The Role of Platelets in Glomerulonephritis and Transplantation

E. N. WARDLE, MB, MRCP, Department of Medicine,
Royal Victoria Infirmary, Newcastle upon Tyne

Electron microscopic studies have shown that platelets are deposited in the kidney during various types of renal transplant rejection (Porter, 1967). Firstly, they are deposited when there is immediate rejection of the graft because of blood group incompatibility between host and donor, which reflects the presence of preformed agglutinating antibodies, the isohaemagglutinins. Similarly, platelets accumulate in the renal vasculature when there is presensitisation of the donor, as can be shown by the presence of cytotoxic antibodies (Lowenhaupt and Nathan, 1968). In both these types of rejection urine excretion soon ceases and microscopic examination reveals microthromboses in the arterioles and glomerular capillaries (Kissmeyer-Nielsen *et al.*, 1966; Williams *et al.*, 1968). Animals rendered thrombocytopenic do not show this hyperacute rejection even though they are presensitised (Clark *et al.*, 1968), but other investigators stress an important role for the polymorph neutrophils that fill the glomerular and peritubular capillaries in hyperacute rejection. Secondly, when rejection occurs after the first week, accumulations of platelets and fibrin are found on the intima of the arterioles. These deposits become covered with endothelium and are incorporated into the intima (Porter *et al.*, 1967), on which deposition of immunoglobulins and complement also occurs (McKenzie and Whittingham, 1968), resulting in the 'endarteritis obliterans' of chronic vascular rejection. Additionally, renal allografts between inbred strains of rats, differing only at weak histocompatibility loci, have demonstrated that fibrin deposition is in part responsible for the glomerulopathy of longstanding grafts (Lindquist *et al.*, 1970). This late proliferative glomerulonephritis begins as an autoimmune response to antigens of the graft which are also shared by the recipient, and is therefore not technically a rejection (Milgrom *et al.*, 1970, 1971). Thirdly, platelet deposition is inevitable when there is early ischaemic damage to a kidney because of a prolonged warm ischaemia time (Clarkson *et al.*, 1970a).

These morphological observations have been reinforced by functional studies showing the disappearance of chromium-tagged platelets into the graft at the time of rejection (Mowbray, 1967), an elevation of serum fibrin

degradation products both in the post-transplant period and at the time of subsequent rejection (Wardle *et al.*, 1971; Bouma *et al.*, 1971), and fibrin derivatives in the urine (Braun and Merrill, 1968; Clarkson *et al.*, 1970b). No evidence, however, has yet been found to prove that a generalised intravascular coagulation is part of rejection (Colman *et al.*, 1969) but there is local fibrin accumulation in the kidney (Salaman, 1970; Straub, 1971). Moreover, there is a favourable response to heparin when given in rejection (McMillan, 1968) and heparin with aspirin has been used for treatment of hyperacute rejection (MacDonald *et al.*, 1970).

More work needs to be done on the effects of the passage of platelet aggregates through the renal circulation. Hansson (1965) studied the effects of platelet aggregation on renal function in the cat. He found that perfusion of a kidney with cold autologous blood at 20°C produced conditions in which sympathetic and myogenic smooth muscle responses no longer influenced vascular tone; the threefold increase of renal vascular resistance observed could be explained by an increase of blood viscosity primarily due to aggregation of platelets. Clumped platelets were observed to be blocking the glomerular capillaries and under normothermic conditions infusion of ADP or collagen extract caused a marked increase of renal vascular resistance. A mechanical plugging of small vessels was the cause of the renal flow resistance and was accompanied by a drop in the number of platelets in the renal venous blood.

Mustard and his co-workers have extended these observations by infusing ADP into the renal circulation of pigs and rabbits (Packham *et al.*, 1968). Infusion of ADP for only five minutes caused the appearance of erythrocytes, protein, and casts in the urine after a lag of three to five hours. Twenty-four hours later histology showed focal areas of injury to the renal cortex, in which there were collapsed glomeruli infiltrated by leucocytes, and the proximal tubules showed evidence of degeneration and casts. Fibrin was apparent in the glomerular capillaries. It was concluded that arrest of bloodflow by platelet aggregates for as little as five minutes might be sufficient to induce tissue injury. This situation is not unique, for it has been shown that similar passage of platelet aggregates can cause myocardial damage (Hughes and Tonks, 1956).

