.545	0.300	0.580	0.440	0.570	0.560	0.535	0.490
2	0.420	0.390	0.240	0.515	0.565	0.565	0.525	0.280	0.580	0.380	0.540	0.550	0.520	0.465
2.30	0.395	0.360	0.190	0.510	0.565	0.562	0.510	0.260	0.580	0.350	0.540	0.545	0.525	0.445
3	0.375	0.340	0.180	0.505	0.565	0.560	0.500	0.255	0.580	0.320	0.535	0.540	0.525	0.440
3.30	0.370	0.335	0.170	0.505	0.562	0.560	0.485	0.270	0.580	0.320	0.535	0.540	0.520	0.430
4	0.365	0.325	0.175	0.505	0.562	0.560	0.475	0.285	0.580	0.315	0.535	0.540	0.520	0.425
4.30	0.360	0.320	0.175	0.505	0.562	0.557	0.470	0.300	0.580	0.300	0.535	0.545	0.520	0.420
5	0.365	0.325	0.185	0.510	0.562	0.557	0.460	0.320	0.580	0.310	0.540	0.550	0.520	0.420
5.30	0.370	0.330	0.210	0.515	0.562	0.557	0.560	0.340	0.580	0.310	0.540	0.555	0.520	0.420
6	0.375	0.335	0.230	0.520	0.562	0.557	0.460	0.355	0.580	0.310	0.540	0.557	0.520	0.420
6.30	0.385	0.340	0.250	0.525	0.562	0.557	0.460	0.370	0.580	0.315	0.540	0.560	0.520	0.420
7	0.400	0.345	0.280	0.530	0.562	0.557	0.460	0.375	0.580	0.370	0.545	0.560	0.520	0.425
7.30	0.410	0.350	0.290	0.535	0.562	0.557	0.460	0.385	0.580	0.330	0.550	0.560	0.520	0.425
8	0.420	0.355	0.305	0.540	0.562	0.557	0.465	0.390	0.580	0.350	0.560	0.560	0.525	0.430
8.30	0.430	0.355	0.315	0.540	0.562	0.557	0.465	0.400	0.580	0.360	0.560	0.560	0.525	0.430
9	0.440	0.360	0.325	0.540	0.562	0.560	0.465	0.410	0.580	0.370	0.560	0.560	0.525	0.435
9.30	0.450	0.360	0.330	0.540	0.562	0.560	0.465	0.415	0.580	0.385	0.560	0.560	0.525	0.440
10	0.455	0.365	0.340	0.540	0.562	0.560	0.470	0.420	0.580	0.400	0.560	0.560	0.525	0.450
10.30	-	-	-	-	-	-	0.470	-	-	-	-	-	-	-

Table 35. The effect of varying the stirring speed and the incubation temperature on ADP-induced platelet aggregation.
Turbidimetric method.

Experiment		Platelet aggregation				Disaggregation	
		30-60	30-90	Maximum	MAT	Time	Percentage
1	High speed	0.090	0.150	0.285	120	150	78.9
	Ordinary speed	0.160	0.225	0.41	150	180	71.9
	Low speed	0.180	0.265	0.47	210	240	29
2	High speed	0.040	0.075	0.150	120	150	96.7
	Ordinary speed	0.130	0.205	0.400	150	180	85
	Low speed	0.090	0.140	0.220	180	210	81.8
3	Room temperature	0.130	0.190	0.315	150	180	74
	37°C	0.030	-	0.23	60	90	76

Table 35 shows that when the stirring speed was increased, less platelet aggregation and more complete disaggregation occurred. With low speed, although the effect on aggregation was not constant in comparison to the results from ordinary speed, yet less and more delayed disaggregation resulted.
The results from an experiment carried out at 37°C showed diminished aggregation and early and more complete disaggregation as compared to an experiment done at room temperature.

Table 36. The kaolin cephalin clotting time in normal controls.

Subject No.	Clotting Time
1	53.6
2	51
3	55
4	50.5
5	56
6	54.8
7	40.6
8	43.6
9	55.8
10	48.3

Table 36 shows the kaolin cephalin clotting times in 10 normal controls. The test was carried out in duplicate and the results shown are the mean clotting time of 2 readings.

The mean clotting time was 50.9 $\pm$ 5.3 seconds the range was 40.3 - 61.5 seconds.

Table 37. The white cell count of platelet-rich plasma in normal controls and uraemic patients.

Normal controls			Uraemic patients			
Subject No.	Platelet count/c.mm.	White count/c.mm.	Subject No.	Platelet count/c.mm.	White count/c.mm.	
1	301,000	293	15	253,000	300	Pre-dialysis
2	430,000	122	15	249,000	193	Post-dialysis
3	350,000	310	22	211,000	200	
4	422,000	355	29	440,000	200	
5	236,500	192	30	260,000	180	
6	390,000	270	31	320,000	300	
7	255,000	200	32	325,000	150	
8	415,000	180	33	212,000	198	
9	271,000	150				

Table 37 shows that there was no significant difference in the white cell count of platelet-rich plasma between normal controls and uraemic patients ($t = 0.334$, $P > 0.1$).

Table 38. The effect of lowering the haematocrit in vitro on ADP-induced platelet aggregation.
Turbidimetric method.

		Platelet aggregation				Disaggregation			
	PCV	30-60	30-90	Maximum	M.A.T.	Time	Percentage	Platelet count per c.mm.	
1	28	0.075	0.110	0.170	120	150	64.7	274,000	diluted
	40	0.105	0.155	0.230	120	150	85.7	405,000	undiluted
2	21	0.080	0.115	0.200	120	180	60	301,000	diluted
	35	0.090	0.135	0.245	120	150	65.3	374,000	diluted
	44	0.110	0.170	0.310	120	150	64.5	420,000	undiluted
3	27	0.065	0.110	0.160	120	180	65.6	296,000	diluted
	41	0.110	0.165	0.305	120	150	73.8	415,000	undiluted
4	25	0.050	0.075	0.125	120	150	72	179,000	diluted
	38	0.080	0.120	0.190	90	120	78.9	241,000	undiluted
5	24	0.050	0.085	0.130	150	210	69.2	148,000	diluted
	37	0.110	0.155	0.335	150	180	70.1	725,000	undiluted
6	38	0.080	0.120	0.205	150	180	65.8	310,000	diluted
	43	0.120	0.180	0.355	150	180	64.8	365,000	undiluted

Table 38 shows the results of 7 experiments in which the haematocrit was lowered in vitro by addition of platelet-poor plasma to citrated blood.

Table 39. The abnormalities in ADP-induced platelet aggregation in uraemic patients.

Subject No.	Platelet aggregation				Disaggregation		Bleeding	Diagnosis
	30-60	30-90	Maximum	M.A.T.	Time	Percentage		
1	N	N	N	P	P	D	-	Chronic renal failure (dialysis)
2	I	I	I	P	P	D	-	Chronic renal failure (dialysis)
3	N	N	N	N	N	N	-	Chronic renal failure (dialysis)
3	N	N	N	N	N	N	-	Chronic renal failure (dialysis)
4	D	D	D	P	P	D	-	Chronic renal failure
5	D	N	N	N	N	D	-	Chronic renal failure (dialysis)
6	N	N	N	N	N	N	-	Acute renal failure
6	D	D	N	P	P	D	-	Acute renal failure
7	N	N	N	P	P	D	-	Acute renal failure
7	D	D	D	P	P	D	+	Acute renal failure
8	N	N	N	N	N	D	-	Acute renal failure
9	D	D	N	N	N	N	-	Chronic renal failure
10	N	N	N	N	N	D	-	Acute on chronic renal failure
11	N	N	N	P	P	D	-	Chronic renal failure
12	D	D	D	P	P	D	+	Chronic renal failure
13	N	N	N	N	N	N	+	Chronic renal failure
14	D	D	N	P	P	D	+	Acute on chronic renal failure
14	N	N	N	P	P	D	-	Acute on chronic renal failure
15	D	D	N	P	P	D	+	Chronic renal failure
15	N	N	N	P	P	D	-	Chronic renal failure (dialysis)
16	N	N	N	N	N	N	-	Chronic renal failure
17	D	N	N	P	P	D	-	Chronic renal failure
19	D	D	D	P	P	D	-	Acute on chronic renal failure
20	N	N	N	P	P	D	-	Chronic renal failure

Table 39 (continued)

Subject No.	Platelet aggregation				Disaggregation		Bleeding	Diagnosis
	30-60	30-90	Maximum	M.A.T.	Time	Percentage		
21	D	N	N	N	N	N	-	Chronic renal failure (dialysis)
22	N	N	N	P	P	D	-	Acute on chronic renal failure
23	N	N	N	N	N	N	-	Acute renal failure
24	N	N	N	P	P	D	-	Chronic renal failure
25	D	D	N	P	P	D	+	Acute renal failure
26	D	D	D	P	P	N	-	Chronic renal failure
27	D	D	D	N	P	D	+	Acute on chronic renal failure
28	N	N	N	N	P	D	+	Acute renal failure
28	N	N	N	N	N	N	-	Acute renal failure
29	N	N	N	N	N	N	-	Acute on chronic renal failure post renal
29	D	D	D	N	N	N	-	Acute on chronic renal failure
30	N	N	N	N	N	N	+	Acute on chronic renal failure post renal
31	D	D	D	P	P	N	-	Acute on chronic renal failure
32	D	D	D	N	N	N	-	Acute on chronic renal failure
33	D	D	N	N	N	N	-	Chronic renal failure
34	D	D	N	P	P	D	-	Acute on chronic renal failure
35	D	D	N	P	P	D	-	Chronic renal failure
36	N	N	N	P	P	D	-	Acute on chronic renal failure
37	D	D	N	P	P	D	+	Acute renal failure

Table 39 (continued)

Subject No.	Platelet aggregation				Disaggregation		Bleeding	Diagnosis
	30-60	30-90	Maximum	M.A.T.	Time	Percentage		
38	D	D	D	PP	P	N	+	Acute renal failure
39	N	N	N	P	P	D	-	Acute renal failure
39	N	D	D	N	N	N	-	Chronic renal failure

N = Normal: D = Diminished: I = Increased
P = Prolonged: + = Bleeding: - = Not bleeding.

Table 39 shows the abnormalities in the parameters used to measure platelet aggregation and disaggregation in 39 uraemic patients. The results of each experiment were compared to the mean values of normal controls $\pm$ 2 standard deviations in the four ranges of platelet count. Any result falling outside the normal range was considered abnormal.

Table 40. Abnormalities in ADP-induced platelet aggregation in uraemic patients

	30-60 platelet aggregation			30-90 platelet aggregation			Maximum aggregation			Maximum aggregation time		Disaggregation time		Percentage disaggregation	
	N	D	I	N	D	I	N	D	I	N	P	N	P	N	D
	(percent)			(percent)			(percent)			(percent)		(percent)		(percent)	
39 uraemic patients	49	48	3	54	43	3	74	23	3	44	56	39	61	39	61
30 chronic uraemic patients	43	53	4	50	46	4	70	26	4	43	57	40	60	37	63
9 acute renal failure	67	33	-	67	33	-	89	11	-	44	56	33	67	33	67
7 chronic renal failure with haemodialysis	57	29	14	72	14	14	72	14	14	57	43	57	43	43	57
11 uraemic patients with bleeding	36	64	-	45	56	-	64	36	-	55	45	9	91	36	64

Table 40. N = normal: D = diminished: I = increased: P = prolonged (The first experiment in each patient was considered in calculating the abnormality). Uraemic patients were subdivided into four clinical categories as shown in the table. The results were compared with the mean values of normal controls $\pm$ 2 standard deviations and the percentage abnormality in all uraemic patients and their sub-divisions are given in this table. Analysis of the results show that the highest percentage diminution in platelet aggregation occurred in patients with a clinical haemostatic failure while it was lower in chronic renal failure on haemodialysis and in acute uraemia. Patients with bleeding also showed the highest abnormality in the disaggregation time, i.e. their platelets took a longer time to disaggregation. The diminution in percentage disaggregation was similar in all groups but was slightly lower in the dialysis patients.

Table 41. Platelet adhesiveness in normal controls
Modified Hellem's method.

Subject No.	Percentage adhesiveness		Percentage Packed cell volume	Platelet count/c.mm.
	uncorrected	corrected		
1	60.8	56.5	43	214,500
2	60.7	58.2	42	232,500
3	53.5	51	42	224,500
4	53.2	50.7	42	241,250
5	62.4	59.9	42	163,750
6	53.8	56.7	39	163,000
7	33.3	47	32	141,750
8	49.4	55.5	44	167,500
9	46.7	52.8	44	211,750
10	55.8	61.9	44	157,250
11	52.6	53.7	40	122,500
12	55.3	49.2	44	208,500
13	51	52.1	40	220,250
14	59.1	53	44	199,500
15	50.6	46.3	43	219,750
16	31.6	43.3	33	157,250
17	45.4	50.1	38	315,000
18	45.4	48.3	39	211,000
19	44.2	48.9	38	127,750
20	50.7	53.6	39	281,750
21	43.2	46.1	39	159,500
22	59.3	60.4	40	161,000
23	50.2	51.3	40	205,000
24	46.6	42.3	43	152,500
25	52.7	48.4	43	243,750
26	36.9	34.4	42	156,500
27	50.9	55.6	38	189,000

Table 41 shows the results of percentage platelet adhesiveness of citrated whole blood in 27 normal controls. The mean percentage adhesiveness was 50.2 $\pm$ 7.8 and the packed cell volume was 40.6 $\pm$ 3.1. A significant correlation existed between the packed cell volume and percentage adhesiveness ($r = 0.651$, $P < 0.001$), as shown in figure 32. The platelet counts presented in this table are in citrated blood. This table also shows the results of percentage platelet adhesiveness after being corrected for variation in PCV.

Table 42. Relationship between platelet adhesiveness and ADP-induced platelet aggreg tion and disaggregation in normal controls.

Subject No.	Platelet aggregation			Percentage disaggregation	Percentage adhesiveness (corrected)	Percentage adhesiveness (uncorrected)	Platelet count/c.mm.
	30-60	30-90	Maximum				
1	0.120	0.180	0.380	51.3	56.5	60.8	301,000
2	0.120	0.180	0.365	46.5	51	53.5	345,000
3	0.180	0.280	0.480	41.6	59.9	62.4	360,000
4	0.130	0.170	0.310	72.6	48.8	33.3	375,000
5	0.170	0.230	0.425	87	43.3	49.4	340,000
6	0.190	0.300	0.485	81.4	40.6	46.7	400,500
7	0.130	0.190	0.380	44.7	49.7	55.8	315,000
8	0.085	0.150	0.245	57.1	48.9	44.2	240,500
9	0.120	0.195	0.380	65.1	53.6	50.7	442,500
10	0.185	0.275	0.450	57.7	46.1	43.2	435,000
11	0.215	0.305	0.565	78.3	37.6	40.1	460,000
12	0.180	0.310	0.530	62.2	36.5	37.6	410,000
13	0.110	0.170	0.290	72.4	49.5	52	380,000
14	0.165	0.240	0.480	47.9	51.3	50.2	390,000
15	0.115	0.190	0.315	50.8	42.3	46.5	255,000
16	0.145	0.215	0.410	65.8	48.3	52.7	422,000
17	0.090	0.130	0.230	76	34.5	36.9	350,000

Table 42 shows no correlation between percentage platelet adhesiveness and ADP-induced platelet aggregation as measured by 30-60, 30-90 seconds and maximum aggregation ($r = 0.0317$, $P > 0.1$; $r = 0.014$, $P > 0.1$; $r = 0.056$, $P > 0.1$). However, a significant correlation existed between percentage platelet adhesiveness and percentage disaggregation ($r = 0.549$, $0.05 > P > 0.02$) as shown in figure 35 and when the results of platelet adhesiveness were corrected for variation in PCV ($r = 0.611$, $0.01 > P > 0.001$) (figure 36).

Table 43. Platelet adhesiveness in uraemic patients
Modified Hellem's method.

Subject No.	Percentage platelet adhesiveness (uncorrected)	Packed cell volume (per-cent)	Percentage platelet adhesiveness (corrected)	Citrated blood platelet count/c.mm.	Blood urea mg. per 100 ml.	Diagnosis
1	38.1	22	71.6	243,500	153	Chronic renal failure
2	39.4	24	69.3	78,500	150	Chronic renal failure
3	28.4	24	58.3	220,250	168	Chronic renal failure
4	40	21	75.3	164,500	217	Chronic renal failure
5	14.1	21	49.4	172,500	155	Chronic renal failure
6	30.8	25	58.9	220,750	290	Acute renal failure
7	56.3	48	43	121,500	340	Acute renal failure
8	48.3	28	71	279,250	310	Acute renal failure
9	37.9	38	42.6	129,750	85	Chronic renal failure
10	18.3	23	50	125,500	131	Chronic renal failure
11	27.9	27	52.4	170,000	120	Chronic renal failure
12	25.4	23	57.1	159,250	87	Chronic renal failure
13	24.7	20	61.8	134,500	180	Chronic renal failure
14	54.8	23	86.5	229,000	203	Chronic renal failure
15	11.6	25	39.7	176,500	169	Chronic renal failure
16	33.5	38	38.2	214,000	106	Chronic renal failure
17	25.7	20	62.8	168,750	252	Chronic renal failure
19	19.8	47	8.3	29,000	110	Chronic renal failure
20	46.6	34	58.5	118,500	120	Chronic renal failure
21	25.9	20	63	151,250	262	Chronic renal failure
22	34.2	20	71.3	178,000	605	Chronic renal failure
23	39.2	25	67.3	68,750	205	Acute renal failure
24	35.1	23	66.8	157,750	162	Chronic renal failure
25	40.4	18	81.1	136,000	121	Acute renal failure

Table 43 (continued).

Subject No.	Percentage platelet adhesiveness (uncorrected)	Packed cell volume (per-cent)	Percentage platelet adhesiveness (corrected)	Citrated blood platelet count/c.mm.	Blood urea mg. per 100 ml.	Diagnosis
26	31.3	34	43.2	105,250	272	Chronic renal failure
27	15	18	55.7	55,000	220	Acute renal failure
28	22.3	36	30.6	61,500	365	Acute renal failure
29	33.4	20	70.5	427,000	300	Chronic renal failure
30	50.5	35	60.6	159,250	650	Chronic renal failure
31	32.7	41	32	153,250	920	Chronic renal failure
32	15.5	30	34.6	231,000	360	Chronic renal failure
33	23.6	23	55.3	132,500	165	Chronic renal failure
34	39.7	33	53.4	155,500	270	Chronic renal failure
35	33.2	24	63.1	177,750	280	Chronic renal failure
36	21.1	19	60	149,000	370	Chronic renal failure
37	10.4	23	42.1	53,000	347	Acute renal failure
38	4.5	22	38	231,250	190	Chronic renal failure
39	20.8	24	50.7	103,000	300	Acute renal failure
40	25.6	20	62.7	196,000	154	Chronic renal failure

Table 43 shows the results of platelet adhesiveness in 39 uraemic patients. The mean platelet adhesiveness was 30.1 $\pm$ 12.2 per cent and the mean packed cell volume 26.6 per cent. When the results were corrected for the variation in packed cell volume the mean platelet adhesiveness was 55.3 $\pm$ 15.9 per cent.

Table 44. Relationship between platelet adhesiveness and ADP-induced platelet aggregation and disaggregation in uraemic patients.

Subject No.	Platelet aggregation			Percentage Disaggregation	Percentage adhesiveness (uncorrected)	Percentage adhesiveness (corrected)	Platelet count/c.mm.
	30-60	30-90	Maximum				
1	0.085	0.160	0.410	19.5	38.1	71.6	254,000
3	0.090	0.160	0.280	42.8	28.4	58.3	250,250
5	0.070	0.135	0.270	33.3	14.1	49.4	427,000
6	0.040	0.090	0.240	37.5	30.8	58.9	384,000
8	0.140	0.230	0.430	33.7	48.3	71	362,000
9	0.020	0.060	0.160	50	37.9	42.6	245,000
10	0.105	0.170	0.315	38.1	18.3	50	320,000
11	0.075	0.120	0.260	27.9	25	52.4	300,000
14	0.060	0.140	0.360	0	54.8	86.5	305,000
16	0.170	0.240	0.420	72.6	33.5	38.2	325,000
29	0.130	0.195	0.345	56.5	33.4	70.5	440,000
30	0.105	0.165	0.280	64.3	50.5	60.6	260,000
31	0.030	0.075	0.185	48.6	32.7	32	320,000
32	0.030	0.075	0.165	60.6	15.5	34.6	325,000
34	0.035	0.808	0.220	15.9	39.7	53.4	266,000
35	0.025	0.070	0.245	10.2	33.2	63.1	253,000
38	0.040	0.080	0.175	31.4	4.5	38	270,500
40	0.075	0.115	0.190	50	25.6	62.7	270,000

Table 44 shows the results of percentage platelet adhesiveness and percentage platelet disaggregation in 18 uraemic patients. The results in this table are shown in figures 35 and 36.

Table 45. Relationship between platelet adhesiveness and the packed cell volume in vitro.

	1	2	3	4	5	6	7	8
Citrated blood ml.	5	4.5	4	3.5	3	2.5	2	1.5
Platelet-poor plasma ml.	0	0.5	1	1.5	2	2.5	3	3.5
Packed cell volume per cent	40	36	33	28	25	20	17	12
Percentage adhesiveness	54.4	38.1	38.5	29.9	23.4	15.4	11.4	1.2

Table 45 shows the effect of changing the PCV in vitro on platelet adhesiveness. The results of platelet adhesiveness were significantly correlated with their corresponding PCV values ($r = 0.989$ $P < 0.001$). A correction factor was obtained from the curve (figure 33). For every 1 per cent reduction in PCV, the platelet adhesiveness was increased by 1.8 per cent.

Table 46. Platelet adhesiveness before and after haemodialysis treatment in chronic renal failure patients.

Subject No.	Sample	Percentage adhesiveness	Packed cell volume	Corrected percentage adhesiveness	Blood platelet count/c.mm.	Blood urea mg. per cent
1	Pre-dialysis	38.1	22	71.6	243,500	153
	Post-dialysis	41.1	22	74.6	262,500	54
2	Pre-dialysis	39.4	26	65.7	78,500	150
	Post-dialysis	44.5	34	56.4	73,500	55
3	Pre-dialysis	28.4	25	56.5	220,250	168
	Post-dialysis	47	26	73.3	230,250	52
4	Pre-dialysis	14.1	21	49.7	172,500	155
	Post-dialysis	39.7	27	64.2	126,500	22
5	Pre-dialysis	25.9	20	63	151,250	262
	Post-dialysis	34.3	20	71.7	150,750	112
6	Pre-dialysis	40.3	23	70.2	177,000	160
	Post-dialysis	50.3	24	80.2	159,500	80
7	Pre-dialysis	25.6	20	62.7	196,000	154
	Post-dialysis	37.8	20	74.9	226,000	80

Table 46 shows the effect of haemodialysis on platelet adhesiveness (modified Hellem's method). The percentage platelet adhesiveness increased significantly after dialysis ($t = 2.834$, $0.02 > P > 0.01$). It was in correlation with blood urea level in mg. per 100 ml. ($r = 0.636$, $0.02 > P > 0.01$). However, when the correlation was repeated after correcting for PCV, it became insignificant.

Table 47. The effect of haemodialysis on platelet adhesiveness in acute renal failure patients.
Modified Hellem's technique.

Subject No.	Sample	Percentage platelet adhesiveness (uncorrected)	Percentage packed cell volume	Percentage platelet adhesiveness (corrected)	Platelet count/c.mm.	Blood urea mg. per 100 ml.
6	Pre-dialysis	30.8	25	58.9	220,750	290
6	Post-dialysis	26.7	24	56.6	239,750	185
7	Pre-dialysis	56.3	48	43	121,500	340
7	Post-dialysis	14.5	43	10.2	38,000	154
8	Pre-dialysis	48.3	28	71.0	279,250	310
8	Post-dialysis	34.5	29	55.4	231,250	130
23	Pre-dialysis	39.2	25	67.3	68,750	205
23	Post-dialysis	23.6	24	53.5	36,000	140
28	Pre-dialysis	22.3	36	30.6	61,500	365
28	Post-dialysis	38.8	36	47.1	63,000	195
37	Pre-dialysis	10.4	23	42.1	53,000	347
37	Post-dialysis	38.6	30	57.7	66,000	161
39	Pre-dialysis	20.8	24	50.7	103,000	300
39	Post-dialysis	22.3	24	52.2	89,500	219

Table 47 . The mean percentage platelet adhesiveness was 32.58 before dialysis and 22.91 after dialysis; the difference between the two mean values was insignificant ($t = 0.994$, $P > 0.1$).

