

BLOOD FLOW AND THROMBOSIS IN ARTERIOVENOUS FISTULAE

David Tempest Reilly

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SUMMARY

An increasing number of patients are being maintained on long-term haemodialysis for end-stage renal failure, despite the renal transplantation programme and the introduction of peritoneal dialysis. Until prevention or cure of chronic renal failure becomes a possibility patients will continue to require long-lasting vascular access via surgically created arteriovenous fistulae. The Brescia-Cimino radiocephalic fistula at the wrist has remained the best procedure, and many patients use one satisfactorily for several years; even if later complications occur such as thrombosis or stenosis, the fistula can often be salvaged for further long-term use. In a group of patients, however, recurrent thrombotic problems occur, which can be life-threatening if access proves impossible. This study was aimed at identifying risk factors for thrombosis in these patients, and to improving clinical management of fistula complications.

Initially the size of the problem was assessed by studying over 150 patients admitted to the dialysis programme over five years, from which two main facts emerged. (1) Analysis of management showed that more fistulae could be salvaged than was previously thought. (2) Although more problems occurred in patients with small vessels, it was impossible to predict which fistulae would fail.

A prospective study of blood flow through the fistula at the time of operation showed that thrombosis occurred equally often in high and low flow fistulae, and fistulae with low initial flow could develop adequately for dialysis. This suggested that technical factors alone could not explain failure of fistulae. Patients

maintained on dialysis with and without thrombotic problems were then studied, and significant differences in parameters of rheological and platelet function emerged. A prospective study was made of a group of patients undergoing fistula construction, who had blood taken pre-operatively for estimation of rheological and haemostatic factors, including platelet function. The later incidence of thrombosis after one year of follow-up was related to the pre-operative tests. It was possible on the basis of these tests to separate the thrombotic from the non-thrombotic patients, which has important implications for prophylaxis.

CHAPTER 1

CHAPTER 1

VASCULAR ACCESS FOR HAEMODIALYSIS : THE PROBLEM

1.1 INTRODUCTION

Failure to achieve satisfactory vascular access is still a major problem in a number of patients requiring haemodialysis for chronic renal failure. Even in patients with apparently adequate vessels there are sometimes unexplained primary failures, and thrombosis of established fistulae occurs often enough to be a significant problem. For unexplained reasons failure of established arterio-venous fistulae is common after surgical operations, including transplantation.

Many causes for fistula failure can be adduced; in early failure technical inadequacy, small vessels and hypotension may contribute to low flow, and in late failure venous stenosis due to intimal hyperplasia (Stehbens, 1968) may predispose to thrombosis. After operations fistula thrombosis may be caused by pressure on the fistula arm, or by venepuncture or hypotension, and after transplantation the improvement in coagulation and the increase in haematocrit may play a part; the precise mechanism remains unclear. Most dialysis units now use an arteriovenous fistula as the chosen access procedure, the commonest being the radiocephalic fistula (Brescia, 1966) which has a much lower rate of thrombosis than an implanted arteriovenous shunt, and also better survival and fewer complications than a bovine heterograft (Hammill, 1980). However, the failure rate is still significant. In Leicester a prospective study of 157 primary radiocephalic fistulae between 1976 and 1981 showed a failure rate of 35% at four years (Reilly et al, unpublished data) and other units report similar figures.

In the Leicester study an attempt was made to correlate apparant adequacy of vessels at the time of operation with success or failure, but it was notable that failure occurred even in fistulae where there had been no apparent problem with the size or mobilisation of the vessels, although failure was more common when it had been noted that the vessels were small. The conclusion to be drawn is that failure of fistulae cannot simply be ascribed to failures of technique, especially in the case of late thrombosis, and other reasons must be sought.

A host of factors, unconnected with the adequacy of the fistula operation, may affect the flow or constituents of the blood. At first sight, uraemic patients with end-stage chronic renal failure should have few thrombotic problems; they have a low haematocrit and hence a low whole blood viscosity, and there is a well-known bleeding tendency in uraemia which has been related to a platelet defect (Rabiner, 1972). However, fistulae do thrombose, and in order to define the relative importance of factors likely to affect fistula success, studies in the following areas were undertaken:-

- (1) Blood flow
- (2) Viscosity
- (3) Red cell deformability
- (4) Platelet function
- (5) Fibrinolysis

1.2 NEED FOR VASCULAR ACCESS

Haemodialysis for end-stage renal failure became a practical proposition in 1960 with the introduction of the Quinton-Scribner external arteriovenous shunt, before which terminal renal failure was untreatable. Since then, numerous advances in dialysis techniques and vascular access procedures have been made; in 1966 a radical improvement occurred with the development of a surgically created arteriovenous fistula (Brescia et al., 1966) which had fewer complications and a longer functional life than implanted plastic shunts. The Brescia wrist fistula remains the standard by which other procedures are judged.

For the foreseeable future, haemodialysis will form the mainstay of the treatment of renal failure. Although home peritoneal dialysis is gaining acceptance, there are problems with infection, and peritoneal adhesions may preclude this technique. Despite advances in renal transplantation there is still a shortage of donor kidneys and the overall success rate nationally is about 50% at two years, which means re-transplantation or a permanent return to haemodialysis for the failures due to rejection. The number of patients on maintenance haemodialysis has increased steadily over the years; in 1979 there were 2,453 patients in the U.K. on maintenance dialysis of whom 69% were back at work (a further 21% were able to work but not in employment) compared with 1,931 patients with functioning renal transplants. Survival of patients has improved to between 75-90% at two years, and of the 4,384 patients in 1979 being treated in UK dialysis and transplantation centres, 491 had started treatment

more than 10 years ago (UKTS Annual Report, 1980). There is thus a need to provide patients with adequate long-term access for haemodialysis. Recurrent problems with access result in a considerable burden on the patient in terms of ill-health and time spent in hospital. If access sites are exhausted and peritoneal dialysis or transplantation are not possible, death is unavoidable.

1.3 TYPES OF VASCULAR ACCESS

Although implanted shunts are still used for access in acute renal failure and sometimes in cases of fistula failure, the commonest primary access procedure is now the Brescia-Cimino side-to-side radiocephalic arteriovenous fistula, being simple to construct and long-lasting. It is usually made some time before dialysis becomes essential so that the vessels can mature in readiness.

In published work primary success with this operation is usually defined as establishment of flow for more than 24 hours although in Leicester it is defined as use of the fistula for dialysis. Primary success rates are of the order of 90% though later complication rates vary between centres. Zerbino describes a 91% primary success rate in establishing such fistulae in 158 patients with a 10% incidence of later complications (Zerbino et al., 1974). Kinnaert reports on 105 fistulae, 84 of which were side-to-side, with an 88% primary success and a 12% incidence of later thrombosis (Kinnaert, 1971). Limet does not mention primary success in his series of 101 side-to-side fistulae but records a 7% later thrombosis rate (Limet, 1974). Cheek's experience with 100 fistulae (84 end-to-side and only 7 side-to-side) was 95% primary success with a mean patency of 13 months (Cheek, 1976). In a review of vascular access techniques Bleyne does not report his numbers but gives a 10% initial failure rate with only 2% later thrombosis, and concludes that initially a Brescia-Cimino fistula should be formed, using as second choice a saphenous vein graft, and as third choice, cadaver saphenous vein or polytetrafluoroethylene (PTFE) (Bleyne et al., 1978).

The experience in Leicester accords well with other studies, with an 11% primary failure rate (meaning either thrombosis before use,

or failure of the flow to develop sufficiently for haemodialysis) in a series of 145 wrist fistulae. It is often difficult to find published details of long term patency and complication rates, and the time between operation and use of the access site for repeated needling is not often quoted, thus reports of fistula survival time may be biased favourably by a long interval before use. In the Leicester series the four year patency rate for side-to-side fistulae was 65% with 38 thrombotic or stenotic complications resulting in loss of the fistula or surgical revision. Mean pre-dialysis time was 1.2 months. Thus for the majority of patients a fistula may work well for many years; it is amongst the 10% with early failure and the 20-30% that thrombose later that the problem lies. If failures of operative technique can be excluded there are still a number of patients who have recurrent thrombotic problems and repeated operations which get progressively more difficult and less likely to be satisfactory.

There are a large number of alternative procedures to choose from once the initial side-to-side fistula has failed, each of which has its own adherents. These include anatomical variations such as side-to-side brachiocephalic fistulae (Someya, 1976) and end-to-side conversion, saphenous vein loops (May et al., 1969), bovine artery heterografts (Chinitz, 1972), PTFE grafts (Elliott et al., 1977), umbilical vein grafts (Kester, 1978) and microvascular fistulae (Mansfield, 1978). However, recent encouraging reports on alternative procedures may have been biased by using grafts as the primary procedure rather than secondarily, following failure of the Brescia-Cimino fistula. In a review of 100 PTFE grafts (Anderson, 1980), 59 were straight grafts in the

forearm between radial artery and an antecubital vein, where there is at least a possibility that a side-to-side fistula might have been performed. In that study there were 3 immediate failures, 9 failures within 3 months and 3 later failures. Average follow-up was six months and the overall patency rate of 75% is not an improvement on the Brescia-Cimino fistula.

The use of bovine heterografts has been reported extensively since their introduction in 1972 (Chinitz et al) and initial experience was favourable. It is apparent now that the incidence of complications is relatively high (Foran, 1975). In a review of 98 bovine grafts, (57 as a primary procedure) compared with 46 arteriovenous fistulae carried out during the same 44 month study period, the mean duration of patency was 17 months for the arteriovenous fistulae and 10.3 months for the bovine heterografts (Hammill, 1980). Bovine grafts had more associated complications though the complication rate of primary versus secondary grafts was not compared. Hammill also culled results on 950 arteriovenous fistula patients and 801 bovine graft patients from the literature and found a four times higher incidence of complications per patient month in the bovine heterograft group. Umbilical vein grafts are similarly prone to trouble, with a survival rate of 50% at one year (Hussey, 1980); in that study patients suitable for Brescia-Cimino fistulae were apparently excluded.

Autologous saphenous vein grafts (May, 1969) have also been used widely as secondary procedures and success varies from 29% (Kestlerova, 1978) to 73% one year patency (Loeprecht, 1978). Again many studies are not comparable in that selection criteria

are unknown, that is whether the vein loop is used as a primary procedure or only in the difficult cases.

Over 20 procedures have been suggested for the problem patients, which suggests that there is no one ideal solution for patients who are unsuitable for a Brescia-Cimino procedure. There is thus a need to study these patients to see whether they have some common factor such as 'hypercoagulability' of the blood, rather than to explain away each failure as due to technique, hypotension, infection or inadequate vessels. Undoubtedly these factors may contribute to failure but that does not explain why other patients do not suffer thrombosis despite such influences. There are no published studies of such prospective investigations of fistula patients; this study constitutes an attempt to examine this problem.

1.4 INFLUENCES ON FISTULA PATENCY

1. Blood Flow

When considering the influences on the success or failure of fistulae, having accepted that surgical technique is a large variable, the most obvious consideration at the time of operation is the adequacy of flow. Flow measurements, using an electromagnetic flowmeter, have been used to determine flow in implanted arteriovenous shunts. Alfrey (1970) studied 25 patients with implanted shunts, 17 of whom had a flow over 200ml/min and 8 of whom had a flow less than 200ml/min. The mean survival of the shunts in the high flow group was 133 days compared with 24 days for the low flow group. These results are not comparable with fistulae since the survival time of implanted shunts is generally so much less, but it does suggest that critical flow levels might exist. That the absolute flow at the time of operation may not be critical is suggested by the work of Mansfield (1978), showing that it was possible to develop flow adequate for dialysis although the initial operation had required microsurgical techniques to perform an anastomosis with a vein less than 1mm in diameter. A flow of around 300ml/min is necessary for haemodialysis but whether early flow much lower than this subsequently develops to this level is not known.

In a side-to-side fistula, the lumen of the anastomosis is usually at least 2-3 times the width of the vessels so that this is unlikely to be a limiting factor determining flow. Previous work has suggested that venous resistance plays a critical role (Wallace & Jamieson, 1978) which may in part be controlled by adequate surgical technique. Angiography is inadequate to assess

flow and various techniques have been used, including dye dilution (Gothlin et al., 1977) and electromagnetic flowmetry (Anderson et al., 1977). Non-invasive techniques using Doppler velocity probes have been used to assess flow in blood vessels (Rushmer, 1966) but these require assumptions to be made concerning the diameter of the vessel in order to calculate volume flow. Newer methods of ultrasonic imaging would allow accurate diameter measurement but these are not generally available. Critical flow levels have not been defined, and one of the aims of this study was to find out what bearing flow rates at the time of operation had on the subsequent performance of the fistula.

2. Viscosity

The resistance to flow, i.e. viscosity, of blood is of obvious importance in determining flow rates. Hyperviscosity has been implicated as a causative or exacerbating factor in a number of low flow states and thrombotic diseases e.g. diabetic micro-angiopathy (Barnes & Bailey, 1979), peripheral vascular disease (Cranley, 1963), cerebral ischaemia (Thomas, 1977) and venous thrombosis (Dormandy, 1973). One of the main determinants of whole blood viscosity is the haematocrit (Begg, 1966), but plasma proteins and the ability of the red cell to deform are also implicated. Controversy exists over the precise contribution of fibrinogen to whole blood viscosity. It has been reported (Nicolaidis et al., 1977) that viscosity is increased in patients with angina, as is the fibrinogen level, and that the rise in viscosity is offset by increased flexibility of red cells in the presence of fibrinogen. An increased rigidity of red cells and a corresponding increase in viscosity has been

described in defibrinated blood (Rampling & Sirs, 1972) but a decrease in whole blood viscosity has been described at very low fibrinogen levels (Blattler, 1979). Other workers find no correlation between red cell flexibility and viscosity or between flexibility and fibrinogen level (Reid, 1976).

Blood is a non Newtonian fluid in behaviour, that is, its viscosity varies with the shear rate. Shear rate is the velocity gradient in a fluid in laminar flow in a tube (Fig.1.1) and its units are $\frac{\text{cm} \cdot \text{sec}^{-1}}{\text{cm}}$, i.e. sec^{-1} ("inverse seconds"). Shear stress is the force applied per unit area, its units being $\text{dynes} \cdot \text{cm}^{-2}$. Viscosity for a fluid in laminar flow is defined as the ratio of shear stress to shear rate, thus its units are $\frac{\text{dynes} \cdot \text{cm}^{-2}}{\text{sec}^{-1}} = \text{dynes} \cdot \text{sec} \cdot \text{cm}^{-2}$. The unit of viscosity, the centipoise (cp) is equivalent to $1 \text{ dyne} \cdot \text{sec} \cdot \text{cm}^{-2}$. These are the same units as viscosity derived from the Poiseuille equation for a Newtonian fluid in laminar flow in a tube, where

$$\text{Volume flow} = \frac{\pi p a^4}{8 \eta L}, \quad \text{where}$$

p = pressure head

a = radius of tube

L = length of tube

η = viscosity

Viscosity can be measured in a number of ways. The standard capillary viscometer (Harkness, 1963) measures the time for a standard volume of fluid to flow under a standard pressure through a fine tube. There is a high rate of shear and a non uniform flow rate so that it is unsuitable for non Newtonian

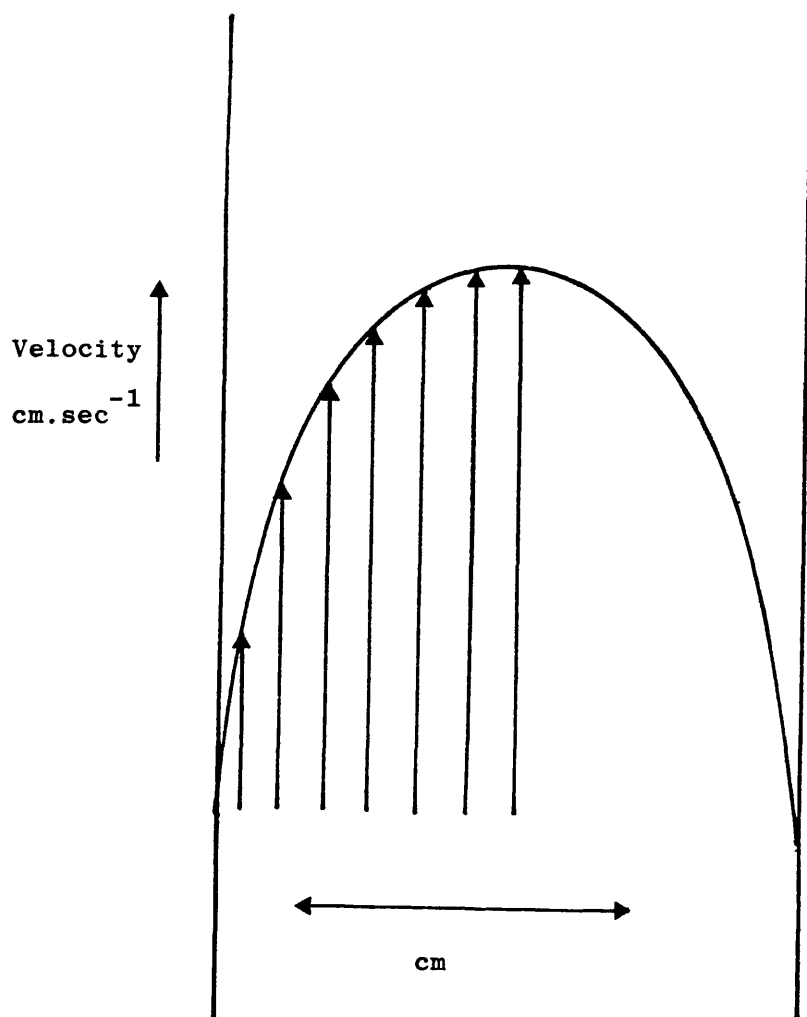


Fig. 1.1

Velocity gradient in a fluid in laminar flow through a vessel : note that velocity decreases towards the wall, so that the shear rate ($\frac{\text{cm.sec}^{-1}}{\text{cm}}$) although high near the wall is zero actually at the wall as well as in the centre of the vessel.

liquids but suitable for plasma. The other standard method is to shear the sample between two closely applied surfaces, the resulting torque being a function of the shear rate. This principle is used in the cup and bob or cone and plate viscometers. Using such instruments allows a range of shear rates to be applied and is suitable for non Newtonian liquids. At low shear rates the viscosity of whole blood becomes high. This is largely mediated by the formation of red cell aggregates, which is fibrinogen dependent (Merrill, 1963). At rest, red cells form branching rouleaux which are gradually broken up as the shear rate increases. These aggregates do not occur in defibrinated blood but small aggregates occur when low concentrations of fibrinogen are present, and these are of a particle size which actually lowers the observed viscosity (Blattler, 1979). The precise delineation of viscosity at very low shear rates is complicated by uncertainty as to the rate and degree of aggregation, which changes with time (Copley, 1975).

Few studies of the prospective value of measurements of viscosity have been performed although preoperative haemoglobin level has been reported to be of value in predicting the outcome of arterial surgery (Bouhoutsos, 1974) and diabetic amputations (Bailey, 1979). Whole blood viscosity was found to be raised in patients who subsequently suffered deep vein thromboses (Dormandy, 1973). In contrast, haemodilution has also been reported as a cause for deep vein thromboses (Janvrin, 1980).

Since the main determinant of whole blood viscosity is the haematocrit, viscosity has to be adjusted to a standard haematocrit, in order to detect the effect of other influences when

comparing two groups. This can be done by resuspending packed cells to a standard haematocrit, or by plotting the regression of log. viscosity against haematocrit (Begg, 1965). If the objective is to determine the importance of the absolute viscosity in individual patients this correction is of course invalid.

In this study absolute viscosities have been measured. The aim has been to investigate the influence of viscosity on fistula flow rate, and also its value in predicting fistula survival.

3. Red Cell Deformability

Red cells, of average size 8.5μ in a wet preparation (Grimes, 1980) have to deform considerably to pass through the microcirculation, the splenic sinusoids being as narrow as 2.8μ , and the capillaries elsewhere of approximately 4μ diameter. The flexibility of red cells makes an obvious contribution to viscosity, in that blood at a haematocrit of 95% is still fluid, whereas if red cells were rigid and unable to slide over each other blood flow would halt at a haematocrit of 60%. If red cells are hardened with acetaldehyde the observed haematocrit is increased and the viscosity becomes very high (Chien et al., 1967). Only in the haemolytic anaemias has it been definitely shown that increased rigidity of the red cell is of pathological importance (Mohandas, 1979).

A measure of the deformability of red cells has been suggested to be of importance in a number of studies. Reduced deformability as measured by filtration has been reported in

diabetic micro-angiopathy (Barnes, 1977), peripheral vascular disease (Reid, 1976), and Raynaud's phenomenon (Dodds, 1979). In haemodialysis patients reduced red cell survival correlates with decreased filterability (Rosenmund, 1975) probably due to splenic sequestration of red cells (Forman, 1973). A decrease in red cell deformability appears to be associated with the integrity of ATP-producing systems of the cell (Weed, 1969) and it has been suggested not only that differences in deformability between normals and patients with vascular disease exist, but that decreased deformability correlates with decreased cellular ATP (Buchanan, 1977) and that pharmacological treatment, e.g. Oxpentifylline may increase ATP content (Stefanovich, 1975) and restore deformability (Ehrly, 1975). This has now been shown to be unlikely by Maughan (1981) who found no difference in ATP content between the red cells of normals and those with occlusive vascular disease, and no change in ATP levels induced by oxpentifylline. The deformation of red cells is complex, depending on membrane flexibility and internal viscosity; different methods of deformability measurement may give conflicting results by measuring differing proportions of these contributory factors. Techniques that have been used are reviewed by Mohandas et al (1979) and Grimes (1980) and include the following:-

(1) Filterability

Teitel (1967) describes a method in which a suspension of washed red cells is passed through a filter paper and the 'filterability' is expressed as the log time for the emergence of half the applied suspension. This suffers from the criticism that the cells may be altered by the washing process, and also that

filter papers contain long branching channels of variable diameter, only some of which permit the passage of red cells (Grimes, 1980). Millipore filters require wetting agents which may lyse red cells (Grimes, 1980). A silver membrane introduced by Baar (1976) has a reticulated structure with an average pore size of $3\text{ }\mu\text{m}$ and is chemically inert. This gives reproducible results but the same membrane would presumably have to be used for all tests.

Polycarbonate filters of uniform pore size have been used in a number of filtration techniques e.g. that of Schmid-Schonbein (1973), Reid (1976), Barnes (1977) and Buchan (1980). Reid passed whole blood anticoagulated with EDTA, under 20cms H_2O pressure, through a 5 μ pore size filter, and expressed the deformability as the volume of red cells filtered per minute. In this method the influence of platelet aggregates and protein precipitates is not excluded. A further refinement of this technique was used by Dodds et al (1979) in which a 5% cell suspension was prepared using autologous pre-filtered plasma and the ratio measured between the amount of red cells filtered and the red cells in a 1ml sample of the suspension.

(ii) Micropipette Technique

In this exacting method the deformability of red cells is measured as the pressure required to draw a standard length of red cell membrane into a micropipette (Evans, 1975). Properties of individual cells can be quantified, but it is not a suitable method for the study of a large number of cells, nor for routine use.

(iii) Ektacytometry

Blood placed in a shear field in a high viscosity medium exhibits a characteristic diffraction pattern when transmitting laser light. This allows detection of subpopulations of undeformable cells, which exhibit a circular instead of ellipsoidal diffraction pattern (Bessis & Mohandas, 1975). Small samples of blood and little time is required but expensive equipment is necessary.

(iv) Resistive Pulse Spectrometry

This depends on the measurement of the spectra of current pulses generated by red cells flowing through a small orifice (Mel & Yee, 1975). At high flow rates the cells are deformed and generate different spectra from undeformed cells flowing slowly. This method can be coupled to Coulter cell sizing equipment with computer analysis. The effect of different variables on the index of deformability are not fully established, and in both this method and ektacytometry internal viscosity more than membrane flexibility may be being measured.

This applies also to the centrifuge packing rate method (Sirs, 1968) where red cell flexibility is derived as a term in the viscosity equation.

The aim of studying red cell deformability in fistula patients was to find:

- (1) whether an increased stiffness occurred in those
fistulae which failed
- (2) whether dialysis improved red cell deformability
- (3) the relationship between red cell deformability and
whole blood viscosity, plasma viscosity and fibrinogen level.

4. Fibrinolysis

Disorders of the blood resulting in hypercoagulability could result either from an increase in clot promoting factors or a decrease in the clearance of microthrombi by the fibrinolytic system resulting in a shift of equilibrium in favour of coagulation. The generally accepted dominant mechanism for clearance of fibrin clots and thrombi is the plasminogen-plasmin system (reviewed by Kernoff & McNicol, 1977) in which the inactive precursor plasminogen is converted by activators to the proteolytic enzyme plasmin. The precise mechanism of fibrinolysis in vivo is controversial. It is suggested that either plasminogen is adsorbed to polymerizing fibrin, and activator then diffuses into the clot in the relative absence of inhibitors (Alkjaersig, 1959) or activator is bound to fibrin, and plasminogen perfusing the thrombus is activated (Chesterman, 1972). Theoretical defects in this system leading to decreased fibrinolytic activity could include lack of activator, lack of plasminogen or increased inhibitor activity, but congenital defects of the system seem to be rare (Kernoff, 1977). Lower fibrinolytic activity with age has been shown (Meade, 1977) together with a raised fibrinogen level. A raised fibrinogen level is not necessarily an indicator of reduced lysis but may often reflect increased synthesis in response to increased lysis (Flute, 1977).

In a number of studies low fibrinolytic activity has been implicated as playing a causative role in thrombosis. The picture is complicated by the fact that fibrinolysis may be secondarily lowered by risk factors such as smoking or enhanced by a thrombotic process, so that a prospective study of patients is needed to

prove whether a low activity is a strong predictor of later thrombosis. In recurrent deep vein thrombosis defective fibrinolytic activity was found in 70% of patients (Isacson & Nilsson, 1972) and in a further study by the same authors, ethyloestrenol, which increases fibrinolysis (Fearnley et al., 1967) was apparently successful in preventing recurrent thrombosis (Hedner, 1976) although this was not a controlled trial. In atherosclerosis a low fibrinolytic activity has also been demonstrated (Wardle, 1972) but the effect of enhancing fibrinolysis has not been adequately tested. In renal disease the situation is confused; some authors report a low activity (Wardle, 1972) and some find normal levels (Remuzzi, 1978). Some of the confusion may stem from the use of such methods of fibrinolytic assay as the euglobulin clot lysis time (ECLT) (Chakrabarti, 1968), dilute blood clot lysis time (DBCLT) (Fearnley, 1964) and fibrin plate assay (Astrup, 1952). These suffer from various criticisms; the ECLT is performed at an unphysiological pH, the DBCLT depends on the relative absence of inhibitors, and the tests may be measuring only a part of the normal spontaneous fibrinolytic system. There is a lack of direct evidence that the plasmin system is dominant and it may be that proteases from leucocytes and platelets are of importance (Plow & Edgington, 1975; Moroz & Gilmore, 1976).

Recent interest has been rekindled in the importance of fibrinolytic activity as a factor in the thrombotic process;

(1) by confirmation that fibrinolytic status, as measured by ECLT, is one of the most important discriminants in the prediction of risk of deep vein thrombosis after gynaecological operations. Since it is difficult to define exactly what ECLT measures, this

casts no light on mechanisms, but indicates areas to study.

(2) by useful clinical results from using the fibrinolytic enhancing anabolic steroid stanozalol in both the postphlebitic syndrome (Burnand et al., 1980) and primary Raynaud's phenomenon (Jarrett, 1978). In the former syndrome the success may be explained by decreased perivascular fibrin cuffing, but in Raynaud's the success is more difficult to explain; although plasma fibrinogen is lowered, no decrease in viscosity occurs, and in fact the haematocrit may increase (Ayres, 1981).

The importance of the fibrinolytic system in determining survival of fistulae has not been investigated, so that a prospective study of the influence of pre-operative fibrinolytic activity was felt to be important.

5. Platelet Function

Platelet dysfunction in uraemia has been extensively documented although the mechanism is still unclear. The bleeding tendency of chronic renal failure has been attributed largely to a thrombocytopathy which can be partly reversed by dialysis (Castaldi, 1966; Eknayan, 1969). The qualitative platelet defects demonstrated by in vitro testing include impaired retention by glass beads (Salzman & Neri, 1966), decreased aggregation in response to ADP (Castaldi, 1966), decreased Factor 3 availability (Lewis, 1956; Rahiner, 1968), increased bleeding time and decreased malondialdehyde formation (Remuzzi, 1978). Increased prostacyclin activity in uraemics may also contribute to a bleeding tendency (Remuzzi, 1978).

In spite of this bleeding tendency, shunts and fistulae often thrombose, and fibrin and platelets are deposited on dialyser membranes despite heparinisation, thus reducing efficiency (Lindsay, 1972). That platelets are implicated in the thrombosis of implanted arteriovenous shunts is suggested by Kaegi (1974) who showed a reduction by a factor of three in the number of shunt thromboses in patients on sulphipyrazone, a platelet inhibitor, without producing bleeding complications. Similar studies on radiocephalic fistulae have not been published.