There is little morphological evidence of the role played by the platelet in glomerulonephritis but the functional evidence is persuasive. Platelet aggregates soon disintegrate unless stabilised by fibrin. Attention has been directed towards the endothelial cell proliferative responses and to changes in the basement membrane and the foot processes of the epithelial cells, rather than to evanescent occurrences that are difficult to detect by biopsy. However, it

can be presumed that fibrin deposition has been preceded by viscous metamorphosis of platelets, a process in which phospholipid procoagulants are released by ADP from the platelet membrane and thrombin is generated. Even in 'minimal change' lipoid nephrosis, platelet aggregates and fibrin formation in the glomeruli have been demonstrated (Duffy *et al.*, 1970). That fibrin plays a role in the proliferative response (Vassalli and McCluskey, 1964) has now been accepted and is supported by immunofluorescent studies (Koffler and Paronetto, 1965; McCluskey and Vassalli, 1969). The basic point is that macromolecules, such as fibrin and immune complexes, which are deposited in the glomeruli, are phagocytosed by mesangial and endothelial cells which, as part of the reticulo-endothelial system, are induced to proliferate.

Whether the platelet plays an important role in glomerulonephritis depends on the demonstration of the immunological mechanisms of that process (Carpenter, 1970; Hardwicke, 1971). Glomerulonephritis is usually caused either by circulating immune complexes, as in acute and chronic serum sickness, or by circulating anti-kidney basement membrane antibody (Dixon, 1968). The model in the latter is obtained by producing an antiserum to renal basement membrane in another animal as in anti-rat nephrotoxic serum glomerulonephritis or duck anti-rat Masugi nephritis (Unanue and Dixon, 1967). In immune complex glomerulonephritis, Cochrane and Dixon (1968) have demonstrated the initial formation of antigen–antibody complexes in the circulation, which then cause platelet clumping and the release of vasoactive amines which, in turn, produce increased vascular permeability, so that the complexes are passively trapped in the basement membrane and there determine complement fixation and attraction of polymorph leucocytes (Fig. 1). The mechanism by which the platelets are involved and the therapeutic implications will be discussed.

It has been known for many years that anaphyllactic reactions cause thrombocytopenia (Roskam, 1927). More recently it was found that this thrombocytopenia might be a prelude to intravascular coagulation and fibrin formation (Eagle *et al.*, 1937; Gans and Krivit, 1961) and this was shown in bovine albumin serum sickness glomerulonephritis (Dixon *et al.*, 1958; Salmon *et al.*, 1968). During the phase of immune elimination of antigen by progressive formation of antibody with immune complex formation, fibrin appears in the renal glomeruli in a proportion of animals (Rich, 1956; Germuth *et al.*, 1957). Fibrin formation is a continuous process but fibrin does not persist in animals whose glomeruli develop spontaneous fibrinolysis (Salmon and Lambert, 1971). The important point is that the whole process starts with platelet damage by circulating immune complexes (Siqueira and Nelson,