Table 48. The mean values of platelet adhesiveness in normal controls and uraemic patients before and after correction for variation in haematocrit.

	Normal controls	39 uraemic patients	30 chronic uraemic patients	9 acute uraemic patients	11 uraemic patients with bleeding
Percentage platelet adhesiveness before correction	50.2	30.1	29.7	31.5	25.9
Percentage platelet adhesiveness after correction	50.2	55.3	55.2	55.6	52.9

	Chronic uraemic patient on haemodialysis		Acute uraemic patient on haemodialysis	
	Predialysis	Postdialysis	Predialysis	Postidalysis
Percentage platelet adhesiveness before correction	30.2	42.1	32.6	22.9
Percentage platelet adhesiveness after correction	65.5	70.7	51.9	47.5

Table 49. Platelet factor-3 availability in normal controls Method of Spaet and Cintron, 1965.

Subject No.	Stypven time (seconds)	Platelet count/c.mm.	Subject No.	Stypven time (seconds)	Platelet count/c.mm.
1	19.6	430,000	17	20.9	283,000
2	23.4	234,000	18	20.5	365,000
3	22.6	450,000	19	17.9	430,000
4	21.4	468,000	20	18.2	390,000
5	22	310,000	21	22.4	255,000
6	15.5	353,000	22	26	353,000
7	19.7	428,000	23	15.3	480,000
8	21.5	426,000	29	23,2	125,000
9	22.8	435,000	25	22.6	245,000
10	21.4	342,000	26	22.1	177,000
11	15.4	423,000	27	27.7	133,000
12	19.1	420,000	28	22.3	215,000
13	20.6	442,000	29	29.3	137,000
14	19.8	380,000	30	29	29,000
15	17.3	260,000	31	48.8	28,000
16	16.3	250,000			

Table 49 shows the results of 23 normal controls tested for platelet factor-3 availability using the method of Spaet and Cintron (1965). Experiments from 24 to 31 were done after diluting the platelet-rich plasma with platelet-poor plasma. The mean stypven time in the normal undiluted control was 19.98 ± 2.8 seconds. No correlation was found between the platelet count of platelet-rich plasma and the stypven time of the undiluted control ($t = 0.131$, $P > 0.1$) or between the former and the stypven time of diluted and undiluted controls ($t = 0.298$, $0.1 > P > 0.05$).

Table 50. Platelet factor-3 availability in uraemic patients
Method of Spaet and Cintron 1965

Subject No.	Stypven time (seconds)	Kaolin cephalin clotting time (seconds)	Platelet count/c.mm.	Subject No.	Stypven time (seconds)	Kaolin cephalin clotting time (seconds)	Platelet count/c.mm.
2	24.1	50.9	201,000	26	27.6	50	151,500
3	24.5	52	375,000	27	43.1	40.5	52,000
10	20.5	46.9	320,000	28	44.5	48.5	81,000
11	23.2	42.9	335,000	29	22.9	54.1	427,000
13	31.5	50.6	145,000	30	33.2	52	260,000
14	29.1	55.2	305,000	31	54.7	72.5*	320,000
15	29.3	53.2	205,000	32	36.6	65.4*	325,000
16	31.1	43.5	325,000	33	30.4	51.4	212,000
18	24	40.6	479,000	34	19.9	47.6	266,000
19	35.3	52.5	52,000	35	24.1	53.3	253,000
20	23.2	50.5	229,000	36	19.5	53	190,000
21	44.4	47.5	160,000	37	25.2	50.7	75,000
22	33.6	43.1	211,000	38	23	33.5	270,500
23	66.9	96.5*	96,000	39	20.3	40	112,750
24	25.2	48.1	204,000	40	23.5	46.5	270,000
25	21.9	33.5	152,000				

*Uraemic patients with prolonged kaolin cephalin clotting time.

Table 50 shows the results of platelet factor-3 availability in 31 uraemic patients. When the results from uraemic patients with prolonged Kaolin cephalin clotting time were excluded there was a significant negative oorrelation between the Stypven time and the platelet count ($r = -0.462$, $0.02 > P > 0.01$). This relationship is illustrated in figure 41.

Table 51. Platelet factor-3 availability in normal controls and uraemic patients (platelet count between 200,000 - 300,000/c.mm.

Normal controls				Uraemic patients			
Subject No.	Stypven time (seconds)	Platelet count/c.mm.		Subject No.	Stypven time (seconds)	Platelet count/c.mm.	
1	16.3	250,000	undiluted	2	24.1	201,500	undiluted
2	21.3	283,000	undiluted	15	29.3	205,000	undiluted
3	20.6	250,000	diluted	20	23.2	229,000	undiluted
4	22.3	236,000	diluted	24	25.2	204,000	undiluted
5	16.8	225,000	diluted	26	33.7	250,000	diluted
6	22.4	255,000	undiluted	29	30.1	250,000	diluted
7	23.4	234,000	undiluted	30	33.2	260,000	undiluted
8	17.3	260,000	undiluted	33	30.4	212,000	undiluted
9	22.6	245,000	diluted	34	19.9	266,000	undiluted
10	22.3	215,000	diluted	35	24.1	253,000	undiluted
				38	23	270,500	undiluted
				40	23.5	270,000	undiluted

Table 51 shows the results of platelet factor-3 availability in normal controls and uraemic patients having a platelet count between 200,000 - 300,000/c.mm. In some experiments the platelet-rich plasma was diluted with platelet-poor plasma. The mean stypven time of uraemic patients was significantly longer than the mean of normal control ($t = 3.764$, $0.01 > P > 0.001$). The mean platelet count of normal controls and uraemic patients were not significantly different ($t = 0.587$, $P > 0.1$).

Table 52. Relationship between platelet factor-3 availability and percentage disaggregation in normal controls and uraemic patients.

Normal controls				Uraemic patients			
Subject No.	Stypven time (seconds)	Percentage disaggregation	Platelet count/c.mm.	Subject No.	Stypven time (seconds)	Percentage disaggregation	Platelet count/c.mm.
1	25.3	78.3	460,000	3	24.5	60.8	375,000
2	17.8	62.2	410,000	10	20.5	38.1	320,000
3	17	57.7	435,000	11	23.2	9.1	335,000
4	23	72.6	375,000	14	29.1	0	305,000
5	20.6	65.1	442,500	15	31.1	72.6	325,000
6	18.2	47.9	390,000	20	23.2	0	229,000
7	22.4	50.8	255,000	29	22.9	56.5	427,000
8	19.8	72.4	380,000	30	33.2	64.3	260,000
9	21.1	65.8	422,000	34	19.9	15.9	266,000
10	26	76	350,000	35	24.1	10.2	253,000
				38	23	31.4	270,500
				40	23.5	50	270,000

Table 51 shows a significant correlation between the Stypven time and percentage disaggregation in normal controls ($r = 0.645$, $0.05 > P > 0.02$), such correlation was insignificant in uraemic patients ($r = 0.389$, $P > 0.1$).

Table 53. The effect of haemodialysis on platelet factor-3 availability in chronic uraemic patients.

	Pre-dialysis				Post-dialysis			
Subject No.	Stypven time (seconds)	Kaolin cephalin clotting time (seconds)	Platelet count/c.mm.	Blood urea (mg. per 100 ml.)	Stypven time (seconds)	Kaolin cephalin clotting time (seconds)	Platelet count/c.mm.	Blood urea (mg. per 100 ml.)
2	24.1	50.9	201,500	194	25.1	54	293,000	46
3	24.5	52	375,000	142	24.6	49.5	420,000	58
21	44.4	47.5	160,000	262	36.5	46.4	217,000	112
15	28.7	44	253,000	160	25.4	43	249,000	80
40	23.5	46.5	270,000	140	21.1	43.5	252,500	80

Table 54. The effect of haemodialysis on platelet factor-3 availability in acute uraemic patients.

	Pre-dialysis				Post-dialysis			
Subject No.	Stypven time (seconds)	Kaolin Cephalin clotting time (seconds)	Platelet count/c.mm.	Blood urea (mg. per 100 ml.)	Stypven time (seconds)	Kaolin Cephalin clotting time (seconds)	Platelet count/c.mm.	Blood urea (mg. per 100 ml.)
23	66.9	96.5	96,000	205	69	104.5	67,000	140
28	44.5	48.5	81,000	365	31	44.6	92,500	195
37	25.2	50.7	75,000	347	26.5	52.1	60,000	161
39	20.3	40	112,750	300	17.9	40. 5	174,000	219

Table 55. Platelet function tests in uraemic patients with clinical haematatic failure

Subject No.	ADP-platelet aggregation				Disaggregation		Platelet count/c.mm.	Platelet adhesiveness			Platelet factor-3 Styven time (seconds)	K.C.C.T. (seconds)	Whole citrated blood platelet count/c.mm.	Blood urea mg.per 100 ml	Bleeding	Diagnosis
	30-60	30-90	Maximum	M.A.T. (seconds)	Time (seconds)	Percentage		Percentage	PCV	Corrected						
7	0	0.010	0.050	210	360	30	74,000	25.6	39	28.5	-	-	54,500	345	Ecchymosis	Acute renal failure perforated peptic ulcer
12	0.01	0.030	0.135	600	600	0	222,000	25.4	23	57.1	-	-	159,250	87	Purpuric spots on cheecks	Chronic renal failure Rheumatoid arthritis
13	0.015	0.040	0.110	210	270	50	145,000	24.7	20	61.8	31.5	50.6	134,500	180	Atraumatic bruising	Chronic renal failure chronic pyelonephritis
14	0.060	0.140	0.360	600	600	0	305,000	54.8	23	86.5	29.1	55.2	229,000	203	Melaena	Chronic renal failure, hydronephrosis, chronic pyelnoephritis
15	0.050	0.110	0.280	600	600	0	205,000	11.6	25	39.7	29.3	53.2	176,500	169	Bruising from minimal trauma	Chronic renal failure chronic pyelonephritis
25	0.030	0.070	0.185	360	600	0	152,000	40.4	18	81.1	21.9	33.5	136,000	121	Haematemesis Melaena	Acute renal failure, post partum haemorrhage
27	0	0	0.020	150	600	0	52,000	15	18	55.7	43.1	49.5	55,000	220	Epistaxis	Acute renal failure Septicaemia
28	0.010	0.015	0.037	150	600	0	81,000	22.3	36	30.6	44.5	48.5	61,500	365	Purpura Vaginal bleeding	Acute renal failure Septic abortion
30	0.105	0.165	0.28	150	240	64.3	260,000	50.5	35	60.6	33.2	52	159,250	650	Epistaxis Haematuria	Chronic renal failure Acute post renal obstruction
37	0	0.050	0.042	300	540	5.8	75,000	10.4	23	42.1	25.2	50.7	53,000	347	Ecchymosis Haematurai	Acute renal failure, incomplete abortion, septicaemia
38	0.040	0.080	0.175	240	330	31.4	270,000	4.5	22	38	23	33.5	231,250	190	Epistaxis Haematemesis	Acute renal failure

Table 56. Inhibition of ADP-induced platelet aggregation and disaggregation by in vitro addition of urea.

Experiment No.	Urea conc. mg. per 100 ml.	30-60 sec platelet aggregation					30-90 sec platelet aggregation				
		Test 1	Test 2	Mean	Control	Difference	Test 1	Test 2	Mean	Control	Difference
1	800	0.060	0.055	0.0575	0.180	0.1225	0.100	0.110	0.105	0.285	0.180
2	750	0.090	0.115	0.1020	0.135	0.0330	0.140	0.175	0.158	0.205	0.047
3	500	0.145	0.170	0.1570	0.205	0.0480	0.230	0.260	0.245	0.300	0.055
4	500	0.080	0.090	0.850	0.120	0.0350	0.130	0.510	0.140	0.170	0.030
5	400	0.160	0.130	0.1450	0.200	0.0550	0.230	0.210	0.220	0.300	0.080
6	375	0.095	0.115	0.1050	0.130	0.0250	0.125	0.160	0.142	0.180	0.038
7	250	0.180	0.170	0.1750	0.210	0.0350	0.260	0.260	0.260	0.305	0.045
8	200	0.155	0.135	0.1450	0.160	0.0150	0.220	0.200	0.210	0.245	0.035

Table 56 (continued).

Experiment No.	Urea conc. mg. per 100 ml.	Maximum aggregation					Percentage disaggregation				
		Test 1	Test 2	Mean	Control	Difference	Test 1	Test 2	Mean	Control	Difference
1	800	0.220	0.280	0.250	0.420	0.170	29.5	23.2	26.3	86.9	60.6
2	750	0.245	0.310	0.277	0.360	0.083	36.7	41.9	39.3	65.3	26
3	500	0.365	0.495	0.430	0.520	0.090	54.8	45.4	50.1	17.1	21
4	500	0.240	0.320	0.280	0.385	0.105	29.2	29.7	29.4	51.9	22.5
5	400	0.370	0.410	0.390	0.540	0.150	67.5	51.2	59.3	67.6	8.3
6	375	0.225	0.310	0.267	0.320	0.053	62.2	54.8	58.5	70.3	11.8
7	250	0.430	0.490	0.460	0.570	0.110	60.4	54	57.2	58.7	0.5
8	200	0.360	0.390	0.375	0.450	0.075	72.2	64.1	68.1	73.3	5.2

The table showed that incubation of urea with platelet-rich plasma caused inhibition of ADP-induced platelet aggregation and disaggregation. Inhibition of platelet aggregation was not significantly correlated with the urea concentration (30-60 = $r = 0.668$, $0.1 > P > 0.05$; 30-90 = $r = 0.261$, $P > 0.1$; Maximum aggregation = $r = 0.403$, $P > 0.1$). However, the urea concentrations were significantly correlated with the inhibition in percentage disaggregation ($r = 0.088$, $0.01 > P > 0.001$).

Table 57. The in vitro effect of creatinine and uric acid on ADP-induced platelet aggregation.
Turbidimetric method.

Experiment	30-60 sec platelet aggregation					30-90 sec platelet aggregation				
	Test 1	Test 2	Mean	Control	Difference	Test 1	Test 2	Mean	Control	Difference
Added creatinine 10 mg. per 100 ml.	0.16	0.15	0.155	0.145	-0.01	0.225	0.2	0.212	0.215	0.003
Added uric acid, 5 mg. per 100 ml.	0.15	0.12	0.135	0.18	0.045	0.23	0.17	0.2	0.27	0.07
	Maximum aggregation					Percentage disaggregation				
Experiment	Test 1	Test 2	Mean	Control	Difference	Test 1	Test 2	Mean	Control	Difference
Added creatinine 10 mg. per 100 ml.	0.39	0.36	0.375	0.4	0.025	79.5	62.5	71	68.7	-2.3
Added uric acid, 5 mg. per 100 ml.	0.47	0.38	0.425	0.475	0.05	61.7	67.1	64.4	53.7	-10.7

This table shows that creatinine added in a c ncentration of 10 mg. per 100 ml. to platelet-rich plasma had no effect on ADP-induced platelet aggregation and slightly increased disaggregation. Uric acid added in a 5 mg. per 100 ml. slightly diminished platelet aggregation but increased platelet disaggregation by 10.7 per cent.

Table 58. The in vitro effect of guanidinosuccinic acid on ADP-induced platelet aggregation
Turbidimetric method.

Experiment	30-60 secs. platelet aggregation					30-90 secs. platelet aggregation				
	Test 1	Test 2	Mean	Control	Difference	Test 1	Test 2	Mean	Control	Difference
1	0.125	0.160	0.142	0.170	0.028	0.175	0.240	0.207	0.240	0.033
2	0.070	0.080	0.075	0.075	0.035	0.100	0.115	0.107	0.180	0.075
3	0.100	0.095	0.097	0.105	0.008	0.145	0.145	0.145	0.160	0.015
4	0.065	0.07	0.077	0.120	0.043	0.135	0.160	0.147	0.195	0.048
5	0.100	0.110	0.105	0.120	0.015	0.155	0.175	0.165	0.180	0.015
6	0.085	0.100	0.092	0.115	0.023	0.130	0.160	0.145	0.180	0.035
7	0.120	0.130	0.125	0.130	0.005	0.185	0.200	0.192	0.220	0.028
	Maximum aggregation					Percentage disaggregation				
Experiment	Test 1	Test 2	Mean	Control	Difference	Test 1	Test 2	Mean	Control	Difference
1	0.395	0.485	0.440	0.500	0.060	72.1	43.3	65.5	59	-6.5
2	0.160	0.195	0.177	0.300	0.123	65.6	82	73.8	60	-13.8
3	0.275	0.305	0.290	0.315	0.025	50.9	14.7	32.8	26.7	-6.1
4	0.225	0.275	0.250	0.315	0.065	64.4	49.1	56.7	31.7	-25
5	0.300	0.305	0.302	0.370	0.068	65	57.4	61.2	54	-7.2
6	0.200	0.270	0.235	0.295	0.060	72.5	64.8	68.6	66.1	-2.5
7	0.370	0.395	0.382	0.38	-0.002	75.6	65.8	70.7	65.7	-5

Table 58 shows that the addition of quanidinosuccinic acid in a concentration of 2×10^{-3} Molar caused a diminution of ADP-induced platelet aggregation and an increase in percentage disaggregation in the 7 experiments performed.

Table 59. The effect of urea on ADP-induced platelet aggregation (optical density readings) Turbidimetric method.

	Test	Control
Start	0.600	0.600
30	0.490	0.430
1	0.405	0.320
1.30	0.350	0.260
2	0.327	0.235
2.30	0.320	0.220
3	0.332	0.215
3.30	0.357	0.235
4	0.380	0.265
4.30	0.390	0.305
5	0.400	0.330
5.30	0.407	0.355
6	0.407	0.380
6.30	0.407	0.400
7	0.407	0.405
7.30	0.402	0.410
8	0.405	0.415
8.30	0.405	0.415
9	0.405	0.415
9.30	0.405	0.420
10	0.405	0.420

Table 59 shows the optical density readings of a representative experiment (No. 4, table 56) in which urea was added in a concentration of 500 mg. per 100 ml. of plasma. The optical density readings of the test are the mean values of 2 test experiments done before and after the saline control.

Table 60. The effect of guanidinosuccinic acid on ADP-induced platelet aggregation (Optical density readings) Turbidimetric method.

	1		2		3		4	
	Test	Control	Test	Control	Test	Control	Test	Control
Start	0.600	0.600	0.600	0.600	0.600	0.600	0.600	0.600
30	0.402	0.390	0.547	0.500	0.507	0.485	0.557	0.545
1	0.260	0.220	0.472	0.390	0.410	0.380	0.480	0.425
1.30	0.195	0.150	0.440	0.320	0.362	0.325	0.410	0.350
2	0.167	0.110	0.422	0.300	0.330	0.300	0.380	0.310
2.30	0.167	0.100	0.440	0.310	0.315	0.290	0.362	0.295
3	0.182	0.110	0.482	0.350	0.310	0.285	0.355	0.285
3.30	0.215	0.130	0.512	0.400	0.312	0.285	0.352	0.285
4	0.247	0.170	0.535	0.445	0.322	0.290	0.365	0.290
4.30	0.282	0.210	0.545	0.475	0.335	0.300	0.382	0.295
5	0.317	0.255	0.552	0.490	0.350	0.315	0.407	0.310
5.30	0.350	0.300	0.552	0.495	0.365	0.325	0.435	0.320
6	0.375	0.330	0.552	0.490	0.377	0.350	0.452	0.335
6.30	0.387	0.360	0.552	0.490	0.387	0.365	0.467	0.345
7	0.400	0.380	0.552	0.490	0.397	0.375	0.480	0.360
7.30	0.410	0.395	0.550	0.490	0.402	0.385	0.487	0.375
8	0.420	0.405	0.550	0.485	0.405	0.400	0.490	0.385
8.30	0.427	0.420	0.550	0.485	0.407	0.405	0.490	0.390
9	0.427	0.425	0.550	0.485	0.412	0.415	0.490	0.390
9.30	0.430	0.430	0.550	0.480	0.415	0.420	0.490	0.395
10	0.432	0.435	0.550	0.480	0.415	0.425	0.490	0.395

Table 60 (continued).

	5		6		7	
	Test	Control	Test	Control	Test	Control
Start	0.600	0.600	0.600	0.600	0.600	0.600
30	0.480	0.455	0.520	0.500	0.490	0.470
1	0.375	0.335	0.427	0.385	0.390	0.340
1.30	0.315	0.275	0.375	0.320	0.330	0.280
2	0.297	0.260	0.365	0.305	0.310	0.260
2.30	0.307	0.265	0.395	0.320	0.320	0.260
3	0.347	0.295	0.447	0.390	0.340	0.290
3.30	0.397	0.335	0.490	0.440	0.380	0.320
4	0.435	0.375	0.515	0.470	0.405	0.350
4.30	0.465	0.410	0.527	0.495	0.430	0.380
5	0.477	0.445	0.530	0.500	0.440	0.400
5.30	0.485	0.450	0.530	0.505	0.460	0.410
6	0.485	0.455	0.527	0.505	0.465	0.420
6.30	0.485	0.460	0.525	0.500	0.470	0.430
7	0.482	0.460	0.525	0.500	0.475	0.430
7.30	0.482	0.465	0.525	0.500	0.480	0.440
8	0.482	0.470	0.525	0.500	0.480	0.445
8.30	0.482	0.470	0.525	0.500	0.480	0.450
9	0.480	0.475	0.525	0.500	0.480	0.455
9.30	0.480	0.475	0.525	0.500	0.480	0.455
10	0.480	0.475	0.525	0.500	0.480	0.455

Table 60 shows the optical density readings in 7 experiments. The optical density re dings of the test are the mean values of 2 tests. The results in this table are used to construct figure 45.

Table 61. Clinical data of uraemic patients.

Subject No.	Sex	Age	Diagnosis	Bleeding	Haemodialysis
1	M	20	Chronic renal failure ? congenital hypoplasia of the kidney	-	+
2	M	33	Chronic renal failure - chronic glomerulonephritis	-	+
3	M	31	Chronic renal failure - chronic glomerulonephritis	-	+
4	F	30	Chronic renal failure - chronic glomerulonephritis - malignant hypertension	-	-
5	F	24	Chronic renal failure ?congenital hypoplasia of the kidney	-	+
6	M	59	Acute renal failure - bronchopneumonia	-	+
7	F	62	Acute renal failure - perforated peptic ulcer	+	+
8	F	53	Acute renal failure - cholangitis and cholycystitis	-	-
9	M	13	Chronic renal failure - chronic pyelonephritis spina bifida	-	-
10	F	25	Chronic renal failure - chronic pyelonephritis	-	-
11	F	57	Chronic renal failure - chronic pyelonephritis myxoedema	-	-
12	F	58	Chronic renal failure - rheumatoid arthritis renal amyloid	+	-
13	F	35	Chronic renal failure - chronic pyelonephritis	+	-
14	M	58	Chronic renal failure - hydronephrosis	+	-
15	F	42	Chronic renal failure - chronic pyelonephritis	+	+
16	F	37	Chronic renal failure - ?renal tuberculosis	-	-

Table 61 (continued)

Subject No.	Sex	Age	Diagnosis	Bleeding	Haemodialysis
17	M	41	Chronic renal failure - polycystic kidney	-	-
18	F	50	Chronic renal failure - chronic pyelonephritis	-	-
19	F	37	Chronic renal failure - renal artery stenosis hypertension	-	-
20	M	32	Chronic renal failure - chronic pyelonephritis polycystic kidney	-	-
21	F	34	Chronic renal failure - malignant hypertension	-	+
22	F	13	Chronic renal failure - chronic glomerulonephritis	-	-
23	M	43	Acute renal failure - post operative peritonitis septicaemia	-	+
24	F	53	Chronic renal failure - chronic pyelonephritis	-	-
25	F	46	Acute renal failure - post partum haemorrhage	+	-
26	M	28	Chronic renal failure - malignant hypertension	-	-
27	M	59	Acute renal failure - subacute proliferative glomerunlonephritis - septicaemia	+	-
28	F	32	Acute renal failure - septic abortion	+	+
29	F	53	Chronic renal failure - acute post renal obstruction	-	-
30	M	63	Chronic renal failure - acute post renal obstruction	+	-
31	M	86	Chronic renal failure - acute post renal obstruction	-	-
32	M	49	Chronic renal failure - polycystic kidney	-	-

Table 61 (continued).