A number of platelet function studies produce conflicting ideas about the nature and extent of platelet abnormalities because the groups studied are not comparable, i.e. chronic renal insufficiency, 'end stage' chronic renal failure and patients on maintenance haemodialysis. The aetiology of renal failure is diverse, and in glomerulo-nephritis, for instance, platelet activation may play a causative role in pathogenesis (Bang, 1973; George, 1974) though antiplatelet drugs and anticoagulants do not affect the natural history of the disease (Trygstad, 1973). Thus there may be a subgroup of patients with increased platelet reactivity who are particularly prone to thrombosis despite the overall platelet depression in the group as a whole. The study of platelet function in fistula patients was designed to test this hypothesis. It was felt that patients with end-stage chronic renal failure requiring fistulae would form a relatively homogeneous group with regard to impairment of renal function, and to compare the platelet function tests at this stage with the later incidence of thrombotic complications would allow conclusions

to be drawn as to whether a hypercoagulable state occurred in a subgroup, and if so, rational prophylaxis could be planned. It has been shown in some studies that urea and creatinine levels do not correlate with bleeding time or platelet retention (Remuzzi, 1978; Salzman, 1966) although previous work has found such a correlation (Rabiner, 1972; Horowitz, 1970) and in chronic renal insufficiency betathromboglobulin levels have been found not to be raised (Adler, 1979) at variance with the study of Green (1979). Many of the tests of platelet function such as adhesion and bleeding time do not correlate with each other (Remuzzi, 1978) suggesting that different aspects of platelet function are being measured. Platelet aggregation has been shown to be reduced in individuals on haemodialysis and patients in chronic renal failure with a creatinine level of above 500 $\mu\text{mol/L}$, but normal or increased aggregation is exhibited by patients with a creatinine below 500 $\mu\text{mol/L}$; it is suggested that platelet function is progressively impaired as the creatinine rises above 500 $\mu\text{mol/L}$ (Lindsay, 1975).

Recently part of the answer to this puzzle of continuing thrombosis or atherosclerosis despite a bleeding tendency has been suggested by Turney & Weston (1981). They found a transferable plasma inhibitor of ristocetin-induced platelet aggregation in dialysis patients which would account for an adhesion defect leading to a prolonged bleeding time, without necessarily affecting platelet aggregation in response to collagen, ADP or thrombin.

A large number of in vitro techniques have been used to test platelet activity. All are subject to the criticism that

platelets are altered on removal from the body. Another difficulty is that abnormal platelet function may be either a cause or consequence of the state being investigated. In vivo observation of platelet activation is difficult, since the process occurs in milliseconds, and while experimental observations may be made using the bat wing membrane or the rabbit cortical vessels (Adams, 1979), in man in vivo testing is limited to calculating the rate of platelet turnover and the sites of platelet deposition. This involves withdrawing, radio-labelling and re-injecting platelets. In this situation ¹¹¹Indium-oxime labelled platelets are likely to have more normal function than ⁵¹Cr-labelled platelets (Heyns, 1980). Other turnover techniques include malondialdehyde assay after platelet cyclo-oxygenase blockade by aspirin (Stuart, 1975), or measuring the rate of reappearance of thromboxane B₂ production by radio-immunoassay (Koh, 1980), also after aspirin blockade. Measurement of proteins in the plasma specifically produced by platelets may give a measure of the degree of platelet activation occurring in vivo provided other factors affecting clearance are known to be constant. Examples of this technique are platelet factor 4 assay (Bolton, 1976) or beta-thromboglobulin assay, which was shown to correlate well with the development of deep venous thrombosis (Ludlam, 1975) provided the rate of thrombus formation was rapid enough.

Laboratory tests on platelets themselves can be grouped into adhesion, aggregation and biochemical techniques. (Table 1.1).

TABLE 1.1

Some techniques for measuring aspects of platelet activity.

Adhesion

Glass bead column (Hellem, 1960)

Glass slide (Page, 1979)

Subendothelium (Baumgartner, 1977)

Aggregation

'In vitro' aggregates - Swank (1968)

- Wu & Hoak (1974)

Induced in vitro aggregation - turbidometry (Born, 1962)

- microscopic (Sano, 1979)

Biochemical

Release markers - betathromboglobulin (Ludlam, 1975)

- platelet factor 4 (Bolton, 1976)

- C₁₄ serotonin uptake/release (Jerushalmy, 1966)

Prostaglandin metabolism products - malondialdehyde (Smith, 1976)

- thromboxane B₂ (Granström, 1976)
(Koh, 1980)

Platelet coagulant activity e.g. PF₃ activity (Walsh, 1972)

(i) Adhesion

Adhesion is a fundamental property of platelets; in the initiation of a thrombus in vivo, platelets adhere to damaged endothelium either in response to basement membrane, to collagen microfibrils or to adsorbed plasma proteins, notably fibrinogen (Lyman, 1974; Kim, 1974). It has been measured by assessing the numbers of platelets adhering to a glass microscope slide (Page, 1979) or by measuring retention of platelets when blood is passed through a glass bead column (Hellem, 1960). Adhesion to biological structures has been assessed by rotating an everted ring of rabbit aorta, denuded of endothelium, in platelet rich plasma (Baumgartner, 1977). These tests give conflicting results presumably because adhesion to glass differs from adhesion to biological material. Aspirin for instance lowers platelet retention in a glass bead column but does not affect adhesion to a glass slide (Page, 1979) or to rabbit aortic endothelium (Weiss, 1975). It thus seems that glass bead systems are also measuring aggregation. It is notable that platelet rich plasma shows no retention in a glass bead column (Hellem, 1960) and therefore either red cells must produce something necessary for adhesion or aggregation e.g. ADP (Born, 1976) or the anticoagulant used (usually citrate) masks platelet responsiveness.

(ii) Aggregation

Following adhesion, a complex series of reactions takes place: the platelet changes shape, producing pseudopodia, platelet factor 3 (the pro-coagulant factor) is made available on the surface, and aggregation promoting substances and vaso-active agents

are produced, following which further aggregation and release reaction occurs (Fig.1.2). Aggregation studies consist of either measuring aggregates already present in the blood, by formalin fixation (Wu & Hoak, 1974) and by screen filtration pressure (Swank 1968; Gimson Weston, 1980), or by observation of aggregation occurring in platelet-rich plasma. This includes stirring the plasma in an optical densitometer, and detecting the decreased turbidity as aggregation occurs (Born, 1962), and microscopical observation of aggregates forming in multiple wells as successive agents are added (Sano, 1979). Obviously the anticoagulants used affects platelet function; in EDTA, calcium is fully chelated so that no aggregation occurs; heparin enhances the effect of ADP on aggregation (Levin, 1978) and citrate lowers the calcium to such small levels that aggregation occurs relatively slowly, and it is possible to detect the so-called second wave of aggregation as initial aggregation is followed by irreversible aggregation after the release phenomenon. This may be an artefact of citrated plasma but the biphasic response has its use in allowing the determination of a threshold level of ADP concentration which just causes the second wave to occur. This could be a measure of the sensitivity to activation of the platelet. A number of aggregating agents have been used, including ADP, adrenaline, arachidonate, thrombin and collagen, some of which use different pathways. Thrombin for instance appears to cause aggregation both by stimulating the thromboxane pathway and by interacting with a platelet activation factor which is not blocked by cyclo-oxygenase inhibitors such as aspirin (Chignard, 1979). ADP is released by both platelets and red cells and appears to be one of the physiological stimuli. For this reason it has been used in this study for maximal stimulation

RELEASE INDUCERS

THROMBOXANE A₂
COLLAGEN
THROMBIN
ADRENALINE
ADP
ARACHIDONATE

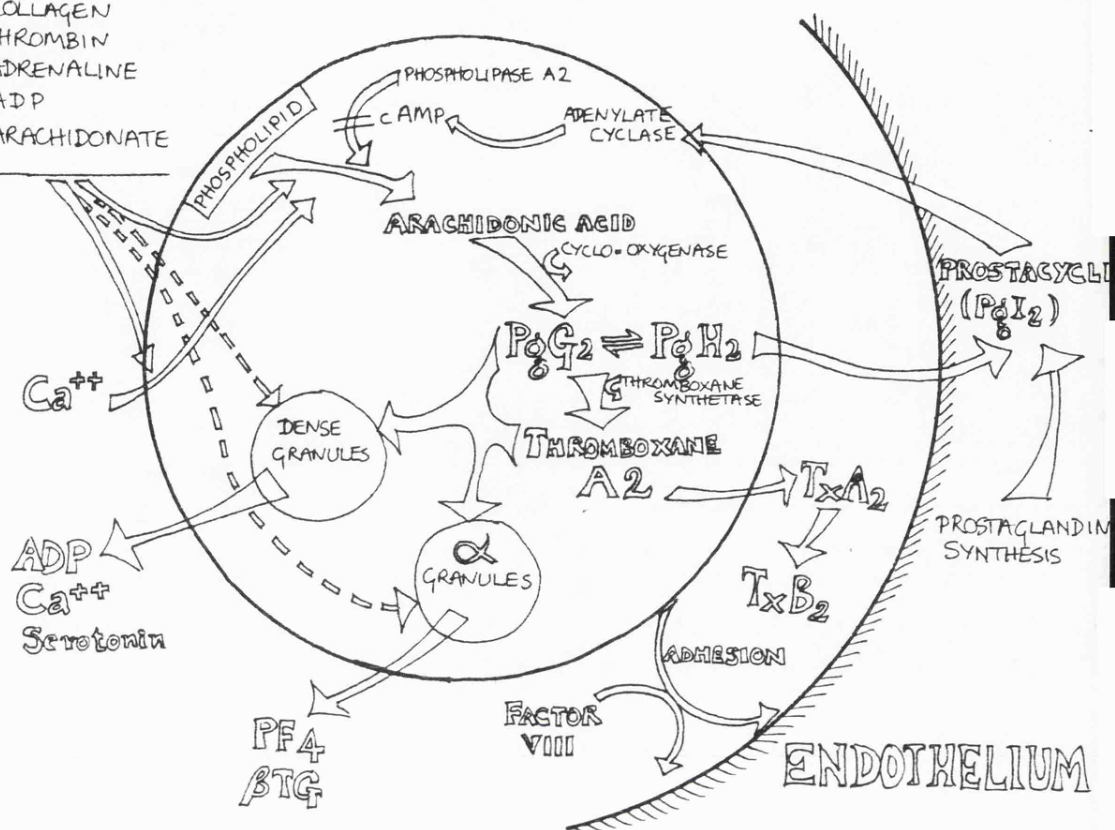


FIG. 1.2

Simplified scheme of platelet activation pathways : note (1) some release inducers may bypass the prostaglandin route (2) final common path may be calcium flux.

of aggregation and also for defining the threshold for a biphasic response, as a possible index for an increased thrombotic tendency.

(iii) Biochemical Tests

Platelets in vitro can be induced not only to secrete substances from the cytoplasmic granules but to take up molecules such as ^{14}C -serotonin to be subsequently released on stimulation (Jerushalmy, 1978). Platelet coagulant activity can be measured in several ways including platelet factor 3 (Walsh, 1972) activity or by platelet factor 4 (anti-heparin factor) release (Bolton, 1976). A measure of the prostaglandin synthetic pathway is the production of an intermediate metabolite, such as malondialdehyde, but the small quantities involved make assay difficult. Since platelet activation is strongly associated with cyclic nucleotide levels measurement of cyclic AMP and cyclic GMP may give some indication of the platelet reactivity. However, platelet content of cyclic AMP or GMP may not reflect the available physiological pool. In platelet rich plasma at least 75% of the cAMP activity is in the plasma rather than the platelets, but washing the platelets may further alter their behaviour.

Platelet Function Tests Used in this Study

In defining precisely the metabolic pathways in platelet dysfunction biochemical tests are necessary, but for the purpose of detecting an overall increase in reactivity of platelets it was felt that screening for increased adhesion, aggregability and in vivo release phenomena would initially prove adequate. In

this study the following tests were used:-

1. Glass bead column platelet adhesion
2. Aggregation in response to ADP.
3. Plasma betathromboglobulin assay

6. Coagulation

In trying to detect a pre-thrombotic state, increased activity of the many components of the coagulation system has been looked for. Because of the many different types of thrombotic disease, both arterial and venous, it is hardly to be expected that some factor common to them all will be detectable and serve as a marker for the risk of thrombosis, and indeed no certain determinant has yet emerged.

As with tests previously discussed, coagulation assays may be secondary to other factors such as atherosclerotic changes in the vessel wall, causing an increased activation or consumption of factors, and again peripheral venous blood may not be representative if the activated factors have been consumed elsewhere. The only way to resolve the importance of coagulation tests is by a prospective study relating incidence of thrombosis to initial blood values. Using a combination of clinical parameters and coagulation tests, a prognostic index for the risk of deep vein thrombosis can be constructed (Clayton, 1976) which is still valid applied prospectively to a subsequent group of patients and allows selective effective prophylaxis of those patients most at risk (Crandon, 1980). The disadvantage of this approach is that the index is only valid for the one centre, for a rigidly defined group

of patients (in Clayton's study all were undergoing gynaecological operations). The same approach can be used for arterial disease, but studies have to be much longer, require greater numbers and end-points are difficult to define. One end-point which can be defined is death from myocardial infarction; in a prospective study of a population of 1500 men, Meade (1980) found a significant elevation of fibrinogen and factors VIIIC and VIIIC in the 27 deaths from infarction, with high values of 2 or 3 of these variables in 63% of the 27. In Meade's study platelet adhesion, fibrinolytic activity and antithrombin III levels were not found to be useful discriminants. One of the ways in which an increased activity of the coagulation pathway could lead to thrombosis is by leading to an increased availability of thrombin which stimulates platelet aggregation and is also necessary for the stabilising of platelet aggregates.

Other tests, for instance of activated factors, are used more in the diagnosis of continuing thrombosis, rather than in diagnosing a pre-thrombotic state. These include fibrinopeptide A (Gerrits, 1974) and fibrin monomer (Alkjaersig, 1973) estimation which detect the action of thrombin on fibrinogen. Neither are of undisputed predictive value.

In renal disease one of the unsolved questions is whether renal patients suffer accelerated atherosclerosis. Certainly they are not at decreased risk, yet with the bleeding tendency of uraemia it is odd that atheroma and thrombosis should continue unabated; as mentioned on page 22 this has been partly elucidated by Turney & Weston (1981) who have demonstrated raised levels of

factor VIII complex and fibrinogen in patients with chronic renal failure and dialysis, and postulate that factor VIII, raised by endothelial damage, may lead to intravascular coagulation and platelet activation by thrombin, due to normal or enhanced aggregation, despite the adhesion defect.

Due to technical limitations, coagulation factors apart from fibrinogen were not measured in the present study. In retrospect it would seem probable that different levels of factor VIII contribute to the postulated prothrombotic state, and could possibly help detect patients likely to have recurrent thrombotic problems with fistulae; it can also be said that, with other factors, fistula thrombosis may be a marker for systemic atherosclerosis.

CHAPTER 2

FIVE YEAR PROSPECTIVE STUDY OF DIALYSIS FISTULAE : PROBLEM PATIENTS AND THEIR TREATMENT

INTRODUCTION

With an increasing number of patients on maintenance haemodialysis treatment, providing satisfactory long-lasting vascular access becomes critically important. There are a large number of reports of the relative success rates of various forms of vascular access, but there is general agreement that the Brescia-Cimino wrist fistula (Brescia et al., 1966) is the most satisfactory. For the patient whose wrist fistula has failed the surgeon has a choice of reconstructing the fistula when possible, constructing another fistula in the contra-lateral wrist, a brachial fistula (Someya et al., 1976) or inserting a graft of biological or synthetic origin. Grafts can be autologous (May et al., 1969) or allogeneic vein (Kestlerova et al., 1978), umbilical vein (Kester, 1978), bovine carotid artery (Chinitz et al., 1972) or synthetics such as polytetrafluoroethylene (PTFE) (Baker et al., 1976) and Dacron (Burdick et al., 1978). Experience has shown that long term results are poor for vein loops (Kinnaert et al., 1979; Haimov et al., 1980) and bovine heterografts (Foran et al., 1975) although with aggressive treatment of the frequent complications of bovine grafts, it is possible to achieve a two-year patency rate of 77% (Vanderwerf et al., 1978). Umbilical vein is relatively untried, although a one-year patency rate of 50% has been reported (Hussey, 1980). Patency rates

for PTFE vary from 62% at one year (Tellis et al., 1979) to 83% (Haimov et al., 1979). Reports of long term patency for PTFE are harder to find; a two-year patency rate of 76% has been reported (Haimov et al., 1980) and a three-year patency rate of 63%.

The difficulty in drawing any conclusions from these studies are that the selection criteria are unknown; grafts have a greater patency rate if inserted as a primary procedure rather than in patients whose access sites are nearing exhaustion. The first aim of the present study was to examine the need for more complex procedures than simple arteriovenous fistulae in a population without previous surgery; thus all patients undergoing first-time fistula operations have been studied, rather than all firstulae, regardless of whether they were first, second or subsequent procedures. The second aim of the study was to attempt to correlate fistula failure with possible aetiological factors, in the hope of predicting those likely to have subsequent thrombotic problems.

PATIENTS AND METHODS

Between January 1976 and May 1981, 157 consecutive patients in chronic renal failure requiring access procedures were accepted onto the dialysis programme. Six patients were excluded from analysis because of previous fistula operations elsewhere. This left 151 patients of whom a further 5 were excluded from analysis because of inadequate data. All 5 had functioning fistulae when lost to follow-up; 3 were transferred to other units and 2 died. Of the remaining 146 patients, one had a primary brachial fistula because of previous bilateral cephalic vein cut-downs in the past. Thus 145 patients undergoing an attempt at wrist fistula construction were available for analysis; this included 2 patients with an unsuccessful exploration

of the wrist, in order to avoid biasing the results by only considering those patients with obviously adequate vessels. Using actuarial analysis (Armitage, 1971) fistula patency curves were calculated for these 145 patients.

Of the 143 patients undergoing a technically successful wrist fistula construction, 136 patients had side-to-side radiocephalic anastomoses and 7 end vein to side artery anastomoses. Details recorded at the time of operation included the age and sex of the patient, size of anastomosis, size of vessels, type of anastomosis, suture material used, and adequacy of flow. From 1976 to 1979 7/0 silk sutures were used for fistula construction; from 1979 onwards 6/0 or 7/0 polypropylene sutures were used. Details recorded during follow up included the date of first haemo-dialysis, and the incidence of complications and their treatment.

Anastomosis size was recorded in millimetres. Vessel size was recorded as good, fair or small for the first 82 operations; for the next 61 operations the diameter was measured with calipers to the nearest 0.5mm, when the vessel was fully dilated. For analysis vessel size was coded for veins as 0 = small (<2mms), 1 = fair (2.1 - 3mms), 2 = good (>3mms), and for arteries as 0 = small (<2mms), 1 = fair (2.1 - 2.9mms) and 2 = good (>2.9mms).

The side-to-side fistulae were constructed under local infiltration anaesthesia (using 1% lignocaine) using the Brescia-Cimino technique (Brescia et al., 1966) modified by using a sigmoid incision for adequate mobilisation of the vessels (see Appendix X). Flucloxacillin was given immediately before the procedure and continued for a week after operation. If the vessels lay too far apart for this technique an end-to-side fistula was constructed (Bell & Calman, 1974) (Fig.2,1).

Fistulae which never developed adequately for dialysis, or thrombosed before use, were regarded as primary failures. Early thrombosis within 24 hours was included in the analysis. Fistulae were regarded as salvaged if thrombectomy, excision of stenosis, or end-to-side conversion, using the same arterialised vein, resulted in satisfactory dialysis.

The local policy for management of chronic renal failure required construction of an AV fistula, once the inevitability of dialysis became apparent, in time for the fistula to mature. Despite this policy, occasional patients required dialysis sooner than expected and the first haemo-dialysis was performed via an implanted shunt at the ankle. For such patients the recorded date of first haemo-dialysis did not correspond with first needling of the fistula. There were 10 such patients in the no problem group and 2 in the problem group for whom the exact time of maturation of the fistula was unknown.

Statistical analysis was performed on the differences between all variables in the problem group and the no problem group; using the two-sample 't' test for normal or log normal distributions (e.g. age, pre-dialysis time), analysis of variance for skewed distributions (e.g. anastomosis size) and χ^2 tables for ranked distributions. Computer discriminant analysis was performed using GLIM software package (Nelder, 1975). As well as the overall comparison, all variables for patients developing early problems (0-6 months) were compared with variables for patients with no problems (follow up 7-70 months).

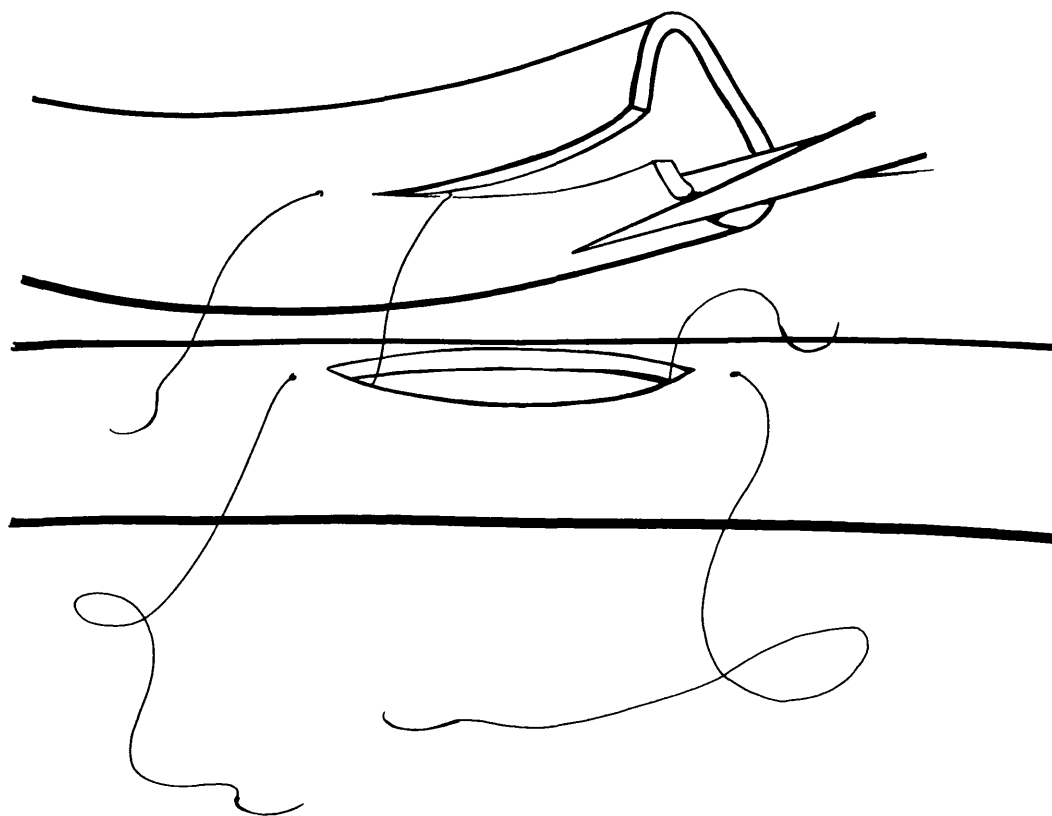


FIG. 2.1 Technique for construction of an end-to-side fistula using scissors to slit and trim the vein and to provide an adequate anastomotic lumen.

RESULTS

Fistula Problems and their Management

Patency

The overall patency rate for wrist fistulae is shown in Figure 2.1. Excluding deaths, the one-year patency rate was 80% and the four-year patency rate 65%. There were 16 primary failures (11%) including 2 failed explorations of the wrist; the patency rate for first time fistulae capable of use for dialysis was 90% at one year. 78% of patients were capable of dialysing on either their first or second wrist fistula at 4 years after the initial operation (Figure 2.2).

Incidence of Problems

An indication of the overall incidence of fistula problems is given by the total number of surgical operations; 252 (145 primary operations + 107 secondary procedures) were performed in the 145 patients over 5½ years, an average of 1.73 operations per patient. 51 patients required further access surgery; the reasons for first access failure are set out in Table 2.1. These patients constituted a problem group, in that many of them required yet further procedures. The total number of procedures performed, including the 26 surgical explorations in Table 2.1, is set out in Table 2.2.

The commonest cause of first access failure was thrombosis, either early or late (Table 2.1). There is an overlap between the categories of poor flow and thrombosis since the one may precede and predispose to the other; the category 'poor flow' was used when the reason for surgical referral was inadequate flow on dialysis. Hyperaemia (venous hypertension of the hand) was not a significant problem, with an incidence of 6 out of 136 side-to-side fistulae (4.4%).

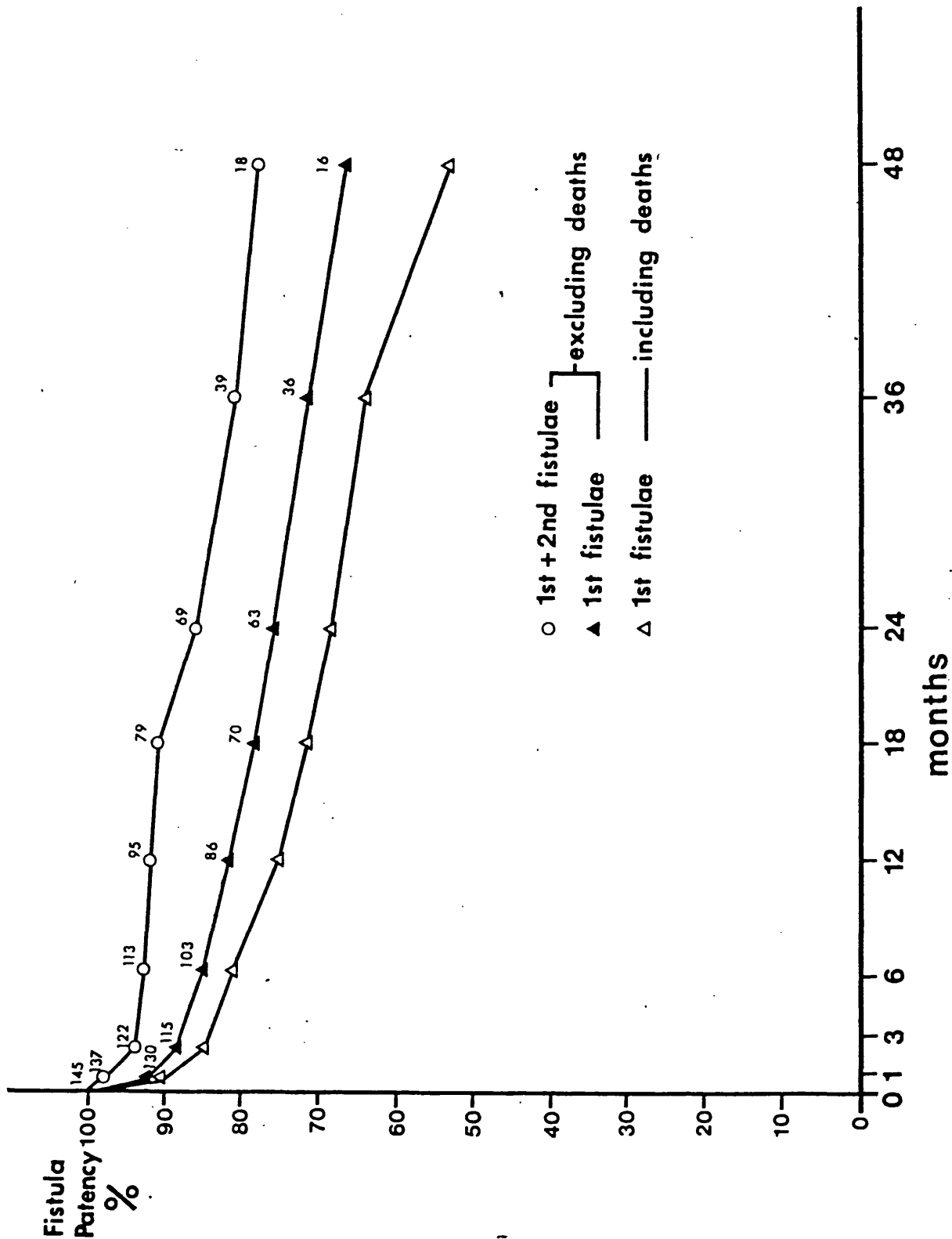


FIGURE 2.2

Patency rate of wrist fistulae in 145 patients undergoing an attempt at fistula construction; this includes 2 patients with an unsuccessful exploration of the wrist. (numbers above the lines indicate patients completing each follow-up interval).

TABLE 2.1

Complications of first fistulae and outcome of surgical intervention.

		<u>Exploration</u>	<u>Satisfactory</u>
Failed wrist exploration (inadequate vessels for fistula)	2	-	-
Primary failure			
(a) failure to develop	3	-	-
(b) thrombosis before dialysis	11	6	2
Late thrombosis	13	3	2
Post-transplantation thrombosis	5	1	1
Hyperaemia (a) early	2	2	2
(b) late	4	4	2
Poor flow on dialysis	10	9	8
Aneurysm	1	1	1
	51	26	18



FIG. 2.3 Late venous hypertension (hyperaemia) of the hand complicating a side-to-side fistula.