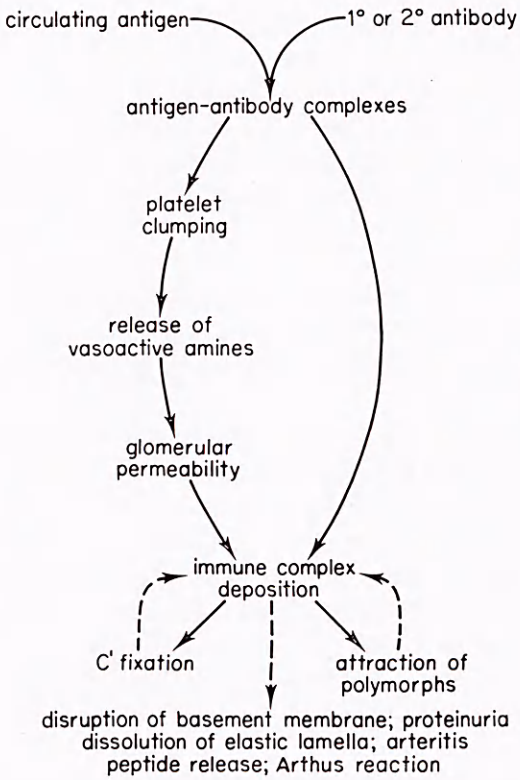

Fig. 1. Role of the platelet in the induction of glomerulitis

1961; Henson and Cochrane, 1969a,b). The release of vasoactive amines from the platelets with the production of vascular permeability is essential to immune complex deposition in the renal vasculature (Cochrane, 1963). Hypertension accentuates this process (Fisher and Bark, 1961). Complexes are otherwise removed by the reticulo-endothelial cells of the liver and spleen. Rabbits treated with antihistamines and then challenged with bovine serum albumin do not show renal localisation of complexes and do not develop glomerulonephritis (Kniker and Cochrane, 1966). Also, prior depletion of the platelets forming the vasoactive amine reservoir can be shown to inhibit immune complex localisation in the vasculature (Kniker and Cochrane, 1968).

Thus, the critical part of the process is damage by immune complexes to the platelets with the induction of viscous metamorphosis (Bettex-Galland *et al.*, 1963). In fact, this ability of antigen–antibody complexes to induce platelet aggregation has been developed as a test for their detection (Pentinnen *et al.*, 1969; Myllyla *et al.*, 1971). Episodes of thrombocytopenia after acute viral infections such as rubella and glandular fever may be brought about in this way. Heat aggregated gamma-globulins have a similar effect (Ishizaka

and Ishizaka, 1962), so long as complement is also activated; this could be relevant to the bleeding diathesis of some dysglobulinaemias. Aggregated IgA which is unable to activate complement does not have this effect on platelets.

Elucidation of the precise action of immune complexes on platelets has been bedevilled by species differences and there has been a long controversy as to whether complement is essential. Movat *et al.* (1965) were the first to show that small immune complexes were phagocytosed by platelets and, in the process, ADP, serotonin and histamine were released. It is now known that large complexes can cause platelet degranulation without phagocytosis (Des Prez and Bryant, 1969). In the rabbit, a heat labile factor in the plasma is essential for these processes (Barbaro, 1961; Gocke, 1965), neither of which require complement. It has also been shown that soluble antigen in antibody excess will cause immune complexes to adhere to platelets and that this 'immune adherence' reaction requires the presence of the third complement component C'3. This is evident because the process is inhibited by C'3 removal using cobra venom. However, C'3 dependent immune adherence of platelets does not seem to occur in man. An additional mechanism of platelet damage in the rabbit is activation of C'6 with resulting platelet lysis (Henson and Cochrane, 1969a; Henson, 1970).

There is also a synergic action of neutrophils on the release of histamine from platelets, in the presence of immune complexes and complement. The presence of neutrophils enhances the release of vasoactive amines (Henson, 1970, 1971), possibly because neutrophil procoagulant substances lead to thrombin activation (Hawiger *et al.*, 1969). Detailed analysis has further revealed that it is basophils carrying reaginic antibody IgE which, on contact with the appropriate antigen, release a 'soluble factor' that makes platelets clump and release their amines (Cochrane, 1971). Under other circumstances platelets from immunised rabbits will release histamine, when in contact with appropriate antigen and in the presence of small lymphocytes that are producers of homocytotrophic antibody (Henson and Cochrane, 1969b). These mechanisms for platelet damage are summarised in Table 1.

TABLE 1. Platelet damage by immune complexes (as in the rabbit)

1. Small immune complexes—phagocytosis by platelets Large complexes—direct damage to platelets
2. 'Immune adherence'—utilising C'3 'Immune lysis'—utilising C'6
3. Damage by neutrophils activated by complexes plus complement and release of basophil 'soluble factor'
4. Histamine release from platelets by antigen combining with homocytotrophic antibody on lymphocytes

By studying human platelets Mueller-Eckhardt and Luscher (1968) have found that consumption of complement is not mandatory but, nevertheless, that cnly immune reagents capable of activating the complement system cause aggregation. It is possible that platelets might have specific receptors for C′-activating agents and that, although serum complement is not required, there may be complement within the platelets themselves. In the final event, antigen–antibody complexes and aggregated gamma-globulin appear to act on platelets in much the same way as thrombin. It is of interest that in other cell types combination of specific antibody with the cell membrane may also unmask phospholipids that have a procoagulant potential, like that of platelet factor 3 (Forrester and Dumonde, 1965).