Subject No.	Sex	Age	Diagnosis	Bleeding	Haemodialysis
33	F	28	Chronic renal failure - malignant hypertension	-	-
34	M	65	Chronic renal failure - bronchopneumonia - bronchial carcinoma	-	-
35	M	31	Chronic renal failure - unknown aetiology	-	-
36	M	51	Chronic renal failure - chronic pyelonephritis congenital bilateral kidneys	-	-
37	F	38	Acute renal failure - incomplete abortion septicaemia	+	+
38	M	55	Chronic renal failure - epidermolysis pullosa	+	-
39	M	32	Acute renal failure - thrombotic microangiopathy	-	+
40	M	23	Chronic renal failure - chronic glomerulonephritis	-	+

FUNCTION OF PLATELETS IN HAEMOSTASIS

1. Platelet plug
2. Vasoconstriction - 5-HT release
3. Contribution to coagulation
4. Clot retraction
5. Capillary integrity

Figure 1 shows the functions of the platelet in haemostasis.

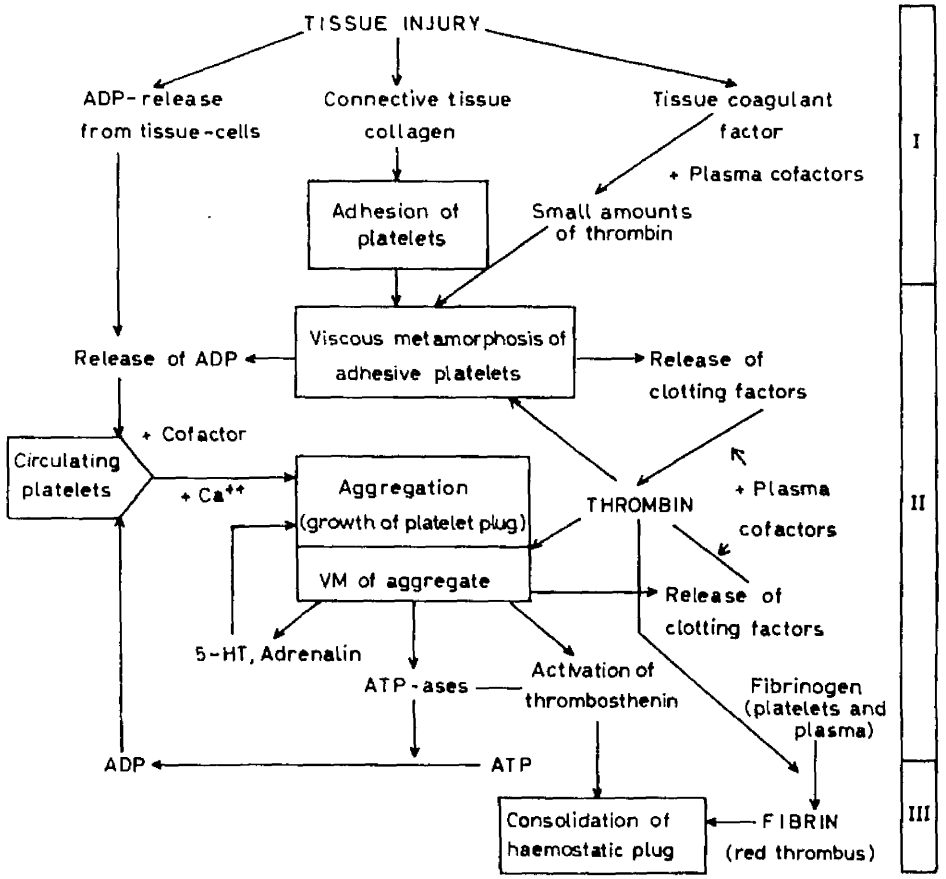

Figure 2 illustrates schematically the mechanism of haemostasis (Luscher, 1967).

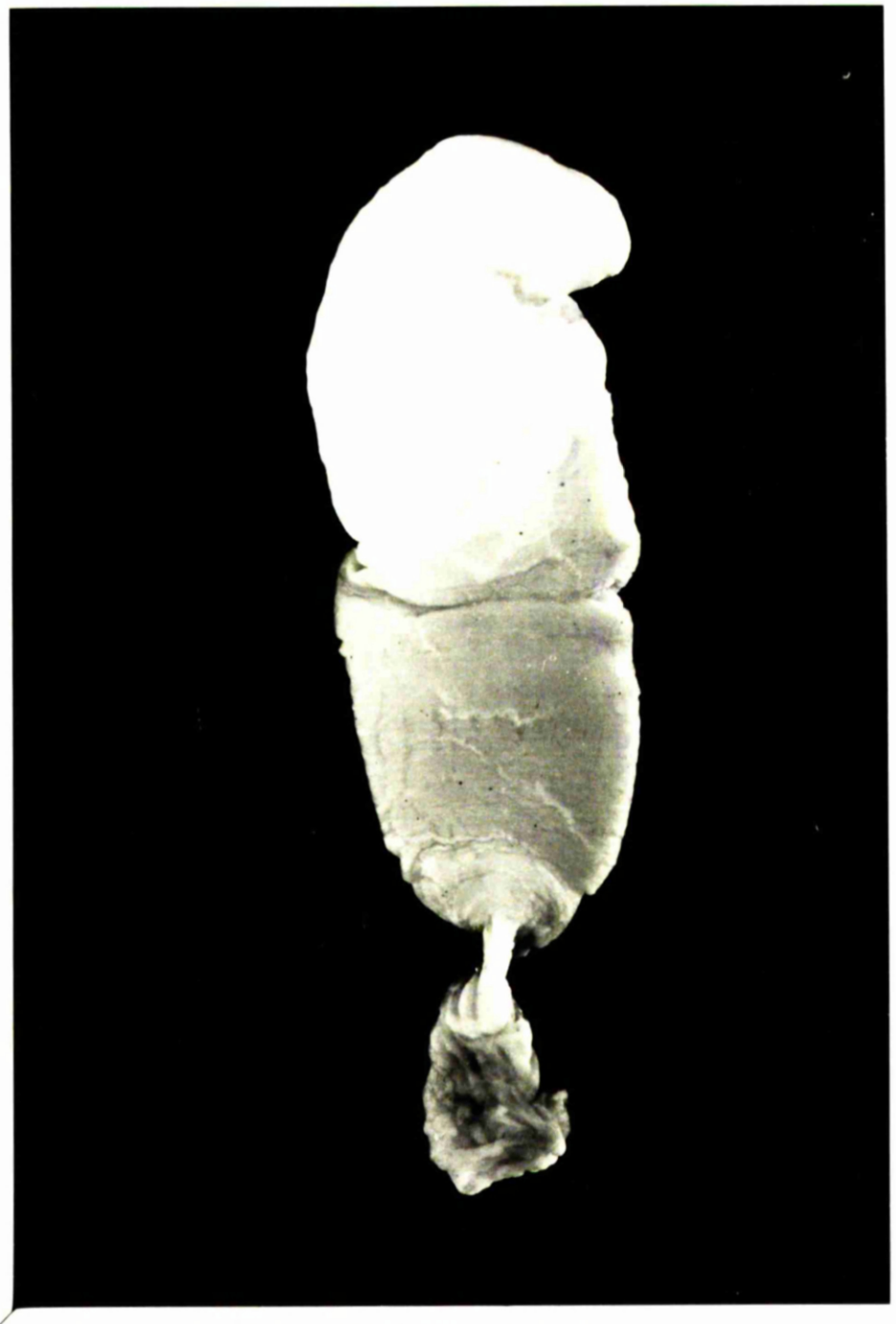

Figure 3 shows an artificial thrombus made in a Chandler tube by rotation of 15 ml. whole blood for 1 hour. The white head and the fibrin tail can be seen.

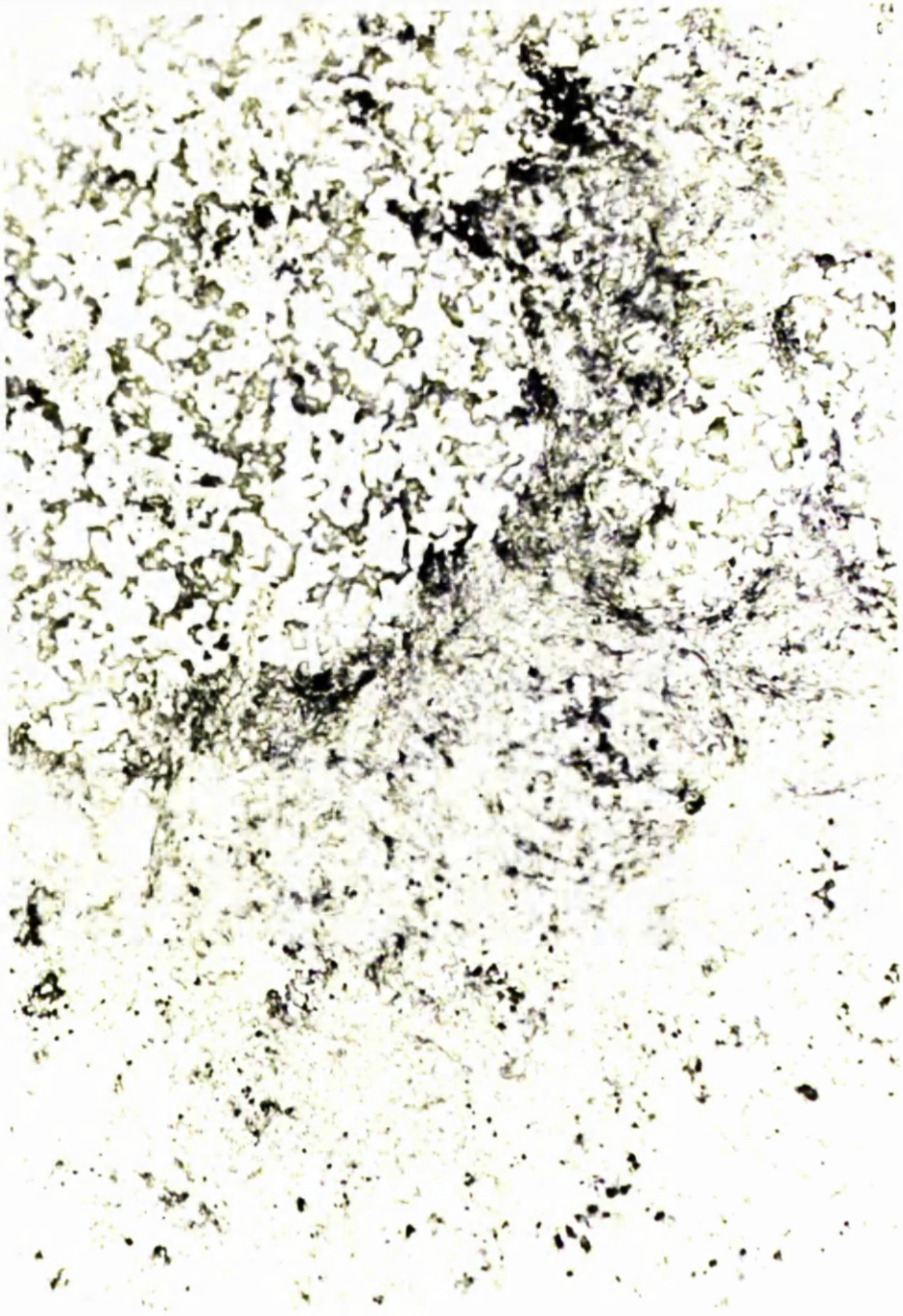

Figure 4 shows a longitudinal section through a Chandler thrombus. The contrast between the platelet head and the fibrin tail can be seen. Original magnification x 500. Stain - Picro Mallory (Photomicrograph prepared by Dr F. Walker)

Figure 5 shows an EEL titrator and a galvanometer (EEL, type 20, Evans Electroselenuim, Ltd., England). This apparatus was used in the study of platelet aggregation by the turbidimetric method.

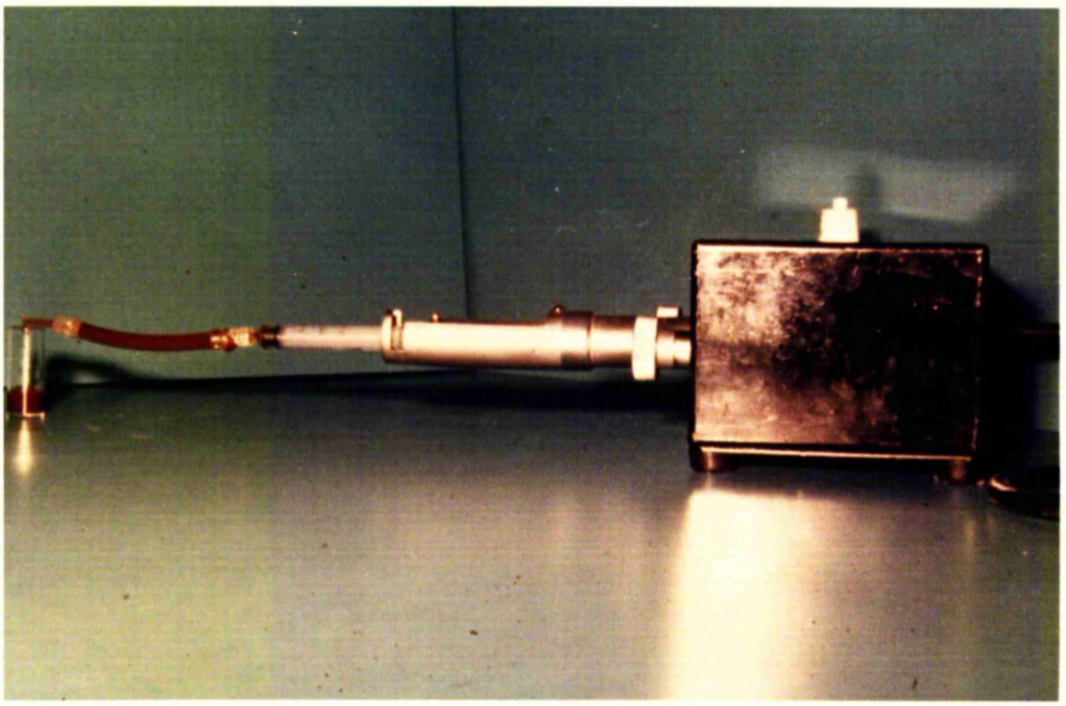

Figure 6 shows the apparatus used for the determination of platelet adhesiveness by a modified Hellem's technique (Hirsh et al., 1966). It consists of a glass bead column through which blood is forced by an electrically driven mechanical pump.

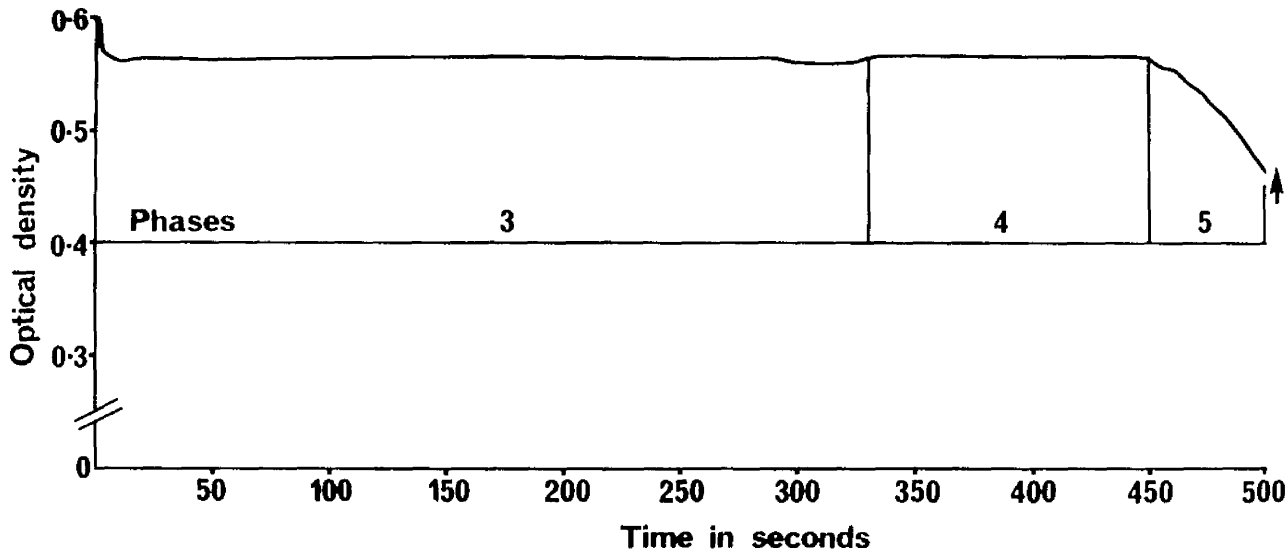

Figure 7 illustrates the optical density changes that took place after recalcification of diluted platelet rich plasma in the turbidimetric system. This is a representative experiment in a normal control (experiment No. 16, table 2). The optical density readings are given in table 6).

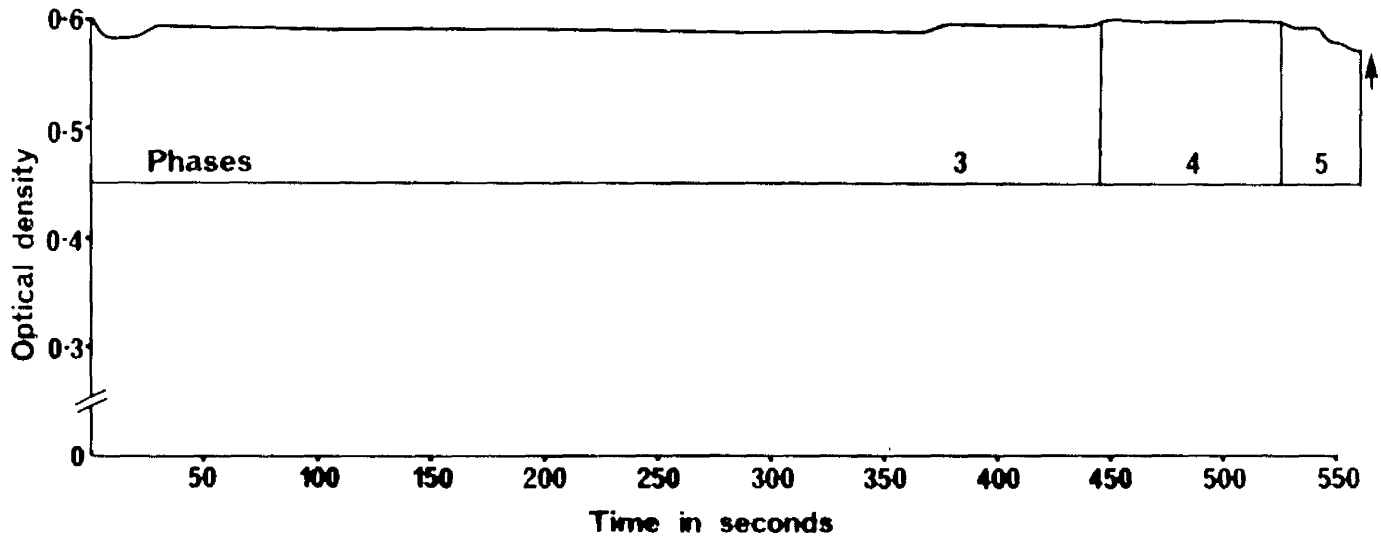

Figure 8 shows graphically the optical density changes after recalcification of diluted platelet-rich plasma in the turbidimetric system. This is a representative experiment in a uraemic patient (patient No. 20, table 4). The optical density readings are given in table 8.

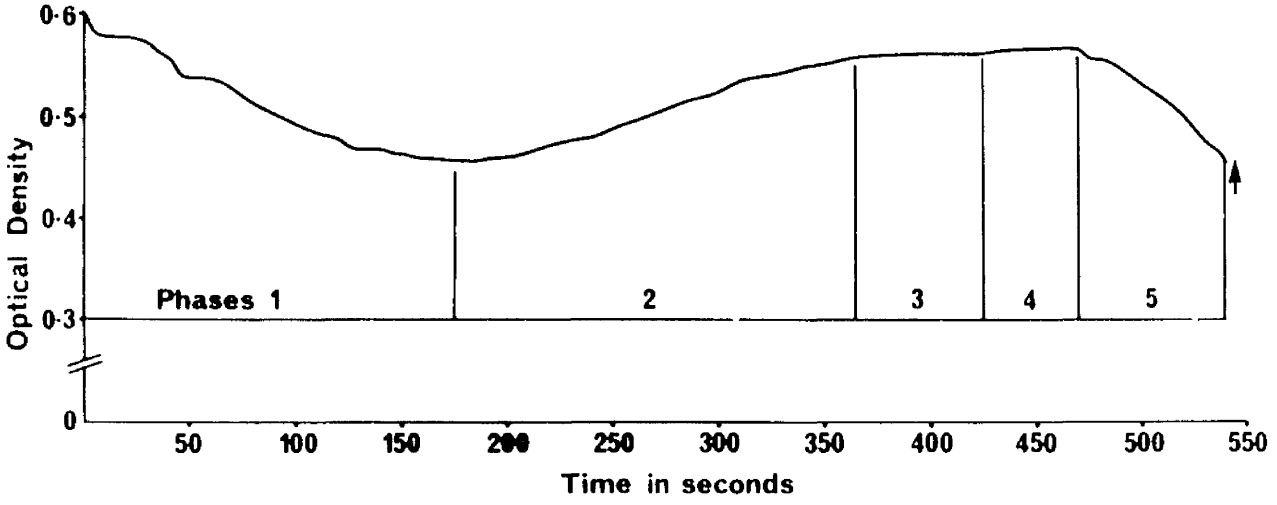

Figure 9 shows the optical density changes after the addition of calcium plus ADP to diluted platelet rich plasma in the turbidimetric system. This is a representative experiment in a normal control (experiment No.16, table 3). The optical density readings are given in table 7.

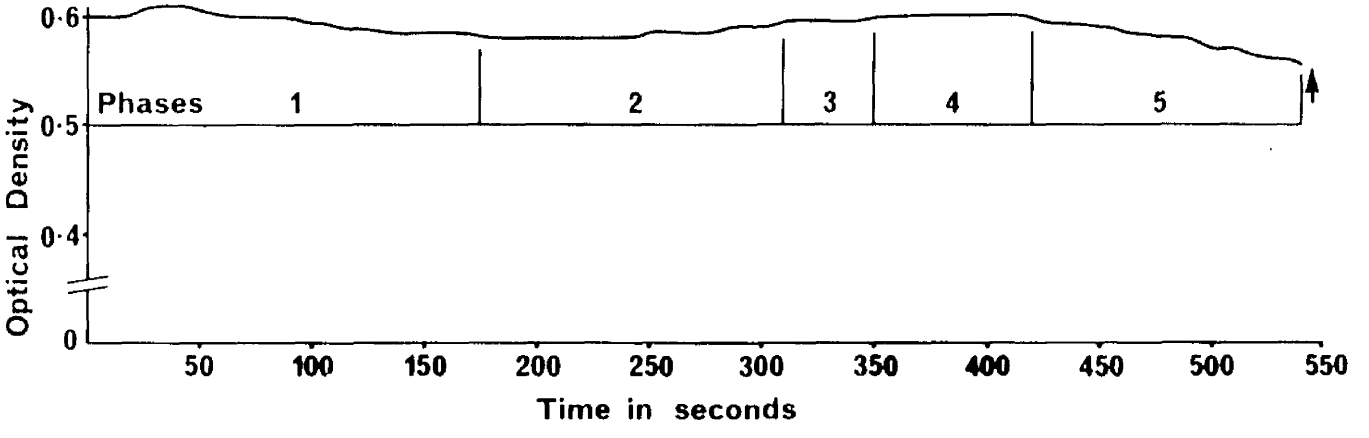

Figure 10 shows the optical density changes after the addition of calcium plus ADP to diluted platelet rich plasma in the turbidimetric system. This is a representative experiment in a uraemic patient (patient No. 20, table 5). The optical density readings are given in table 9.