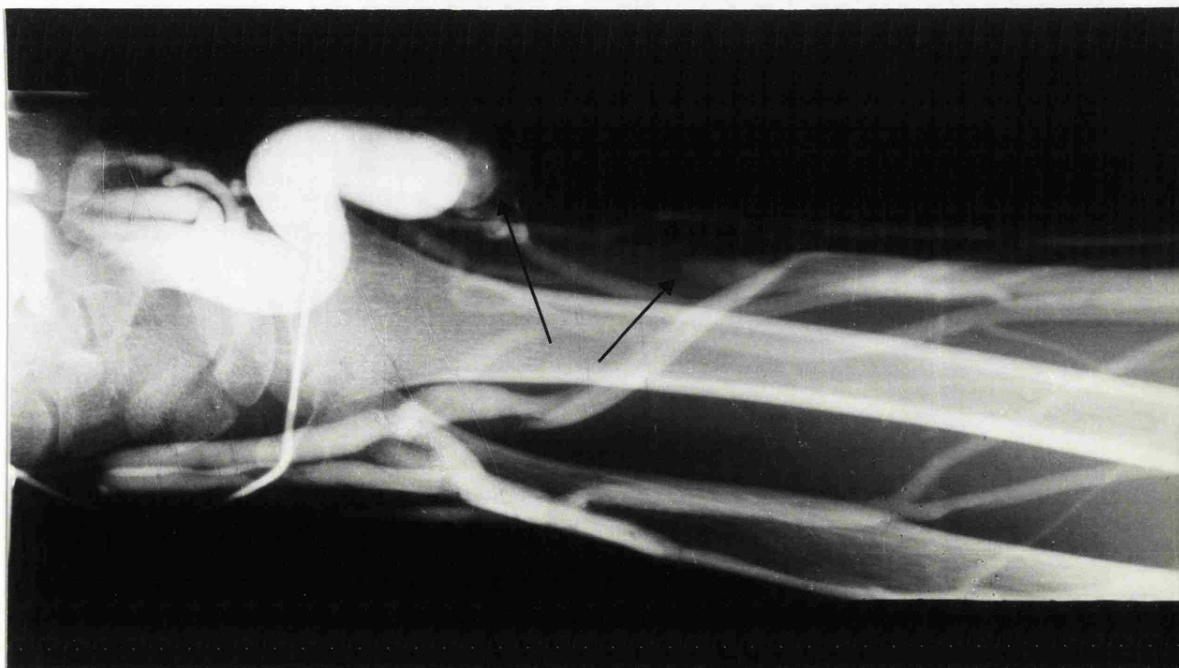


FIG. 2.4 'Fistulogram' of side-to-side fistula with proximal vein stenosis (arrowed) causing dilatation of distal vein and hyperaemia of the hand.

Management of Problems

Excluding the 2 patients with unsuccessful wrist exploration in whom it was not possible to construct a fistula, 26 out of 49 patients (53%) underwent surgical exploration for thrombotic or other problems. This had a successful outcome (patent for at least 3 months afterwards) in 18 (70%) (Table 2.1). Least successful was exploration of early thrombosis, with 2 out of 6 successful thrombectomies, and most successful was revision of fistulae for poor flow on dialysis (9 out of 10 successful revisions) caused by short segment venous stenosis. Early hyperaemia of the hand was successfully treated by ligation of the distal vein in both patients in whom it occurred. The first two patients with late hyperaemia (Figure 2.3) underwent ligation of the distal vein which was soon followed by failure of the fistula, but in 2 patients later in the series phlebography demonstrated a stenosis of the proximal vein (Fig.2.4) which was successfully treated by end-to-side conversion of the fistula with distal vein ligation (Figures 2.5 - 2.8).

The low rate of exploration for primary failure of fistulae (6 out of 14) probably reflects a decision that the initial fistula had a poor prognosis due to small vessels. The low rate of exploration for late thrombosis (3 out of 13) reflects partly delayed referral and partly different policy in the early part of the series when a new access procedure might have been preferred.

Second or Subsequent Access Procedures

94 out of the 145 patients (65%) had no problems with their fistulae, but in the 51 patients (35%) in whom a first time wrist fistula was not possible ($n = 2$) or gave rise to problems ($n = 49$), 107 procedures (2.1 per patient) were required to re-establish satisfactory dialysis by the date of review, May 1981. The variety

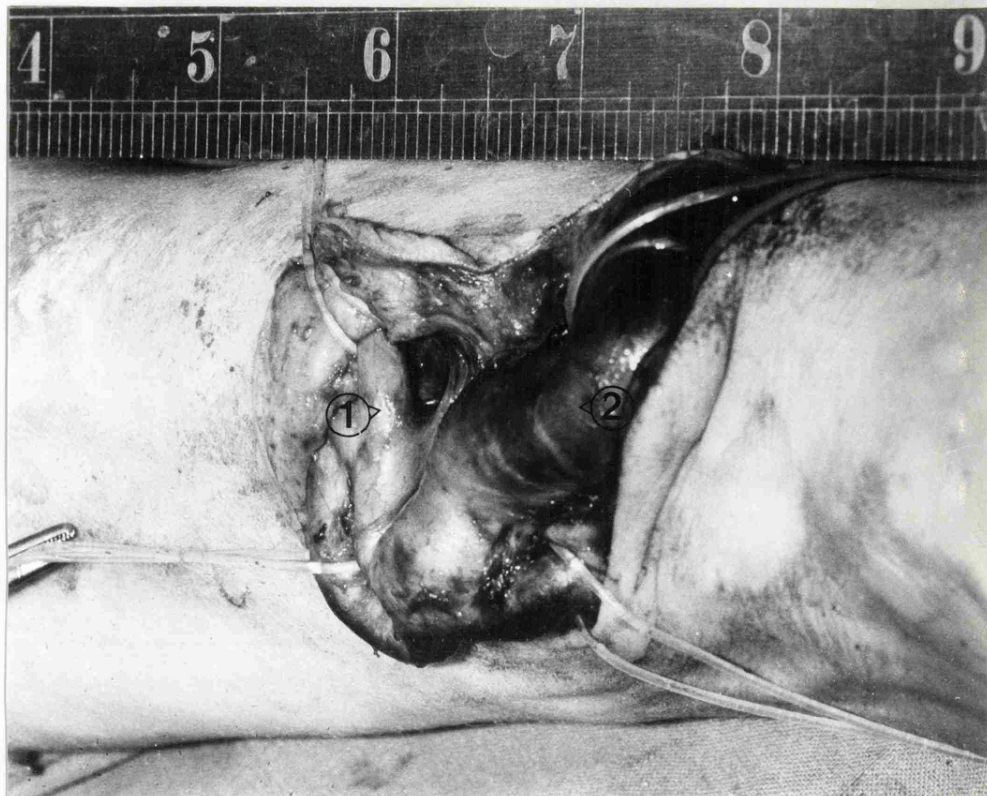


FIG. 2.5 Exposure of side-to-side fistula at wrist showing (1) stenosis of proximal vein (2) dilatation of distal vein (same case as Fig. 2.4).

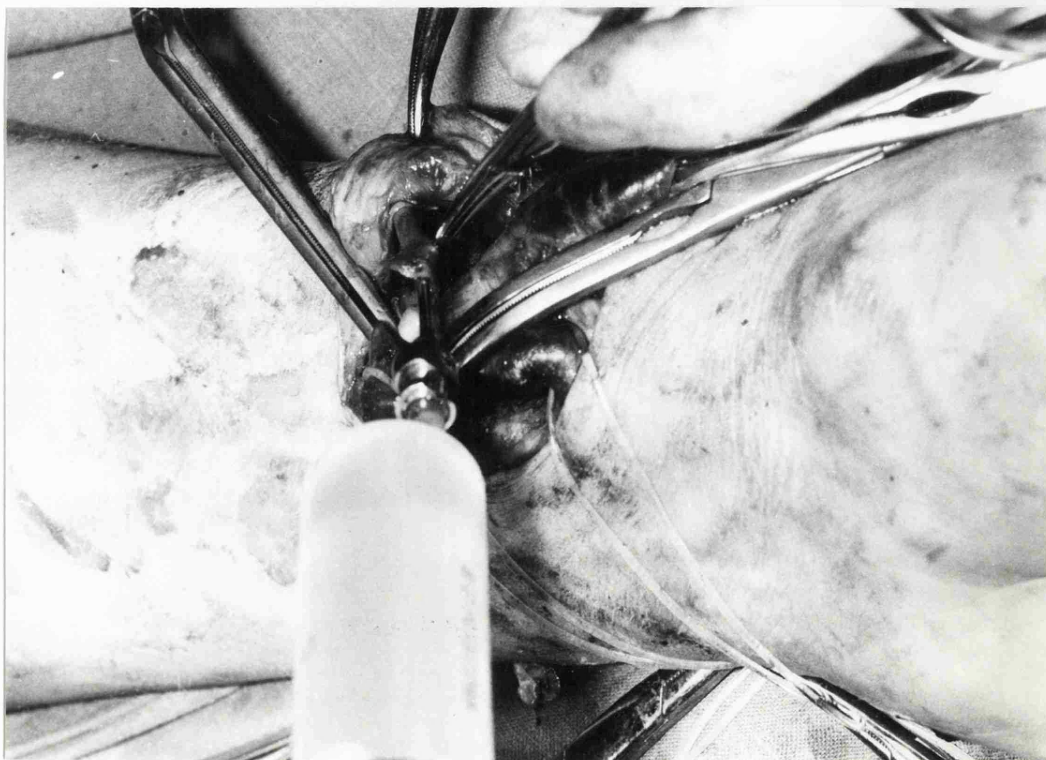


FIG. 2.6 Radial artery held between clamps : proximal vein divided proximal to stenosis and flushed with heparinised saline.

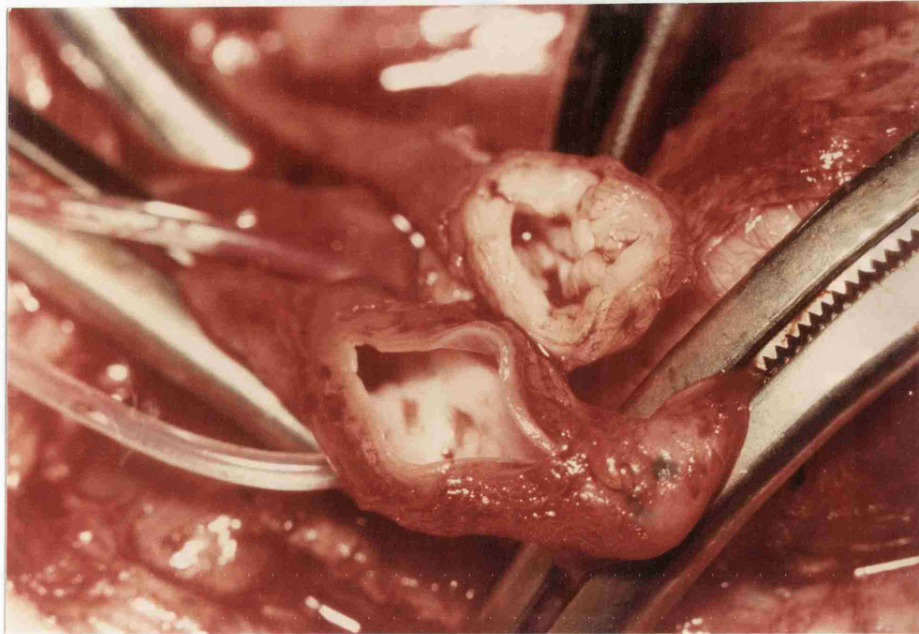


FIG. 2.7 Preparation for anastomosis : a 0.8cm slit made in the artery and the vein end bevelled, Note thin artery wall and thick vein wall.



FIG. 2.8 Completion of anastomosis (arrowed) between end of vein (v) and side of artery (a) using 6/0 Prolene. Remaining step is to tie and divide distal vein.

of procedures is illustrated in Table 2.2. There were 65 new access procedures of which the commonest was another wrist fistula (49%) either in the other wrist or using another vein in the same wrist. 17 (26%) side-to-side brachial fistulae and 4 (6%) autologous saphenous vein loop grafts in the forearm were constructed. The technique for constructing a brachial fistula is depicted in Appendix XI (Fig. XI.3)

42 salvage procedures were performed, 26 of which were on first fistulae, and 19 on second or subsequent fistulae. The commonest procedure was thrombectomy alone of which there were 18 (43%) which includes 5 unsuccessful attempts at declotting loop grafts. Only 3 of these 18 (17%) resulted in patency of 3 months or more, whereas for the next commonest procedure, end-to-side conversion (29%) with or without thrombectomy, 11 out of 12 patients had a patent fistula three months later.

Present Situation of Problem Group

The success of secondary access procedures is reflected by the fact that of the 51 patients in the problem group, 30 were dialysing satisfactorily with either a wrist or brachial fistula at the time of review (Table 2.3). Seven patients died with a functioning fistula and 4 patients with a successful transplant required no further access procedures. The patency rates for the 17 brachial fistulae (showing a 71% success at one year) and the 4 saphenous loop grafts are shown in Figure 2.9.

Influences on Fistula Patency

Age and Sex

There was a similar proportion of females in both the problem

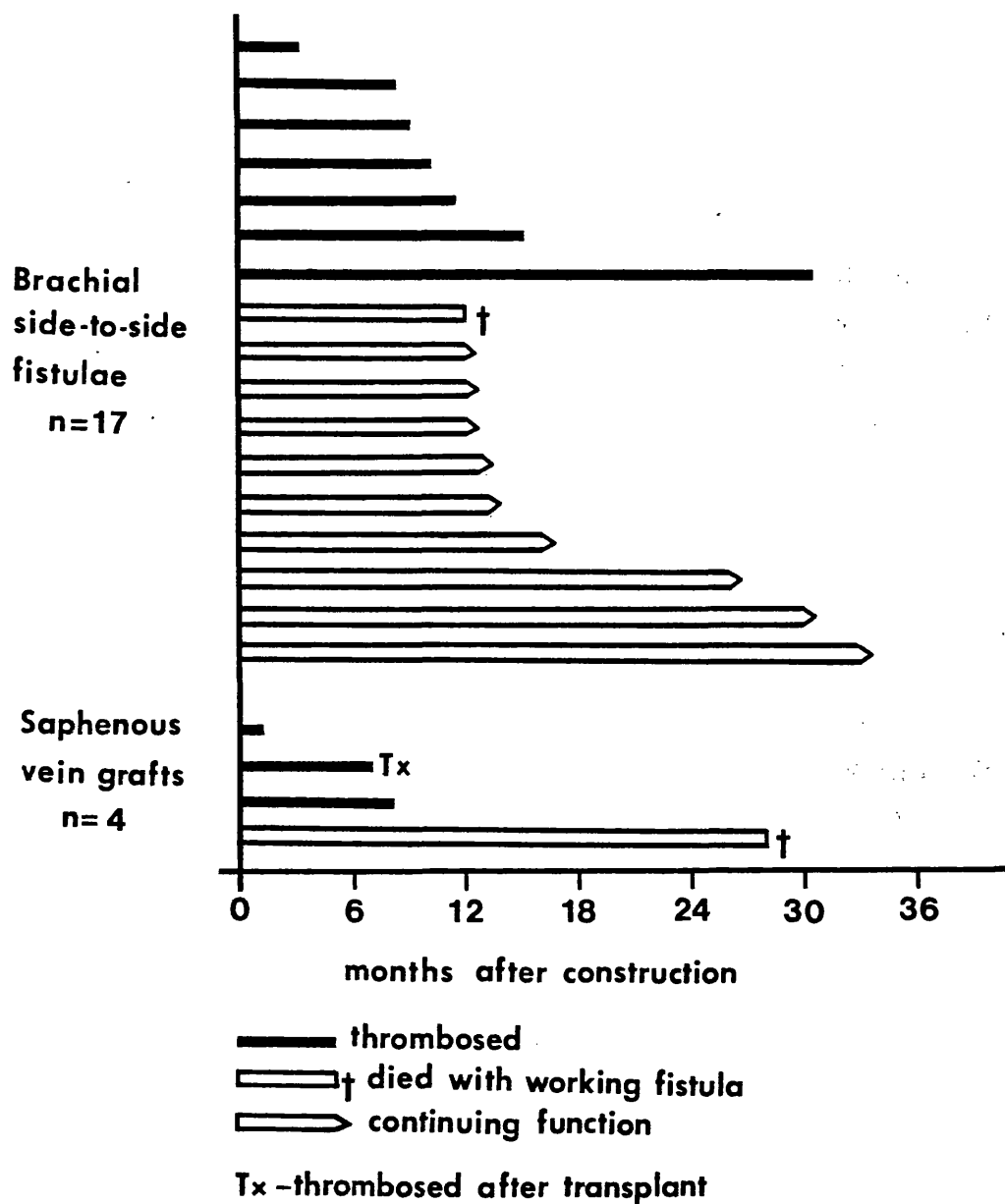


FIGURE 2.9

Secondary access procedures : patency rate of brachial fistulae and autologous saphenous vein graft fistulae.

TABLE 2.3

Present situation of problem group (n = 51).

Deaths

Dialysing on fistula	7
Dialysing on ankle shunt	2
On peritoneal dialysis	1

Dialysing satisfactorily

Wrist fistula	24
Brachial fistula	6
Ankle shunt	3

Not yet used

Brachial fistula	2
Wrist fistula	1

<u>Transferred with working fistula</u>	1
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<u>Functioning renal transplant</u>	4
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and no problem group (41%:40%). The mean age of either group also did not differ significantly (40.6 years : 39.6 years) (Figure 2.10).

Race

11 out of 19 Asian patients (58%) have had fistula problems compared with 40 out of 126 "Caucasian" patients (32%). This is significant ($\chi^2 = 4.9$, DF=1, $p \leq 0.05$) and perhaps reflects poorer nutritional state and smaller vessels in the Asian patients.

Predialysis Time

Excluding 16 failures before use for dialysis, the mean time before use of the fistula in the problem group was 1.0 months compared with a mean time of 1.25 months for the no problem group. This was not significantly different. At the time of review 12 patients had not yet started haemo-dialysis. Three were being maintained on CAPD but their fistulae were judged adequate to support haemo-dialysis.

Cause of Renal Failure

No significant differences existed between the proportions of patients suffering from glomerulonephritis, pyelonephritis or polycystic kidneys in the two groups. There was only one early onset diabetic, who had no thrombotic problems.

Smoking

Cigarette smoking could not be discerned as a significant influence since less than 15% of patients confessed to the habit and the proportion was not greater in the group with thrombotic problems.

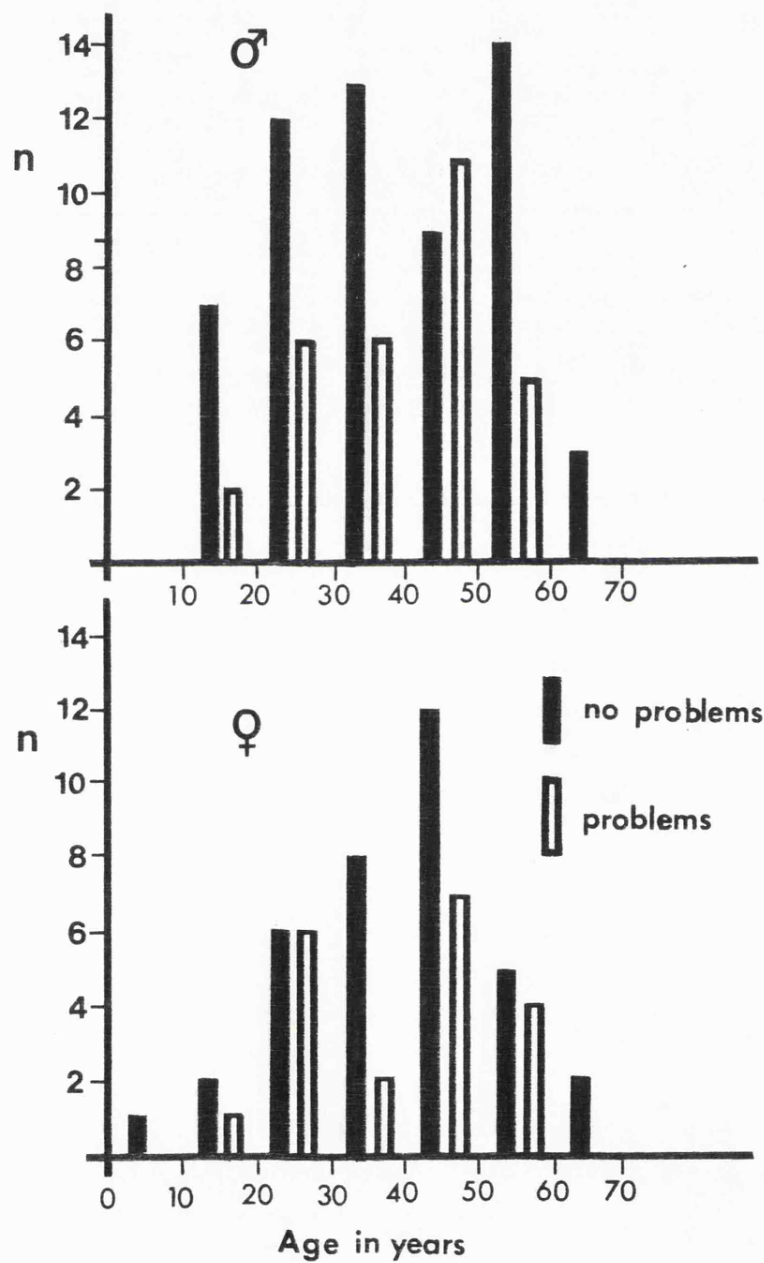


FIGURE 2.10

Age and sex distribution of fistula patients.

Infection

No first time fistula was lost due to infection.

Hypotension

It is difficult to be precise about the influence of hypotension, which has been defined in this study as an episode with a systolic pressure lower than 80mmHg lasting for more than 2 hours. The incidence of hypotension, particularly during dialysis, has not been studied. In 3 patients failure of the first or subsequent fistula was associated with significant hypotension, due to dialysis in one patient and colitis in two patients.

Operations

Two first or subsequent fistulae in this study thrombosed after operation for bilateral nephrectomy. Renal transplantation was a more potent cause of failure; 9 patients suffered thrombosis of a first or subsequent fistula after transplantation, 6 within a week of operation. In 4 no further access was necessary because of continued success of the transplant. 34 patients underwent transplantation without loss of their fistula. At the time of review, 10 of these patients had a functioning transplant and fistula, 19 were dialysing again on their first fistula, and 5 have died with working fistulae.

Technical Factors

At the time of construction of the 143 fistulae, measurements were made of the diameter of the anastomosis in 105 patients (72%), of vein diameter in 125 (86%), of arterial diameter in 119 (83%) and

of the suture material used (silk or Prolene) in 133 (93%). Overall comparison between groups with or without problems revealed 12/40 (30%) with problems to have small veins compared with 12/85 (14%) without problems ($p < 0.05$). Arterial diameter was also important - 15/38 (39%) of the problem group had small or fair-sized arteries ($\leq 2.9\text{mm}$) compared with 20/81 (25%) of the group without problems ($p < 0.05$). The suture material used had no significant effect; 27/46 (59%) problem patients had had 7/0 silk used, compared with 39/87 (45%) of patients without problems (NS). The mean anastomotic diameter (9.5mm) in the problem group was not significantly smaller than that of the no problem group (11mm).

In case influences had been obscured by including patients who developed problems after many months on dialysis, patients developing early problems (within 6 months of fistula construction) were compared with patients without problems (followed up for at least 7 months). This showed significant differences again in vessel sizes. 9/23 (39%) of problem patients had small veins ($\leq 2\text{mm}$ diameter) compared with 11/69 (16%) of patients without problems ($p < 0.01$). 11/23 (48%) of problem patients had small to fair-size arteries ($\leq 2.9\text{mm}$) compared with 11/65 (17%) of patients without problems ($p < 0.001$). Anastomosis diameter was the same (10mm) in both groups and the proportion of patients who had an anastomosis constructed with silk rather than Prolene was the same (52% : 50%).

In order to attempt to construct an index of likelihood of thrombotic problems from these prognostic factors, discriminant analysis was undertaken using the GLIM software package (Nelder, 1975)

on data from 70 patients in whom there was complete information on the following variables: age, sex, time before haemo-dialysis, vein diameter and arterial diameter. The programme assigns a probability for each individual variable of lying in the thrombotic or non-thrombotic group and then weights each value so that when the effects of the variables are added, maximum separation is achieved. If no overlap occurred it would be possible to predict from given data a very strong probability for an individual patient of subsequent thrombosis or not. In this instance such a separation was not possible and adding in age and sex did not improve the discrimination provided by using vessel size and pre-dialysis time (Figure 2.11).

DISCUSSION

The success rate of establishing arteriovenous fistulae at the wrist (89%) in an unselected population compares well with Kinnaert's experience of an 88-92% success rate in establishing a wrist fistulae in a partially selected population, where, in one-third of the series, patients were selected according to the calibre of their forearm vessels (Kinnaert et al., 1977). The patency rate of 80% at one year and 65% at 4 years also compares well with other series, and vindicates the policy of always attempting a wrist fistula first in new patients.

One purpose of the study was to examine the need for alternative access procedures; I have shown that in a population without previous operations four out of five patients will manage on a first or subsequent wrist fistula for at least four years.

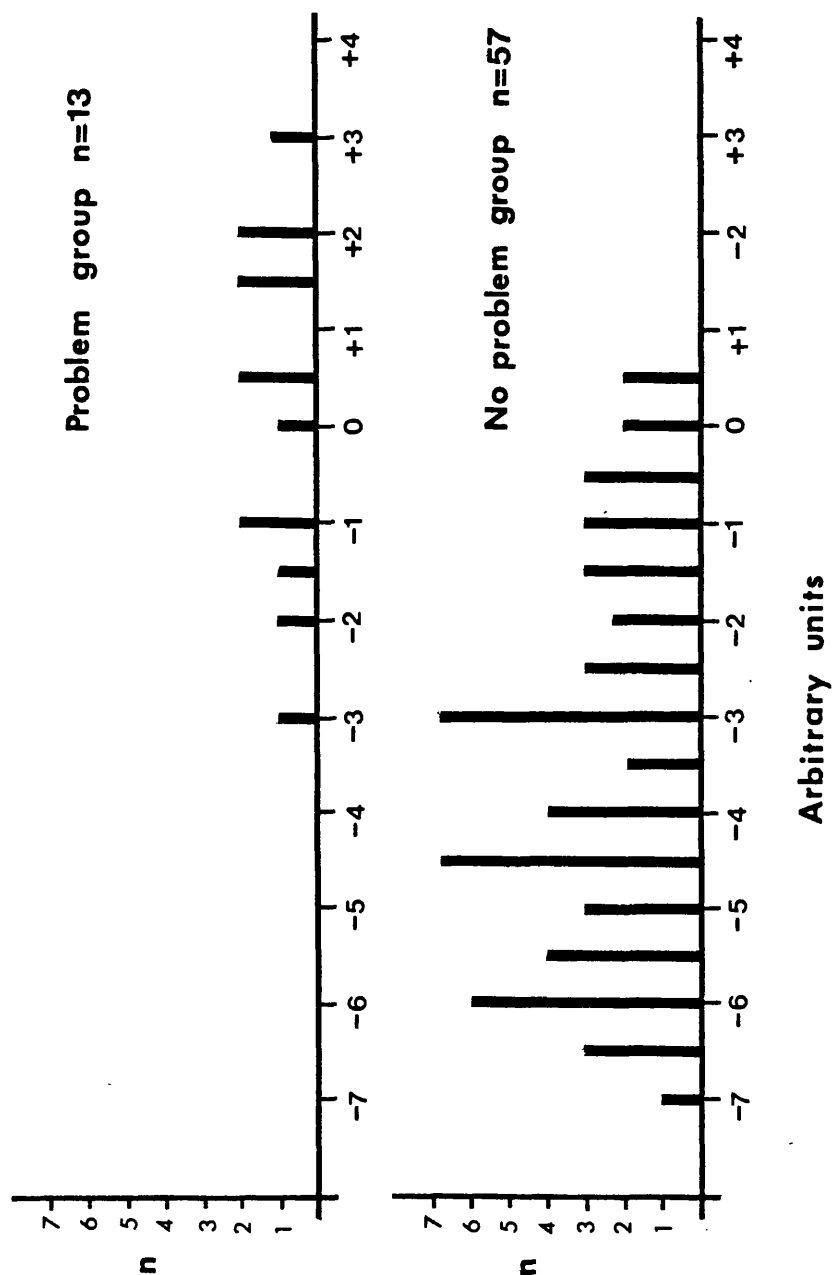


FIGURE 2.11

Discriminant analysis using age, sex, vessel size and predialysis time : failure to separate the two groups.

This of course depends on prior care of the forearm veins with avoidance of intravenous infusions in peripheral arm veins. In such a population the need for access via an implanted conduit has been shown to be very limited; only 4 autologous saphenous vein grafts became necessary in local patients in $5\frac{1}{2}$ years, and none since 1977. The policy for managing dialysis access changed in 1977 because of the problems with loop grafts, and the side-to-side brachial fistula was used more commonly; the 71% one-year patency for this procedure compares well with any other secondary access procedure. Apart from thrombosis there were no complications in the 17 patients except for one patient who developed neuroischaemia of the hand as a result of too large an anastomosis in the brachial artery. This recovered completely on taking down the fistula and constructing another brachial fistula in the other arm.