The process that induces coagulation in the renal glomerulus may be visualised as shown in Fig. 2. It is clear that the 'platelet release reaction' which liberates amines and the 'platelet aggregation reaction' are distinct.

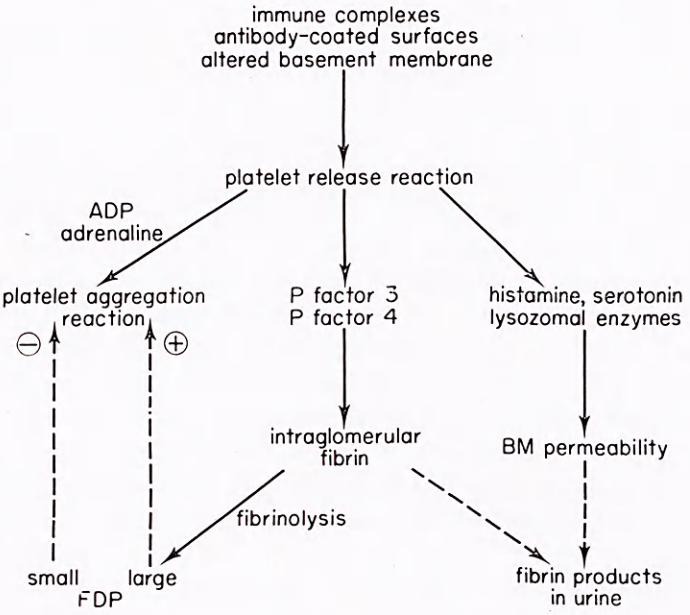

Fig. 2. Induction of glomerular coagulation.

In fact, human platelets contain very little histamine. Apart from their direct effect on the platelet, immune complexes interacting with endothelium may lead to exposure of collagen or basement membrane which will then add to platelet aggregation at the site of injury. Thereafter, there are at least three mechanisms by which platelet aggregation can lead to fibrin deposition.

Firstly, many coagulation factors such as fibrinogen and factors V, VIII, XII and XIII are associated with the platelet membrane. Secondly, the platelet phospholipids PF3 and PF4 are procoagulant substances, and, finally, thrombin is generated near platelet aggregates. Furthermore, both collagen and antigen-antibody complexes are activators of Hageman factor XII which is recognised as a common trigger to activation of thromboplastin, fibrinolysis and the kinin system.

Some of these mechanisms essential to glomerular damage and coagulation, which are induced by immune complex deposition, can be illustrated from experiments with bovine serum albumin nephritis in the rabbit (Fig. 3). If,