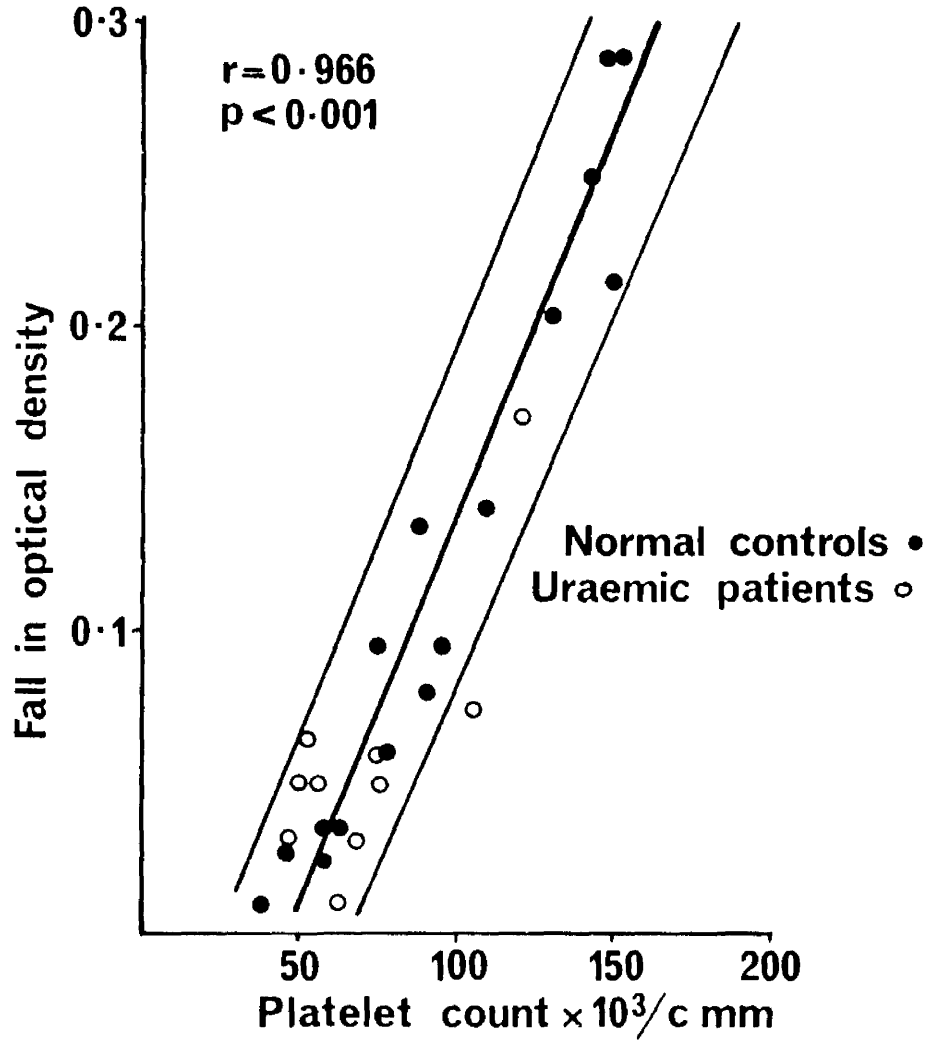

Figure 11 illustrates the relationship between the platelet count and the optical density fall in phase 5 following recalcification in the turbidimetric system. The results from all uraemic patients except one (No. 22) fell within the 95 per cent confidence limit of normal controls. The results from normal controls are shown in table 2, and from uraemic patients in table 4.

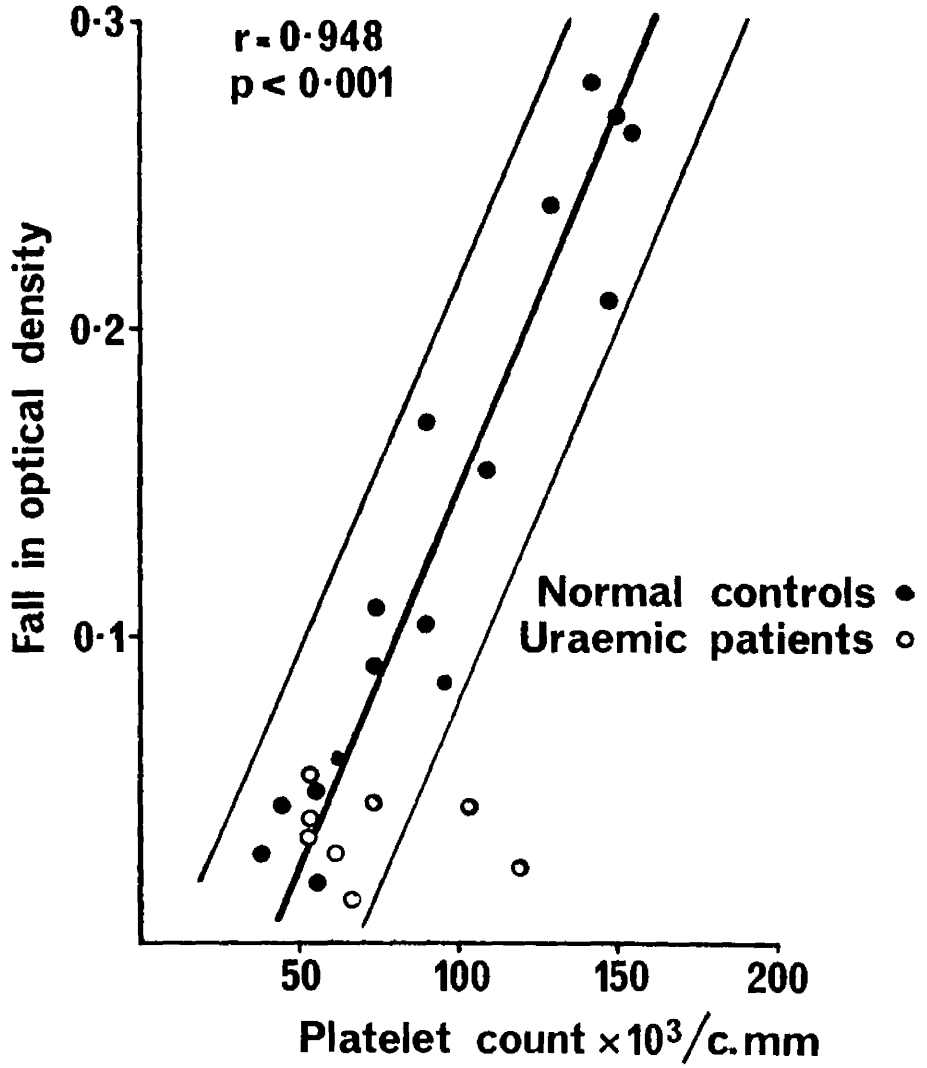

Figure 12 shows the relationship between the platelet count and the optical density fall in phase 5 following the addition of calcium and ADP in the turbidimetric system. The results from two uraemic patients (Nos. 18, 22) fell outside the normal 95 per cent confidence limit. In two patients (Nos. 35 and 36) the fall in optical density could not be determined because of failure of their platelets to disaggregate in phase 2. The results from normal controls are given in table 3 and those from uraemic patients in table 5.

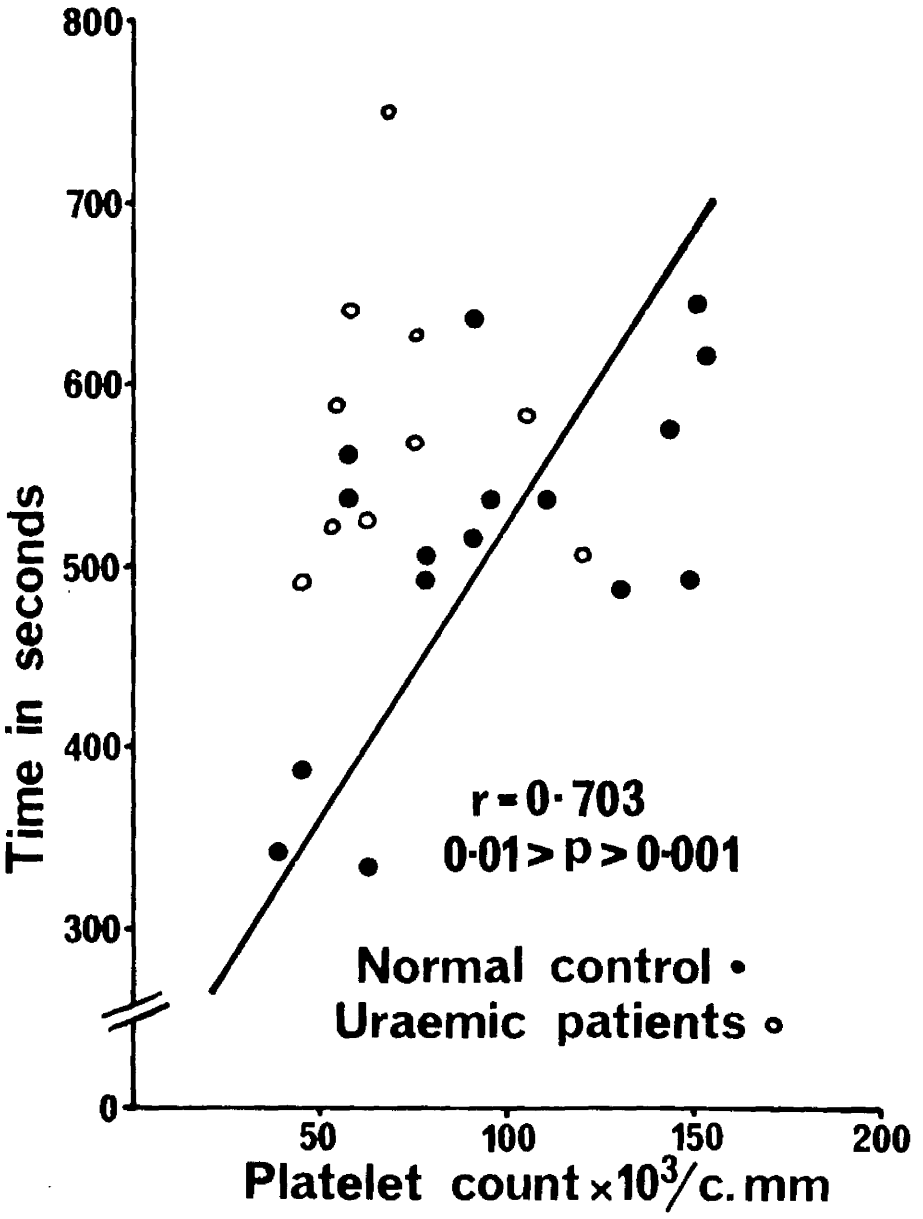

Figure 13 shows the relationship between the platelet count and the aggregation time in phase 5 following recalcification in the turbidimetric system. The results from two uraemic patients fell outside the 95 per cent confidence limit of normal controls (Nos. 21, 38). The results from normal control are shown in table 2, and those from uraemic patients in table 4.

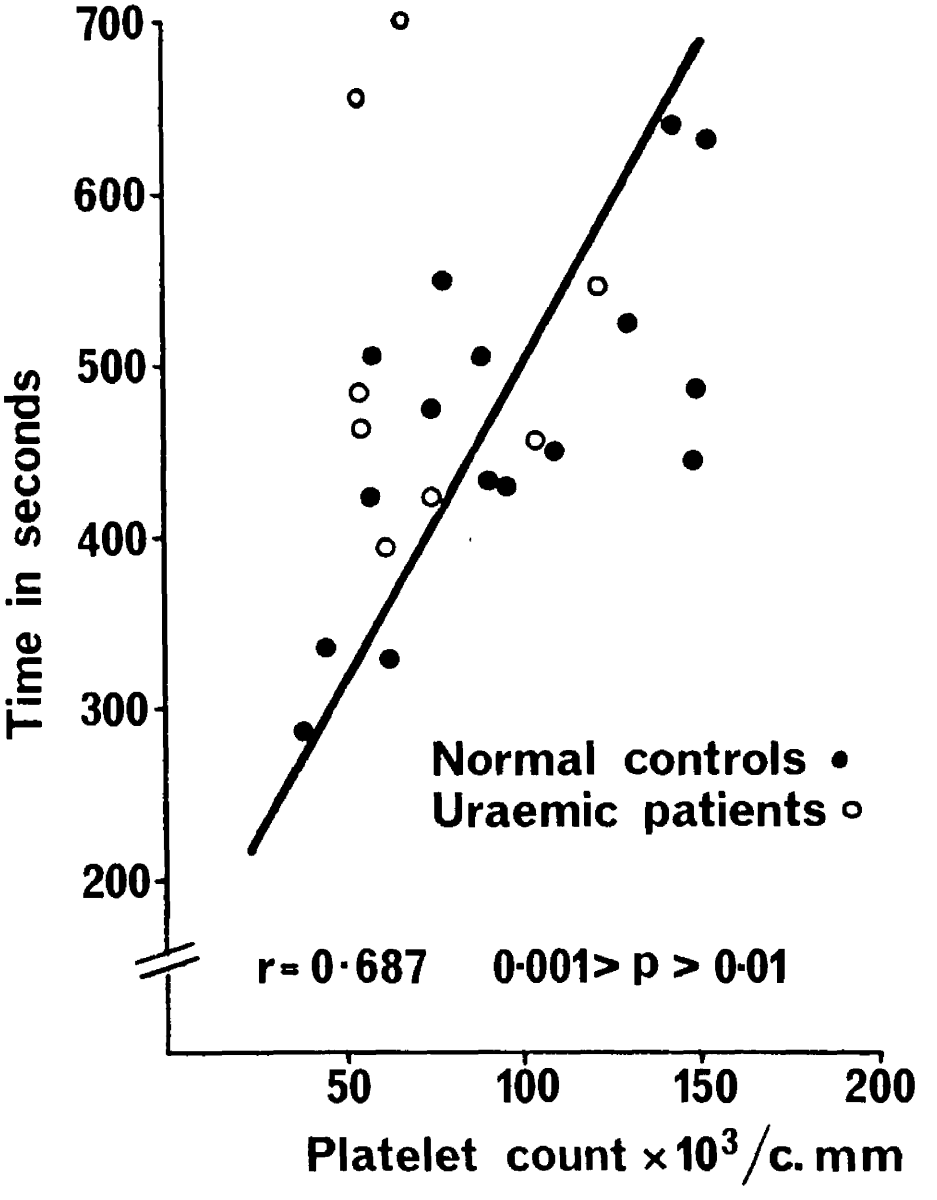

Figure 14 shows the relationship between the platelet count and the aggregation time in phase 5 when calcium and ADP were added in the turbidimetric system. The results from two patients (Nos. 21, 38) fell outside the 95 per cent confidence limit of normal controls. The results from normal controls are shown in table 3 and those from uraemic patients in table 5.

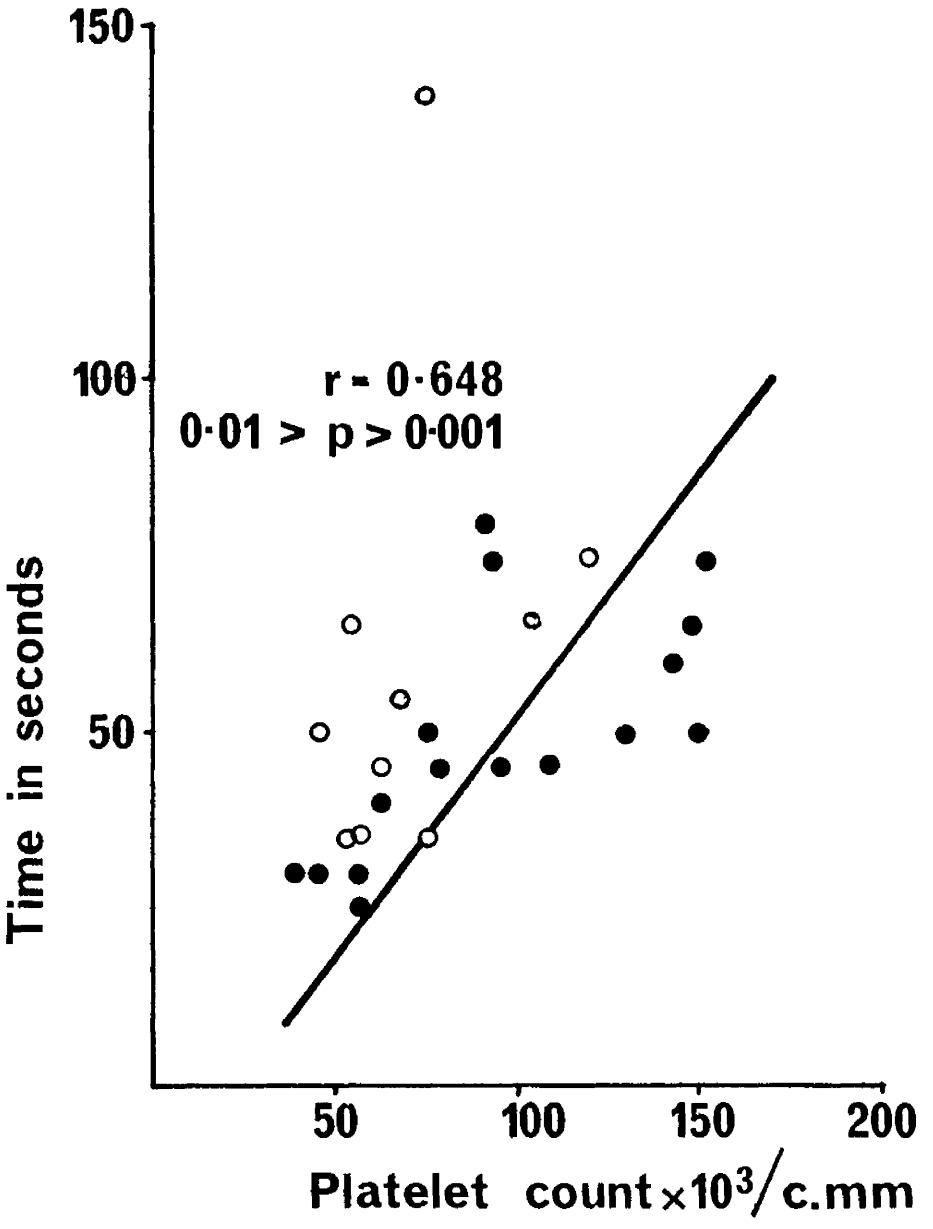

Figure 15 shows the relationship between the platelet count and the duration of phase 5 following recalcification in the turbidimetric system. All uraemic patients shows normal results except one (patient No. 35). The results from normal controls are presented in table 2 and those from uraemic patients in table 4.

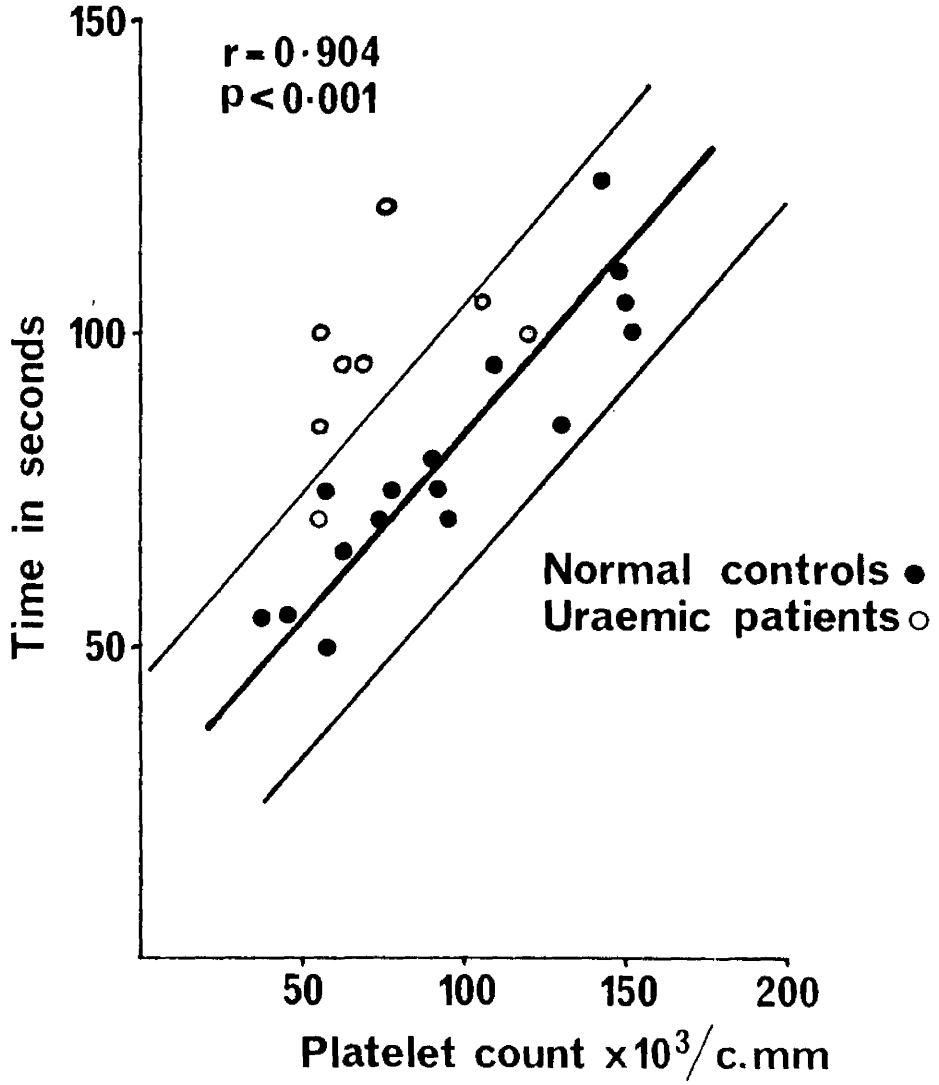

Figure 16 shows the correlation between the platelet count and the duration of phase 5 when calcium and ADP were added in the turbidimetric system. The results from three uraemic patients (Nos. 18, 20, 21) showed normal relationship and the rest (Nos. 12, 15, 17, 35, 38) were outside the normal 95 per cent confidence limit. The results from normal controls are shown in table 3 and those from uraemic patients in table 5.

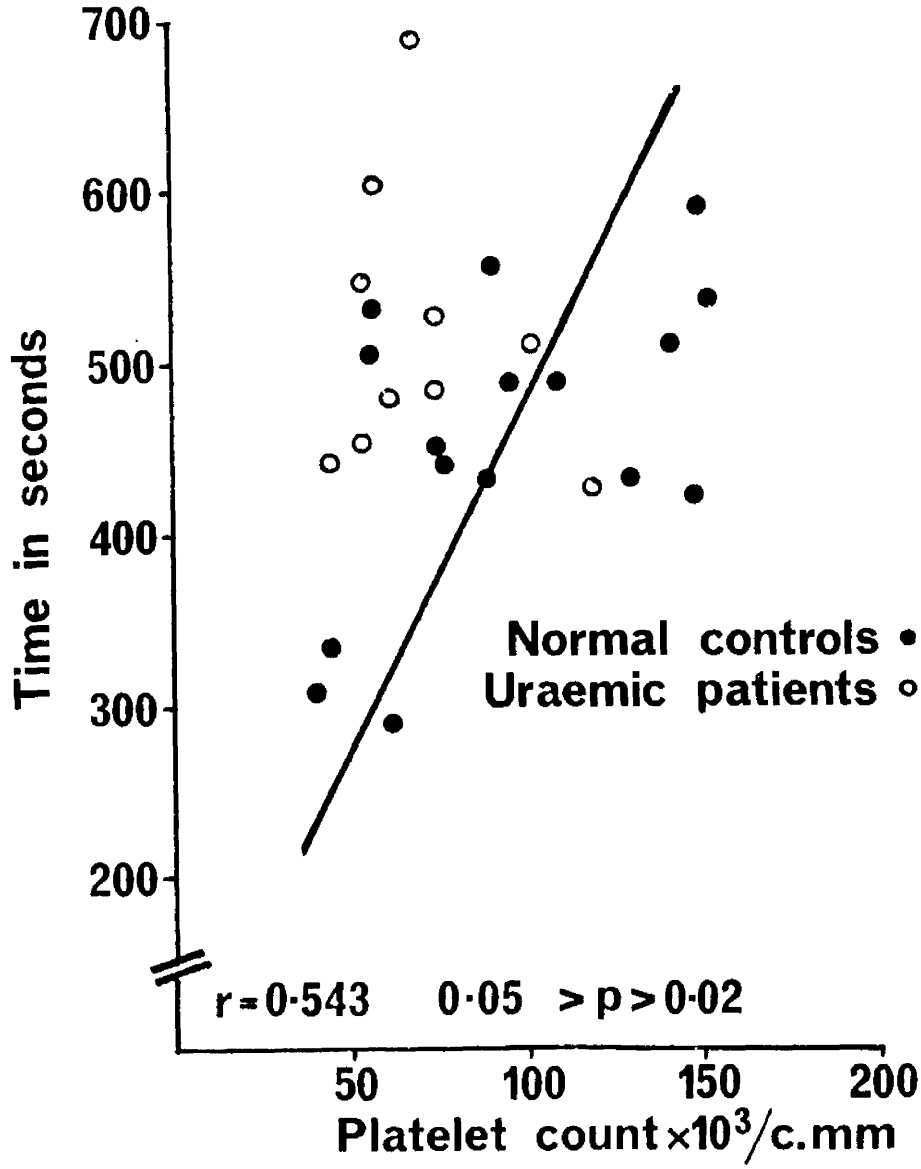

Figure 17 shows the relationship between the platelet count and the clotting time after recalcification in the turbidimetric system. All uraemic patients showed normal results except 3 (Nos. 15, 21, 38). The data for normal controls are shown in table 2 and those from uraemic patients in table 4.