There is a clinical impression that some patients thrombose their fistulae more easily than others despite apparently adequate flow. While it is possible to explain away most failures as due to hypotension, operations, infection and technical error, in many patients fistulae are long-lasting despite small vessels, intercurrent operations or frequent hypotension on dialysis. An attempt has been made in this study to quantify various factors that might be expected to affect the success of fistulae. As expected, the size of vessels is important, which may reflect either the greater relative importance of technique with fine vessels, or that an absolute flow rate is necessary for the survival of fistulae. With the reported success of microvascular fistulae (Mansfield & Cooke, 1978) it may be that the former is crucial, and in fact many fistulae constructed from small

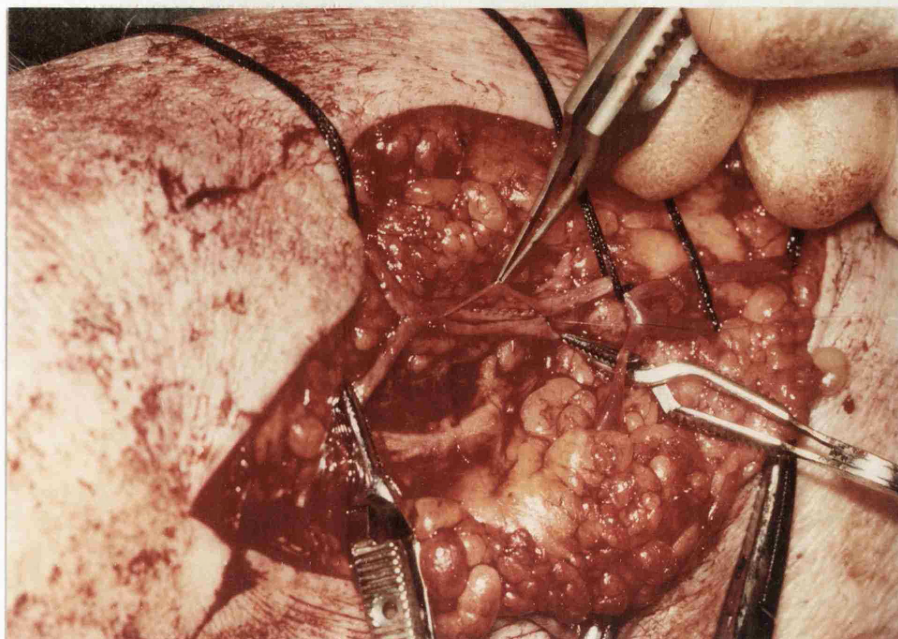


FIG. 2.12 Fistula construction at wrist using 2mm diameter vessels and 7/0 Prolene.

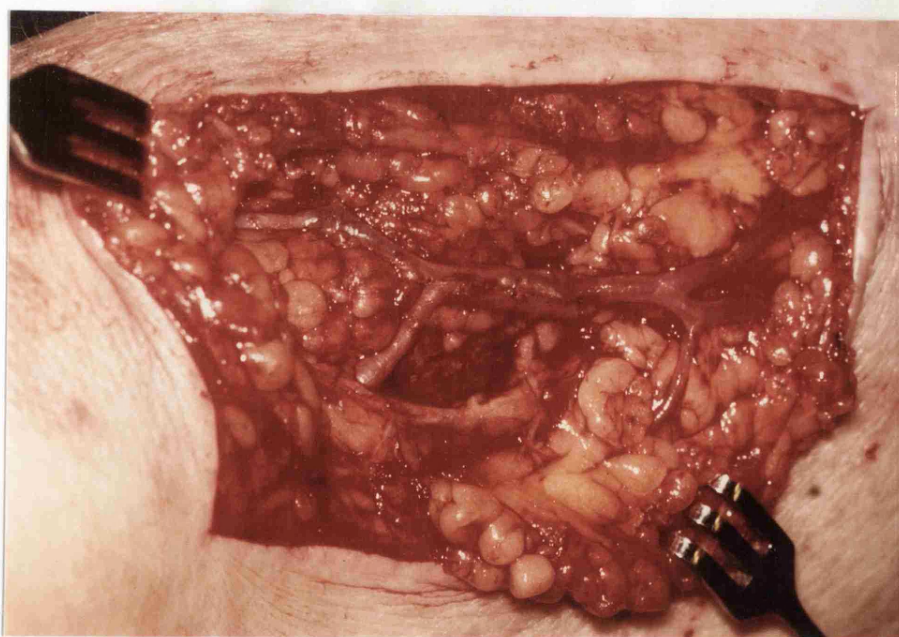


FIG. 2.13 Completed small vessel fistula (successfully used for dialysis). Note preservation of vein branches to maximise flow.

vessels of the size shown in Figures 2.12 - 2.13 in the present study were successful. It was not possible on discriminant analysis to separate without considerable overlap the patients who developed problems from those who did not, which suggests the presence of other influences, such as differences in blood coagulability between patients. The failure of fistulae after renal transplantation previously reported by Kinnaert (1977) has been confirmed in this study with thrombosis occurring in 20% of allografted patients. The cause of thrombosis remains unclear but may be due to the improvement in platelet function after transplantation. Another difference from Kinnaert's study (1977) is that no particular influence of age or sex on fistula failure has been shown; this is similar to Hammill's (1980) findings.

From the analysis of complications and their treatment, it is probable that greater numbers of fistulae could have been salvaged, in that only 3 fistulae out of 13 with late thrombosis underwent surgical exploration. Urgent referral and operation is now the norm. Again, the most common salvage procedure was thrombectomy alone but only in 3 of those resulted in greater than 3 months patency. Bone (1979) also found a poor success rate for thrombectomy alone, and found much better results on using per-operative angiography to display hidden thrombus or stenosis. In this centre thrombectomy alone is seldom attempted. Pre-operative angiography (Fig. 2.14) is now standard practice and thrombectomy is usually combined with an anatomical revision of the fistula, such as end-to-side conversion. A side-to-side fistula is the procedure of choice for vascular access in many units (Kinnaert, 1977; Giacchino, 1979) and many patients find the distal segment of vein invaluable as a needling site when they are on home dialysis. The side-to-side configuration has sometimes been criticised because of

the risks of venous hypertension of the hand but in this series the incidence of this complication was only 4.4%. It is now clear that hyperaemia of the hand indicates the development of proximal vein stenosis which is amenable to corrective surgery.



FIG. 2.14 Fistulogram of 5 year old fistula showing thrombus (arrowed) in upper forearm venous run-off.

the risks of venous hypertension of the hand but in this series the incidence of this complication was only 4.4%. It is now clear that hyperaemia of the hand indicates the development of proximal vein stenosis which is amenable to corrective surgery.

CHAPTER 3

CHAPTER 3

CONTROL STUDIES

3.1 VISCOSITY

In this study the instrument used was the Wells-Brookfield cone and plate microviscometer (Wells, Denton & Merrill, 1961) (Fig.3.1). This consists of a truncated flat cone which is rotated against a plate warmed to 37°C. The torque at each shear rate is read from the dial and a conversion factor gives the viscosity. Five shear rates were used between 11.5 and 230 sec⁻¹. At lower shear rates the measurement becomes relatively inaccurate, the error of the instrument according to the manufacturers being 1% of full scale deflection.

It is difficult to decide what shear rates actually exist in vivo, since the rate varies across the width of the vessel. In arteries it will be high (except near the centre of the lumen), of the order of 1000 sec⁻¹. In capillaries, where the flow is intermittent, the shear rate must often be zero or near zero. It was felt that the shear rates used would probably reflect the range of flows in fistulae.

The Wells-Brookfield viscometer has been used in a number of studies; in patients with Raynaud's phenomenon (Jahnsen, 1977); intermittent claudication (Dormandy, 1973, Di Perri, 1978); in determining the effects of fibrinogen (Blattler, 1979); in studying the effects of operation on viscosity and fibrinogen levels (Harvey Kemble, 1972). It has proved a simple and reliable instrument except at very low shear rates. The coefficient of variation on 12 successive samples of the same blood in Begg's study

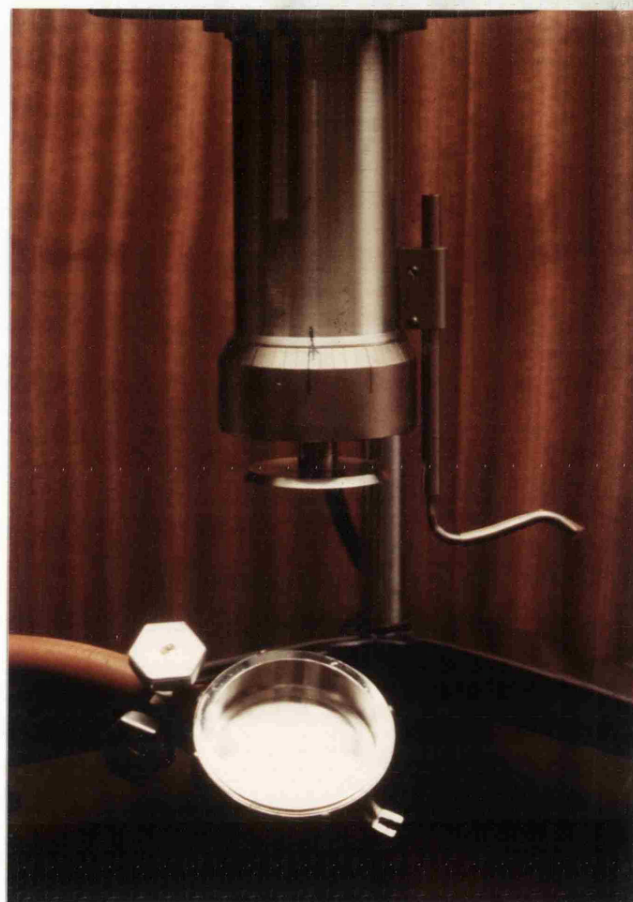


FIG. 3.1 Wells-Brookfield micro-viscometer showing flattened cone which rotates against the warmed cup (below) in which the 1ml blood sample is placed.

was 7.12% at shear rate $5.75s^{-1}$ and 1.52% at shear rate $230s^{-1}$ (Begg, 1966).

Initially the use of the viscometer was evaluated in a control group.

Subjects

22 fit volunteers from the hospital staff or otherwise fit patients admitted for minor procedures such as hernia repair were studied. There were 12 females and 10 males aged 17-70 (mean 39). Five were smokers.

Methods

2ml blood samples were taken in the morning, anticoagulated with EDTA and assayed within 1 hour of venesection. Blood was taken and viscometry performed by the same observer.

Results

Whole blood viscosity was recorded at 5 shear rates. The highest ($230s^{-1}$) and lowest ($11.5s^{-1}$) are here reported. Mean haematocrit was 43.4 ± 3.9 . Mean high shear viscosity was 4.1 ± 0.5 cps, mean low shear viscosity 7.9 ± 1.2 cps. Log. viscosity correlated with haematocrit at high shear rate ($r=0.66$, $p < 0.001$) and low shear rate ($r=0.44$, $p < 0.05$) (Fig.3.2).

Discussion

The close correlation of log viscosity with haematocrit is known (Begg, 1966), and has been used to "correct" viscosity to a standard haematocrit to enable comparisons between patients to be made, and reveal the effect of other influences on viscosity.

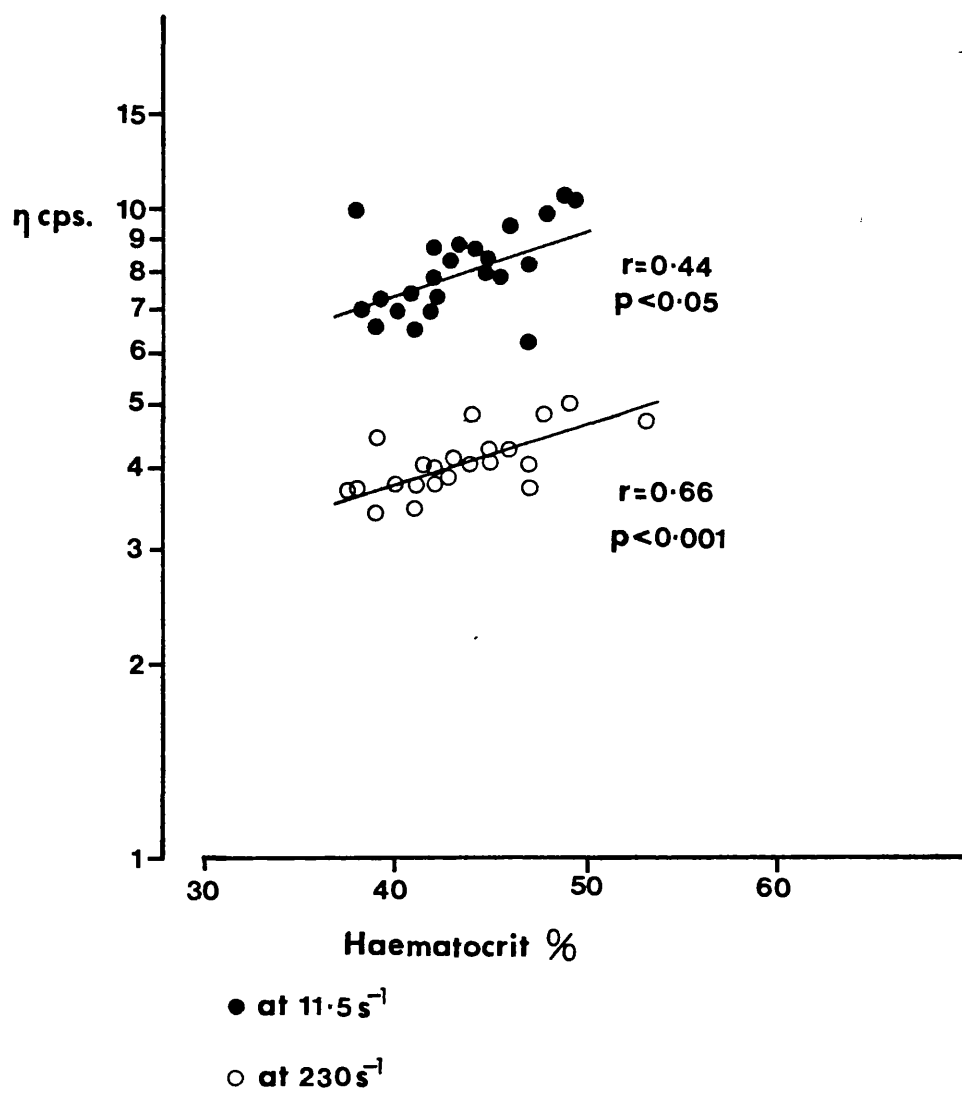


FIG. 3.2

Relationship between whole blood viscosity (η) and haematocrit in controls. Semi-logarithmic plot.

The mathematical validity of this technique is dubious; it assumes that the viscosity of the individual would follow a line parallel to the regression line for the population if its haematocrit were adjusted, i.e. that the factor causing the individual to depart from perfect correlation behaves in a linear manner. For this reason only absolute viscosities have been measured in the patient study, in order to detect any prognostic value for the individual.

3.2 RED CELL DEFORMABILITY, PLASMA VISCOSITY AND FIBRINOGEN

A filtration technique slightly modified from Dodds (1979) for the measurement of red cell deformability was used for the following reasons. Filtration through $5\ \mu$ pores more nearly reflects the condition in the microcirculation than centrifuge packing rate methods or methods involving shear stress in high viscosity media. Also, it is likely that deformation of single cells through orifices is qualitatively different from shear deformation in bulk red cell flow.

Red cells were resuspended in autologous pre-filtered plasma to allow them their native environment (because adsorbed proteins may well affect red cell deformability) without the influence of protein precipitates and platelet aggregates. Techniques using washed red cells resuspended in artificial media may well be measuring membrane flexibility, but artefacts are more likely. Allowing the 1 ml suspension to pass through the filter under its own hydrostatic pressure will probably prevent unphysiological shear rates at the pore orifices.

A major problem with filtration techniques is that the influence of plasma viscosity on the rate of flow is unknown (though a priori a high plasma viscosity would seem to decrease flow), and there is also the possibility of fibrinogen-dependent aggregation of red cells delaying filtration. It has been suggested (Schmid-Schonbein, 1973) that this can be overcome by filtering plasma in parallel with the red cell suspension, and dividing the rate of flow of the suspension by that of the plasma. This is only mathematically sound if the relationship between suspension filtration rate and

plasma viscosity is known; this would require resuspending red cells in plasma of different viscosities.

This gives rise to several problems; pathological cells may have a different relationship with plasma viscosity; the equilibration time for red cells with differing protein concentrations is not known and red cells become significantly less filtrable two hours after venepuncture; different protein concentrations or plasma tonicities may cause osmotic or other effects on the red cell. Because of these difficulties no correction for plasma viscosity was made in this study, although the plasma viscosity was measured to investigate any correlation. Normal plasma on its own passes rapidly through the filter, in about 20% of the time taken for half the red cell suspension to pass, which suggests the limiting factor to be the red cell. Pathological plasma may have a more significant effect.

The technique was initially evaluated in a control population to determine the variability and reproducibility of the test and the effects of plasma viscosity and fibrinogen.

Subjects

The control population consisted of volunteers on no drugs drawn from hospital staff, or otherwise fit surgical patients admitted for minor procedures such as hernia repair. There were 40 males and 40 females aged 17-88.

Method

10ml venous blood samples were taken into EDTA containers for red cell filtration estimation (Appendix II) at the same time as 2ml samples into EDTA for plasma viscosity

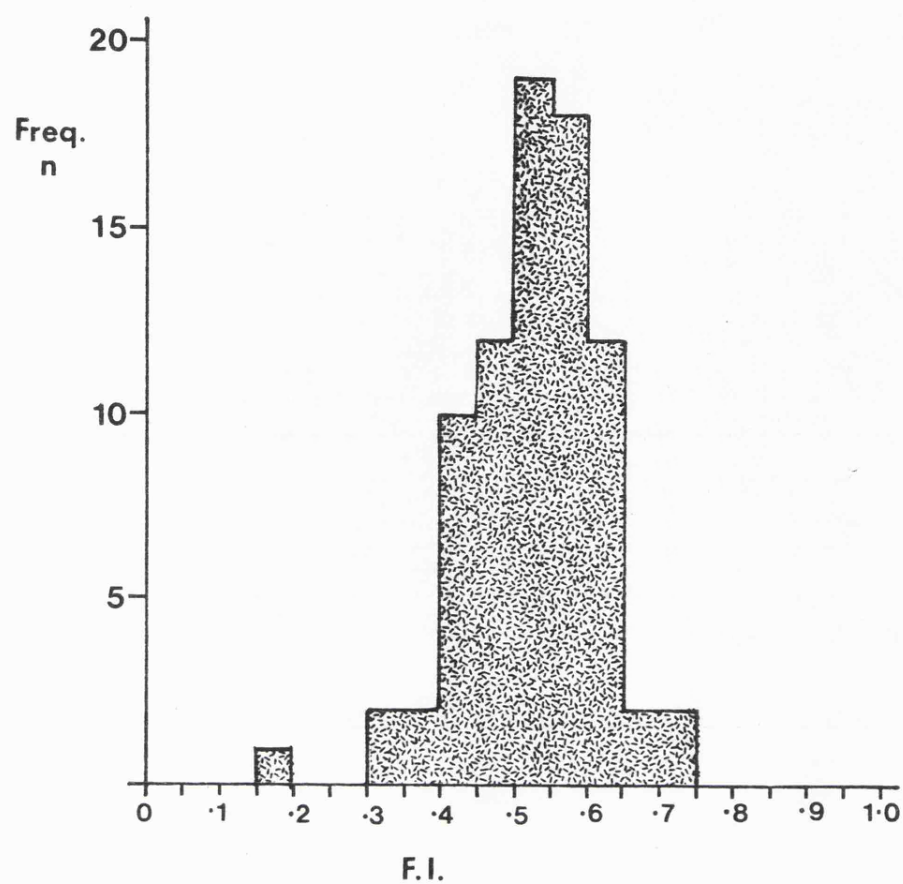


FIG. 3.3

Distribution of red cell filterability (FI) amongst a healthy population.

estimations (Appendix III) and 9ml citrated samples for fibrinogen assay (Appendix IV).

Results - Normal Population

For the 80 controls the filtration index was normally distributed with a mean of 0.53 ± 0.09 (Fig.3.3).

(i) Influence of age and sex

There was no significant difference between the mean filtration index of the males (0.53 ± 0.07) and of the females (0.54 ± 0.07) in the control group, nor did age appear to exert an effect (Fig.3.4).

(ii) Diurnal variation

Blood from 22 males and 22 females was tested at 9am when fasting and then again at 3pm. Filtration index for females was 0.52 ± 0.06 morning and afternoon. Filtration index for males rose from 0.48 ± 0.06 to 0.51 ± 0.08 , although this rise was not significant. Non-fasting specimens were also obtained for seven females and six males at 9am and 3pm. Filtration index rose in both groups from 0.50 ± 0.03 to 0.52 ± 0.05 in the females and 0.47 ± 0.05 to 0.52 ± 0.06 in the males although again these differences were not statistically significant.

(iii) Smoking

Mean filtration index for 25 smokers (mean age 41) was 0.52 ± 0.1 , and for 55 non-smokers 0.53 ± 0.07 (mean age 41.5) (Table 3.1).

(iv) Plasma viscosity and fibrinogen

36 controls had a mean plasma viscosity of 1.60 ± 0.07 cps; 25 of these controls also had plasma fibrinogen estimated with a mean value of 2.9 ± 0.6 g/L. No correlation between filtration and either

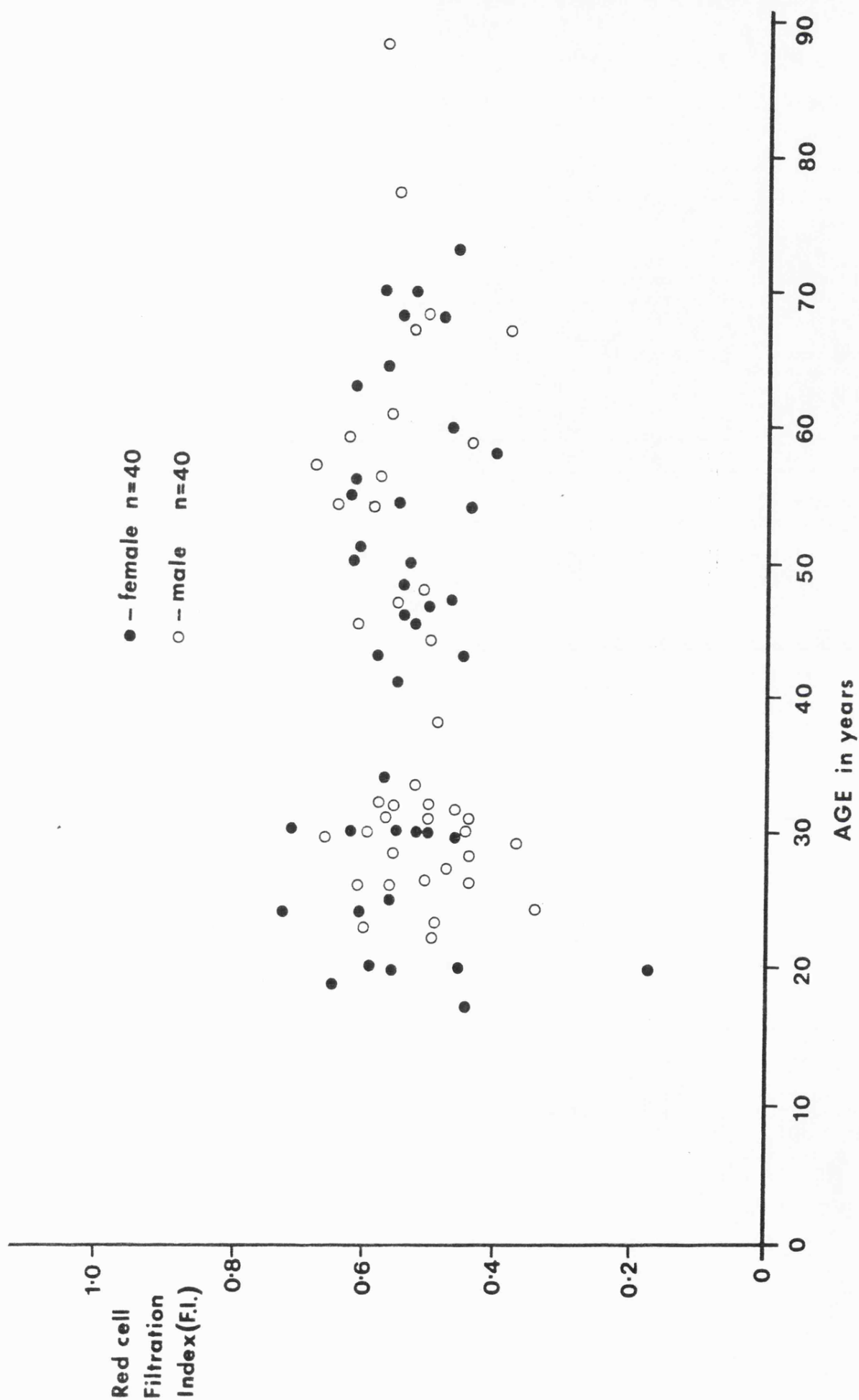


FIG. 3.4

Age and sex distribution of red cell filterability in a normal population.

TABLE 3.1

Filtration index in male and female smokers and non-smokers.

Numbers in sub-groups are given in brackets.

	<u>FEMALE</u>		<u>MALE</u>	
	SMOKERS (15)	NON-SMOKERS (25)	SMOKERS (10)	NON-SMOKERS (30)
Age	42± 17	41± 17	38.5± 15	42± 18
F.I.	0.54±0.12	0.54±0.08	0.52±0.09	0.52±0.07
Plasma Viscosity cps	1.61±0.08(8)	1.61±0.07(15)	1.53±0.12(3)	1.59±0.05(10)
Fibrinogen g/L	3.1 ±0.7 (5)	2.8 ±0.4 (9)	3.0 ±1.0 (4)	2.8 ±0.6 (7)

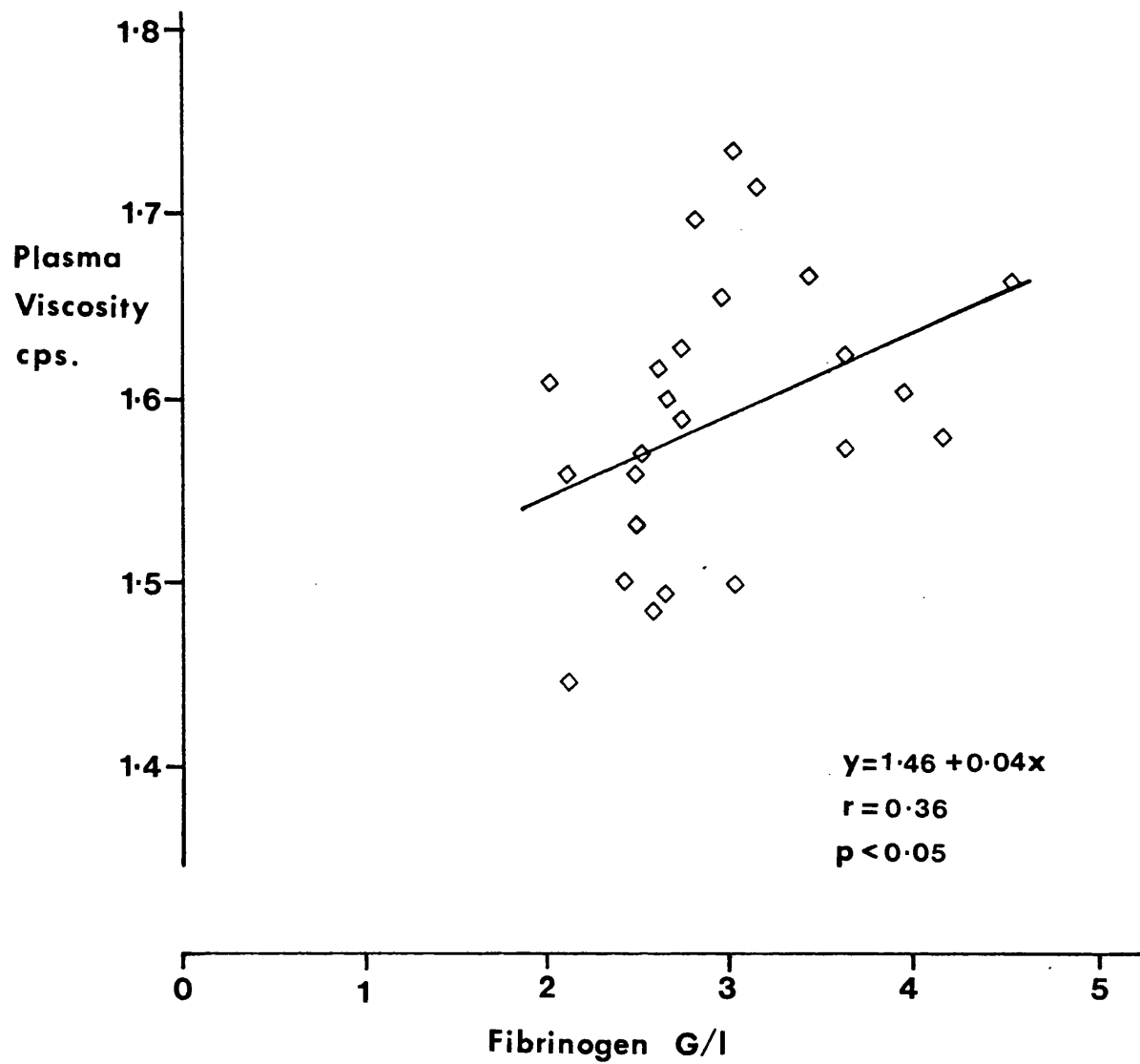


FIG. 3.5

Plasma viscosity and fibrinogen in controls.

viscosity or fibrinogen was found, although plasma viscosity correlated with fibrinogen as expected (Fig 3.5).