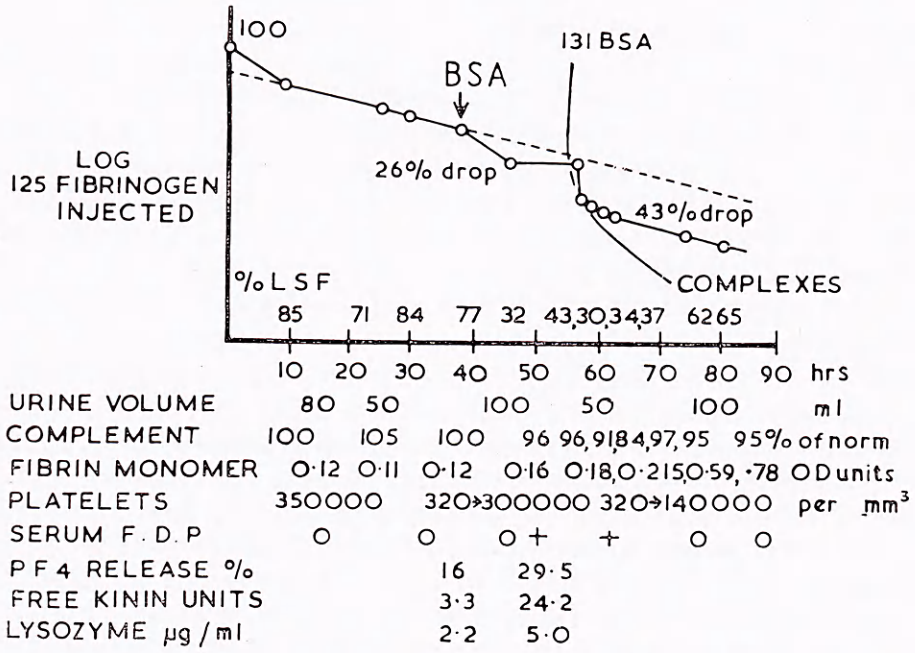

Fig. 3. Results of antigen challenge in BSA rabbits during long-term immunisation, showing 'rebound defibrination' to antigen challenge on two occasions and the accompanying fall of platelets, release of platelet factor 4, rise of fibrin monomer, and appearance of serum FDP, kinins, and lysozyme. There is, also, increased fibrinolysis and a transient fall of complement and urine volume.

during the phase of immunisation to previous antigen challenge, further antigen is given intravenously, the number of platelets decreases and a 're-bound defibrination' takes place. This can be shown when the animal's fibrinogen has been labelled and the drop in fibrinogen corresponds to

deposition of fibrin in the kidneys, lungs and liver, which is caused by coagulation induced by circulating immune complexes involving precipitating antibody. At the same time platelet factor 4 is released into the plasma, as demonstrated by its effect on a heparin-thrombin clotting time (Farbiszewski *et al.*, 1968), and there is also a loss of low-soluble fibrinogen in 8 per cent ethanol (LSF mol wt 340,000) and the appearance of an increased amount of high-soluble fraction (HSF mol wt 270,000), recognised as the first catabolic product (Sherman *et al.*, 1969). The appearance of kinins and a rise of serum lysozyme due to effects on granulocytes can also be shown.

However, although heterologous protein glomerulonephritis is useful as an experimental model, corresponding as it does to human serum sickness, in human glomerulonephritis the amount of antigen that triggers off the response must be very much less. Antigens that have been identified as causing human nephritis are viruses such as hepatitis antigen, streptococci, *plasmodium malariae*, the nuclear antigens in SLE, autologous gamma-globulin in cryoglobulinaemic nephritis and penicillamine. Type 12 nephritogenic streptococci may have a special ability to release soluble antigens (Treser *et al.*, 1969) that may evoke the correct amount of antibody to produce moderate-sized complexes. Streptococcal M protein is also recognised as a procoagulant (Kantor and Cole, 1959; Kantor, 1965; Humair *et al.*, 1969).

Nephrotoxic serum nephritis is used to simulate a nephritis caused by antibody directed against basement membrane. Glomerular coagulation may be induced and fibrin deposition can produce wire loop lesions (McCluskey and Vassalli, 1969). However, this picture is seen only when fresh concentrated, but fully adsorbed, sheep nephrotoxic serum is used in high dosage so that the animals become oliguric and survive only for a further three or four hours (Dr Michael Floyd, personal communication). The first change observed after a period of thirty to sixty minutes is a fall in the circulating platelet count. Thereafter, there is a fall in fibrinogen and a rise in plasma haemoglobin levels, but the fact that free haemoglobin appears in the urine when plasma levels are below those needed to saturate haptoglobins indicates that haemolysis is confined to the glomerular loops. Histology shows blockage of glomerular capillary loops by amorphous eosinophilic material; fibrin occupies the peripheral part of the vessel and entrapped and fragmented red cells the axial position. All this occurs in the heterologous phase of glomerular protein deposition and is presumably triggered off by binding of antibody to the basement membrane, in which complement punches holes, to expose reactive groups that cause platelet adherence.

The detailed mechanisms that may cause the deposition of platelets in a renal allograft are summarised in Table 2. The mechanism of action of immune

TABLE 2. Mechanisms for Platelet Deposition and Damage in the Renal Transplant

1. IgG antibodies coating vascular endothelium
2. Circulating immune complexes
3. Exposure of subendothelial collagen
4. Release of aggregating agents such as thrombin, ADP, serotonin

complexes on platelets has already been discussed. It is probable that the circulating antibodies, produced in response to the graft, bind to transplantation antigens on endothelial cells (Vetto *et al.*, 1971) causing platelet aggregation in the capillaries and adhesion of platelets to the endothelium. In fact, any gamma-globulin coated surface will release platelet serotonin and nucleotides and cause platelet aggregation (Glynn *et al.*, 1965; Packham *et al.*, 1968). Mowbray and Pariyananda (1971) have shown that it is the IgG antibody-forming immune complexes that cause platelet aggregation whereas IgM type antibody protects platelets from damage.