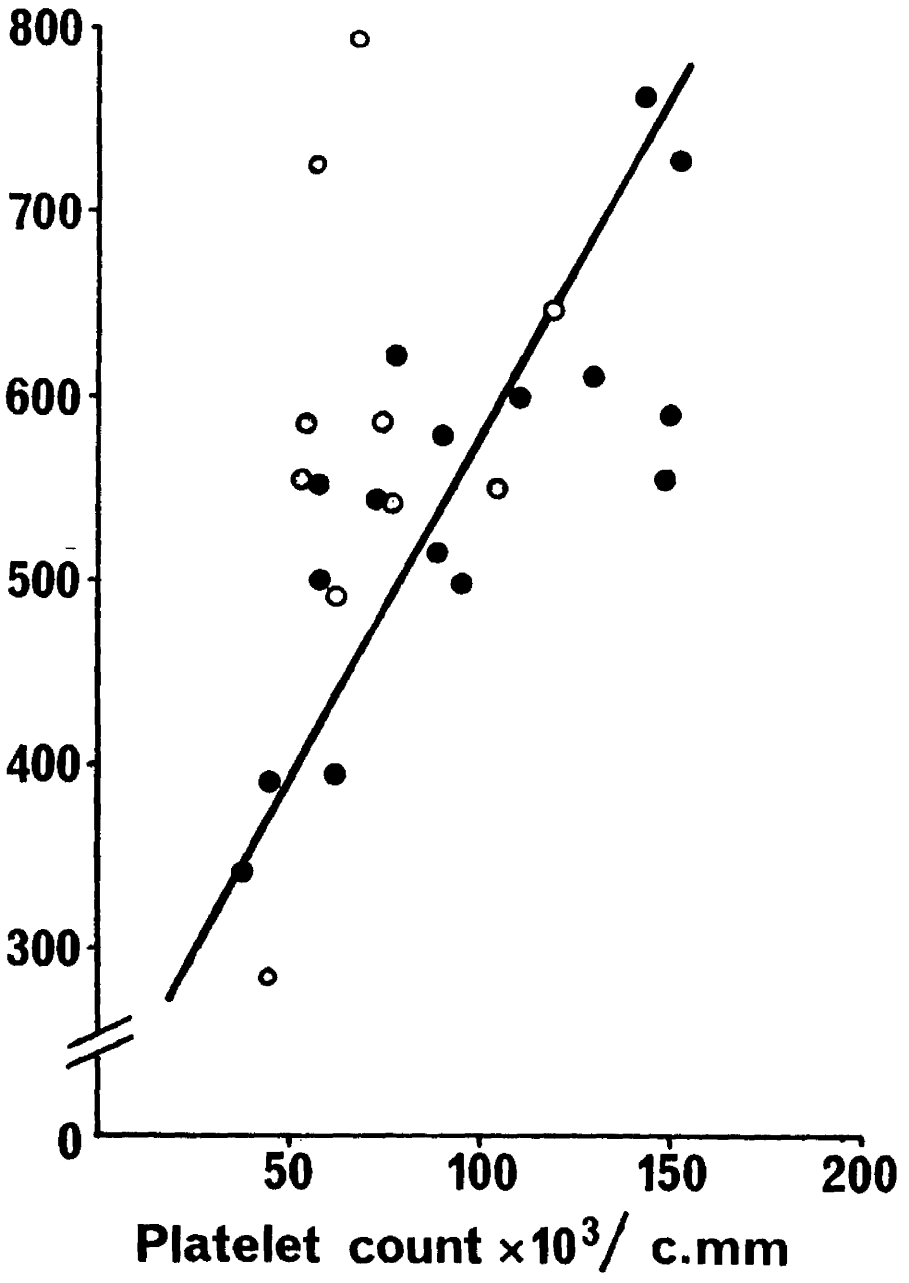

Figure 18 illustrates the relationship between the platelet count and the clotting time when calcium and ADP were added in the turbidimetric system ($r = 0.768$, $P < 0.001$). All uraemic patients showed normal results except two (Nos. 21 and 38). The figure is constructed from the data in tables 3 and 5.

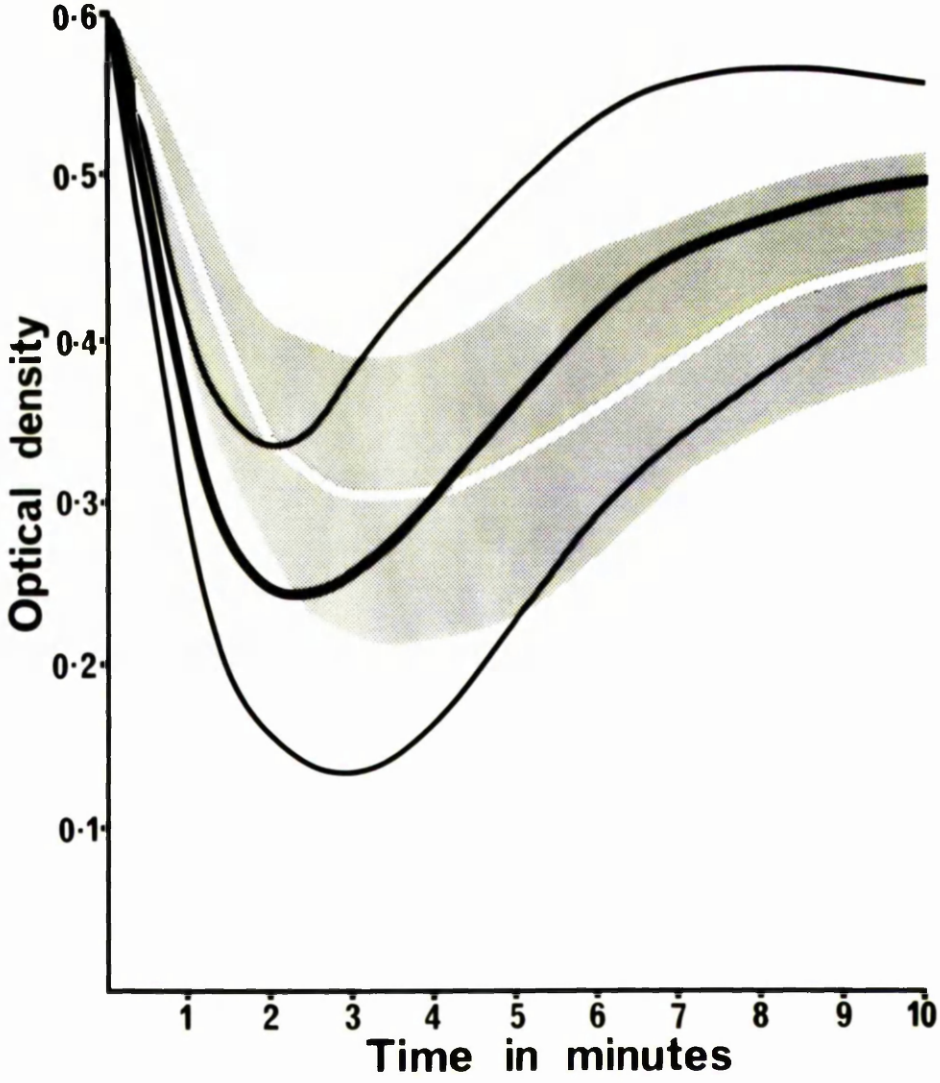

Figure 19 shows the mean optical density changes in ADP-induced platelet aggregation in normal controls (black line), and uraemic patients (white line). The optical density readings are the mean values from 12 normal controls and 5 uraemic patients ± one standard deviation. The platelet counts of both normal controls and uraemic patients ranged between 350,000 - 450,000/c.mm. (group 1). The optical density readings from normal controls are presented in table 25, and those from uraemic patients in table 29.

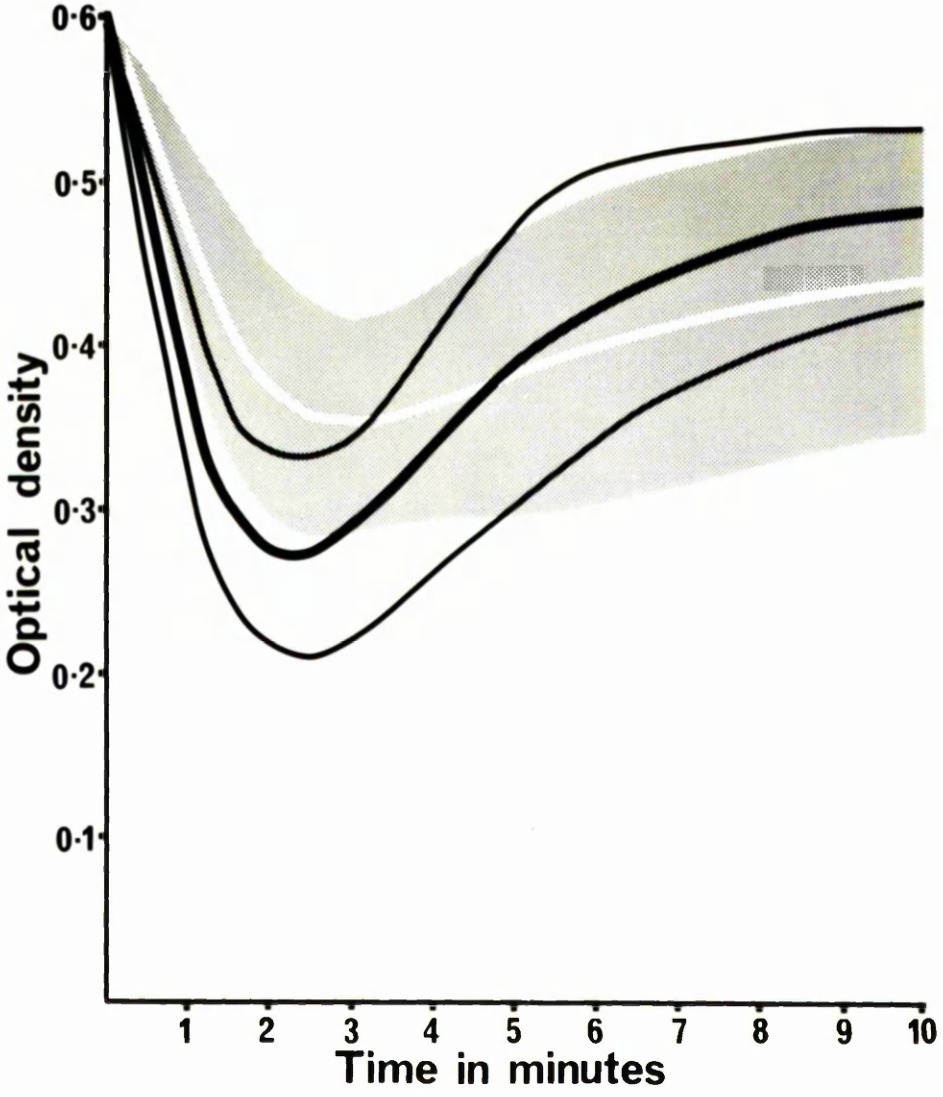

Figure 20 shows the mean optical density change in ADP-induced platelet aggregation in normal controls (black line), and uraemic patients (white line). The optical density readings are the mean value of 9 normal experiments and 17 uraemic patients ± one standard deviation. The platelet counts of both normal controls and uraemic patients ranged between 250,000 - 350,000/c.mm. (group 2). The optical density readings from normal controls are presented in table 26 and those from uraemic patients in table 30.

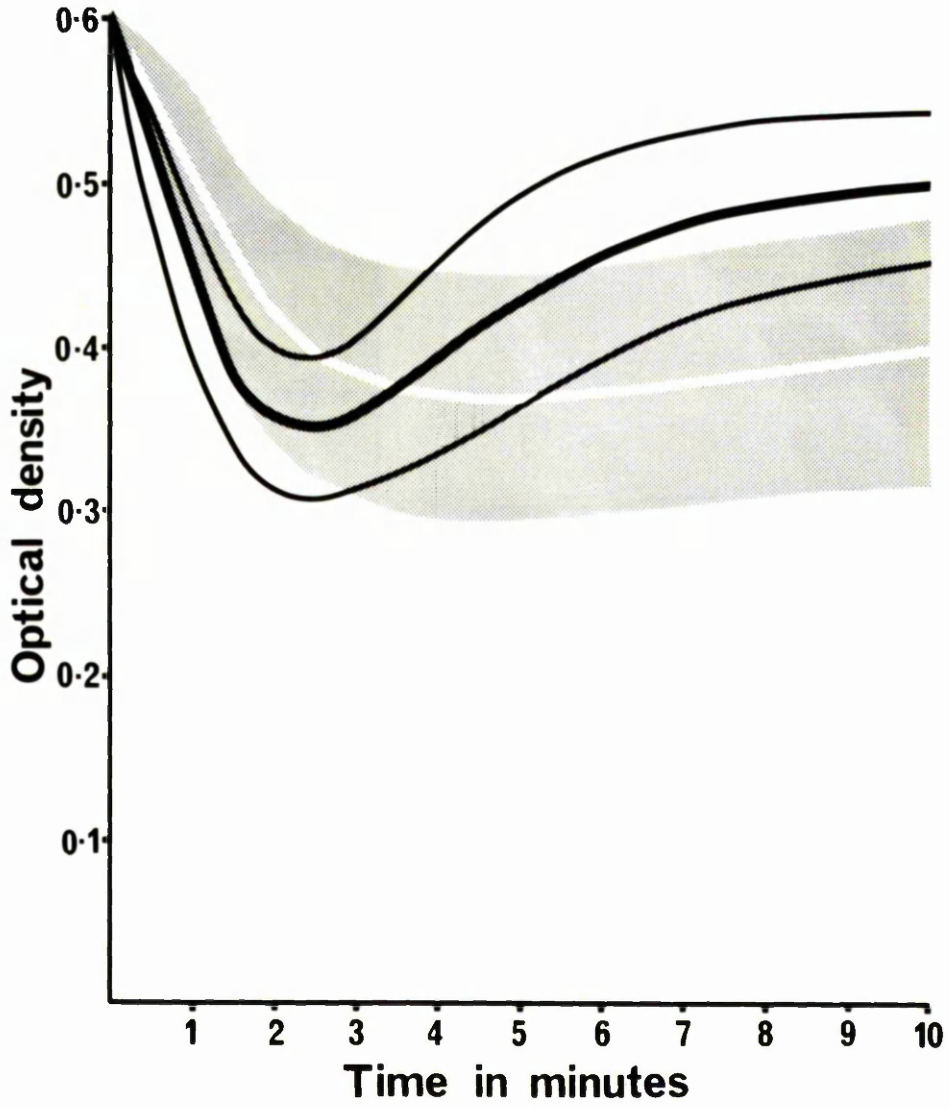

Figure 21 shows the mean optical density changes of ADP-induced platelet aggregation in normal controls (black line), and uraemic patients (white line). The optical density readings are the mean values of 7 normal experiments and 16 uraemic patients ± one standard deviation. The platelet counts of both normal controls and uraemic patients ranged between 150,000 - 250,000/c.mm. (group 3). The optical density readings from normal controls are presented in table 27 and those from uraemic patients in table 31.

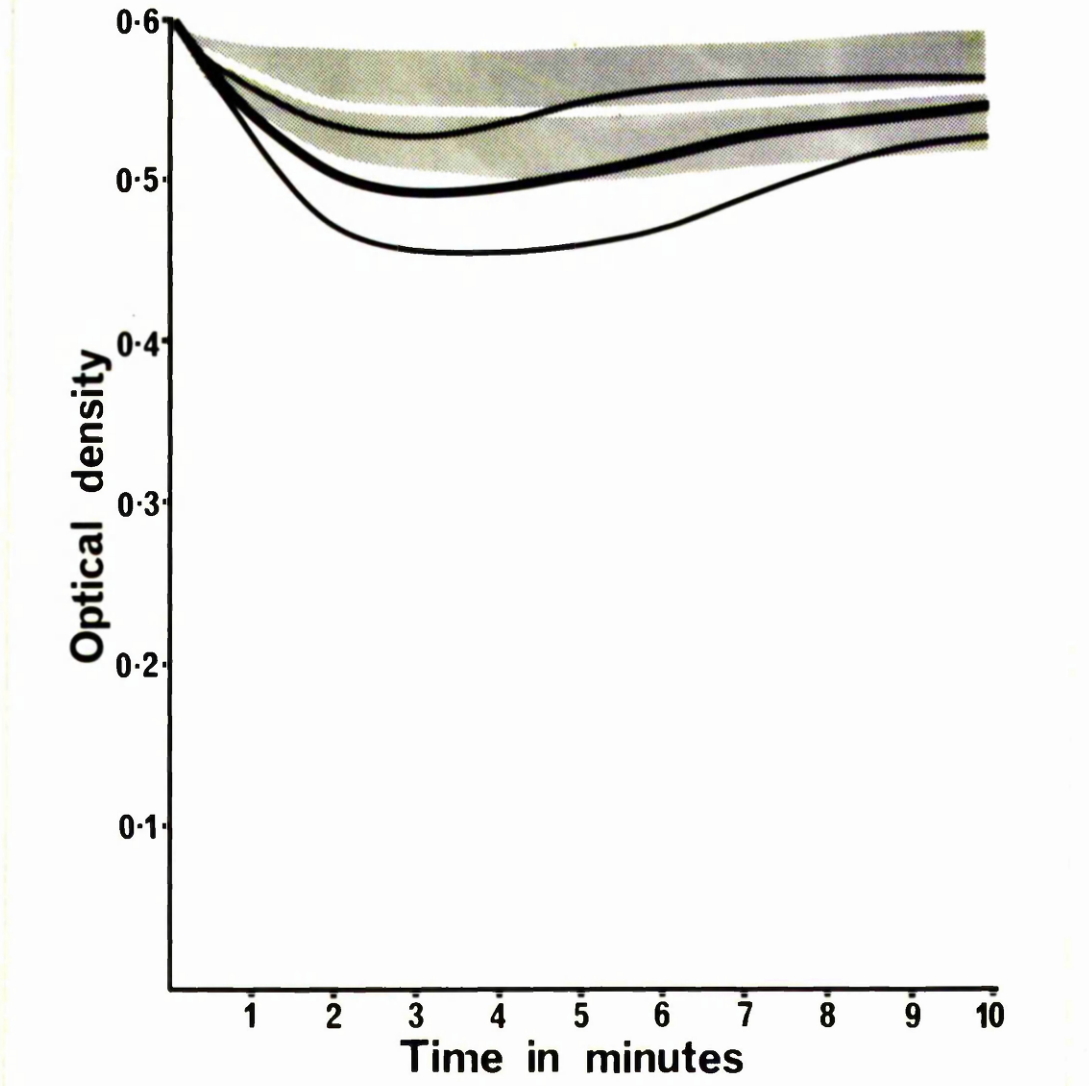

Figure 22 shows the mean optical density changes of ADP-induced platelet aggregation in normal controls (black line), and uraemic patients (white line). The optical density readings are the mean value from 7 normal experiments and 8 uraemic patients ± one standard deviation. The platelet counts of both normal controls and uraemic patients ranged between 50,000 - 150,000/c.mm. (group 4). The optical density readings of normal controls are presented in table 28, and those of uraemic patients in table 32.

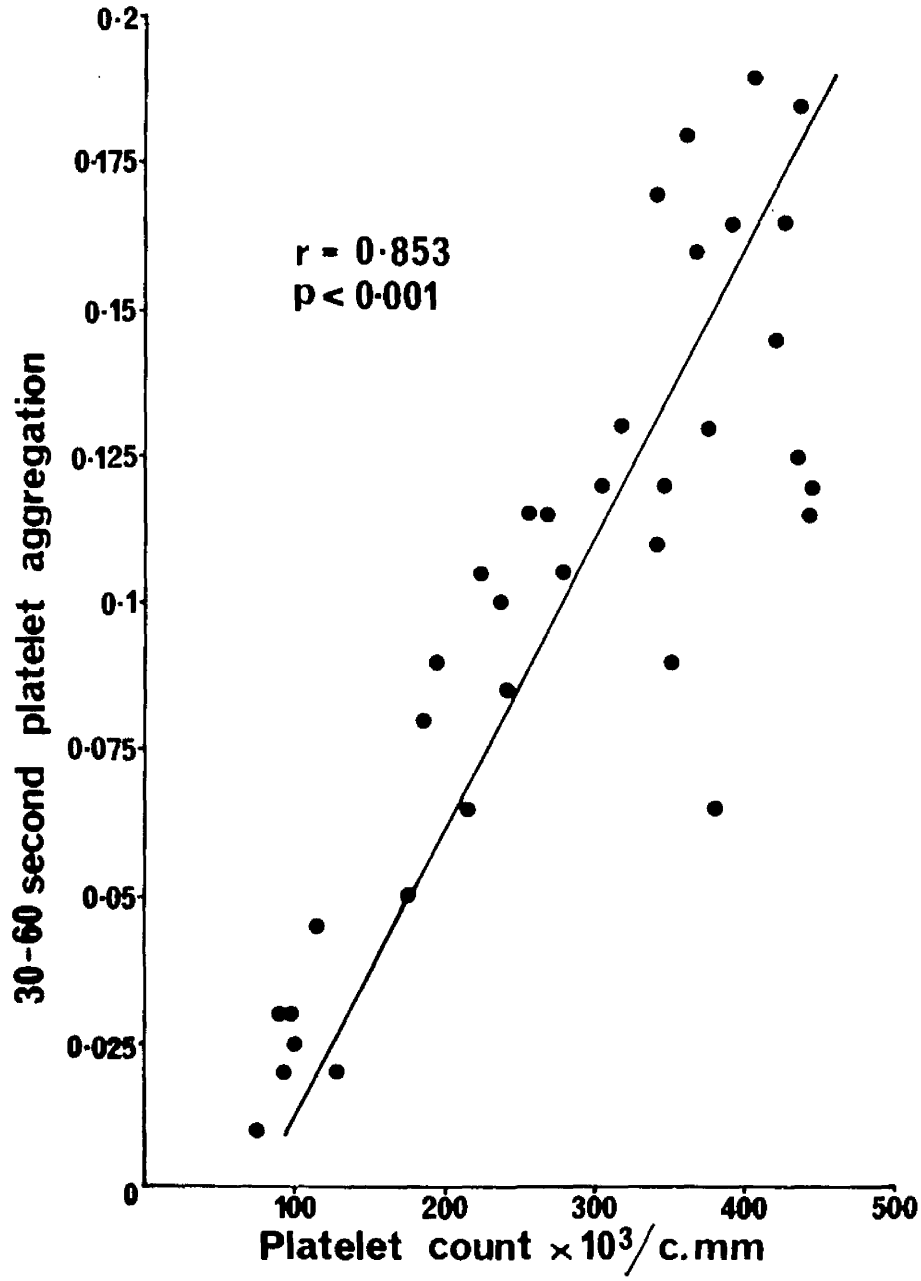

Figure 23 shows the relationship between the platelet count and 30-60 platelet aggregation in the turbidimetric system. The results from 35 normal experiments are presented in this figure, and are shown in table 23.