Discussion

There is a shortage of studies of normal controls using this type of filtration technique. Dodds (1979) reports a mean deformability index of 0.63 ± 0.2 using a technique similar except for a 60 second rather than 45 second filtration period. The index of 0.53 ± 0.09 in this study shows rather less scatter. Some influence of time of day is apparent, although not reaching statistical significance, so that studies on patient groups were all performed in the non-fasting state in the morning. Although smoking did not appear to exert an effect, when groups are analysed it is apparent that there are not enough numbers of the male smokers to draw firm conclusions. No influence of plasma viscosity or fibrinogen on red cell filtration was detected. This may be because a narrow range of viscosity occurs amongst normals; it does not exclude higher levels of fibrinogen or other plasma proteins having a significant effect in pathological groups. These values were accordingly measured in renal patients to assess whether delayed filterability previously reported was a consequence, or independent of, changes in plasma viscosity or fibrinogen.

3.3 FIBRINOLYTIC ACTIVITY

The assay introduced by Moroz & Gilmore (1975) overcomes some of the objections to the ECLT, DBCLT and fibrin plate assays in that the whole blood is only diluted with an equal quantity of buffer, and a physiological pH of 7.4 is used. The spontaneous, rather than stimulated, fibrinolytic activity of whole blood is estimated by incubation in a I^{125} fibrin coated polystyrene tube and the release of radioactivity measured. This allows detection of fibrinolytic activity mediated by cell systems as well as plasmin. Leucocytes were shown to be sources of fibrinolytic activity, which could be enhanced by aspirin, using this method (Moroz & Gilmore, 1976). A modification of this technique introduced by Burden (1981) was used in a study of deep venous thrombosis (DVT) in surgical patients and the pattern of activity was found to correlate well with the onset of DVT (Reilly, 1980). However, only one patient of the 28 studied had extremely low fibrinolytic activity and went on to suffer DVT. The method is of proven sensitivity, detecting plasmin concentrations as low as 0.2 $\mu\text{g/ml}$ (Burden, 1981), and with wider studies it might be possible to detect a subgroup of patients at particular risk from thrombosis. For these reasons, fibrinolytic activity was measured in fistula patients using the fibrin matrix test.

Control values for 89 patients studied by Reilly & Burden (1980) gave a mean fibrinolytic activity of 180 ng fibrin lysed/ml/hr. In the present study further controls were examined.

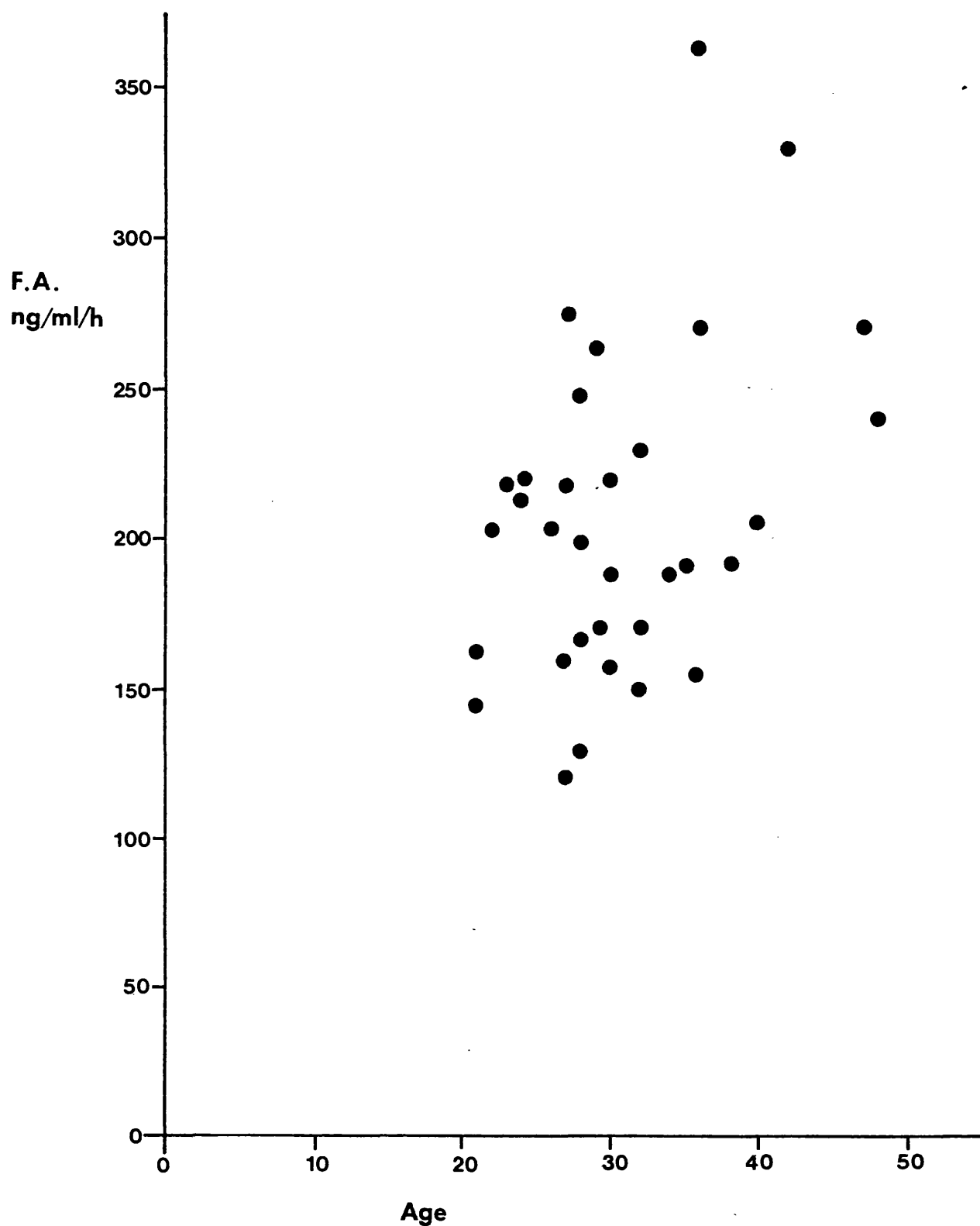


FIG. 3.6

Fibrinolytic activity (FA) in ng fibrin lysed/ml/h and age in controls.

Subjects

33 healthy subjects from amongst the hospital staff, non-smokers on no drugs, formed the control group. There were 20 males and 13 females with a mean age of 30.8 ± 6.8 (range 21-48).

Methods

Blood was taken in the morning, put on ice and assayed within 4 hours (see Appendix V). Tubes from two different batches were also tested using serum stored at -20°C from one subject as a control. Blood was always taken and tested by the same observer.

Results - Controls

Mean fibrinolytic activity was 207 ± 55 ng fibrin lysed/ml/hr. No correlation with age on this small sample was shown (Fig. 3.6).

Evaluation of Assay

The differences in fibrin lysed in two different batches are shown in Table 3.2. Batch 2 shows a greater activity by a factor of 1.4, and also a lower coefficient of variability for activity in serum, i.e. 9% compared with 24%. No significant change in activity with age of assay tubes was found up to an age of two months, when activity started to fall off. No correlation was found between absolute counts (DPM) of the assay tubes and the value of fibrinolytic activity assayed.

Discussion

The control values were similar to previous studies but there was greater variability between batches. This may have represented variability in the biological activity or purity of the

TABLE 3.2

Comparison of batches. Each estimation in quadruplicate.

Fibrinolytic activity in ng fibrin lysed/ml/hr

	Batch 1	Batch 2
Buffer blanks	48.6 $\pm$ 6.7 (n=9)	47.25 $\pm$ 10.5 (n=12)
Stored serum	225 $\pm$ 53 (n=11)	319 $\pm$ 30 (n=12)

fibrinogen used, or may have reflected differences between polystyrene tubes used.

In view of the differences between batches, the stored serum was used to provide a standard to convert all subsequent results to those of the first batch for comparability.

3.4 PLATELET AGGREGATION

Platelets aggregate with such rapidity in vivo that the process is extremely difficult to follow, but in vitro studies induce large artefacts. In vivo, platelets are in equilibrium with the endothelium, which produces the disaggregatory prostaglandin, prostacyclin (Moncada, 1977). This is unstable, with a half-life of 2-3 minutes (Moncada, 1978), so that in vitro studies measure the reactivity of platelets with decreasing amounts of this important biological inhibitor present, and with activity altered by a far lower than physiological calcium ion concentration, and with the platelet changing in activity as it becomes acidotic. Cooling platelets further damages them, so that platelet rich plasma is best stored covered at room temperature, for as short a period as is practicable. If further dissection of individual platelet function is required, density gradient fractionation is performed, but in this study overall 'aggregability' was measured. The relative importance in vivo of the various physiological aggregating agents, which include collagen, thrombin, adenosine diphosphate (ADP), serotonin and thromboxane A₂, is unknown. It has been suggested that ADP from erythrocytes is of importance (Born, 1976) and it has been shown that concentrations likely to be achieved in vivo are sufficient to cause aggregation (Aursnes, 1981). Larger quantities of ADP are needed in vitro because of the inhibitory effect of citrate. In this study ADP has been used as the aggregatory stimulant. The major drawback of measuring one aspect of platelet behaviour without measuring endothelial activity is recognised.

The principles of the turbidometric assay of platelet aggregation were described by Born (1962). As platelets in platelet rich plasma aggregate, the optical density decreases; a beam of light passing through such a sample will produce an e.m.f. on striking a photo-electric cell. This impulse can be amplified and thus the pattern of aggregation displayed on a chart recorder. This allows the investigation of the extent and rate of aggregation in response to various stimuli, and the threshold dose of stimulus to be determined, above which irreversible aggregation occurs. ADP can induce aggregation with, or without, the production of thromboxanes via a release reaction. Reversible aggregation releases no thromboxane but neither does monophasic irreversible aggregation. Only the biphasic response has been shown to correlate with the production of thromboxane A₂, rapidly degraded to thromboxane B₂ (Koh, 1980).

Some of the problems with assessing the response of platelets in such an in vitro system are that not only may the platelets be unrepresentative of a more active in vivo population but also they will be altered in activity by citrate concentration and haematocrit. The higher the haematocrit, the smaller the volume of platelet rich plasma and the greater the concentration of platelets. Since the concentration of platelets is likely to affect the degree of aggregation for physical reasons, many investigators dilute the platelet rich plasma (PRP) with platelet poor plasma (PPP) if the platelet count is above 300,000. This does not correct for the opposite effect of haematocrit on citrate concentration; one reason for apparently more reactive platelets could be a lower haematocrit and thus a greater dilution of citrate by the plasma. This has been

shown to explain the apparently greater platelet reactivity of women compared with men (Kelton, 1980).

In this study it was not possible to perform platelet counts on PRP. Whole blood platelet counts were recorded to allow later correlation, and the effect of 50% dilution of PRP on ADP threshold and extent of aggregation was studied. It was felt that since the test was being used to see whether it held predictive value for the individual the absolute rather than the adjusted aggregability was important; the purpose was not so much to draw comparisons with normal controls, but to compare renal patients developing thrombosis with other renal patients. The technique was initially evaluated in control studies.

Studies on Controls

Subjects

19 healthy volunteers on no drugs were selected from amongst the hospital staff. 12 patients with peripheral vascular disease were also studied.

Methods (see Appendix VI)

14 subjects had paired studies performed at 9am and 3pm to investigate diurnal variation. 5 further subjects were tested in the morning only, one of whom was also tested on 7 other occasions to determine day to day variability. 12 subjects with peripheral vascular disease had studies on both undiluted and diluted platelet rich plasma. The platelet aggregation studies were completed within 2 hours of venesection. Aggregation extent and ADP threshold in $\mu\text{mol/L}$ were recorded. The platelet count of whole blood was measured by counting in a Neubauer chamber with a light

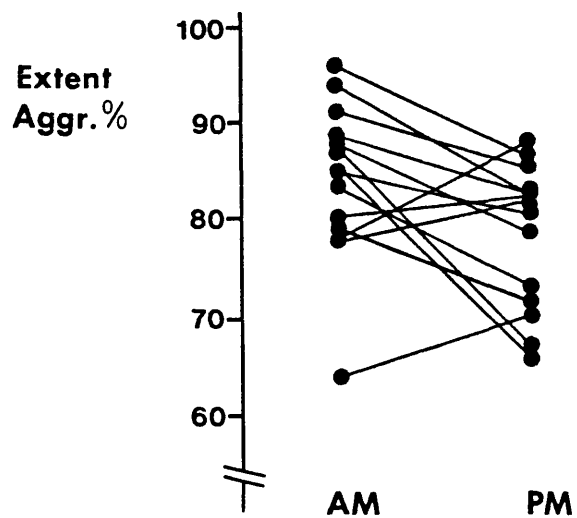


FIG. 3.7

Extent of platelet aggregation : diurnal variation amongst controls.

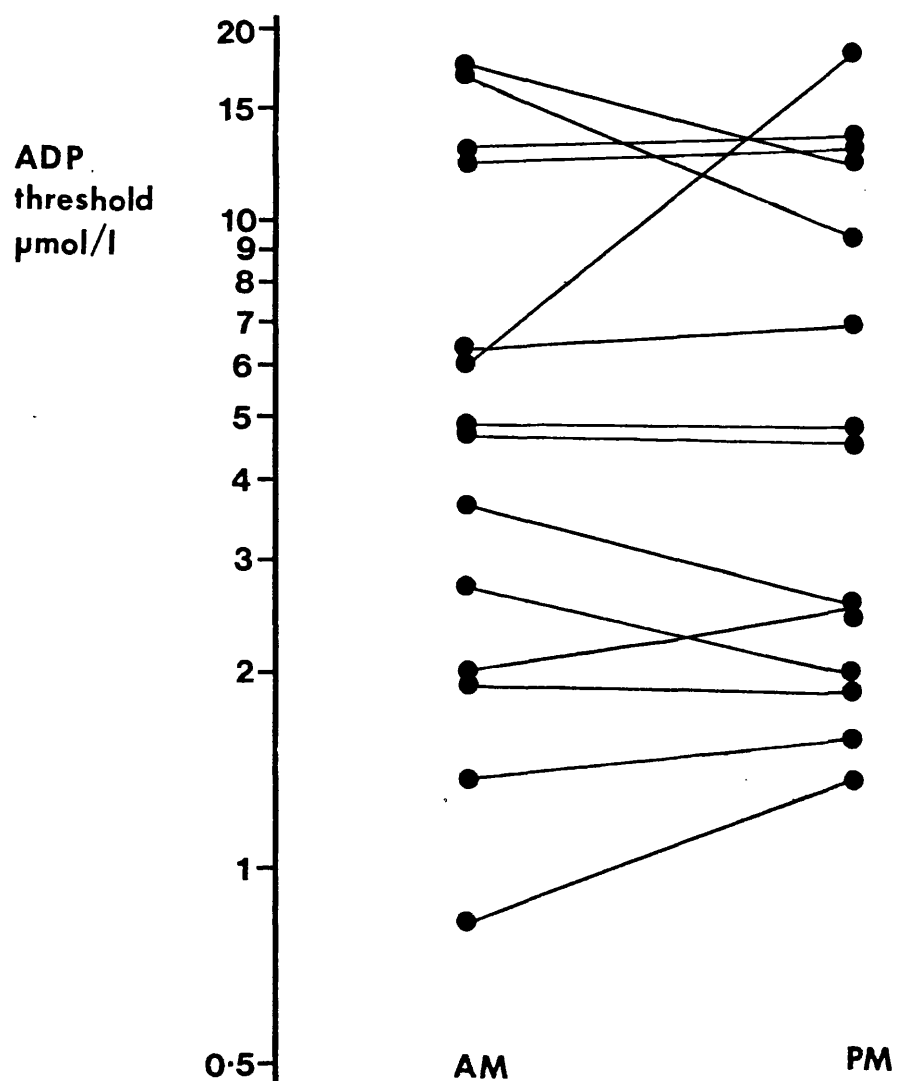


FIG. 3.8

ADP threshold : diurnal variation amongst controls (logarithmic scale)

microscope. Platelet count and aggregation extent were analysed using paired 't' tests. ADP thresholds being determined between limits were analysed using non-parametric tests. Blood was always taken and assayed by the same observer.

Results - Paired studies (14 subjects)

The mean extent of aggregation to 10 μ molar ADP was $78.4 \pm 7.4\%$ in the afternoon, compared with $84.4 \pm 8.1\%$ in the morning ($p < 0.05$ paired 't' test) (Fig.3.7). Mean platelet count was 186 ± 44 platelets/ 10^{-9} L at 3pm compared with 195 ± 65 at 9am (not significant). ADP thresholds did not differ significantly from morning to afternoon (Fig.3.8).

Results - Unpaired studies (32 observations on 19 subjects)

Extent of aggregation correlated positively with platelet count ($r=0.63$, $p < 0.001$) (Fig.3.9). ADP threshold correlated negatively with platelet count (Kendall's $\tau = -0.35$, $p < 0.05$).

Within person variability

The variability of platelet count aggregation extent and ADP threshold in one subject over a four month period is shown in Table 3.3. Mean and coefficient of variation for ADP threshold is approximate and for illustration only.

Effect of dilution of PRP

The extent of aggregation fell significantly from 77 ± 14 to 68 ± 18 (paired 't' test, $p < 0.05$) on diluting the PRP. Although there was a tendency for the ADP threshold to rise, this was not invariable, and tended to occur with the lower platelet counts. On these numbers the threshold is not significantly raised after dilution. (Table 3.4).

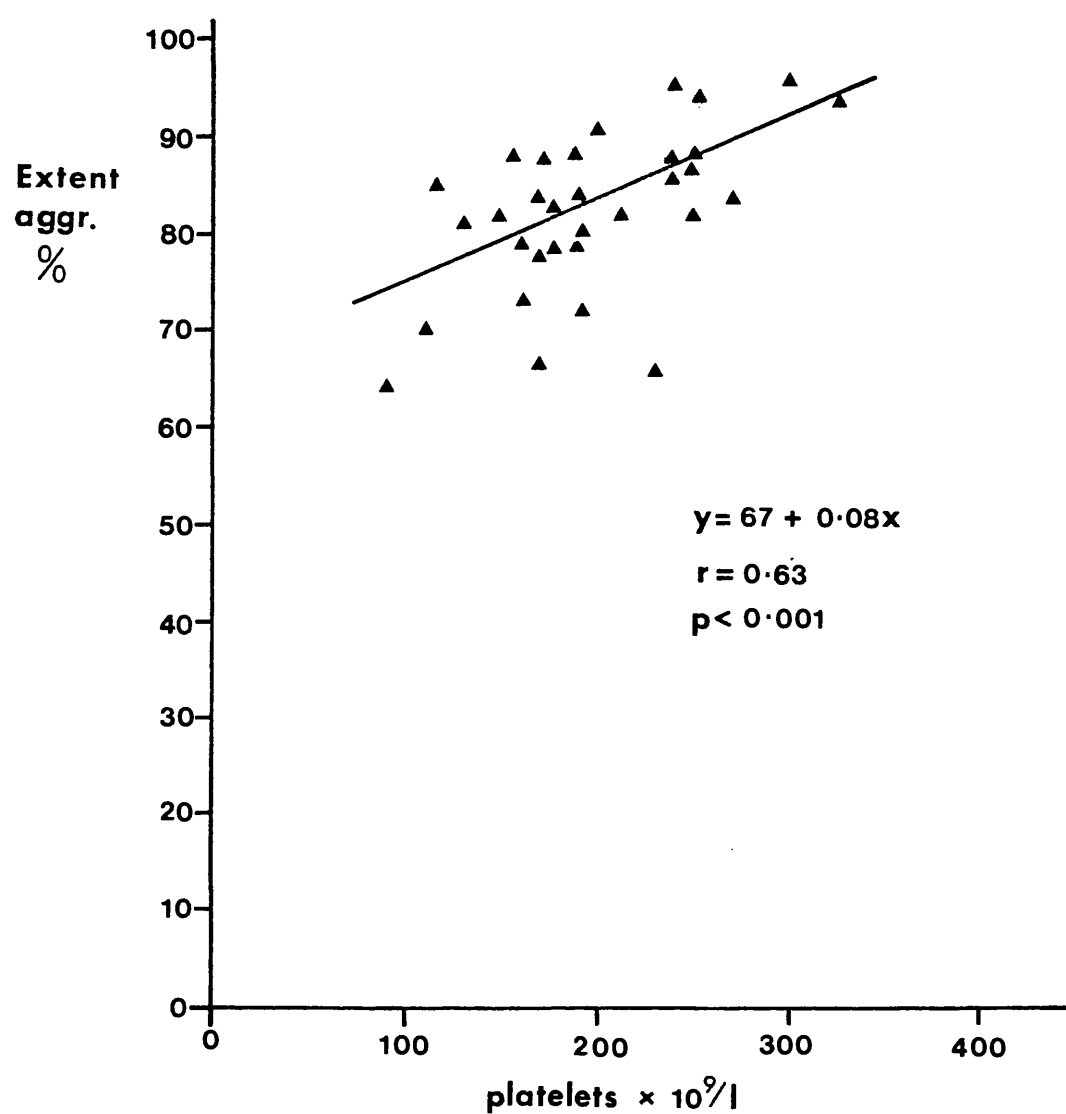


FIG. 3.9

Extent of platelet aggregation and whole blood platelet count in controls.

TABLE 3.3

Variability over four months in one subject.

	Aggregation Extent (%)	Threshold (μ mol/L ADP)	Platelet Count/ 10^{-9} L	Platelet Adhesion (%)
14.3.80	79	1.5	-	-
16.4.80 am	94	4.5	325	57
pm	82	4.5	251	44
15.7.80	87	0.8	279	32
16.7.80	91	0.8	246	41
17.7.80 am	87	0.8	227	36
pm	83	1.3	197	15
18.7.80	95	3.3	254	-
Mean $\pm$ SD	87.3 $\pm$ 5.7	2.2 $\pm$ 1.6	254 $\pm$ 40	37.5 $\pm$ 14
Coefficient of Variation (%)	6.6	75	16	37

TABLE 3.4

Platelet Aggregation : influence of dilution of PRP 50:50 with PPP.

SUBJECT	PLATELETSx10 ⁹ /L	AGGREGATION EXTENT %		ADP THRESHOLD μ mol/L	
	OF WHOLE BLOOD	UNDILUTED	DILUTED	UNDILUTED	DILUTED
1	279	55	64	1.75-2.0	1.75-2.0
2	268	76	55	5.5 -6.0	5.5 -6.0
3	232	51	45	1.25-1.5	2.0 -2.25
4	188	98	99	0.75-1.0	1.0 -2.0
5	226	78	55	2.0 -2.25	-----
6	283	79	70	2.0 -2.25	1.75-2.0
7	286	93	79	1.0 -1.25	1.5 -2.0
8	262	82	82	0.5 -0.75	0.75-1.0
9	179	74	52	1.0 -1.25	2.0 -2.25
10	131	62	50	3.0 -3.25	3.0 -3.25
11	272	89	66	3.25-3.5	3.25-3.5
12	328	82	96	1.25-1.5	1.5 -2.0
Mean	236 $\pm$ 58	76.6	67.8	2.1	2.4

3.5 PLATELET ADHESION

A modification of the method of Hellem (1960) was used to assess the degree of retention of platelets by a glass bead column. The test has particular sensitivity to hyperadhesive states due to reduction in the surface area of the glass beads (Adeplat T, Immuno Ltd). It is therefore not possible to measure hypoadhesive states accurately with this system. Lowered adhesion has been shown in bleeding orders such as von Willebrand's disease (Hellem, 1970); raised adhesion has been shown in thrombotic disorders by a glass-wool technique (Moolten, 1949) and a rolling-tube technique (Bicher, 1974) but whether the tests have any predictive value for thrombotic events, or measure response to thrombosis, is unclear.

The test was initially assessed by studying normal controls.

Subjects

14 healthy volunteers on no drugs from amongst the hospital staff.

Methods

Paired studies were performed at 9am and 3pm simultaneously with the aggregation studies. Platelet adhesion was assessed by the Adeplat T test (see Appendix VII). One subject had adhesion measured on 6 occasions to assess within person variability. Blood was always taken and passed through the glass bead column by the same observer. Platelet counts were performed by a technician.

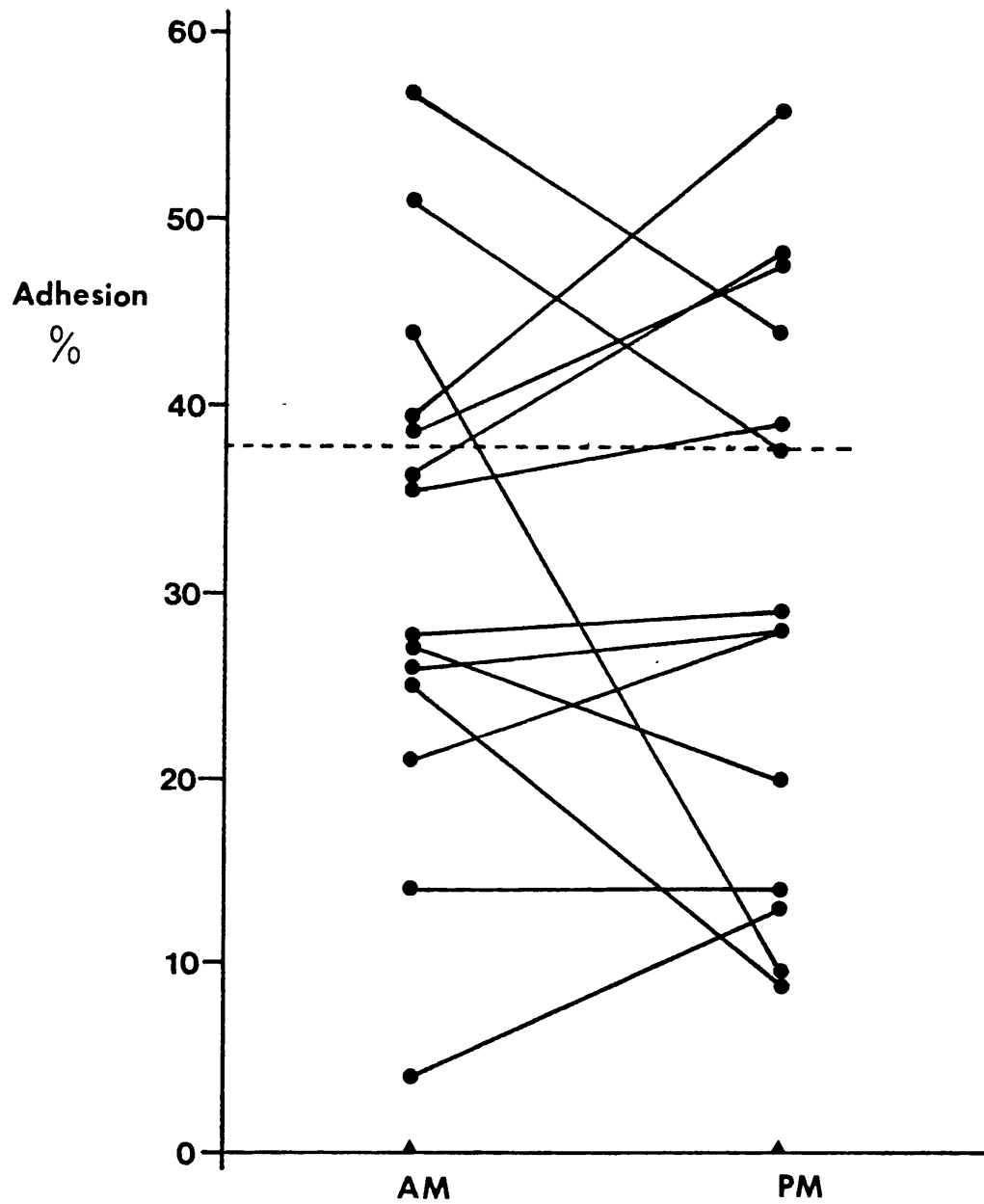


FIG. 3.10

Platelet adhesiveness - diurnal variation in controls. Dotted line represents suggested upper limit of normal.