Bounameaux first noted in 1959 that platelets would adhere to subendothelial collagen fibres and later it was found that purified collagen would cause platelet aggregation (Zucker and Borrelli, 1962). The factor released from platelets by collagen and inducing their further aggregation is ADP. Collagen also releases histamine, serotonin, and platelet factors 3 and 4 which trigger off the clotting mechanisms (Niewiarowski *et al.*, 1968).

Electron micrographs of platelets in contact with collagen fibres show a pattern of breaks in the platelet membrane matching the cross-striations of the collagen fibres (Hovig *et al.*, 1968) and in some situations the platelets appear to be attempting phagocytosis of the collagen (Mustard *et al.*, 1967). Treatment of collagen with collagenase, or heating the collagen so that the helical structure is lost, destroys the ability to aggregate platelets. Collagen may also act indirectly on platelets by activating Hageman factor XII to generate thrombin (Wilner *et al.*, 1968) and this action of collagen in the activation of clotting is possibly of greater importance than the effect on the platelet.

Both glomerular and tubular basement membrane are similar to collagen (Kefalides *et al.*, 1966), but it has been found that on a dry-weight basis purified rat basement membrane has a less pronounced reaction on the platelet than bovine collagen (Chung-Hsin Tao, 1971). It therefore seems that the number of 'platelet aggregating sites' may be less in basement membrane than in collagen, or else that they are hidden or protected. It is known that the epsilon-amino-groups of lysine have to be exposed if collagen is to act as an aggregating agent (Wilner *et al.*, 1968) and it will be of interest to know

how the platelet aggregating molecular configuration compares with the composition of the antigenic site (Mahieu *et al.*, 1971) for anti-glomerular basement membrane antibodies. Indications are that these are two separate moieties of the basement membrane, which consists of a collagen-like component and a non-collagen glycoprotein antigen (Shibata *et al.*, 1969; McIntosh *et al.*, 1971).

Control of these processes in transplantation and glomerulonephritis is of interest to the physician. Table 3 indicates some basic approaches. A few

TABLE 3. Approaches in therapeutics

1. *Agents that protect the platelet*
   (*a*) Drugs that maintain platelet cyclic 3-5 AMP content—
   aminophylline, prostaglandin EI, Persantin
   (*b*) Drugs acting on the platelet membrane—
   antihistamines, tranquillisers and antidepressants
   aspirin which acetylates the membrane
   hydrocortisone, azathioprine
   phenylbutazone, indomethacin, sulfinpyrazone, mefanamic acid
   (*c*) Late FDPs
2. *Anticoagulation*
3. *Promotion of fibrinolysis*
   especially by agents that are also inhibitors of platelets and complement—
   (*a*) salicylate derivatives—salicylaldoxime, di-iodo-salicylic acid
   (*b*) indomethacin, flufenamic acid

facts about the drugs that could be used to inhibit the aggregation of platelets are worthy of specific mention. Although antihistamines are useful in preventing nephritis in the rabbit, concentrations that effectively block ADP-induced platelet aggregation also cause haemolysis of erythrocytes and the release of serotonin (Mustard, 1970) so that any benefit is negated. Heparin has to be used at the high concentration of 20 units/ml to inhibit platelet adhesion but at this level it interferes with the patency of the vascular wall (Rowsell *et al.*, 1967). This might be an explanation of the rare reports of heparin-induced thrombocytopenia (Natelson *et al.*, 1969). However, concentrations of 10 units/ml are enough to inhibit the action of immune complexes, gamma-globulin and collagen. What ought to be advantageous with heparin is the fact that it also inhibits complement (Ecker and Gross, 1929) and inflammatory reactions. Being the only true antithrombin it is a better anticoagulant than dicoumerol or warfarin (Wessler, 1953). Fibrinogen degradation products (FDP) might be used to inhibit platelets, and their preparation for infusion has been suggested. Salmon (1971) has shown in

rabbits that streptokinase-induced fibrinolysis will not only remove fibrin deposited in the glomeruli but the FDP formed will protect platelets from immunological damage. The use of Arvin to produce FDPs must also be considered, but its use could be dangerous in the patient who lacks a normal fibrinolytic mechanism.