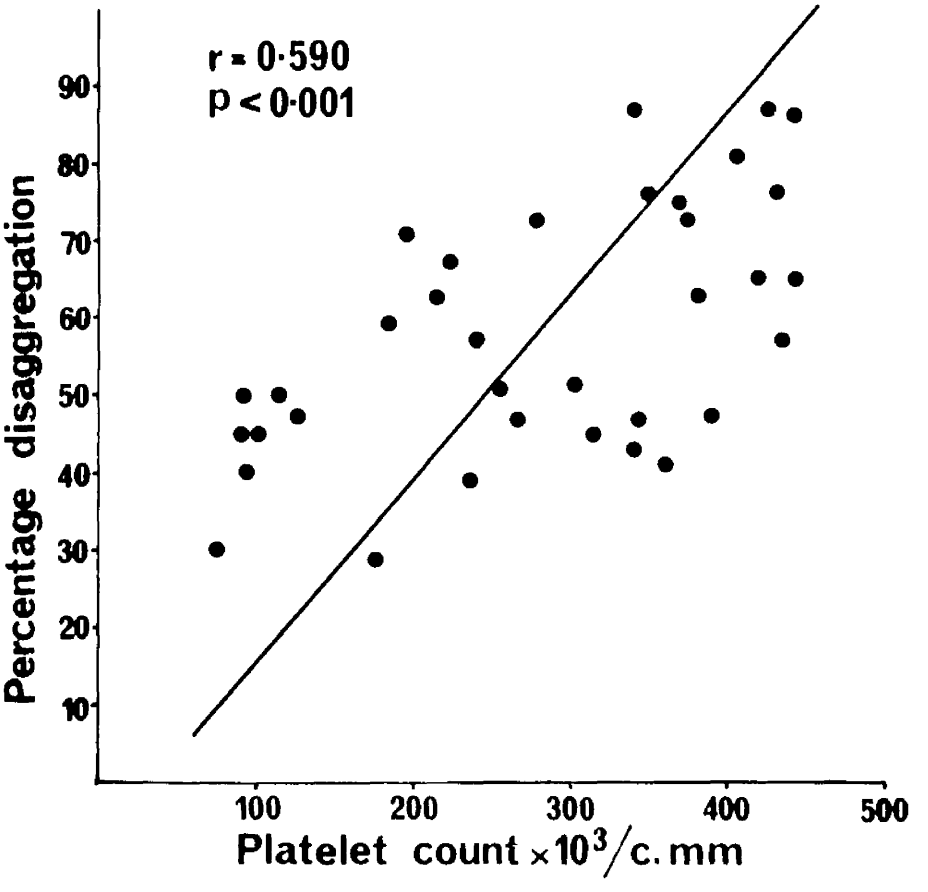

Figure 24 illustrates the relationship between the platelet count and percentage disaggregation after ADP has been added to platelet rich plasma in the turbidimetrio system. The results from 35 normal experiments are presented in this figure and are shown in table 23.

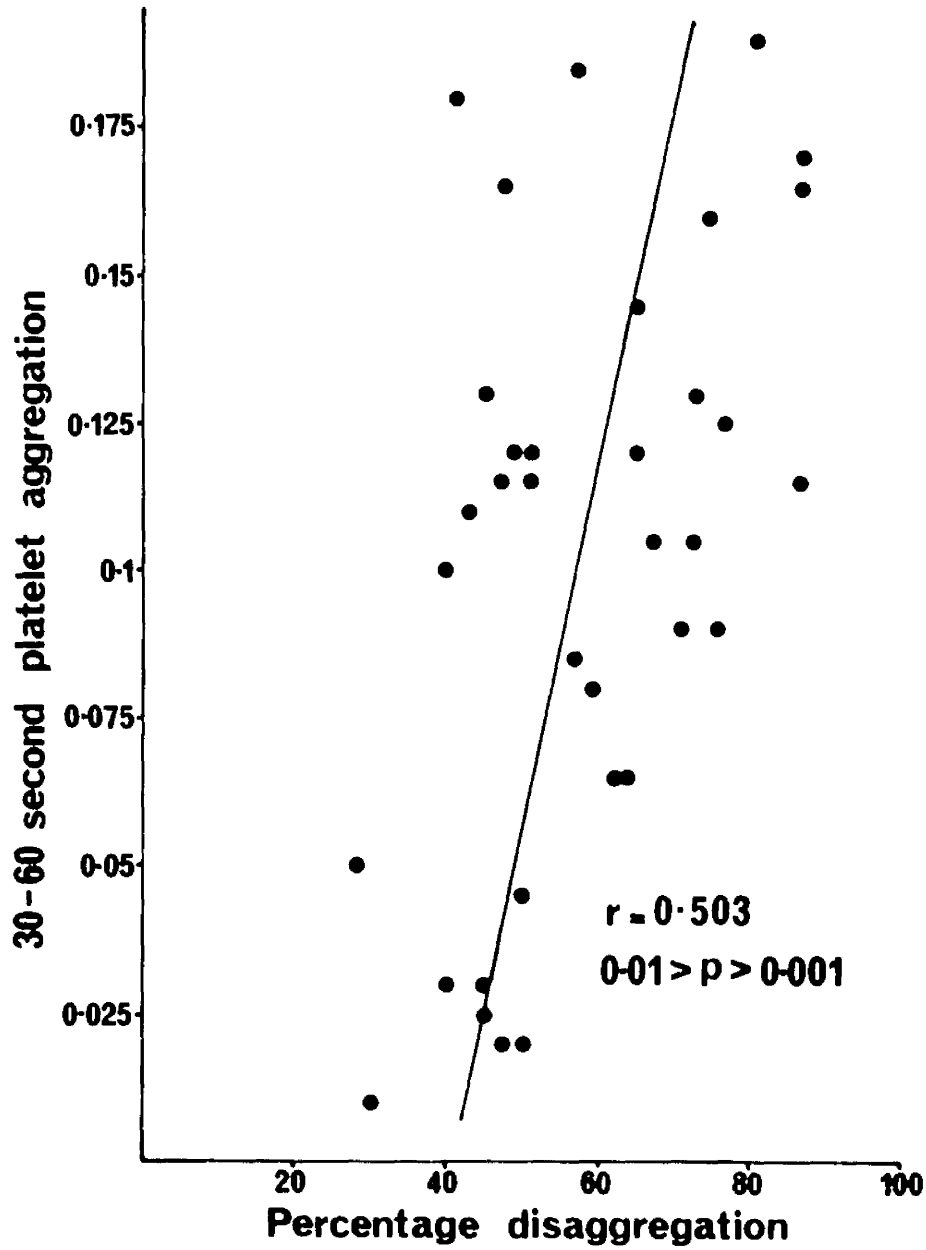

Figure 25 shows the relationship between 30-60 platelet aggregation and percentage disaggregation in the turbidimetric system. This figure is compiled from the data of 35 normal experiments and these are shown in table 23.

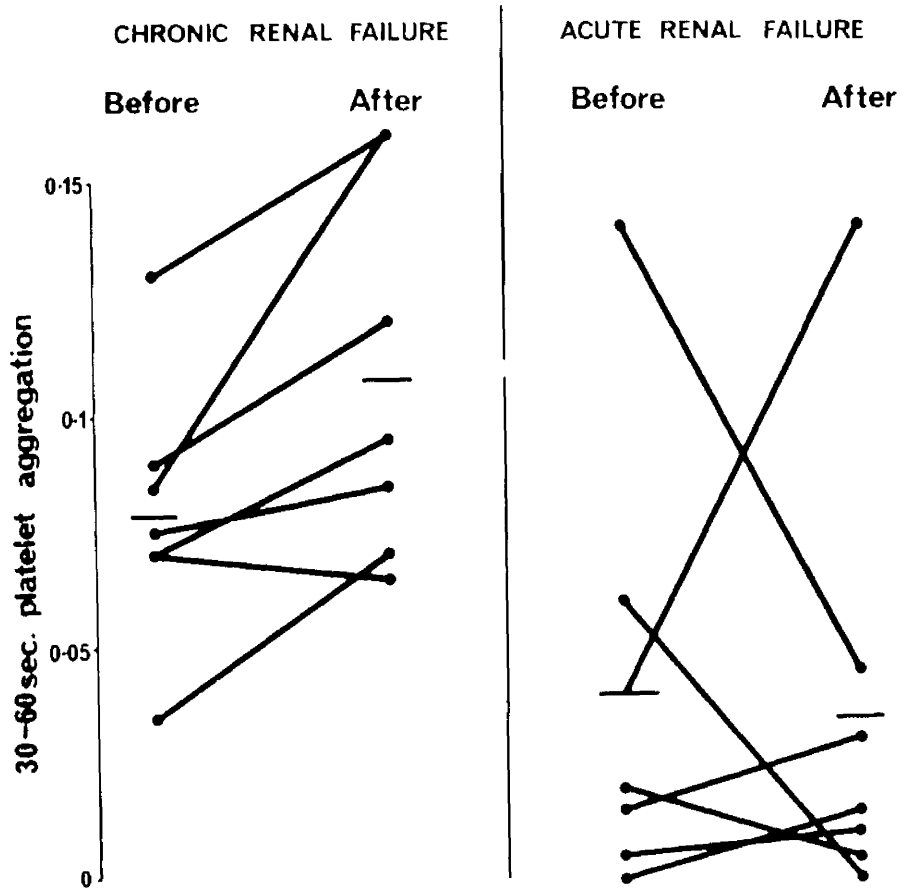

Figure 26 shows the effect of haemodialysis on 30-60 platelet aggregation in chronic and acute renal failure patients. The results are given in tables 20 and 21.

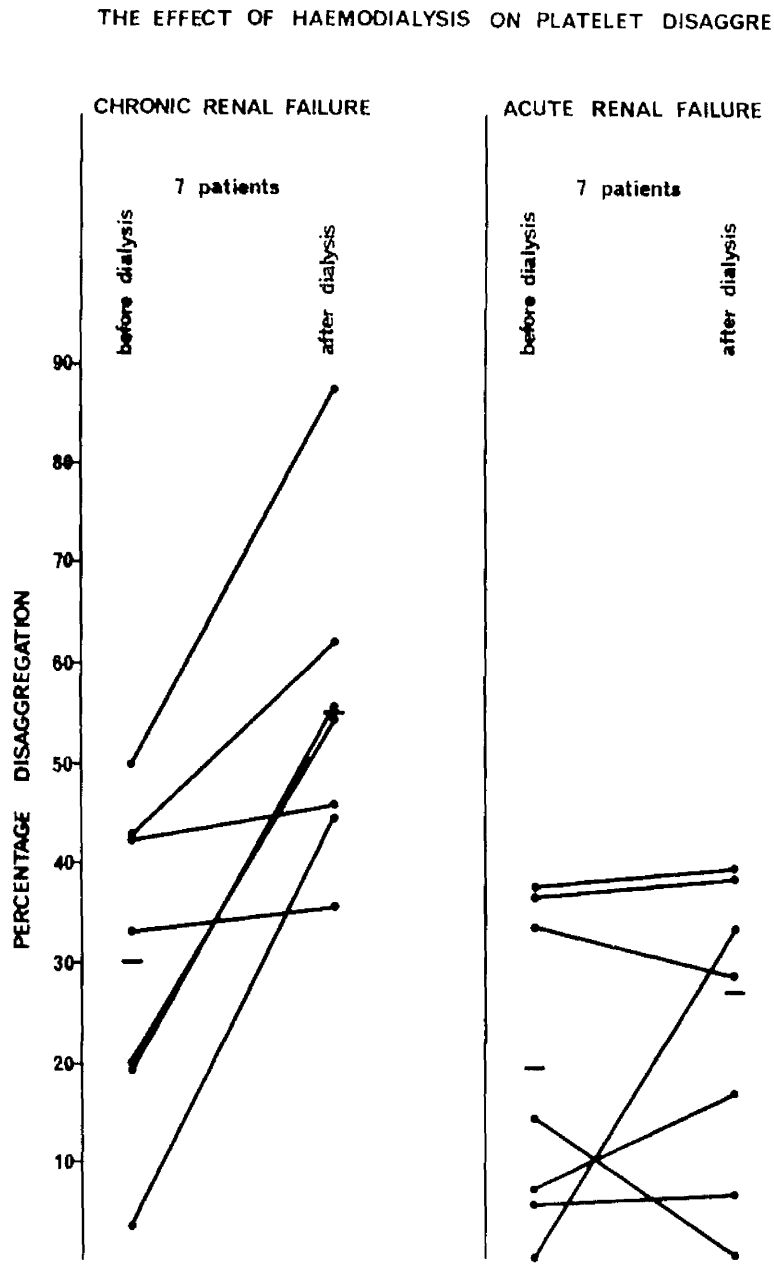

Figure 27 illustrates graphically the effect of haemodialysis on percentage platelet disaggregation in chronic and acute uraemic patients. The results are shown in tables 20 and 21.

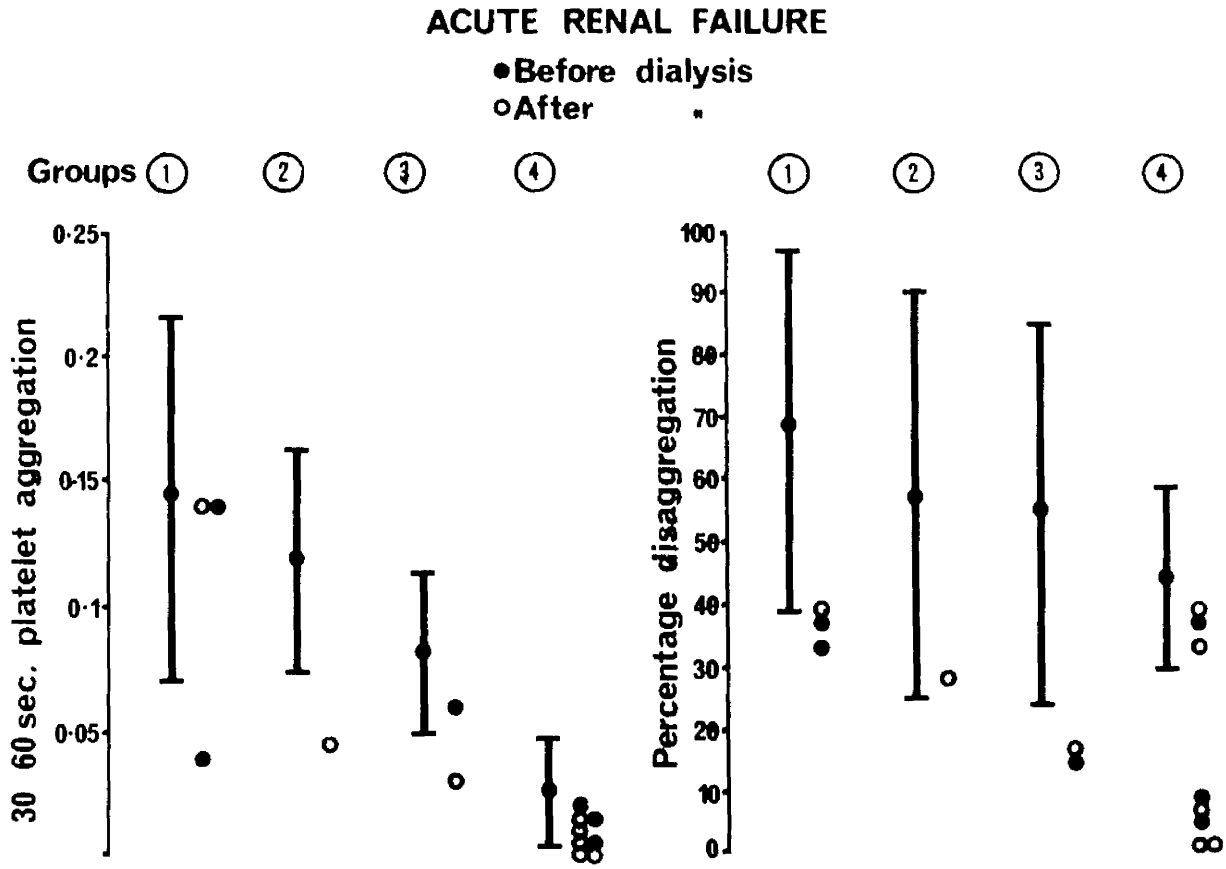

Figure 28 shows the effect of haemodialysis on 30-60 platelet aggregation and disaggregation in 7 acute uraemic patients. Results are presented in 4 groups according to their platelet count and in relation to the normal mean values ± 2 standard deviations in each group. This figure is compiled from data shown in table 21.

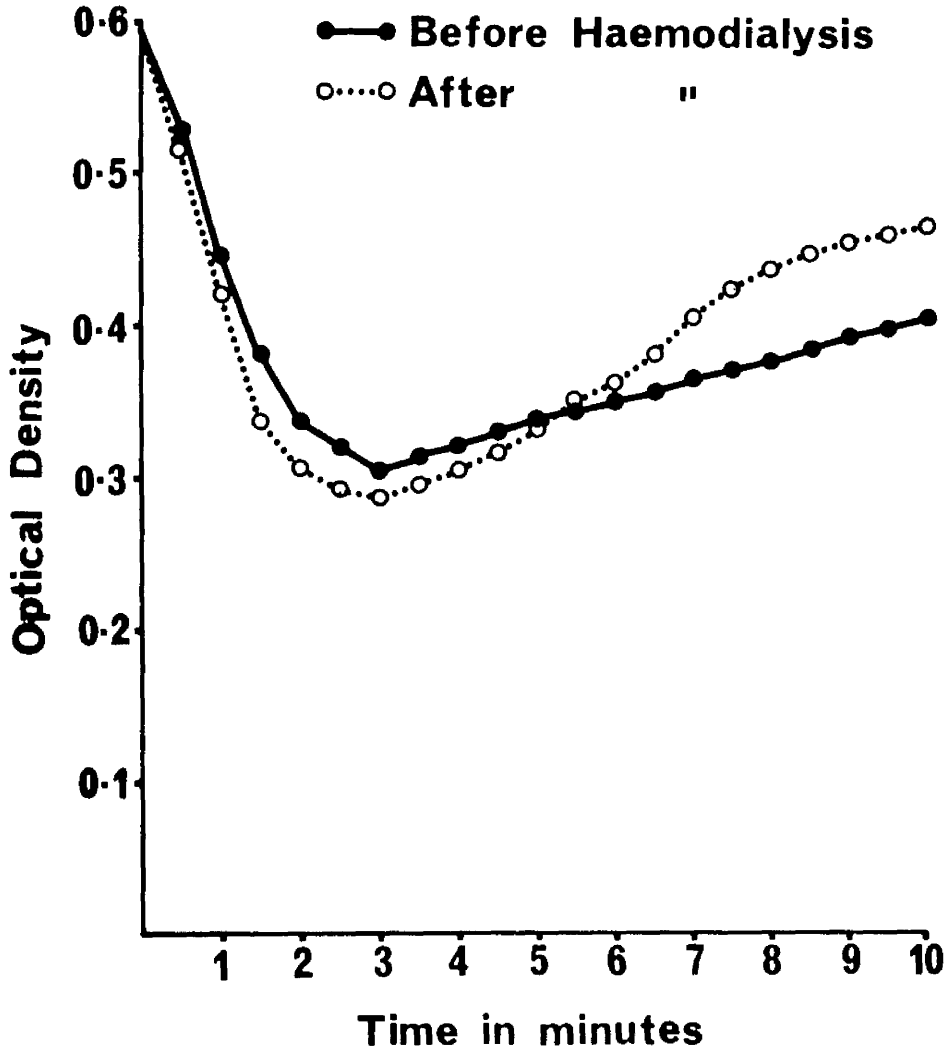

Figure 29 shows the effect of haemodialysis on ADP-induced platelet aggregation. The mean optical density readings from 7 chronic uraemic patients before and after haemodialysis were used to construct the curves and are given in table 33.

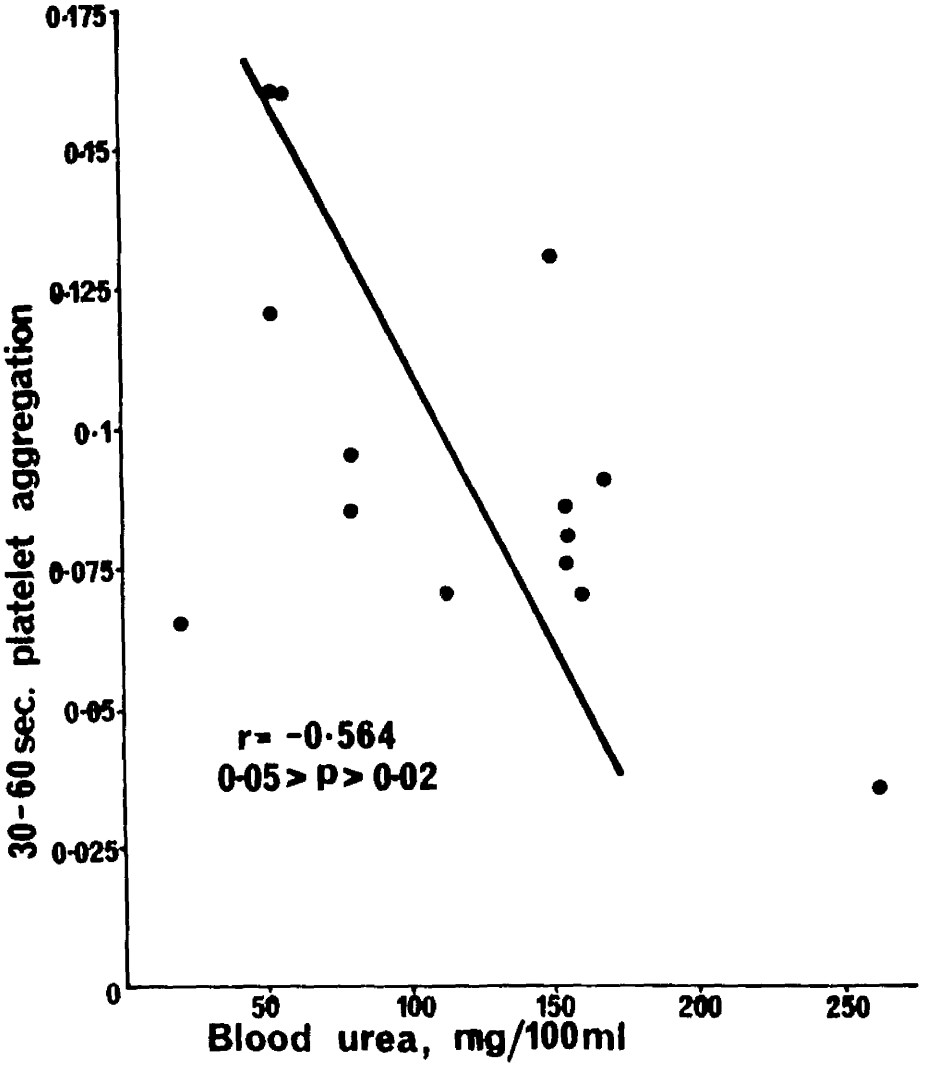

Figure 30 illustrates the relationship between 30-60 platelet aggregation and the corresponding blood urea level before and after haemodialysis in chronic renal failure. The figure is constructed from data in table 20.