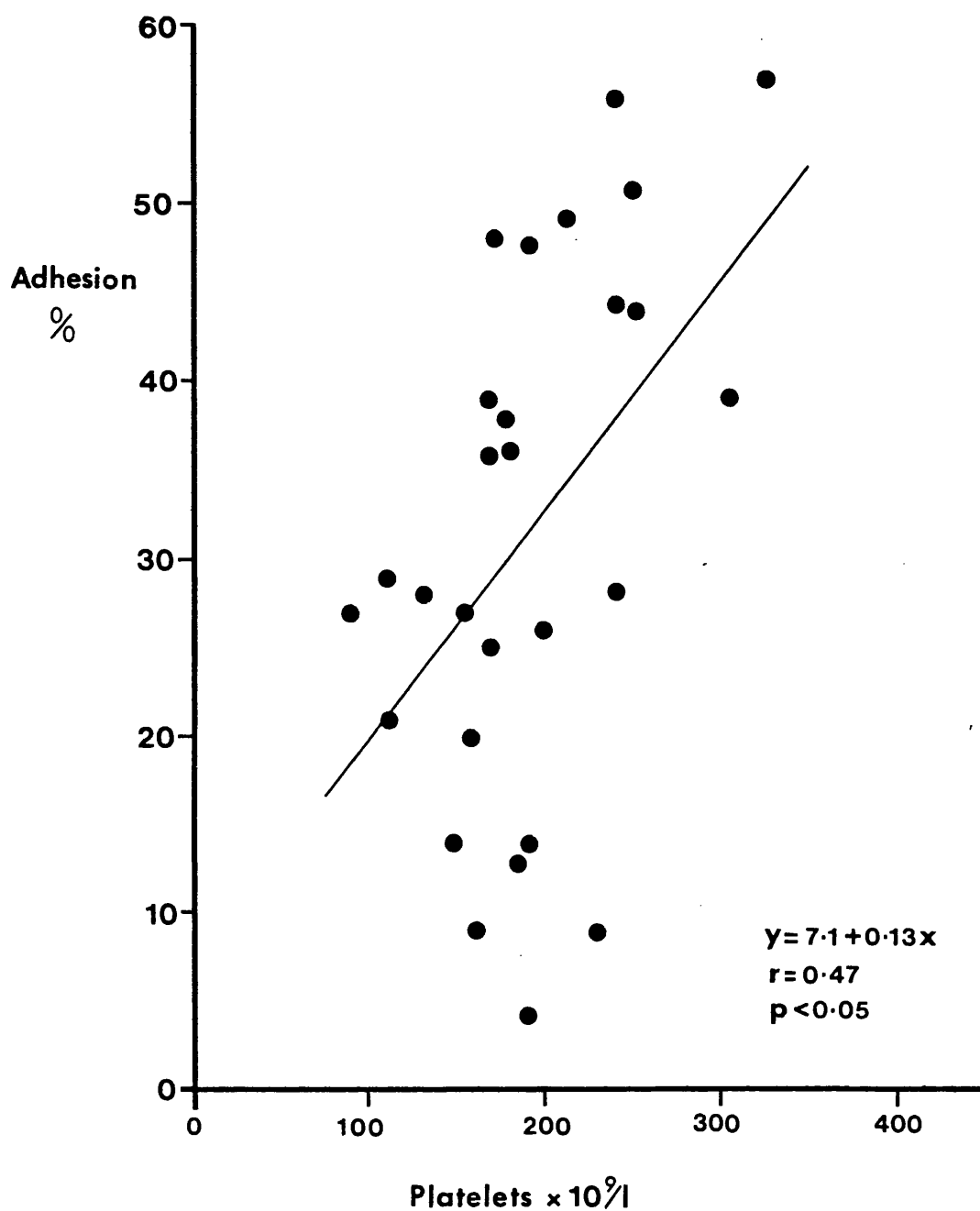


FIG. 3.11

Platelet adhesiveness and whole blood platelet count in controls.

Results

Platelet adhesion was very variable (Fig.3.10) and the mean value for the afternoon $31 \pm 16\%$ was scarcely different from the morning value, $32 \pm 14\%$. Five individuals had an adhesion value greater than 38%.

Platelet adhesion rose with platelet count. The correlation was significant ($r=0.47$, $p<0.05$) (Fig.3.11) although more marked at higher adhesion values. There was no correlation with extent of aggregation or ADP threshold values.

Discussion

The extent of platelet aggregation has been shown to be affected by platelet count, as expected on physical grounds and also to depend upon the time of day. This latter effect is probably not mediated solely by the platelet count, which although lower in the afternoon, is not significantly so. It is possible that post prandial lipaemia might be the determinant. For these reasons blood was taken in the morning when studying patients.

The adhesion test was also shown to differ according to the time of day, although in no constant fashion. Glass bead retention measures both adhesion of the platelet to the glass surface and the trapping of aggregates, so that in view of the dependency of aggregation on platelet count it is not surprising that adhesion is also proportional to platelet count. No correlation between adhesion and aggregation can be shown however; this may be because of small numbers, but also because of the variability of adhesion measurements. The poorer correlation of adhesion and platelet count, compared with aggregation and platelet count, seems

to be partly due to a subgroup with low adhesion but a normal platelet count. It is possible that undisclosed aspirin intake, for instance, might explain this.

The overall values for platelet adhesion were higher than the normal range associated with the Adeplat T system, where a value of 38% is regarded as indicative of a hyperadhesive state. Five normal controls exceeded this value. None has so far succumbed to a thrombotic event.

3.6 BETATHROMBOGLOBULIN ASSAY

Betathromboglobulin (β -TG) is a protein of uncertain function stored along with platelet factor 4 in the α -granules of platelets, and appears in the plasma when platelets undergo the release reaction. Functions suggested for it are as a granule-packing protein (Begg, 1978) or possibly as a prostacyclin antagonist (Hope, 1979). It provides a marker for the magnitude of in vivo release reaction, provided that in vitro release during sample handling is excluded. It is affected by renal clearance, but may provide an index of continuing thrombosis, or thrombotic risk, if renal clearance is uniform. Clinical applications investigated include the diagnosis of deep vein thrombosis (Ludlam, 1975) and the prognosis of vascular disease (Jones, 1979). It has been measured in this study by the radioimmunoassay method of Ludlam (1975) using a commercial kit (β TG-RIA, Radiochemical Centre, Amersham). It was felt that patients with end-stage renal failure would have a relatively uniform degree of renal impairment, which would allow comparison of β -TG values between individuals (Method: see Appendix VIII).

Control Data

Subjects

Fit volunteers amongst hospital staff on no medication.

Details

Male	21
Female	11
Age (mean $\pm$ SD)	30.3 $\pm$ 6.3 (range 20-53)
Smokers (6 male, 3 female)	9
Non-smokers	<u>23</u>
Total	32

Method (see Appendix VIII)

Blood was taken in the morning, always by the same observer who also performed all the assays.

Results

<u>Group</u> -----	β -TG ng/ml	
	<u>Mean</u> ----	<u>Median</u> -----
Males	50.4 $\pm$ 17.5	50
Females	35.1 $\pm$ 23.6	26
Smokers	53.2 $\pm$ 24	60
Non-smokers	42 $\pm$ 19	38
Total	45.2 $\pm$ 20.8	40

Discussion

The overall value of 45.2 ng/ml is the same as that found in a small study by Adler (1979) but higher than other larger series, such as that of Kaplan (1981) who found 90% of values between 6.6 ng/ml and 47.9 ng/ml, with a median (since the distribution was logarithmic) of 17.8 ng/ml. My control values suggest a logarithmic distribution (Fig. 3.12) with a median of 40 ng/ml, and 90% of values lying between 15 and 70 ng/ml. These differences probably reflect differences in sample handling technique, such as the absence of PGE₁ in the anticoagulant mixture (Randi, 1981).

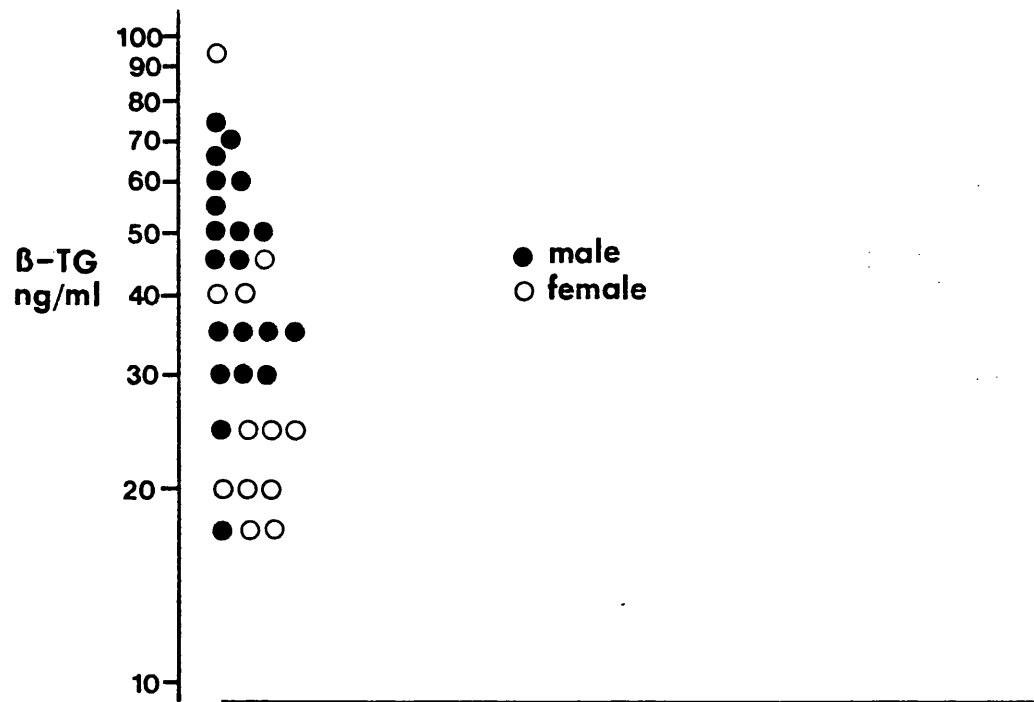


FIG. 3.12

β -TG values in a normal population. (n = 32)

The value for males is higher than that for females (though not significantly so on these numbers), as found by Dewar (1979) although he noted significant differences only in the 20-40 age group. He also showed a rise of about 6 ng/ml in plasma β -TG in the age group 60-70, with a further rise in the 70-80 age group. No influence of age or smoking is detectable in this small study.

Problems with evaluating the assay results are determining whether a raised β -TG value is the result of poor renal clearance, in vitro release, or a true increase in platelet release in vivo. The first problem can only partially be solved by looking for a correlation between β -TG levels and creatinine as found by Depperman (1980) and adjusting the β -TG level for creatinine level if the correlation is significant. In vitro release accounts for occasional high values; if these occur, the test should be repeated. If facilities are available another method of distinguishing in vivo from in vitro release is to measure platelet factor 4 levels simultaneously. This protein, also released from platelets, is cleared much more rapidly from plasma in vivo than β -TG; thus a plasma with a high ratio of β -TG to PF4 would suggest true increased release in vivo, whereas a lower ratio would suggest an increased contribution of in vitro release (Kaplan & Owen, 1981).

CHAPTER 4

CHAPTER 4

RHEOLOGY AND HAEMOSTASIS IN RENAL FAILURE PATIENTS : AN EVALUATION OF THE EFFECT OF THE CONSTRUCTION OF AN ARTERIOVENOUS FISTULA, AND A COMPARISON WITH A CONTROL POPULATION

INTRODUCTION

The aim of this study was to investigate a range of rheological and haemostatic tests in patients with chronic renal failure undergoing construction of an arteriovenous fistula, in the hope of identifying factors which might indicate a pre-disposition to thrombosis of their fistula.

The tests studied were platelet function tests (adhesion, aggregation and plasma β -thromboglobulin level), fibrinolytic activity in whole blood, red cell deformability, whole blood viscosity and haematocrit. By performing pre- and post- operative tests the effect of fistula construction on these variables has been assessed. In addition the results in the patient group have been compared with values obtained in normal individuals.

An arteriovenous fistula from the moment of construction will have a flow of about 100-300 mls/min, which will gradually increase. There is no way of avoiding turbulence within the fistula, which presumably predisposes to atherogenesis and thrombosis. Since it is likely that such turbulent flow might affect platelet activation or fibrinolytic activity, values of these haemostatic variables were estimated on the day before and the day after

fistula construction. It was not expected that whole blood viscosity and red cell filterability would be altered, but paired observations would give an indication of variability.

PATIENTS AND METHODS

Over the period December 1979 to April 1981, 42 patients in end-stage chronic renal failure requiring arteriovenous fistula construction were studied prospectively. There were 18 females and 24 males; mean age was 42 ± 12 years (15-63); 37 patients had had no previous access surgery; 5 had had previous fistula operations. 3 of the latter had failing renal allografts. In 42 patients blood was taken for platelet function tests, fibrinolytic activity, red cell filterability and whole blood viscosity on the day before operation (See Appendix I - VIII). The tests were repeated the day after operation in 16 patients. Plasma viscosity (see Appendix III), fibrinogen (see Appendix IV) and serum creatinine (see Appendix XII) were estimated before operation only. The cause of renal failure was recorded with details of drug therapy and smoking habit. 19 patients had blood flow measurements at operation and these results are described in Chapter 7.

Statistical analysis included paired t tests for the studies on the effect of operation (with Wilcoxon's signed rank sum test for skewed distributions), two-sample t tests for comparison between renal and control values, linear regression analysis for correlation of numerical variables and Spearman's correlation for ranked populations.

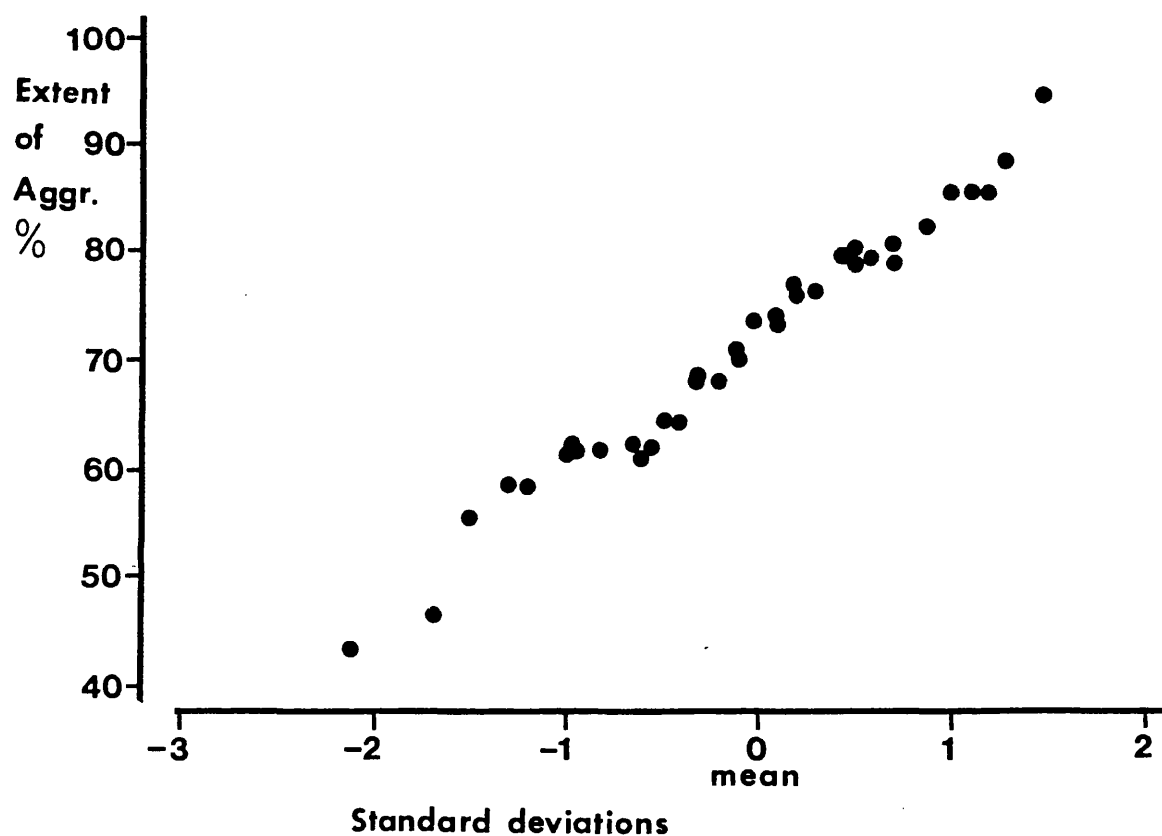


FIGURE 4.1

Example of computer normal probability plot:

Extent of platelet aggregation % compared with theoretical deviations of each value from the mean ($r = 0.97$).

The normality of distributions was assessed by computer probability plotting; if the theoretical distribution and real distribution fitted perfectly, then there would be a straight line correlation between the numerical value of the variable and its theoretical deviation from the mean (Fig. 4.1). If, for example, a distribution is skewed towards the origin, then values below the mean will be closer to the mean than theoretically predicted and the correlation will be concave upwards. The degree of fit was assessed by the correlation coefficient r . For a perfect correlation $r = 1.0$; for all distributions in this study r varied from 0.94 - 0.99, allowing the use of parametric tests; non-parametric tests were used however if there was any obvious departure from a straight line correlation.

4.1 Influence of Fistula Operation : Results

Table 4.1 shows the mean values obtained from 16 patients studied before and after operation. There was no significant change in the majority of variables. There was a slight rise in platelet count, paralleled by a fall in ADP threshold and an increased extent of aggregation, although these were statistically insignificant, the fact that there was a significant rise in β -thromboglobulin supports the idea of increased platelet activation after operation. In vivo release phenomenon without aggregation might also explain the results, as well as the fact that the 'aggregability' in vitro of platelets from peripheral blood may not reflect aggregation occurring in vivo.

TABLE 4.1

Pre- and post-operative values $\pm$ SD in 16 patients undergoing arteriovenous fistula construction.

<u>Platelet Function</u>	<u>Pre-Op</u>	<u>Post-Op</u>	<u>Paired t-test</u> p value (= 2 α)	
Platelet count $\times 10^8$ /L	201 $\pm$ 75	209 $\pm$ 101	p = 0.74	NS
Platelet adhesion %	22 $\pm$ 12	18 $\pm$ 12	p = 0.26	NS
Aggregation-extent %	78 $\pm$ 13	81 $\pm$ 10	p = 0.46	NS
-ADP threshold μ mol/L	2.28 $\pm$ 2.1	2.19 $\pm$ 1.8	p = 0.82	NS
β -Thromboglobulin ng/ml	89 $\pm$ 30	104 $\pm$ 20	p = 0.0125	*
<u>Fibrinolytic Activity</u>				
ng fibrin lysed/ml/h	202 $\pm$ 109	226 $\pm$ 76	p = 0.37	NS
<u>Red Cell Filtration Index</u> <u>(FI)</u>				
	0.44 $\pm$ 0.17	0.42 $\pm$ 0.16	p = 0.18	NS
<u>Whole Blood Viscosity (cps)</u>				
11.5s ⁻¹	5.39 $\pm$ 1.47	5.56 $\pm$ 1.3	p = 0.72	NS
230s ⁻¹	2.88 $\pm$ 0.28	2.79 $\pm$ 0.34	p = 0.13	NS
<u>Haematocrit %</u>	25.7 $\pm$ 4.1	24.9 $\pm$ 4.1	p = 0.034	*

The slight rise in fibrinolytic activity is not significant; neither is the slight fall in red cell filterability. Whole blood viscosity rose slightly but not significantly at a shear rate of 11.5s^{-1} . These changes might be explained by a postoperative rise in fibrinogen. Haematocrit fell slightly but significantly; this was accompanied by a slight and apparently insignificant fall in viscosity at 230s^{-1} . These results may reflect increased fluid intake while the patient was in hospital.

Since the operation made little difference to the variables studied in these 16 patients except perhaps to platelet release, subsequent patients had blood taken before operation only.

4.2 Rheological and Haemostatic Values in Renal Failure

Table 4.2 summarises the comparison between values for patients in renal failure and normal controls.

Platelet Function

Platelet adhesiveness and extent of aggregation to ADP were significantly lower in the patient group. β -thromboglobulin was significantly raised, but platelet number in whole blood was not significantly higher in the renal patients.

The decrease in responsiveness of platelets was as expected from numerous previous reports (Lewis, 1956; Castaldi, 1966; Eknoyan, 1969; Rabiner, 1972; Lindsay, 1975; Remuzzi, 1978). Adhesion showed no correlation with either creatinine level or haematocrit, at variance with Eknoyan (1969), although Lindsay (1975) and Remuzzi (1978) also found no correlation between in vitro platelet adhesion

TABLE 4.2

Comparison between patients with renal failure (age 42 ± 12 y) and normal controls. (mean age 36 : range 19-88 years)

	<u>Patients</u>		<u>Controls</u>		<u>p_value</u> ($=2\alpha$)
	<u>Mean</u> $\pm$ <u>SD</u>	<u>(n)</u>	<u>Mean</u> $\pm$ <u>SD</u>	<u>(n)</u>	
Platelet count $\times 10^9$ /L	231 $\pm$ 101	(41)	200 $\pm$ 57	(18)	0.1 < p < 0.2
ADP threshold μ mol/L	2.02 $\pm$ 1.59	(38)	6.05 $\pm$ 5.2	(20)	< 0.005 (Wilcoxon)
Platelet adhesion %	17 $\pm$ 11.5	(39)	32 $\pm$ 14	(15)	< 0.005
Aggregation extent %	71 $\pm$ 13	(39)	84 $\pm$ 8	(21)	< 0.005
β -thromboglobulin ng/ml	121 $\pm$ 45	(40)	45.2 $\pm$ 20.8	(32)	< 0.001
Creatinine μ mol/L	1028 $\pm$ 274	(39)	- - -	-	-
Haematocrit	25.3 $\pm$ 5.5	(42)	43.4 $\pm$ 3.9	(22)	< 0.001
Whole blood viscosity: (cps)					
11.5s ⁻¹	5.09 $\pm$ 1.68	(41)	7.9 $\pm$ 1.2	(22)	< 0.001
230s ⁻¹	2.84 $\pm$ 0.43	(41)	4.1 $\pm$ 0.5	(22)	< 0.001
Fibrinolytic activity ng/ml/h	234 $\pm$ 93	(38)	207 $\pm$ 55	(33)	> 0.2 NS
Fibrinogen g/L	4.8 $\pm$ 1.6	(34)	2.9 $\pm$ 0.6	(25)	< 0.0005
Plasma viscosity cps	1.66 $\pm$ 0.17	(39)	1.60 $\pm$ 0.07	(36)	< 0.05
Red cell Filtration Index (FI)	0.43 $\pm$ 0.12	(39)	0.53 $\pm$ 0.09	(80)	< 0.0005

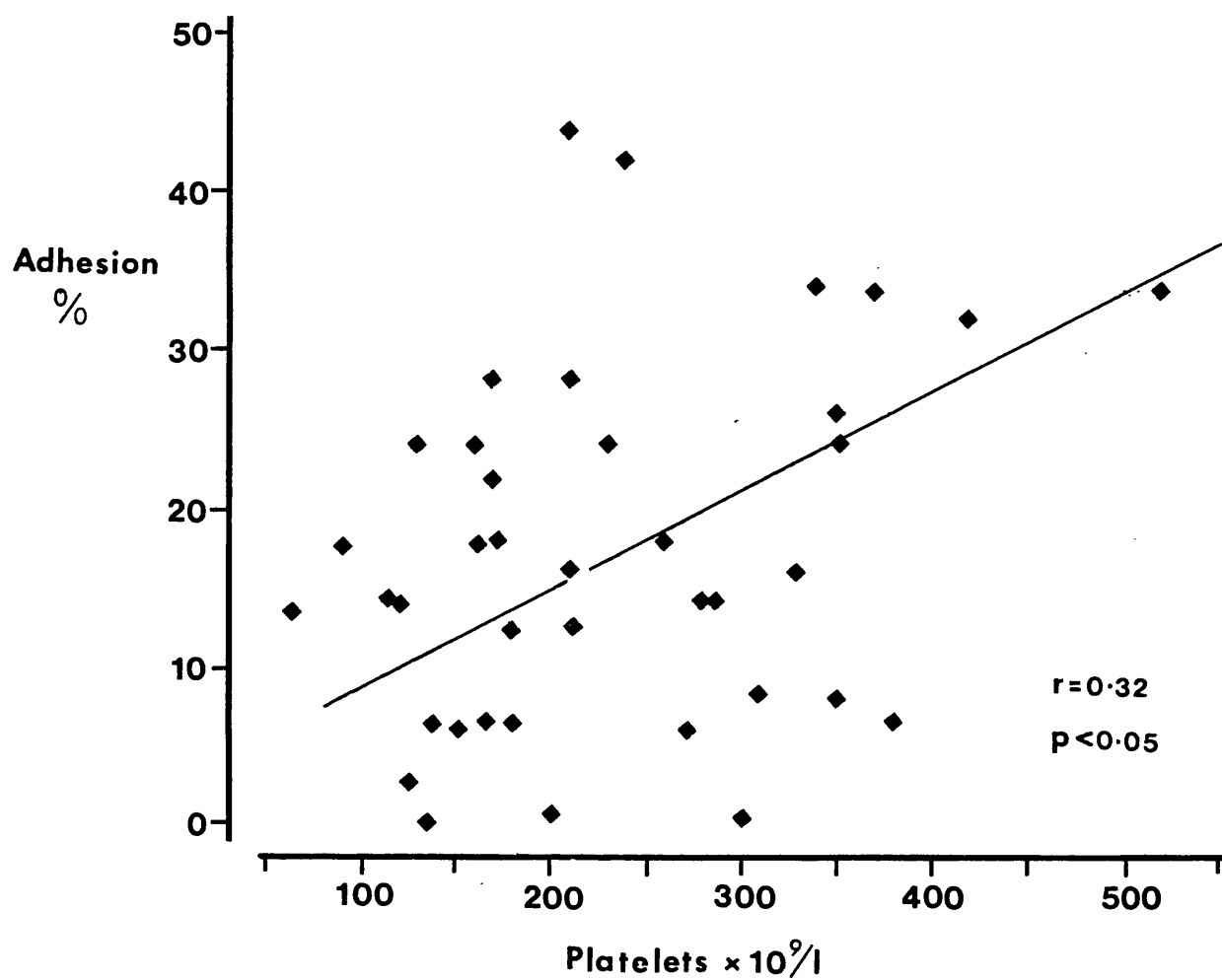


FIGURE 4.2

Relationship of platelet adhesiveness to whole blood platelet count.

and creatinine. It is possible that there was insufficient variability in haematocrit to demonstrate a correlation, or that the variability in creatinine level masked the correlation; deletion of one point (haematocrit 42, adhesion 0%) does reveal a significant correlation ($r = 0.33$, $p < 0.05$). Adhesion did however correlate with platelet count ($r = 0.32$, $p < 0.05$) (Fig. 4.2) as it did amongst the controls (see p 87); this is probably due to the aggregation component of the test; platelet aggregation (on physical grounds) would be expected to occur more readily the greater the concentration of platelets. This is shown by the correlation of aggregation extent and whole blood platelet count in renal patients ($r = 0.41$, $p < 0.05$) (Fig. 4.3) and in controls (see p 82).

Defective adhesiveness cannot be accurately detected on the Adeplat T test, since the lower limit of the normal range is 3%; 3 renal patients had adhesion lower than this (0%). Two patients had adhesion above 38%, the manufacturers' upper limit of normal, despite creatinine levels of over $1200 \mu\text{mol/L}$.

β -thromboglobulin (β -TG) levels were significantly raised, as expected, in the patients with renal failure. Depperman (1980) found a strong correlation between β -TG levels and creatinine and deduced that β -TG was handled by the kidney in a similar fashion to other microproteins such as β_2 -microglobulin. A correlation was not found in this study, perhaps because of the narrower range of creatinine levels studied, and also because of smaller numbers. β -TG did not correlate with the whole blood platelet count, unlike adhesiveness or aggregation; this can be explained on the basis that release can occur without aggregation, and adhesion can occur without

release. The platelet count itself showed a wider variability in the patient group than in the control (231 ± 101 compared with $200 \pm 57 \times 10^9/L$); this has been also shown in a much larger series by Giles (1981). Using Coulter cell sizing and counting equipment, he showed that renal patients had a mean platelet volume similar to normal; but whereas 1.4% of normals had a count above $450 \times 10^9/L$, and 0.4% below $150 \times 10^9/L$, for renal patients these figures were 29.4% and 6.5% respectively.

The threshold level of ADP required to give a biphasic response was lower in the renal patients; as mentioned previously (p 78) this may be because of lowered citrate concentration in the platelet rich plasma (PRP) because of a lowered haematocrit. Since the platelet concentration in PRP is also reduced by a lower haematocrit, this suggests that ADP threshold measurement is less dependent on platelet count than is extent of aggregation. This is supported by the positive correlation between extent of aggregation and the platelet count, and the lack of correlation between ADP threshold and platelet count (Spearman's $r = 0.26$, NS).