Methylxanthines such as aminophylline could have some application because they inhibit the antigen-induced release of histamine from leucocytes (Lichtenstein and Margolis, 1968). The use of prostaglandin E1 seems to be limited by its adverse effects (Carlson *et al.*, 1968). It might also be difficult to achieve *in vivo* the high concentrations of dipyridamole (Persantin) required to inhibit the platelet aggregation and the release reaction, although claims have been made for its use as an inhibitor of thrombosis. Moreover, its efficiency does not compare with that of aspirin, three 600 mg tablets of which have a platelet inhibitory effect that lasts from four to seven days (Weiss *et al.*, 1968; Stuart, 1970) in spite of its short half-life in the circulation, because it acetylates the platelet membrane (Al-Mandhiry *et al.*, 1970). Aspirin will not, however, inhibit platelet adhesion to collagen, but it is still among the most effective of the drugs that act against the platelet (Zucker and Peterson, 1970; O'Brien *et al.*, 1970).

The other drugs that have a good claim for consideration are the non-steroidal anti-inflammatory drugs such as phenylbutazone, indomethacin, sulfinpyrazone and flufenamic acid, since not only do they inhibit antigen–antibody reactions (Brown and MacKay, 1968) but they have the added advantage of being inhibitors of complement (Jobin and Tremblay, 1969; Acki and von Kaulla, 1969). Some remarkable results have been reported of the use of indomethacin in glomerulonephritis (Vermylen *et al.*, 1971); it has been shown to inhibit the loss of urinary FDP, which has been claimed as an index of the activity of glomerulonephritis (Clarkson *et al.*, 1971), as well as diminishing the proteinuria.

Heparinisation in transplant rejection (Starzl *et al.*, 1968) and for anuric glomerulonephritis (Kincaid-Smith *et al.*, 1968) has become standard practice in some centres and certainly produces results in some patients; careful laboratory control is, however, essential. Intrarenal coagulation can be assumed in patients who are rapidly becoming anuric but better laboratory procedures are required to select other patients in whom glomerular fibrin and platelet deposition are taking place. Serum and urine FDP estimations have their advocates but simpler procedures such as estimation of changes in the level of circulating fibrin monomer (Lipinski *et al.*, 1968; Niewiarowski and Gurewich, 1971) and platelet factor 4 may well turn out to be as reliable.

Meanwhile, trials of anti-platelet drugs continue. The simplest and probably

most effective is aspirin. Preliminary analysis of our own trial of Persantin in transplant rejection (Wardle *et al.*, 1971) has shown that this agent confers no benefit. However, its action might well be used synergistically with other inhibitors; there has been a single case report of effective reversal of thrombotic micro-angiopathy using aspirin and Persantin in combination (Giromini *et al.*, 1972). It is likely that a therapeutic cocktail consisting of one or more platelet inhibitors and two or more immunosuppressant drugs will soon be recognised as standard treatment for progressive glomerulonephritis and transplant rejection.

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The Yellow Jaundice

Foul remedies for foul diseases. The earliest treatment of jaundice seems to have concentrated on the patient's urine. Paracelsus recommended making cakes of urine, freshly voided by the patient, mixed with wood ash. The cakes were buried in a dunghill and the jaundice would slowly fade. The key to this treatment was making an odd number of cakes, preferably seven or nine. This type of therapy appealed to one President of the College. Edward Browne wrote to his famous father, Sir Thomas, of . . . 'a magical cure for the jaundice. Burn wood under a leaden vessel filled with water; take the ashes of that wood and boil with the patient's urine; then lay nine long heaps of the boiled ashes upon a board in a rank and upon every heap lay nine spears of crocus.' If the authority of a President is not enough to endorse this line of treatment Edward Browne assured his father that his remedy 'hath greater effects than is credible to any one that shall barely read this receipt without experiencing.'