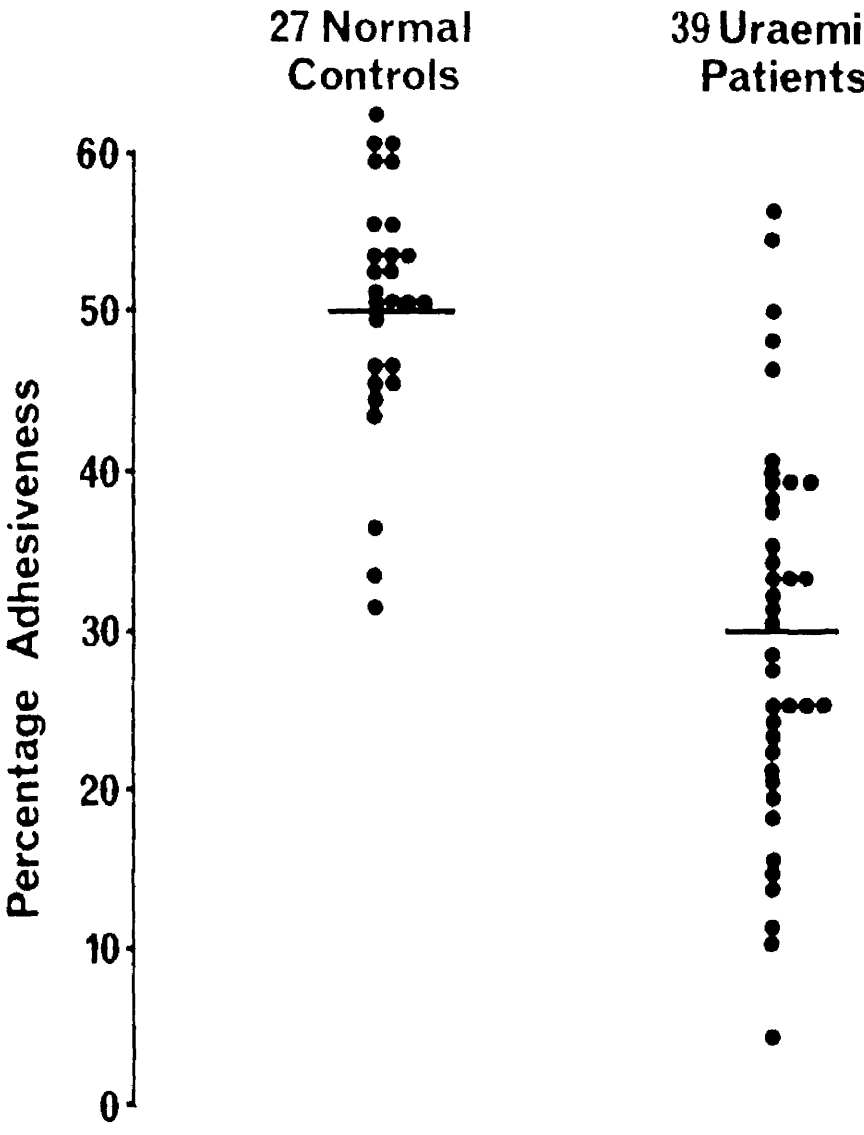

Figure 31 shows percentage platelet adhesiveness in 27 normal controls (table 41) and 39 uraemic patients (table 43). The results are presented without correction for the packed cell volume. The mean percentage platelet adhesiveness in the uraemic patient is significantly lower than the mean value of normal controls ($t = 7.483$, $P < 0.001$).

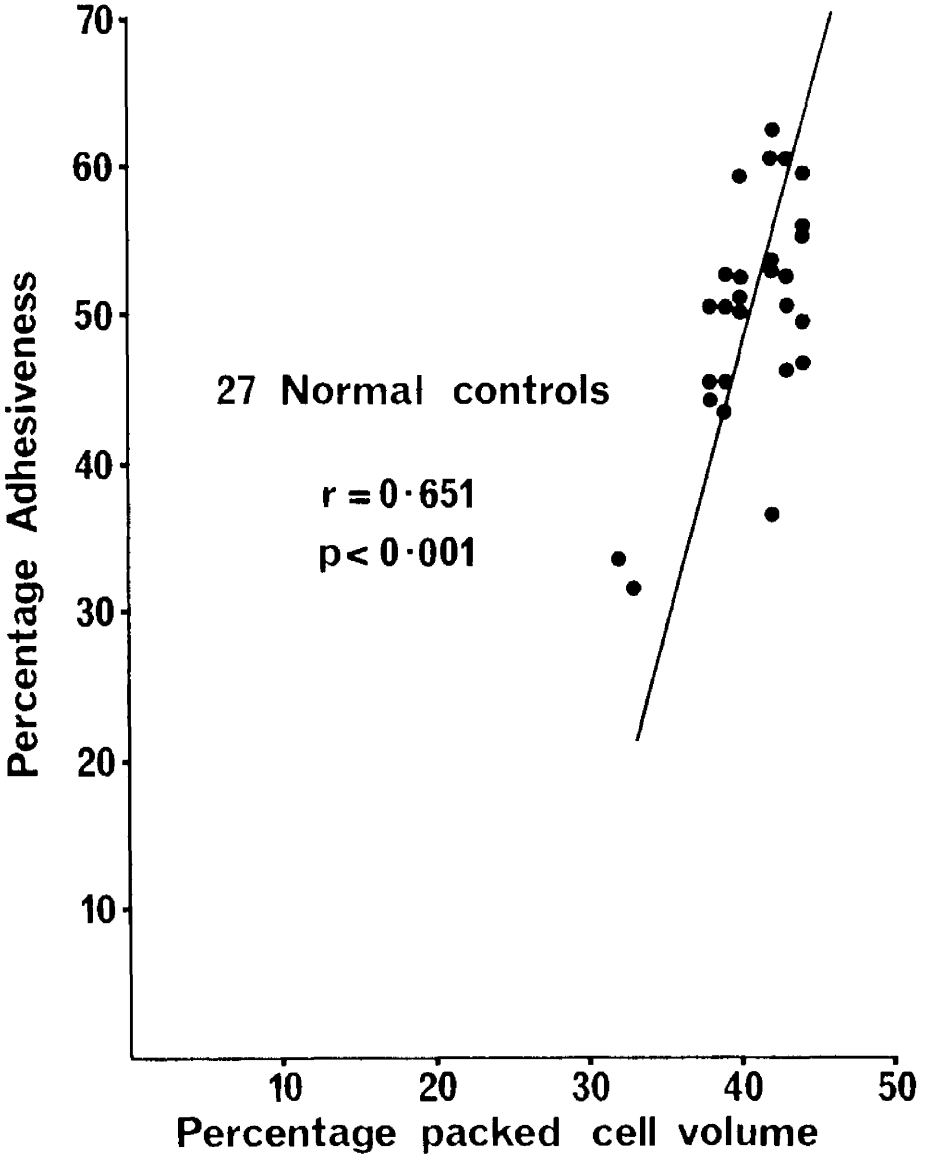

Figure 32 illustrates the relationship between the percentage platelet adhesiveness and the packed cell volume in 27 normal controls. The figure is constructed from data in table 41.

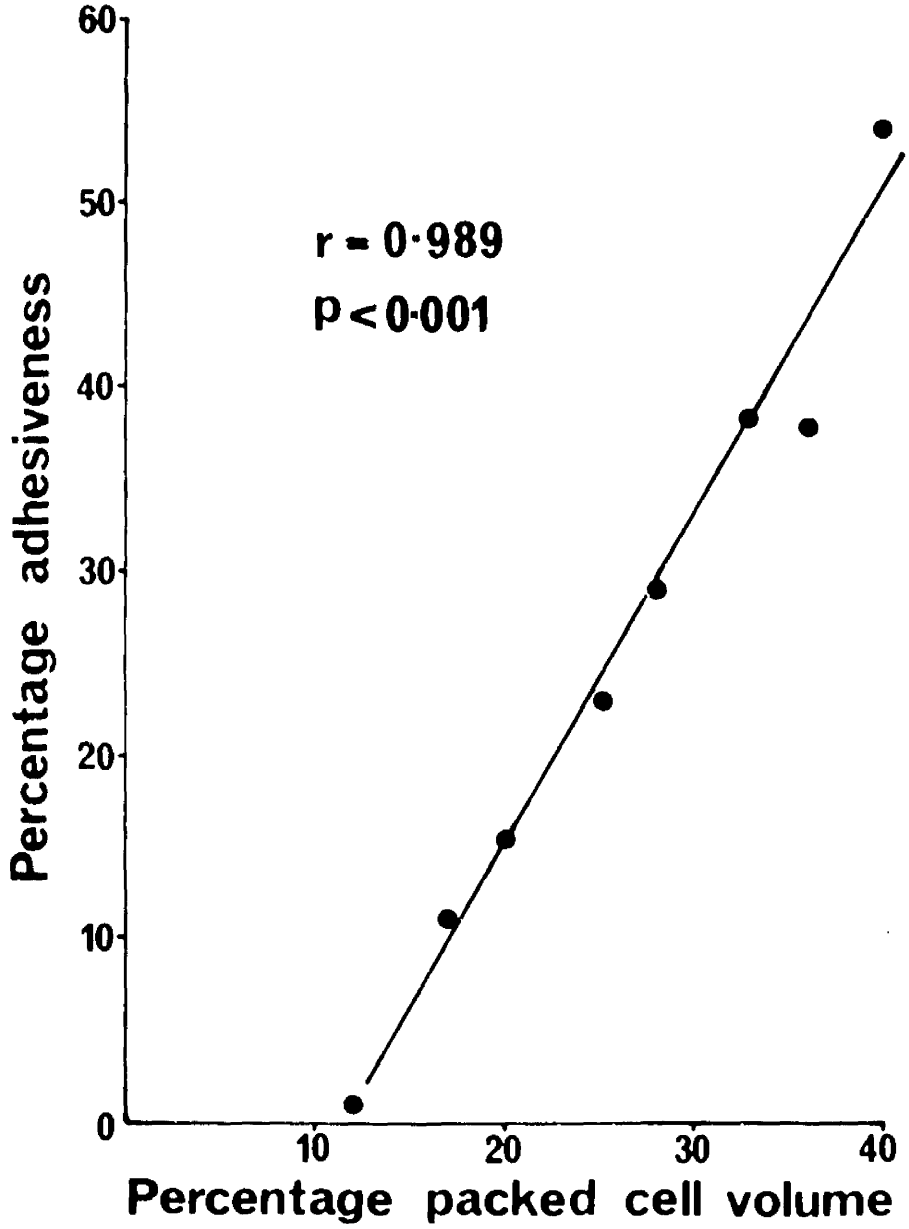

Figure 33 illustrates the correlation between percentage platelet adhesiveness and the haematocrit values when the packed cell volume was lowered in vitro by addition of platelet-poor plasma to whole citrated blood. The results are shown in table 45.

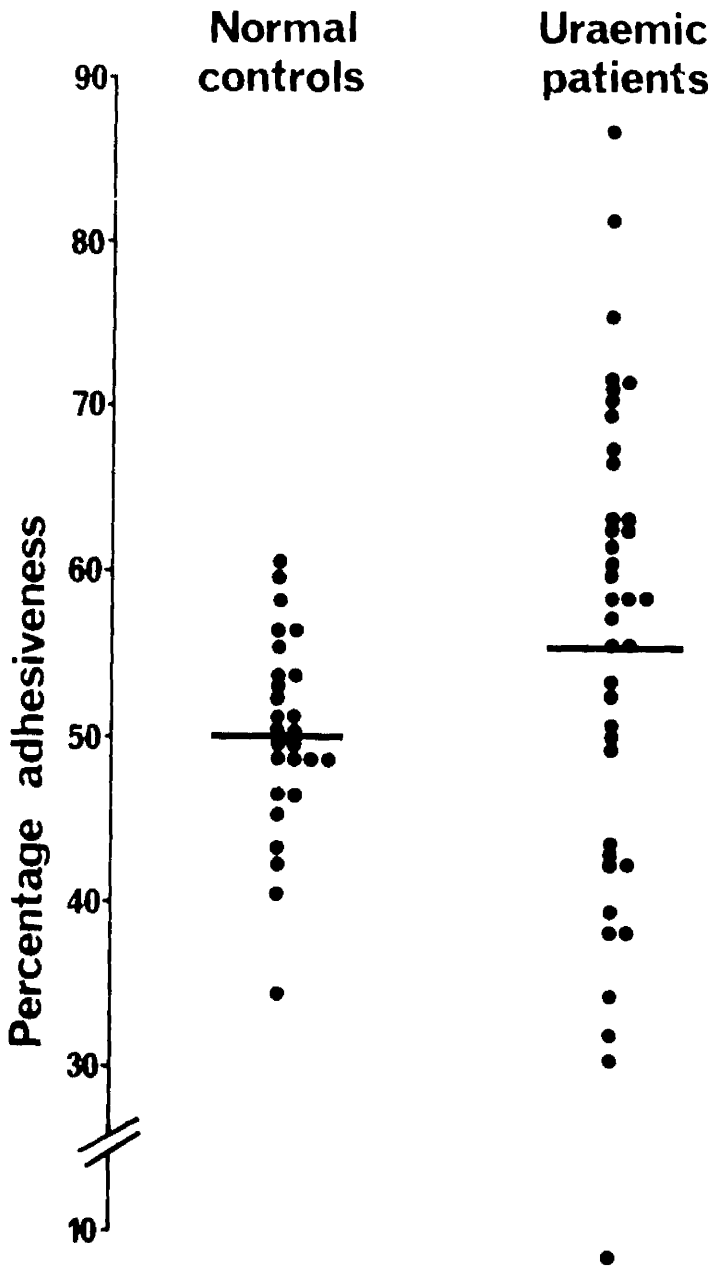

Figure 34 shows the percentage platelet adhesiveness in normal controls and uraemic patients after correcting the results for the packed cell volume. The mean percentage platelet adhesiveness in uraemic patients is not significantly different from the mean value of normal controls ($t = 1.630$, $P > 0.1$). The results from normal controls are shown in table 41, and those from uraemic patients in table 43.

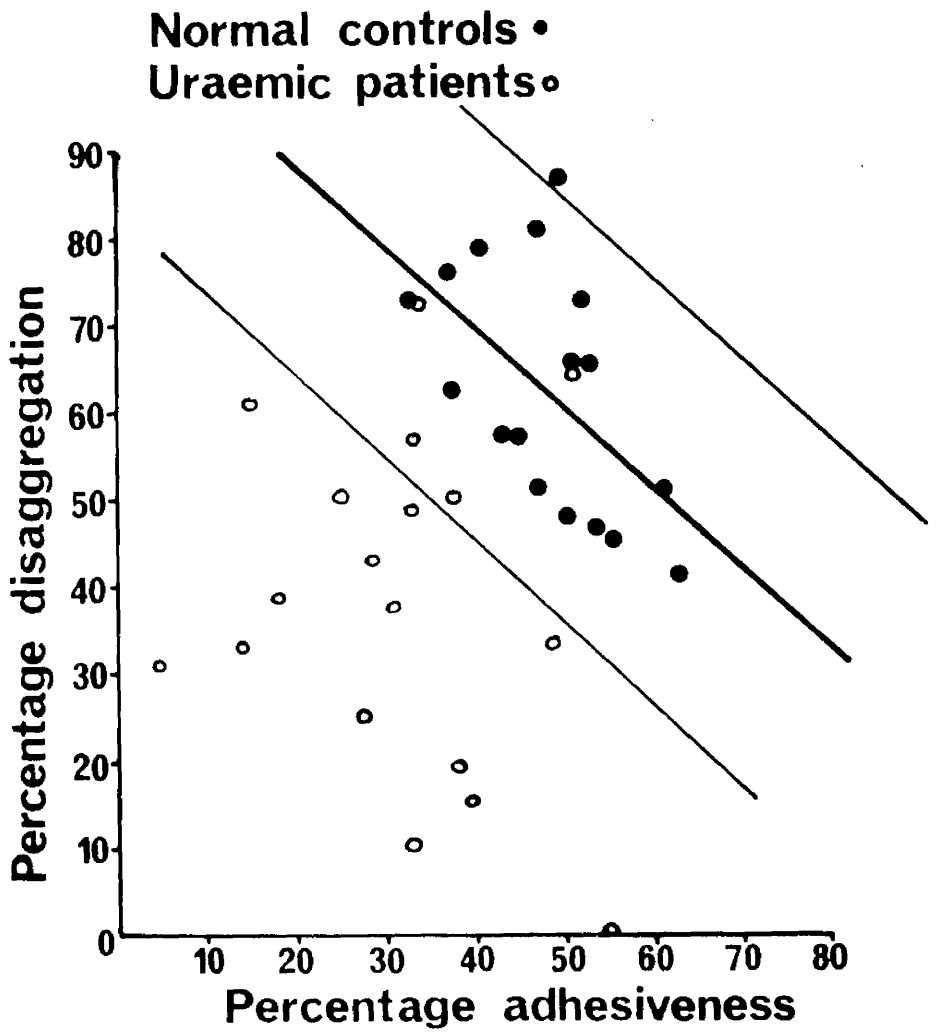

Figure 35 shows the relationship between percentage platelet adhesiveness and the percentage platelet disaggregation after addition of ADP in the turbidimetric system. The results of percentage platelet adhesiveness are not corrected for the haematocrit. The figure is compiled from normal data shown in table 42. The results from uraemic patients are given in table 44. Both normal controls and uraemic patients fell within the same range of platelet count (365,970 ± 62,690/c.mm.)

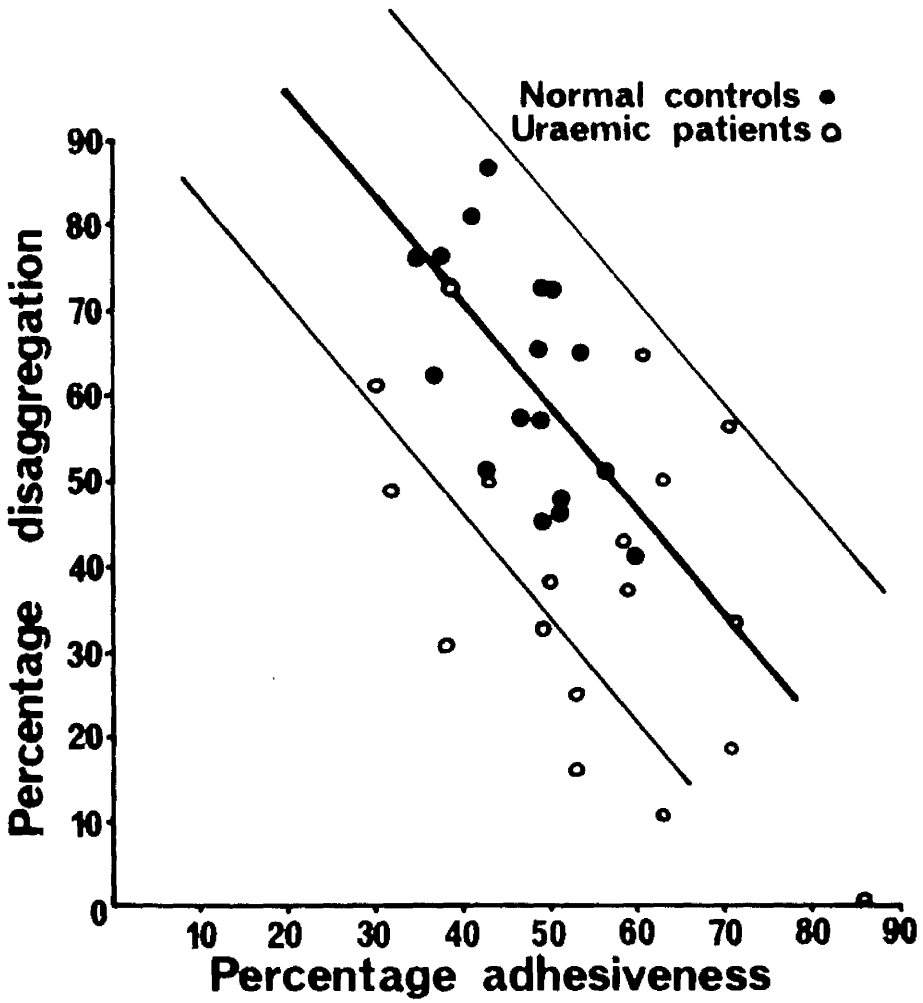

Figure 36 shows the relationship between percentage platelet adhesiveness and the percentage platelet disaggregation after addition of ADP in the turbidimetric system. The results of percentage platelet adhesiveness presented in this figure are corrected for variations in haematocrit. The figure is compiled from normal data shown in table 42. The results from uraemic patients are given in table 44. Both normal controls and uraemic patients fall within the same range of platelet count (365, 970 $\pm$ 62,690/c.mm.)

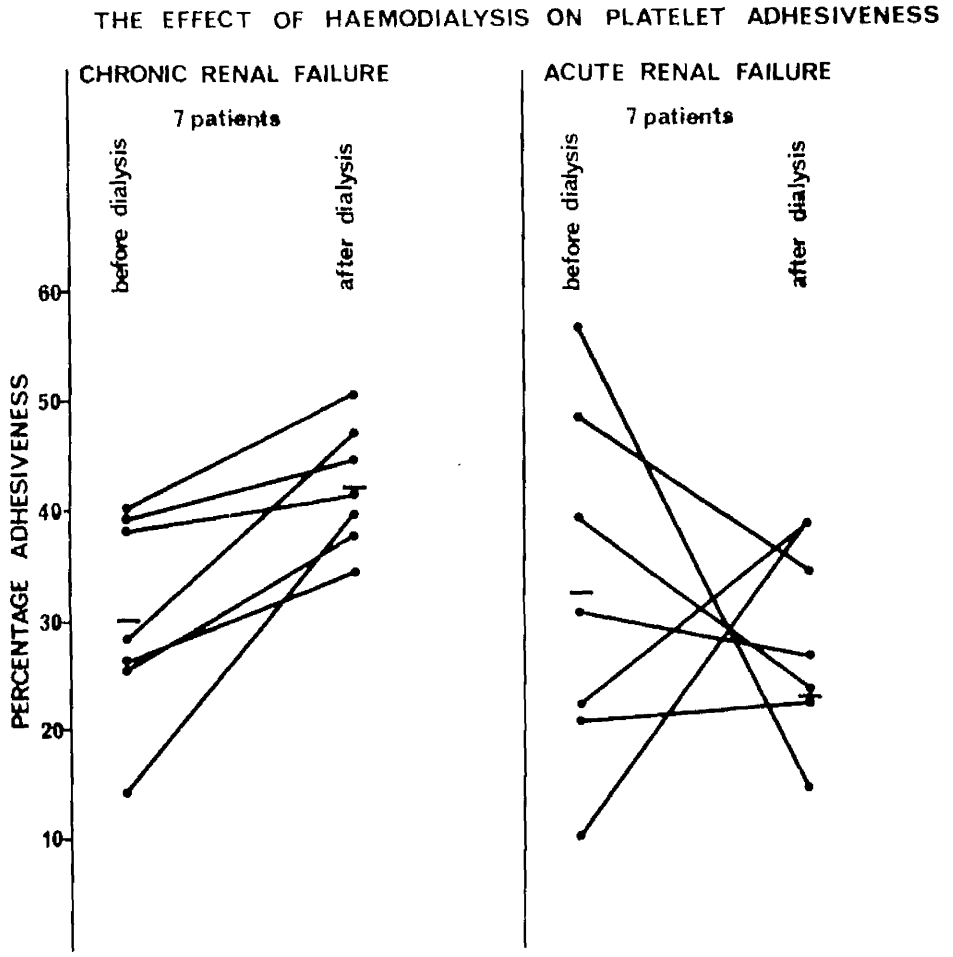

Figure 37 illustrates the effect of haemodialysis on percentage platelet adhesiveness in patients with acute and chronic renal failure. The results are not corrected for variations in packed cell volume. The figure is constructed from data in tables 46 and 47.