Fibrinolytic Activity

The mean fibrinolytic activity (FA) for the renal patients, 234 ± 93 ng/ml/h was not significantly greater than for the normal controls (207 ± 55 ng/ml/h). This confirms earlier work by Remuzzi (1978) using englobulin clot lysis time as a parameter of fibrinolytic activity, although earlier it had been suggested that fibrinolysis was depressed in renal failure (Wardle, 1972). FA did not correlate with fibrinogen levels; the high plasma fibrinogen of

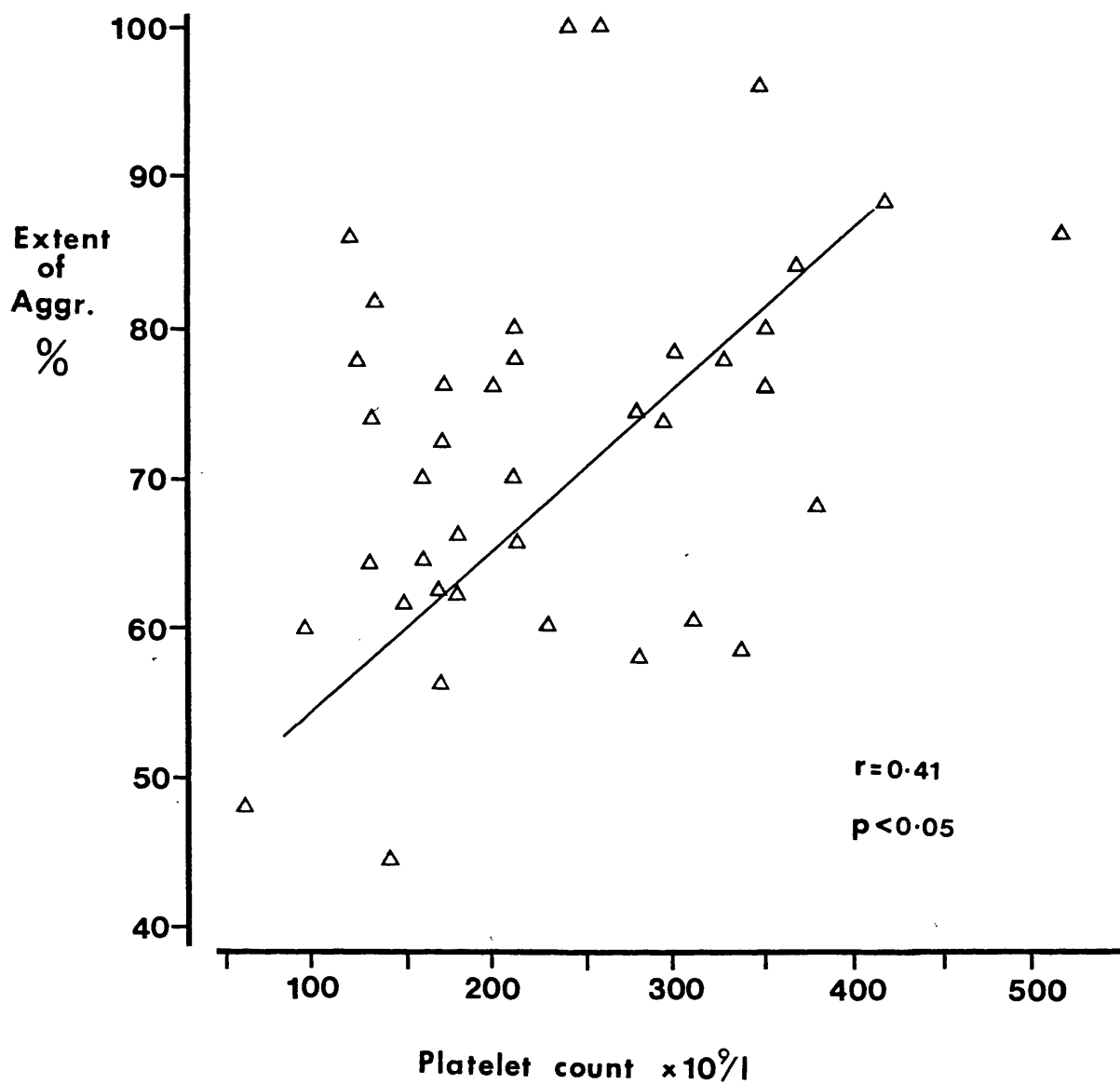


FIGURE 4.3

Relationship of platelet aggregation extent to whole blood platelet count.

the renal patients (4.8 ± 1.6 g/L) is more likely to be due to stress than reduced fibrinolysis, since it is an acute phase reactant. The fibrinogen level correlated strongly as expected with plasma viscosity ($r = 0.8$) (Fig. 4.4) and this is reflected in the raised plasma viscosity of the renal failure group, 1.66 ± 0.17 cps compared with 1.60 ± 0.07 cps for the controls.

Red Cell Filterability

The filtration index (FI) of the renal patients was significantly lower than that of the controls ($p < 0.005$). As plasma viscosity rose, filtration fell ($r = 0.55$, $p < 0.001$) (Fig. 4.5). Even for the patients with plasma viscosity in the normal range, however, the FI was still significantly lower, suggesting that a true decrease of flexibility of the red cell was being measured, which might be due to the morphological characteristics of the red cell. It seemed unlikely that plasma viscosity per se was slowing the filtration rate significantly; it seemed more likely that it was a marker for fibrinogen, which at high values could cause significant red cell aggregation despite the dilute suspension, and so delay filtration. This is supported by a similar negative correlation with fibrinogen ($r = 0.53$, $p < 0.01$) (Fig. 4.6). These relationships were not apparent in the control group, suggesting that fibrinogen only exerted an effect at high values or on pathological cells.

Since previous studies had suggested that reduced red cell flexibility contributed to the anaemia of renal failure, (Forman, 1973), a correlation between FI and haematocrit was sought. This

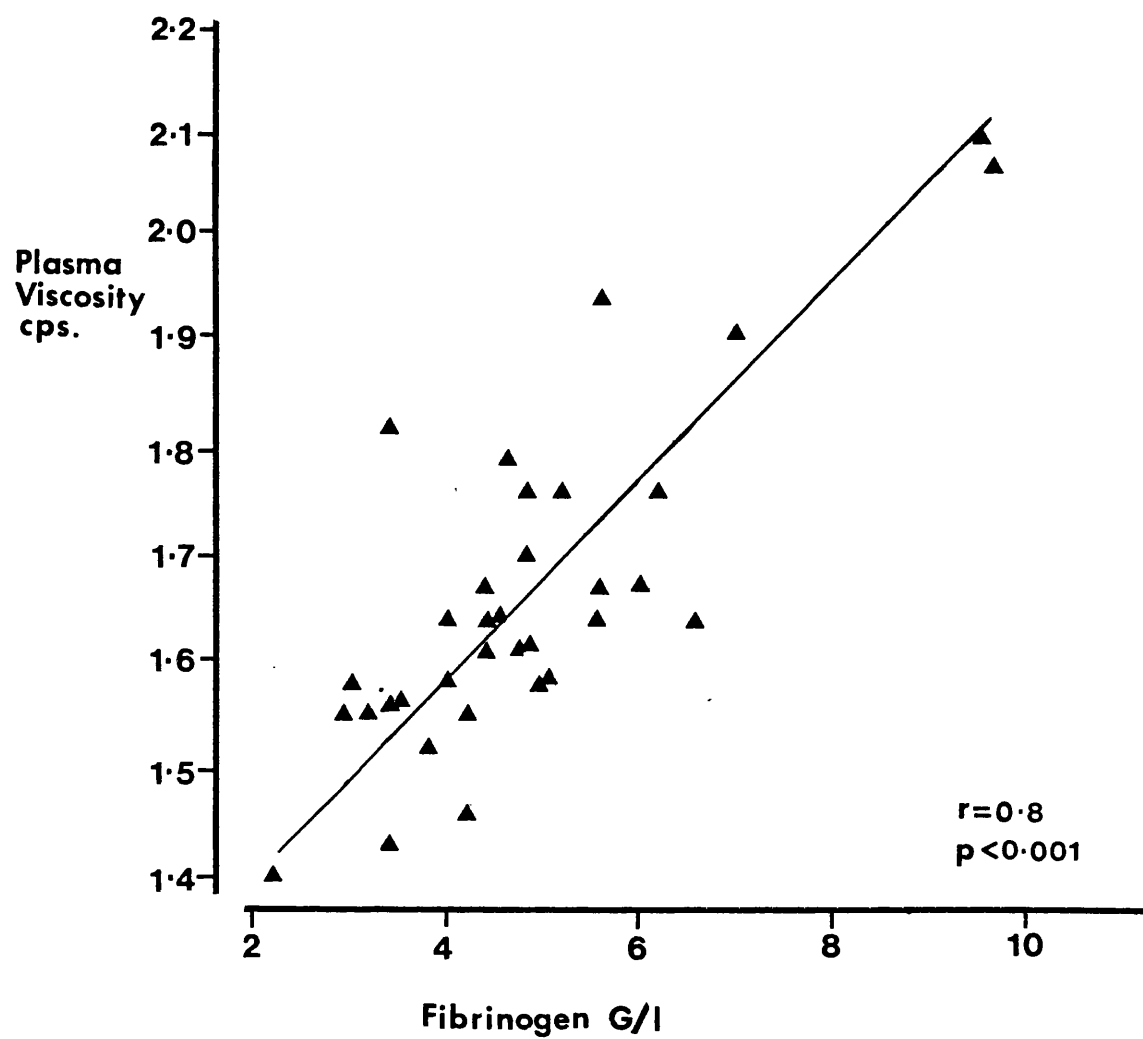


FIGURE 4.4.

Correlation of plasma viscosity with fibrinogen level.

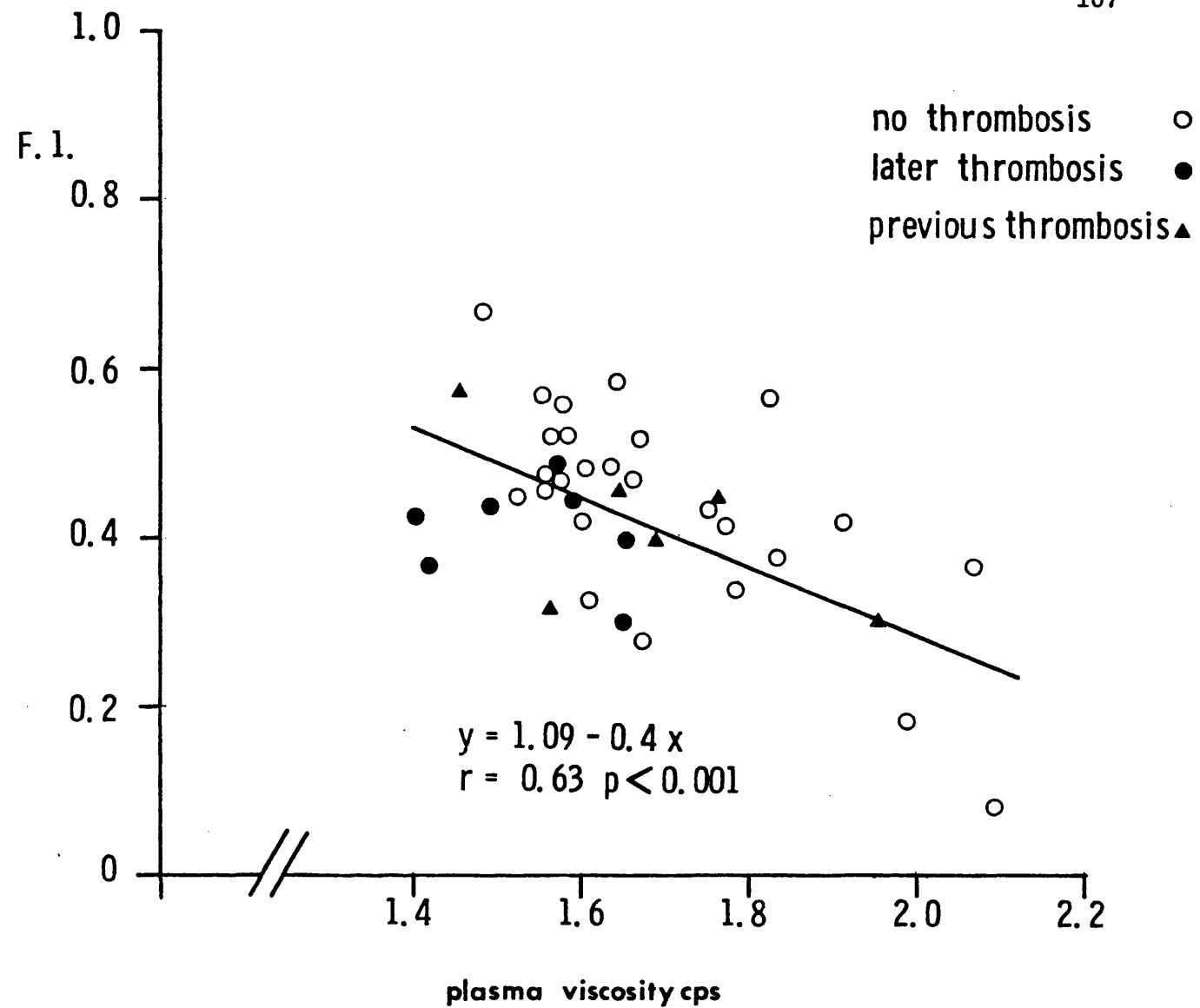


FIG. 4.5

Red cell Filtration Index (F.I.) and plasma viscosity in patients undergoing arteriovenous fistula construction.

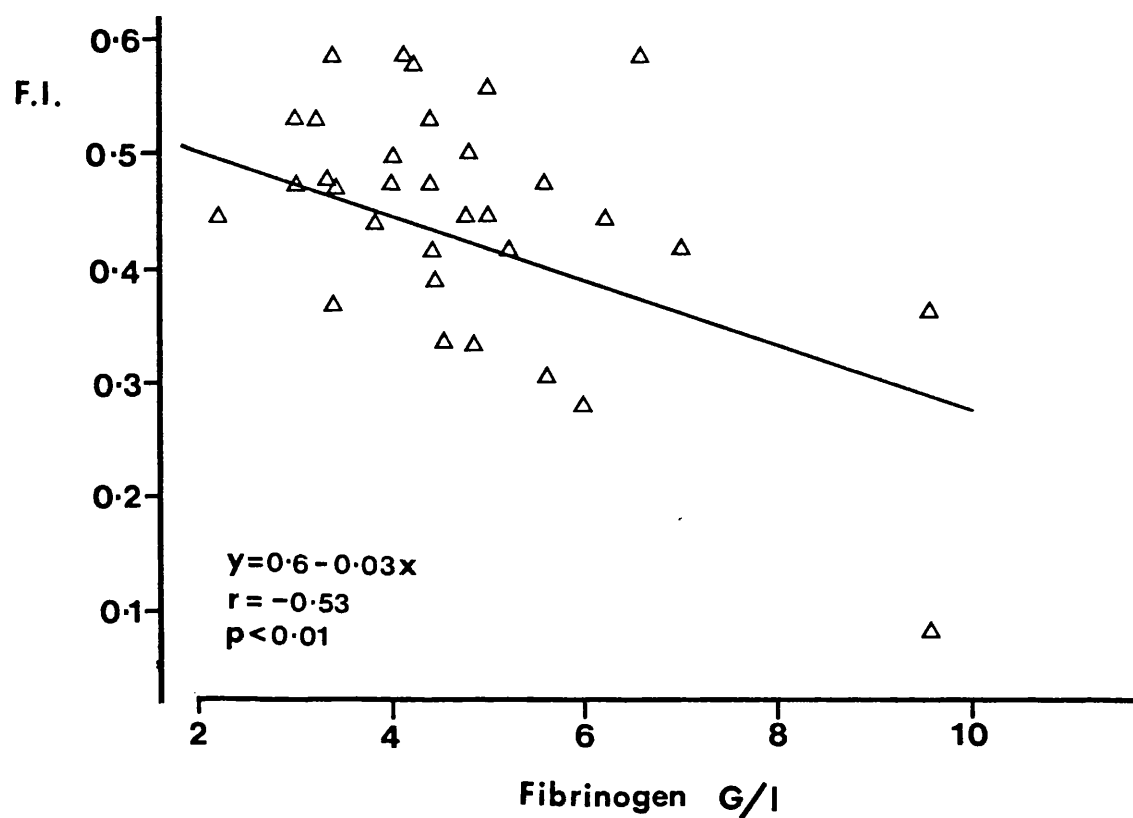


FIGURE 4.6

Relationship of red cell filtration index (FI) to fibrinogen level.

was not apparent; neither was any correlation found between filterability and whole blood viscosity, although theoretically there could be a negative correlation, if stiffer red cells increased viscosity. Alternatively there is a suggestion that there should be a positive correlation with an increase in red cell flexibility compensating for increased viscosity (Nicolaidis, 1977).

As a result of the apparent influence of fibrinogen a further study in normal controls was performed, which foundered on technical difficulties. Ten normal controls were studied; 60ml of venous blood was removed from each and the plasma separated and filtered. Fibrinogen was dissolved in aliquots of the plasma to give final concentrations of 2, 4, 6 and 8 mg/ml. A further blood sample was taken from each person and the packed cells were resuspended in these aliquots and the filtration test performed. No constant relationship of the filtration rate to the fibrinogen concentration was found, but in some cases red cell aggregation was macroscopically apparent and filtration occurred very slowly. There were insuperable problems to overcome in this line of investigation; despite overnight agitation, it was often impossible to get the fibrinogen into solution; also the fibrinogen preparation (Sigma) contained salts which increased the osmolarity of the plasma and so would alter the red cells by shrinkage. Dialysing the fibrinogen to remove salts would still leave the problem of diluting the other plasma proteins on adding the fibrinogen solution. Fibrinogen may well have conflicting effects on red cell filtration by not only causing aggregation but also increasing membrane flexibility by adsorption to the cell

surface (Sirs, 1972). The equilibration time for this latter process would need to be known.

Whether the reduced filtration of red cells in renal failure is caused by surface effects from the constituents of uraemic plasma, or due to changes in internal viscosity and biochemistry, or due to altered cellular morphology, is unknown, although the latter seems the most likley. Whatever the cause, one reason for investigating this property was to see whether it contributed to whole blood viscosity at any shear rate, and whether despite anaemia the red cells could raise the whole blood viscosity; this has been shown not to be the case. The effect, if any, is far outweighed by the haematocrit. Another possibility could be that the flexibility of the red cell is related to the release of ADP which may be a physiological stimulus to platelet activation. The remaining idea to be investigated was that the filtration test, like the erythrocyte sedimentation rate, might be a magnifier of small effects, and thus could possibly form part of a 'thrombotic profile'.

Whole Blood Viscosity

At both high and low shear rates (Table 4.2) viscosity measurements were significantly lower than for the controls. This was associated with a significantly lower haematocrit (25.3 ± 5.5), to be expected in renal failure. There was a strong linear correlation between log. viscosity and haematocrit at all shear rates (Fig. 4.7) as with the controls. Using this regression line it is possible to extrapolate to other haematocrits and predict

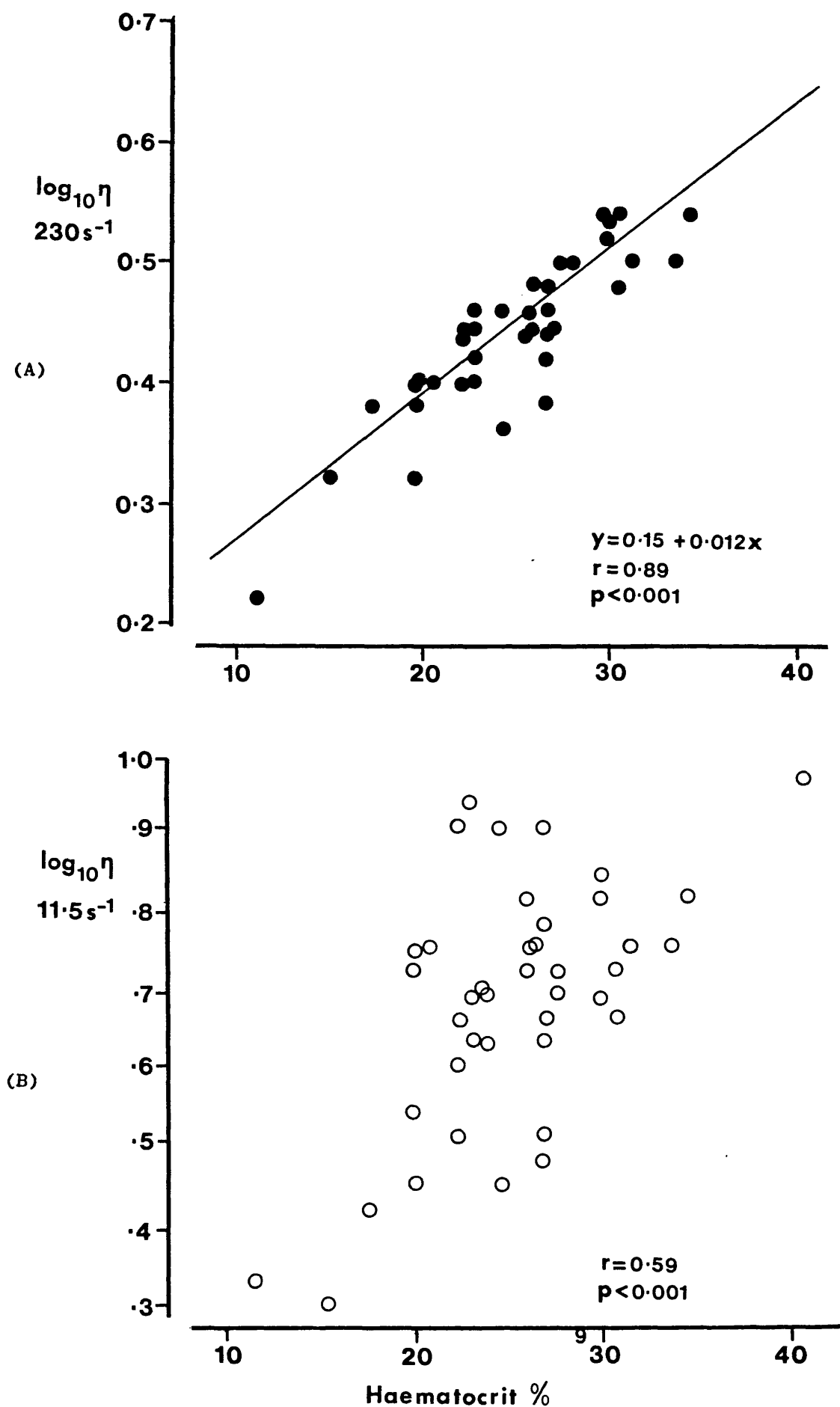


FIGURE 4.7

Relationship between log. viscosity (η) at 230 s^{-1} (A) and 11.5 s^{-1} (B) to haematocrit.

corresponding mean viscosities for a population, as a guide as to whether haematocrit is the only difference, although such transformed data cannot be subjected to statistical analysis; as explained previously, viscosity for individuals cannot be 'corrected' to a standard haematocrit. Such extrapolation would suggest that the only difference at high shear rates between renal patients and controls is the haematocrit, but at low shear rates the viscosity is proportionately higher in the renal failure patients. This could be explained by the higher fibrinogen level of the renal patients.

Influence of Other Factors

No differences between the sexes or correlation with age was found for any of the variables studied. Patients received a variety of drugs, from vitamins only to multiple therapy with, for example, beta-blockers, vasodilators, diuretics and prednisone. The largest common factor was a beta-blocker; 19 out of 42 patients were on regular treatment with a beta-blocker, but no differences in, for example, platelet responsiveness, were detected, although it might be expected that beta-blockade would render platelets more likely to aggregate, by reducing cyclic AMP content.

SUMMARY

A number of differences in the variables studied has been found between patients with renal failure and controls. The mean creatinine value of 1000 $\mu\text{mol/L}$ is indicative of end-stage

renal failure, yet the platelet count overall was not reduced, ADP threshold was decreased, and although platelet adhesion and extent were reduced compared with normal controls they were by no means grossly inhibited. Beta-thromboglobulin is a fair index of platelet release reaction provided renal function is constant; it is no surprise, therefore, that the overall level was much higher in patients with renal damage. It is still possible that amongst patients with similar impairment of renal function different β -TG levels might indicate different levels of platelet excitation and therefore risk of thrombosis. Fibrinogen levels were higher in the renal patients, and plasma viscosity was also slightly but significantly raised. Red cell filterability was found to be significantly lowered in renal patients, but its determinants were unknown. Whole blood viscosity at high shear rates gave an indication of haematocrit, and at low shear rates a measure of fibrinogen level; the prognostic value of all these measurements remained to be assessed.

CHAPTER 5

CHAPTER 5

PATIENTS ON MAINTENANCE HAEMODIALYSIS : THROMBOTIC AND NON-THROMBOTIC

PATIENTS

There are two ways of examining the possibility of a pre-thrombotic state; one is to study a group of patients prospectively, as described in Chapter 6, and relate later thrombosis to the pre-operative blood results; the other is to examine a group of patients who have had recurrent problems in the past, and compare them with similar patients who have had no trouble over an equal or greater follow-up time. Such a study is described here.

The process of haemodialysis will cause alteration in most haematological variables assayed because of contact of blood with an artificial surface. Differences between dialysis patients and either normal individuals or patients with end-stage renal failure could be due to the effects of dialysis, or of thrombosis, or could reflect a pre-thrombotic state. The object of the present study was to compare two groups of long-term dialysis patients, those with no access thrombosis ever, and those who had had two or more fistula thromboses.

PATIENTS AND METHODS

20 patients aged 23 to 60 on maintenance haemodialysis treatment using an arteriovenous fistula were studied. There were 17 males and 3 females. The patients were recruited on attending the home dialysis clinic on the day after dialysis. 10 patients with a mean follow-up time of 48.5 ± 25 months (range 11-84 months) whose fistula had never been affected by thrombosis, were studied. 10 patients with a mean follow-up time of 34.5 ± 18 months (range 15-77 months) who had had two or more fistula thromboses were investigated over the same 8 month time interval, between October 1980 and May 1981. The degree of age matching between the two groups is shown in Table 5.1.

Blood was taken as described in Appendix X and platelet function, fibrinolytic activity, red cell filterability and whole blood viscosity were estimated (Appendix I - VIII). In addition a sample of serum was sent for creatinine estimation in the biochemistry laboratory (Appendix XII).

RESULTS

An overall comparison of the two groups (Table 5.2) shows that despite a similar platelet count (222 : 217) the thrombotic group had a higher extent of platelet aggregation, a lower threshold response to ADP, and a higher beta-thromboglobulin level (Fig. 5.1) although only the difference in B-TG levels reached statistical significance. Platelet adhesion was similar in both groups. The mean age of the thrombotic group (45.7) was lower than the non-thrombotic group (51.8). This reflects the selection procedure; looking for patients who have been on dialysis for a

TABLE 5.1

Patients on haemodialysis - Age and Sex matching

<u>Thrombotic Group</u>		<u>Non-Thrombotic Group</u>	
M	60	M	60
M	54	M	59
M	52	F	58
M	51	M	56
M	48	M	56
M	46	M	54
M	46	M	53
M	42	M	50
F	35	F	44
M	23	M	28
Mean	45.7		51.8
age			

TABLE 5.2

Patients on maintenance haemodialysis : comparison between those with
and without past thrombotic problems

	Past Thrombosis (n = 10)	No Thrombosis (n = 10)	Two-sample t test p value (=2 α)
Age	45.7	51.8	
Platelets x 10 ⁹ /L	222 $\pm$ 64	217 $\pm$ 66	0.87 NS
Aggregation extent %	79 $\pm$ 7.6	69.5 $\pm$ 13.4	0.08 *
ADP threshold μ mol/L	1.1 $\pm$ 0.7	1.9 $\pm$ 1.3	0.12 NS
β -TG ng/ml	176 $\pm$ 24	151 $\pm$ 22	0.04 *
Adhesion %	12.9 $\pm$ 14.3	13.6 $\pm$ 7.0	0.89 NS
Creatinine	825 $\pm$ 182	698 $\pm$ 259	0.24 NS
PCV	32.5 $\pm$ 6.2	29.1 $\pm$ 8.6	0.33 NS
Whole blood viscosity-cps:			
11.5s ⁻¹	7.70 $\pm$ 1.83	5.3 $\pm$ 2.4	0.03 *
230s ⁻¹	3.48 $\pm$ 0.64	3.25 $\pm$ 0.65	0.43 NS
Fibrinogen g/L	4.2 $\pm$ 1.1	4.8 $\pm$ 0.6	0.16 NS
FA ng/ml/h	254 $\pm$ 81	262 $\pm$ 81	0.83 NS
Plasma viscosity cps	1.73 $\pm$ 0.13	1.80 $\pm$ 0.11	0.23 NS
Red Cell Filtration	0.45 $\pm$ 0.08	0.44 $\pm$ 0.13	0.87 NS

long time without problems will tend to select a higher age group.

Creatinine values did not follow the platelet function tests although the mean creatinine level was higher in the problem group (925 : 698 $\mu\text{mol/L}$) (not significant). Haematocrit and whole blood viscosity were higher in the thrombotic group, but the differences in viscosity were only significant at low shear rates (Fig. 5.2). However, plasma viscosity and fibrinogen were lower in the thrombotic group, (Fig. 5.3) although the differences were not significant. Red cell filterability was the same for both groups.