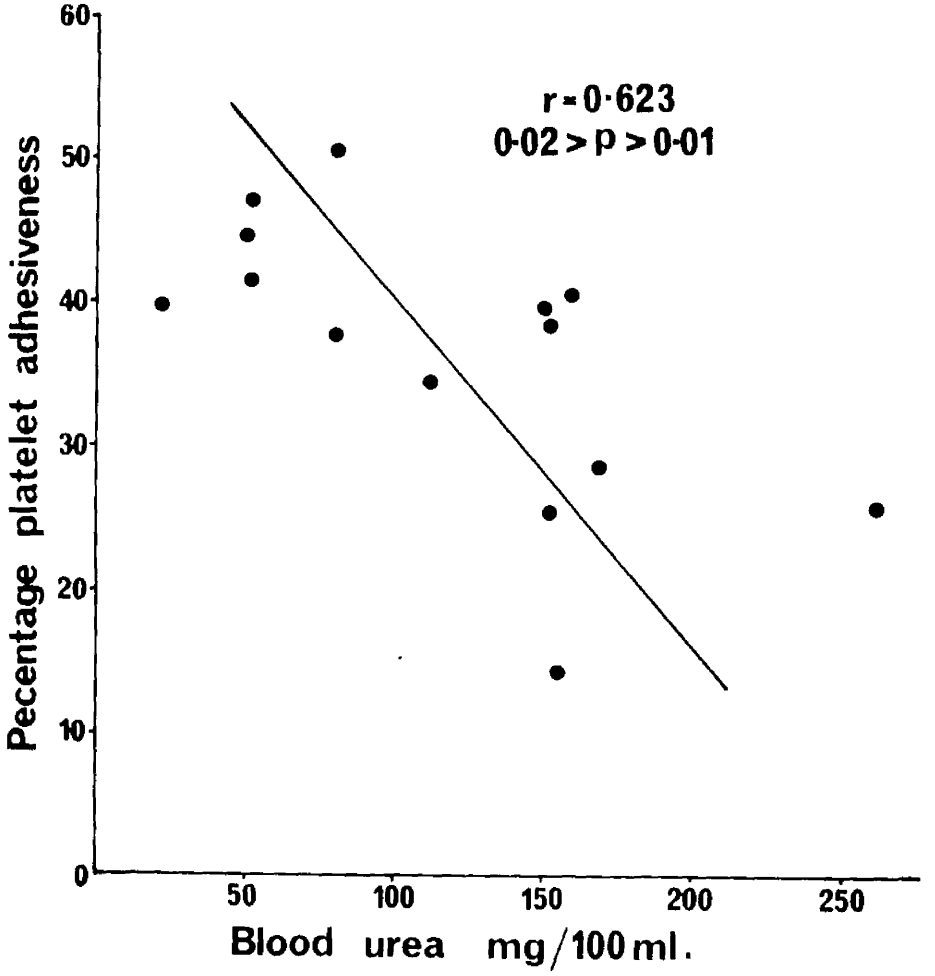

Figure 38 shows the correlation between the percentage platelet adhesiveness and the corresponding blood urea levels before and after haemodialysis in chronic uraemic patients. The results are given in table 45.

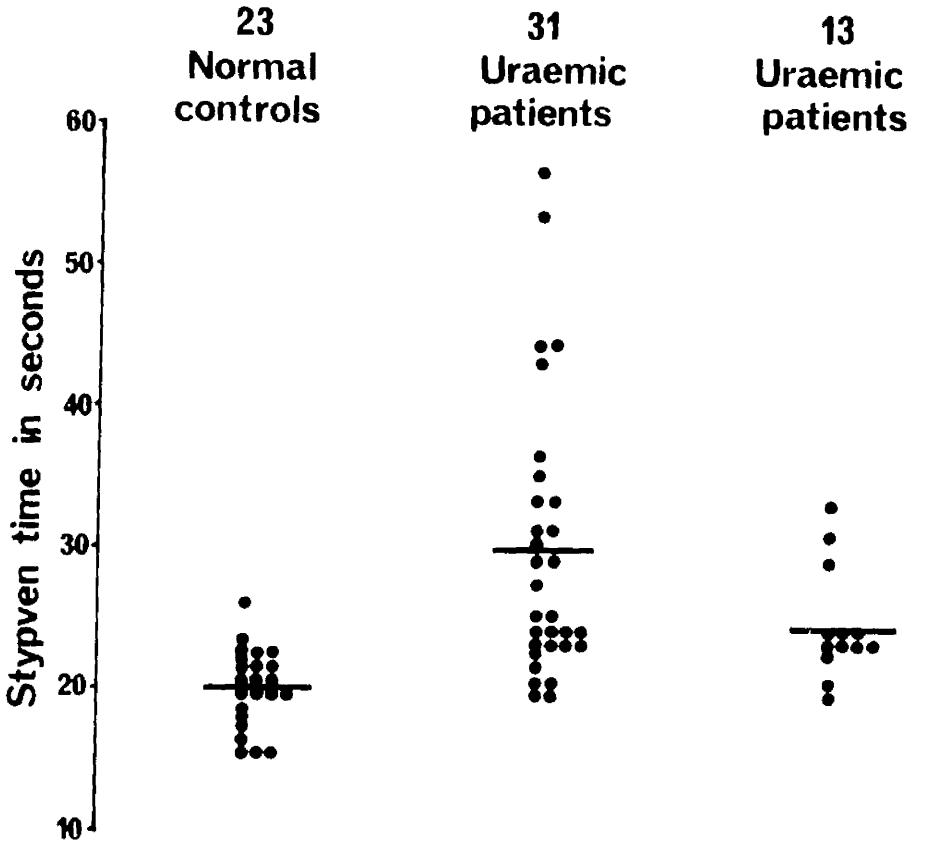

Figure 39 shows the results of platelet factor 3 availability tests in normal controls and in uraemic patients (method of Spaet and Cintron, 1965). The "Stypven times" of 23 normal controls are presented in table 49. The results from 31 uraemic patients shown in this figure (table 50) include all patients regardless of the platelet count or the abnormality in their Kaolin cephalin clotting time. The "Stypven times" of 13 uraemic patients with a platelet count within the normal mean value $\pm$ 2 standard deviations, and having a normal Kaolin cephalin clotting time are also shown in this figure.

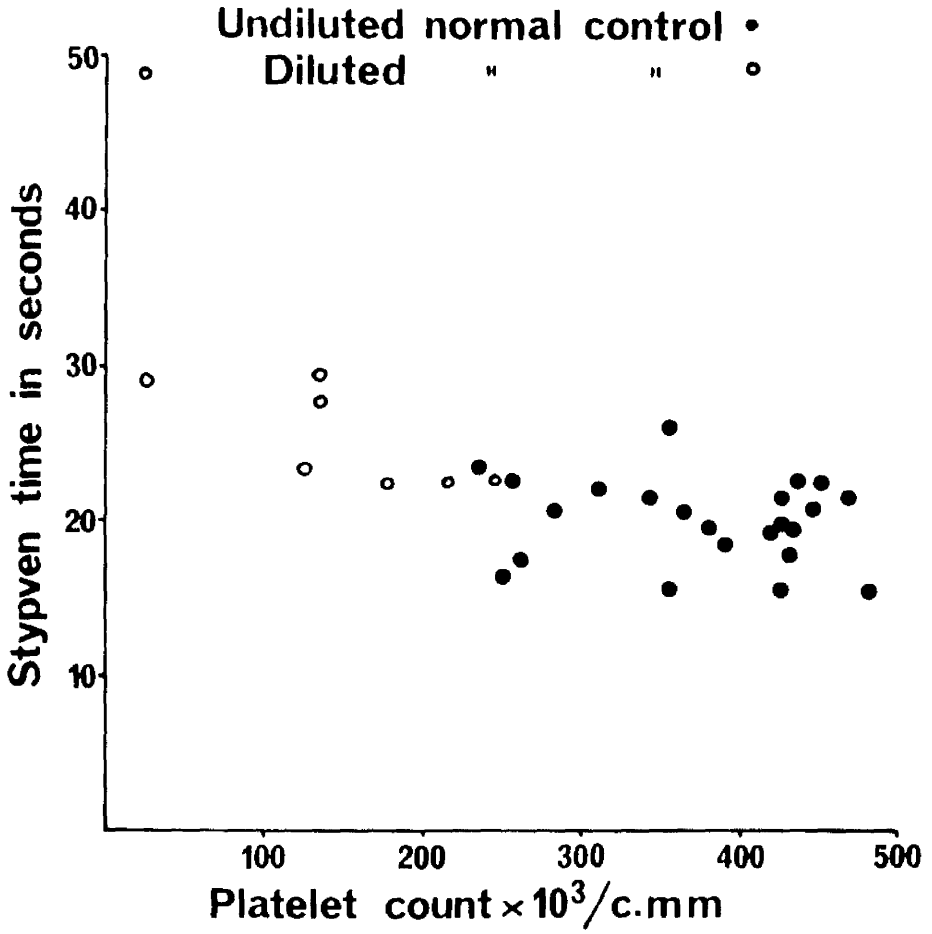

Figure 40 shows the relationship of "Stypven" times in relation to the platelet count in normal controls. There is no significant correlation between the platelet count and the "Stypven" time ($r = 0.131$, $P > 0.1$); the correlation is still insignificant when the results from 8 experiments are included in which the platelet-rich plasma was diluted with platelet-poor plasma ($r = 0.298$, $0.1 > P > 0.05$).

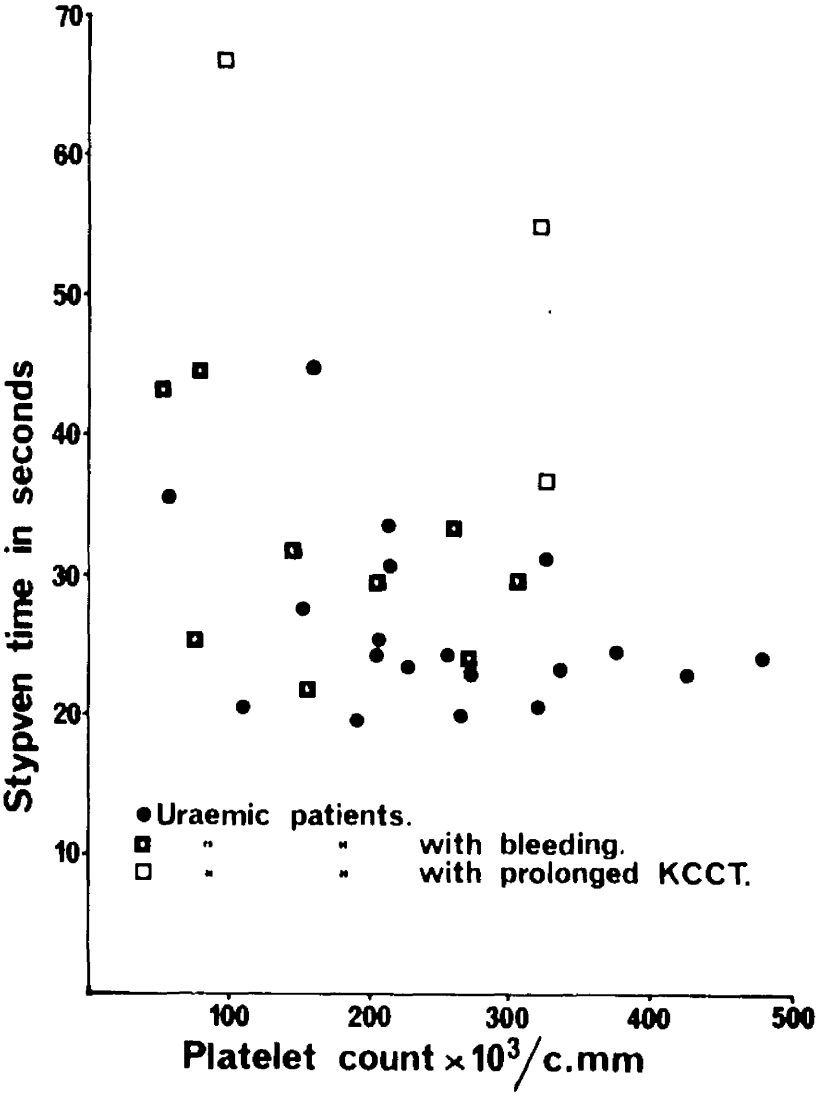

Figure 41 shows the relationship between "Stypven" times and the platelet count in uraemic patients. When the results from uraemic patients with prolonged Kaolin cephalin clotting time are excluded, there is a significant correlation between the "Stypven" time and the platelet count ($r = -0.462$, $0.02 > P > 0.01$). The results from uraemic patients are presented in table 50.

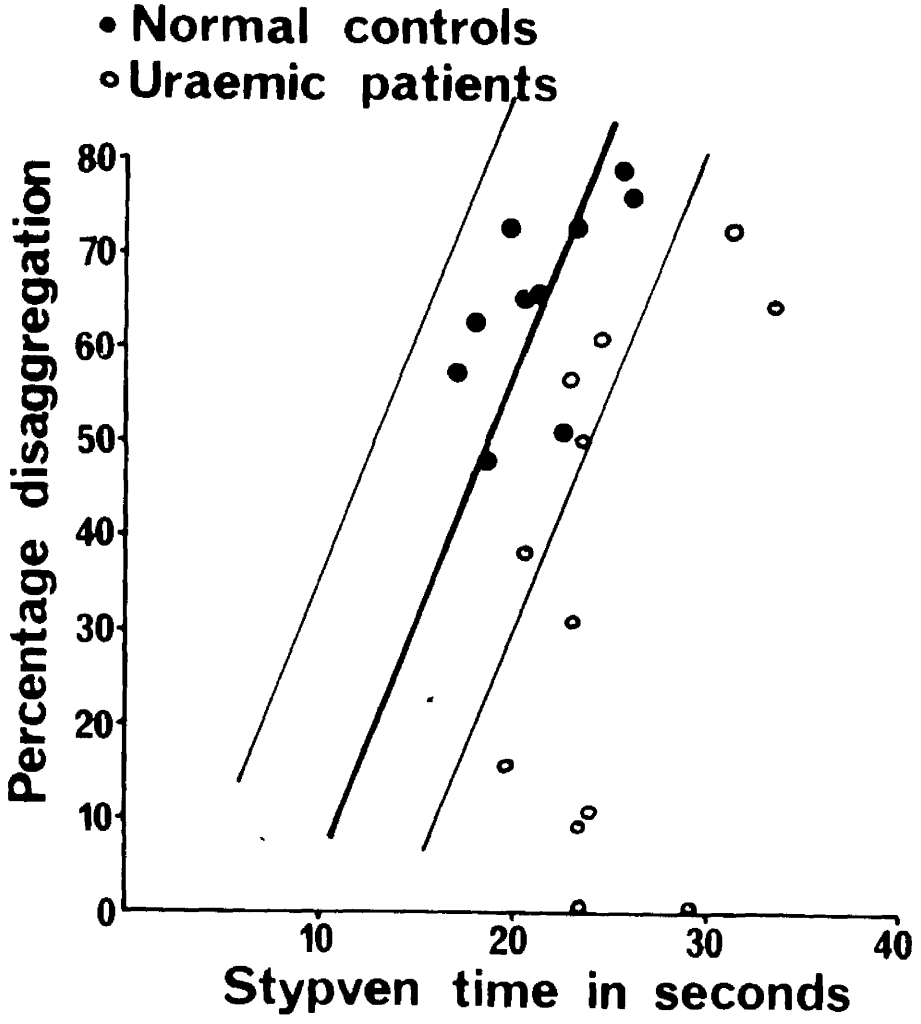

Figure 42 illustrates the relationship between the "Stypven time" in the platelet factor-3 availability test and the percentage disaggregation after ADP has been added to platelet-rich plasma in the turbidimetric system. A significant correlation was obtained between the "Stypven time" and percentage disaggregation in 10 normal controls ($r = 0.655$, $0.05 > P > 0.02$). The results from 12 uraemic patients are shown in this figure. These uraemic patients had normal Kaolin cephalin clotting times and a platelet count within the normal range. The results of 8 uraemic patients fell outside the normal 95 per cent confidence limit around the regression line. The results of normal controls and uraemic patients are given in table 52.

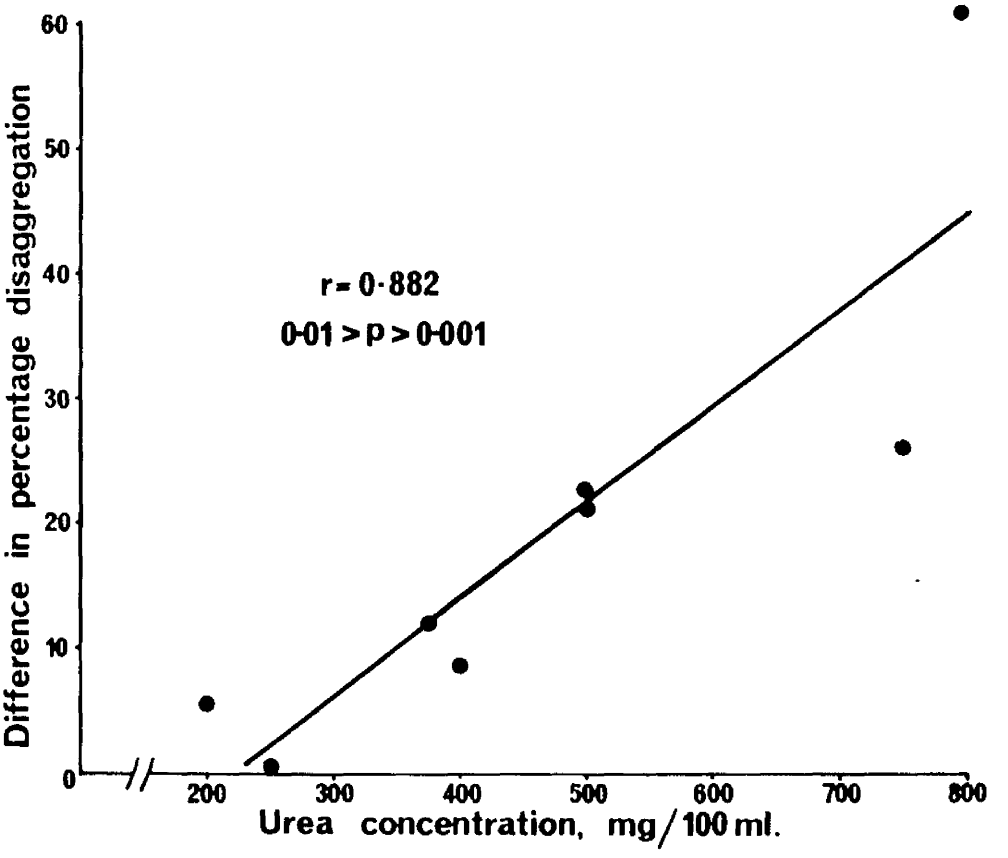

Figure 43 shows a significant correlation between the percentage platelet disaggregation and urea concentration in vitro. The figure is constructed from data in table 56.

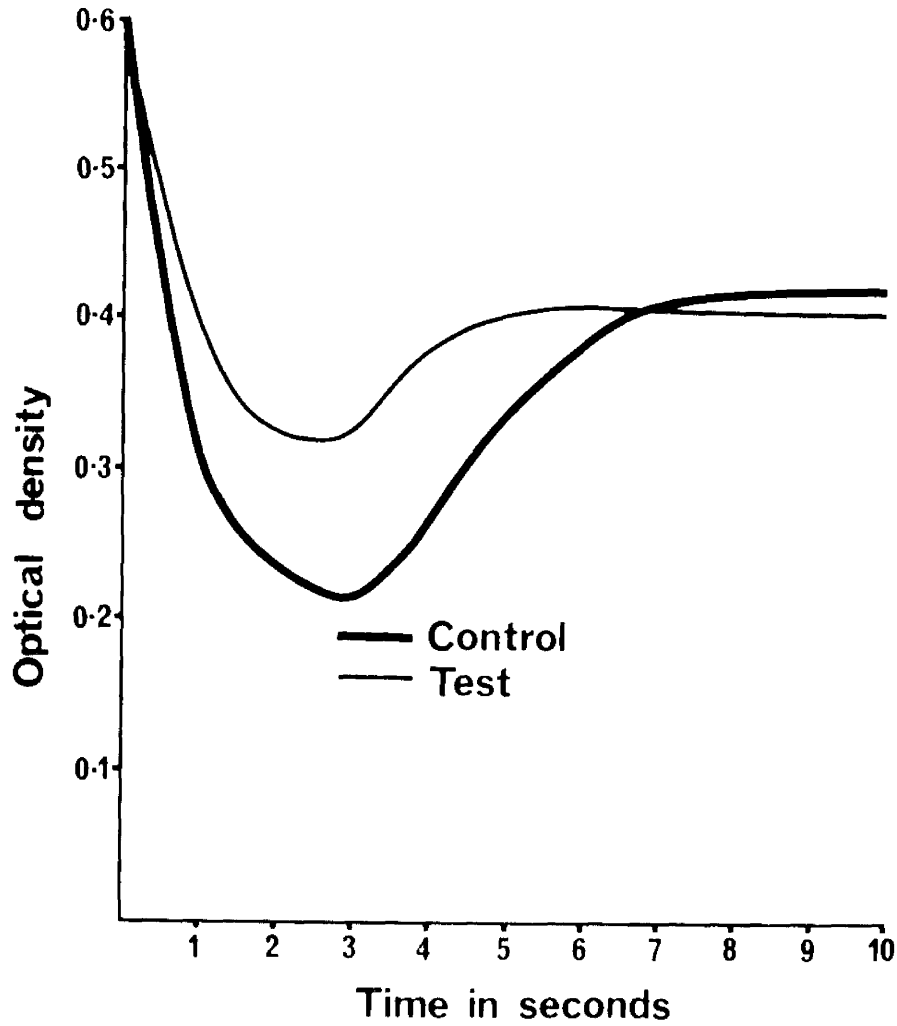

Figure 44 shows the effect of urea on ADP-induced platelet aggregation. This is a representative experiment (No. 4, table 56). The optical density readings of the test are the mean values of two tests done before and after the saline control. The concentration of urea added was 500 mg. per 100 ml. of plasma. The test shows a diminution in both aggregation and disaggregation as compared to the control. The optical density readings are given in table 59.

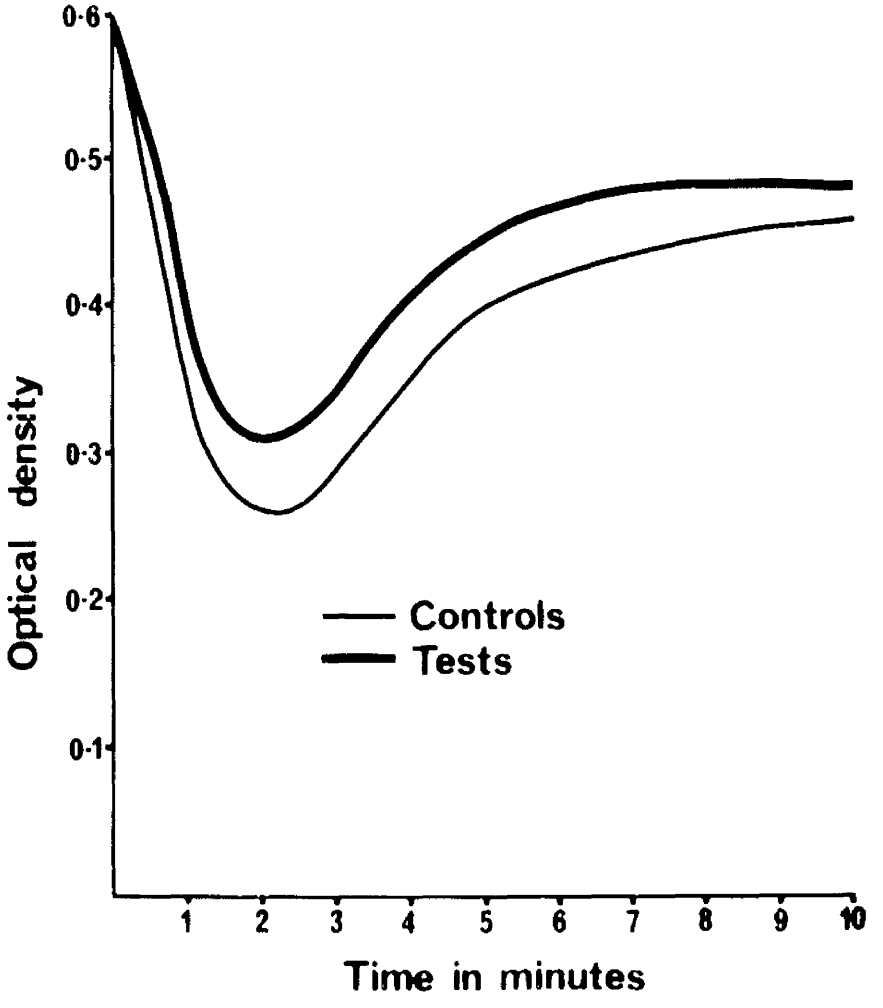

Figure 45 illustrates the effect of guanidino succinic acid on ADP-induced platelet aggregation. The optical density readings of the test and the controls are the mean values of 7 experiments and are shown in table 60.