The differences between the 20 patients on maintenance haemodialysis and the 42 in end-stage renal failure are set out in Table 5.3. As one would expect, creatinine levels are lower in the dialysed patients. Although platelet count is hardly different there is some increase in reactivity of the platelets with an increased extent of aggregation and lower threshold to ADP, although these differences are not significant. Platelet adhesiveness is significantly lower in the patients on dialysis (13% : 17%) and β -thromboglobulin is significantly raised (165 : 121 ng/ml).

Haematocrit and whole blood viscosity at both shear rates were significantly raised in the dialysed patients; plasma viscosity was also raised (1.77 : 1.66 cps) despite slightly lower mean fibrinogen level. Fibrinolytic activity was slightly but not significantly raised in the dialysis group, and red cell filterability was slightly improved.

A comparison between patients on maintenance haemodialysis and normal controls is set out in Table 5.4 which shows lower

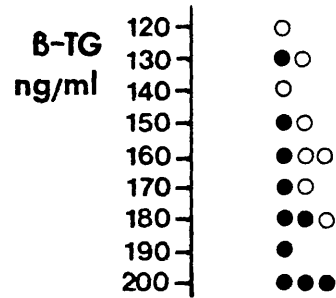


FIG. 5.1

β -thromboglobulin distribution in patients on haemodialysis.

● = patients with thrombotic problems

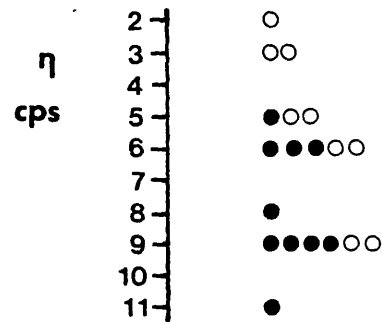


FIG. 5.2

Whole blood viscosity at $11.5s^{-1}$ distribution in patients on haemodialysis.

● = patients with thrombotic problems

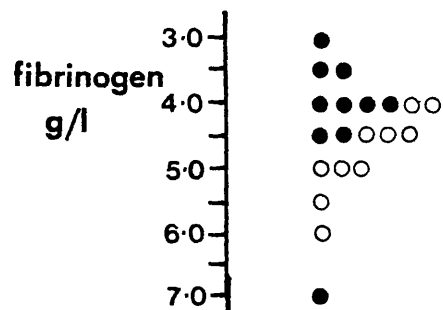


FIG. 5.3

Fibrinogen distribution in patients on haemodialysis.

● = patients with thrombotic problems

TABLE 5.3

Comparison of patients with end-stage renal failure (RF) with those on maintenance haemodialysis (MHDT)

	CRF (n = 42)	MHDT (n = 20)	Two-sample t test p value (=2α)
Age	42 ± 12	49 ± 10	-
Platelets x 10 ⁹ /L	231 ± 101	219 ± 63	NS
Aggregation: extent %	71.5 ± 13	74 ± 12	NS
ADP threshold umol/L	2.02 ± 1.6	1.54 ± 1.1	< 0.1 (NS)
α ₂ -thromboglobulin ng/ml	121 ± 45	165 ± 26	< 0.01
Platelet adhesion %	17 ± 11.5	13.3 ± 11	< 0.1 (NS)
Serum creatinine μmol/L	1028 ± 274	765 ± 225	< 0.001
Haematocrit %	25.3 ± 5.5	31 ± 7.5	< 0.01
Whole blood viscosity cps 11.5s ⁻¹	5.09 ± 1.68	6.56 ± 2.4	< 0.025
230s ⁻¹	2.84 ± 0.43	3.39 ± 0.64	< 0.01
Plasma fibrinogen g/L	4.83 ± 1.6	4.46 ± 0.9	NS
Plasma viscosity cps	1.66 ± 0.17	1.77 ± 0.12	< 0.01
Fibrinolytic activity ng/ml/h	234 ± 93	258 ± 79	NS
Red cell filtration index (F.I.)	0.43 ± 0.12	0.45 ± 0.1	NS

platelet adhesiveness (13% : 33%) and aggregation (74% : 84%) in the dialysed patients, despite a similar platelet count and a lower ADP threshold. β -thromboglobulin is much higher (165 : 45 ng/ml) in the dialysed patients. Haematocrit is lower (13% : 43%) and so is whole blood viscosity, although not so much at low shear rates; plasma fibrinogen is much higher (4.46 : 2.9 g/L) as is plasma viscosity (1.77 : 1.60 cps). Fibrinolytic activity is raised (258 : 207 ng/ml/h) and red cell filtration is lower (0.45 : 0.53) in the dialysed patients.

DISCUSSION

Comparison of the two groups of dialysing patients

In any study of multiple variables it is to be expected that occasional 'significant' differences at the 5% level should emerge; however, the differences in platelet function in the thrombotic group, apart from adhesion and actual platelet number, all show a trend in 'thrombotic' direction, with the increase in β -thromboglobulin significant at the 4% level. The creatinine level of the thrombotic group is not significantly higher than that of the non-thrombotic, so that the raised β -TG cannot be explained on this basis. If the creatinine level were the sole explanation, one would also expect reduced platelet aggregability as a result of a higher creatinine.

The increased whole blood viscosity is explained partly by the higher haematocrit of the thrombotic group; at low shear rates the influence of fibrinogen on red cell aggregation is usually

TABLE 5.4

Comparison between patients on maintenance haemodialysis (age 49 ± 10 y) and normal controls (age 36 : range 19-58)

	MHDT (n = 20)	CONTROLS	n	Two-sample t test p value (=2 α)
Platelets $\times 10^9$ /L	219 $\pm$ 63	200 $\pm$ 57	18	NS
Aggregation : extent %	74 $\pm$ 12	84 $\pm$ 8	21	< 0.01
ADP threshold $\mu\text{mol/L}$	1.54 $\pm$ 1.1	6.05 $\pm$ 5.2	20	< 0.01
β -thromboglobulin ng/ml	165 $\pm$ 26	45.2 $\pm$ 20.8	32	< 0.01
Platelet adhesion %	13.3 $\pm$ 11	32 $\pm$ 14	15	< 0.01
Serum creatinine $\mu\text{mol/L}$	765 $\pm$ 225	---	--	--
Haematocrit %	31 $\pm$ 7.5	43.4 $\pm$ 3.9	22	< 0.01
Whole blood viscosity: 11.5s^{-1}	6.56 $\pm$ 2.4	7.9 $\pm$ 1.2	22	< 0.05
(cps) 230s^{-1}	3.39 $\pm$ 0.64	4.1 $\pm$ 0.5	22	< 0.001
Plasma fibrinogen g/L	4.46 $\pm$ 0.9	2.9 $\pm$ 0.6	25	< 0.0001
Plasma viscosity cps	1.77 $\pm$ 0.12	1.60 $\pm$ 0.07	36	< 0.001
Fibrinolytic activity ng/ml/h	258 $\pm$ 79	207 $\pm$ 55	33	< 0.05
Red cell filtration index (FI)	0.45 $\pm$ 0.1	0.53 $\pm$ 0.07	80	< 0.01

more apparent, but in this case despite a higher low shear viscosity, both plasma viscosity and fibrinogen are decreased in the thrombotic group. The other possible explanation would be a decrease in red cell flexibility, but there is no apparent difference in filtration index between the two groups. Whatever the mechanism, such an increase in whole blood viscosity could contribute to a predisposition to thrombosis during a hypotensive period, when an increased resistance to flow would slow peripheral circulation further; combined with a greater tendency of platelets to aggregate and undergo the release reaction the difference in thrombotic tendency in the two groups might be partly explained. The likelihood is, of course, that any pre-thrombotic state is multi-factorial; this will represent a potential ability to thrombose which is not apparent until triggered by hypotension, poor surgery, or other factors. There was no difference in fibrinolytic activity between the two groups, although it may be that small differences were obscured by variability of the test system.

Comparison with controls and patients with terminal renal failure

As previously explained, studies comparing different sized groups that are not very well age or sex matched do not provide as hard data as a more tightly controlled study; however, some pointers towards areas of interest may be provided by such a comparison. It has been reported that platelet function is restored to normal by dialysis (Castaldi, 1966; Eknoyan, 1969) although other studies have shown that platelet function still remains somewhat below par (Remuzzi, 1978); the results of the comparison of patients on dialysis with

normal controls and individuals in end-stage renal failure not yet on dialysis suggest that dialysis does not return platelet function to normal, although aggregation extent increases and the threshold to ADP falls. Platelet adhesiveness is lower in dialysing patients than patients with renal failure or normal controls. Taken with the increased levels of beta-thromboglobulin, this perhaps suggests that the platelets culled from the peripheral blood in dialysing patients may be partly exhausted due to damage by contact with the dialysis membrane.

Haematocrit is apparently improved in the dialysing patients, and this is accompanied by an increase in whole blood viscosity, although not to normal levels. Low shear rate viscosity is higher than would be expected from extrapolation of the regression line for normal controls. This may be explained by the high fibrinogen level, probably a response to dialysis and reflected in a high plasma viscosity. The plasma viscosity in the chronic renal failure (CRF) group is not so high, despite a higher mean fibrinogen level. This is explained by the wider scatter of fibrinogen, with some very high levels in the CRF group. The filterability of red cells is improved in dialysed patients compared with the renal failure patients, but not to normal levels, suggesting continuing morphological abnormalities in the red cell or possibly as the result of the high fibrinogen. Fibrinolytic activity in dialysed patients is raised compared with controls, which again may be related to the higher fibrinogen, since an increased synthesis of fibrinogen may be a response to increased breakdown.

SUMMARY

The existence of significant differences between 'thrombotic' and 'non-thrombotic' patients on dialysis has been shown; these findings suggest an increased platelet activity in the thrombotic group despite a similar platelet count, accompanied by a raised whole blood viscosity, despite lower fibrinogen and plasma viscosity. Platelet function as measured by aggregation is improved by dialysis although it remains impaired in comparison to normal individuals. However, the levels of β -TG indicate that dialysis also produces significant platelet activation.

It is not clear whether the differences reported in this chapter between non-thrombotic and thrombotic patients reflect a predisposition to, or the result of, thrombosis. For this reason, the prospective study reported in the next chapter was undertaken.

CHAPTER 6

CHAPTER 6

A PROSPECTIVE STUDY OF PATIENTS UNDERGOING ARTERIOVENOUS FISTULA

CONSTRUCTION : EVIDENCE FOR A PRE-THROMBOTIC STATE

The underlying hypothesis to be tested in this study was that the patients developing later thrombotic problems would have 'hypercoagulable' blood compared with patients who remained free of trouble. The main difficulty in such a study is the exclusion of extraneous influences; some failures of fistulae may be unavoidable whatever the thrombotic potential of the patient, due to technical problems or mishandling of the fistula. If the constituents of the supposed 'hypercoagulable' or 'pre-thrombotic' state could be identified, this would allow rational prophylaxis using anticoagulation, platelet inhibition, fibrinolytic enhancement or viscosity lowering drugs depending on the cause found. Any treatment carries a certain risk, for example anticoagulation in renal patients (Biggers, 1977), and accurate definition of the sub-group likely to suffer thrombosis is necessary.

PATIENTS AND METHODS

The 42 patients in end-stage chronic renal failure described in Chapter 4, whose blood had been tested on the day before fistula construction, had been followed up for a mean period of 8.7 months (range 1-18) at the time of analysis in June 1981. Of these 42 patients, 37 had first time fistulae and 5 had had previous access procedures (3 of these patients were in end-stage renal failure due to failing renal allografts). Four patients died during the follow-up

period with functioning fistulae. No patients were lost to follow-up. The majority of procedures were wrist fistulae; the length of follow up, type of fistula and incidence of complications are illustrated in Fig. 6.1. 18 patients completed a year of follow up of whom 3 suffered complete failure of their fistula. In 5 patients fistulae were salvaged by thrombectomy or end-to-side conversion. 9 patients with first time fistulae and no thrombotic problems completed a year of follow up and so could be compared critically with the 8 patients suffering thrombotic episodes. The 3 patients with secondary access procedures who had been followed up for over a year were not included in the analysis because there was no way of knowing whether the previous thrombotic problems were related to surgical technique or a thrombotic tendency. The degree of similarity of operative details including vessel size, blood pressure in the fistula arm, and cause of renal failure is shown in Table 6.1.

RESULTS

The pre-operative assay results for the 8 'thrombotic' patients and the 9 trouble-free patients are set out in Table 6.2. In both groups the mean age, creatinine level and haematocrit were the same. Whole blood viscosity was lower in the thrombotic group at both low and high shear rates, although this did not reach significance. The patients with thrombosis had a significantly lower plasma viscosity (1.52 : 1.74 cps) ($p = 0.03$) (Fig. 6.2) and a lower plasma fibrinogen, although in contrast their fibrinolytic activity was raised. In view of the variability of this measurement no definite conclusion could be drawn; the possibility exists that a raised fibrinolytic activity is a marker also for an activated coagulation system.

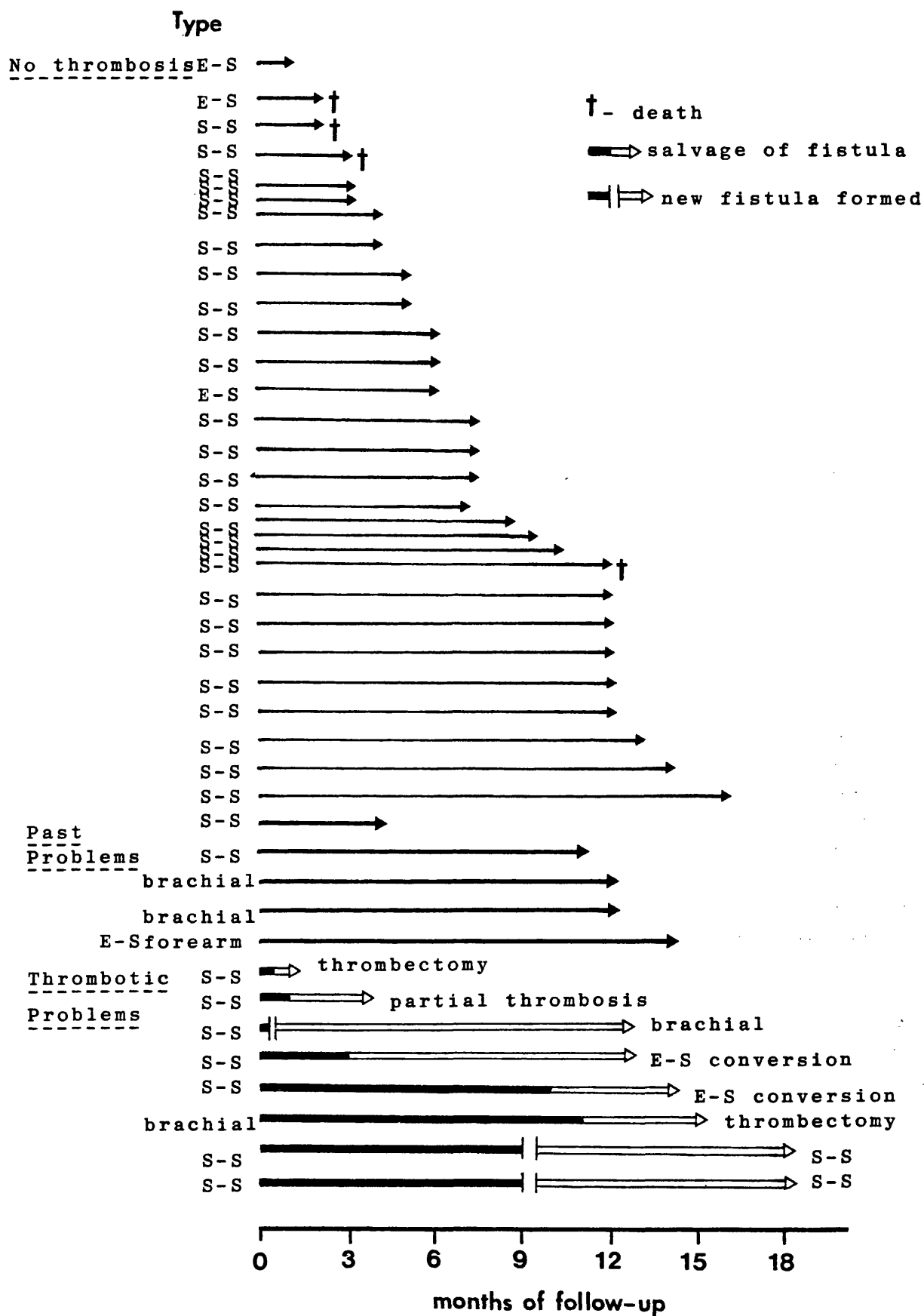


Fig. 6.I. Patency of 42 arteriovenous

fistulae Nov. 1979- May 1981

TABLE 6.1

Operative details of 9 patients with no thrombosis followed up for over a year (Group 1) and 8 patients with thrombosis of their arteriovenous fistula (Group 2)

Patient	Anastomosis diameter (mm)	Vein diameter (mm)	Artery diameter (mm)	Suture gauge (Prolene)	Fistula type	Cause of CRF
<u>Group 1</u>						
MW	14	2.5	3.0	7/0	Wrist side-to-side	GN
MJW	11	4.0	3.0	6/0	"	CPN
NH	17	4.0	2.0	7/0	"	PCK
AH	15	3.5	3.5	6/0	"	GN
EC	14	2.5	2.0	7/0	"	GN
JF	12	2.5	3.0	6/0	"	CPN
HA	15	4.0	3.0	6/0	"	GN
JEF	10	3.5	2.0	6/0	"	VUR
TB	15	4.0	3.0	6/0	"	VUR
	mean 13.7	3.39	2.72			CPN
<u>Group 2</u>						
BR	15	3.0	1.5	7/0	"	GN
AG	15	4.5	3.5	7/0	"	GN
GR	17	3.0	2.5	7/0	"	GN
MW	15	5.0	3.0	7/0	"	CPN
GP	8	4.0	5.0	7/0	Brachial side-to-side	Ca/DXT
DD	15	3.0	2.5	6/0	Wrist side-to-side	CPN ¹²
UM	13	3.0	2.5	6/0	"	GN ⁸
JN	10	2.0	3.0	7/0	"	
	mean 13.5	3.44	2.94			

TABLE 6.2

Chronic renal failure patients followed up for over a year : comparison between patients with and without later thrombotic problems.

	No Thrombosis n = 9	Thrombosis n = 8	two-sample t test p value (=2 α)
Age	43 $\pm$ 14	40 $\pm$ 15	0.7 NS
Platelets $\times 10^9$ /L	171 $\pm$ 55	277 $\pm$ 88	0.01 *
Aggregation : extent %	72 $\pm$ 14	79 $\pm$ 14	0.35 NS
ADP threshold μ mol/L	2.53 $\pm$ 1.69	1.28 $\pm$ 1.61	0.18 NS
thromboglobulin ng/ml	90 $\pm$ 21	123 $\pm$ 65	0.27 NS
Platelet adhesion %	21 $\pm$ 14	14 $\pm$ 11	0.27 NS
Creatinine μ mol/L	1039 $\pm$ 239	1012 $\pm$ 256	0.84 NS
Haematocrit %	25.8 $\pm$ 4.5	25.8 $\pm$ 5.5	0.99 NS
Whole blood viscosity : 11.5s ⁻¹ (cps)	5.36 $\pm$ 1.5	4.43 $\pm$ 1.62	0.24 NS
	2.87 $\pm$ 0.32	2.71 $\pm$ 0.43	0.40 NS
Plasma fibrinogen g/L	4.87 $\pm$ 2.36	3.98 $\pm$ 1.38	0.46 NS
Plasma viscosity cps	1.74 $\pm$ 0.21	1.52 $\pm$ 0.1	0.03 *
Fibrinolytic activity ng/ml/h	198 $\pm$ 102	291 $\pm$ 121	0.16 NS
Red cell filtration index	0.45 $\pm$ 0.15	0.37 $\pm$ 0.11	0.27 NS

The results of the platelet function tests showed that there was apparently greater reactivity in the group that later suffered thrombosis; there was a higher extent of aggregation, a lower threshold to ADP (Fig. 6.3) and a higher β -thromboglobulin level. Platelet adhesiveness, a very variable function, was lower in the thrombotic patients but not significantly so. The whole blood platelet count was significantly higher in the thrombotic group ($277 : 171 \times 10^9 / L$) ($p = 0.01$) (Fig. 6.4).

An attempt was made to estimate the risk of thrombosis by multivariate regression analysis using the GLIM software computer programme (Nelder, 1975). The programme examines each variable for its value in discriminating between group 1 (the patients with no problems) and group 2 (the patients with thrombotic problems); having picked out the best discriminants, it assigns each variable a certain weighting, such that when the variables are added the maximum possible separation is achieved between group 1 and group 2. The useful discriminants were whole blood platelet count, β -thromboglobulin level and plasma viscosity. These values were assembled into a formula which allowed the two groups to be separated without overlap:-

$$x = 76.7 + 0.19 \times \text{platelet count} - 100.6 \times \text{plasma viscosity} + 0.43 \times \beta\text{-thromboglobulin}$$

The values of "x" were calculated for each patient, and are shown in Fig. 6.5.

DISCUSSION

The final numbers of patients in this study which could be analysed with validity are small, because of the need for adequate follow

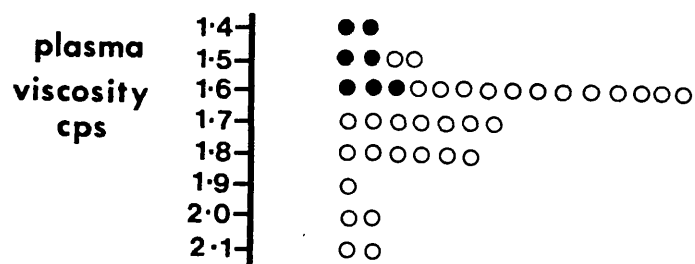


FIG. 6.2

Plasma viscosity distribution in patients undergoing

fistula construction. ● = patients with later thrombosis

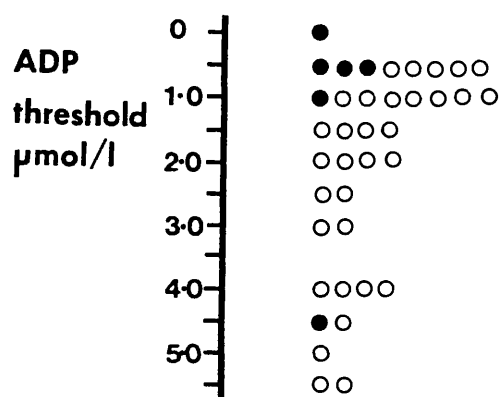


FIG. 6.3

Threshold for ADP stimulated platelet aggregation

in fistula patients. ● = patients with later thrombosis

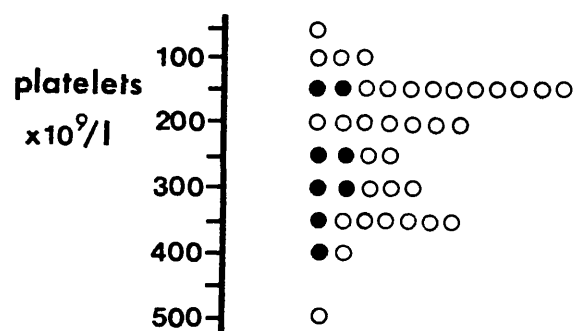
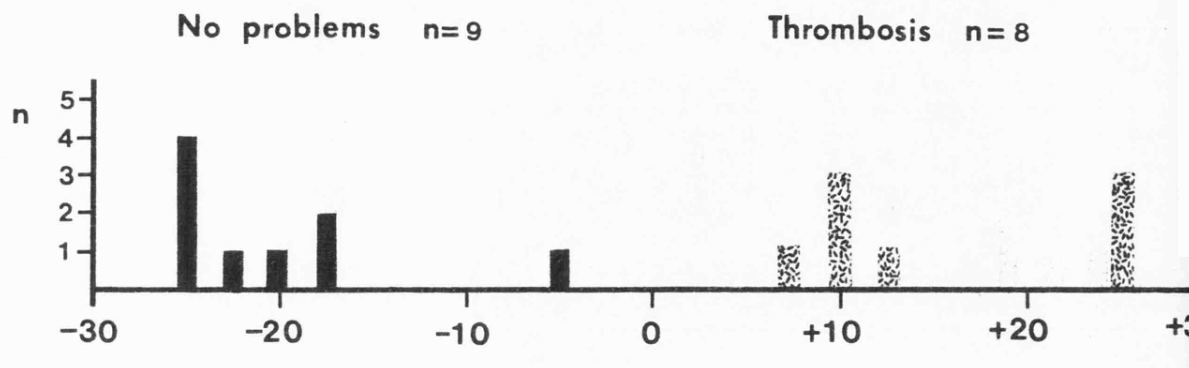


FIG. 6.4

Whole blood platelet count in fistula patients.

● = patients with later thrombosis.



Values for $x = 76.7 + 0.19 \times PC - 100.6 \times PV + 0.43 \times \beta_{TG}$

FIG. 6.5

Graphical representation of separation of fistula patients into thrombotic and non-thrombotic groups using GLIM multivariate analysis.

up before assigning a patient to the group at low risk of thrombosis. Despite these small numbers, there were two significant differences between the two groups; the patients who went on to suffer thrombosis had a higher platelet count and a lower plasma viscosity. Other differences did not achieve significance, but might be pointers; the higher β -thromboglobulin level suggests a greater activation of platelets in vivo; the greater extent of platelet aggregation in vitro to ADP may be explained by the higher platelet count, but the lower threshold to ADP may be a better index of increased sensitivity of the platelet, because it is less dependent on platelet count (Chapter 4, p103). It seems reasonable that the concentration of platelets in whole blood should be taken into account in assessing thrombotic risk; the fact that the platelet counts are significantly higher in the thrombotic patients, whilst the differences in the platelet function tests do not achieve significance, suggest an over-riding importance of absolute platelet number. In this study patients had blood assayed before operation and were followed prospectively so that the results of the tests are likely to reflect the spontaneous activity of platelets and the fibrinolytic system, rather than the effect of thrombosis; studies which have suggested that, for example, platelet malondialdehyde assay is an index of thrombotic risk, may be measuring the result rather than the risk of thrombosis.

Whole blood platelet count has been described as an index of thrombotic risk in several studies, e.g. in peripheral vascular disease (Bouhoutsos, 1974; Morris-Jones, 1981). It is more difficult to explain why the thrombotic patients should have a lower plasma viscosity (and a slightly lower whole blood viscosity at the same haematocrit).

Plasma viscosity correlates strongly with fibrinogen but is also affected by other plasma proteins. Fibrinogen is lower in the thrombotic group, but not greatly so; other proteins were not measured and it is a possibility that hypoalbuminaemia, for instance, might explain the difference. Although fibrinogen has been implicated as a risk factor for myocardial infarction (Meade, 1980) studies on the outcome of peripheral arterial reconstruction have shown no influence of fibrinogen (Greenhalgh, 1981); there is also the interesting observation that dilution of blood leads to faster clotting, and that haemodilution during surgery may correlate with the onset of deep vein thrombosis (Janvrin, 1980). It is known that with artificial surfaces adsorption of albumin in preference to fibrinogen reduces platelet deposition (Lyman, 1974). It may be that the lowered plasma viscosity reflects an alteration in albumin concentration which renders platelet deposition more likely. Further investigation is needed to confirm or disprove this finding.

Red cell filterability, despite the previously shown negative correlation with plasma viscosity, is lower in the thrombotic group, although the difference does not reach a significant level. Less flexible red cells should theoretically increase the likelihood of thrombosis by increasing resistance to flow, however, no association between whole blood viscosity and red cell filterability could be shown in this study; the likely reason is the over-riding importance of haematocrit in determining viscosity, which may disguise any small contribution from red cell flexibility (Chapter 4, p110).

The data on these two groups of patients are sufficient to conclude that there is a strong probability of intrinsic differences in

coagulability between patients who will have later thrombosis of their arteriovenous fistulae and those with a trouble-free course. The patients do not otherwise differ in age, creatinine level or haematocrit, nor in details of their fistula operation; all operations were performed by the same surgeon and similar size vessels were used in both groups; an earlier study showed that while failures were more common in patients with small vessels, failure could not be predicted by analysis of factors such as age, sex, size of vessels, size of anastomosis, suture material or time before needling of the fistula (Chapter 2).

The construction of a formula separating the thrombotic from the non-thrombotic patients does not of course establish the validity of the supposed difference between the two groups; in order to prove the formula it should then be applied prospectively to another group of patients to confirm whether the thrombotic risk could be accurately predicted. This could be done by applying the formula to the data from the 25 fistula patients who had not completed a year's follow-up - this provides a table of likelihood of thrombosis (Table 6.3). An arbitrary cut-off point is taken at +5 : this gives 12 patients with a greater probability of thrombotic problems; in 2 months since the time of review, 1 patient in the high risk group has had a thrombosed fistula, and none in the low risk group.

The remaining study to be performed was to assess the importance of blood flow at the time of operation in determining success or failure of the fistula. Despite the lack of difference

TABLE 6.3

Prediction of future thrombosis (derived from $x = 76.7 + 0.19PC - 100.6PV + 0.43\beta - TG$) in 25 patients with arteriovenous fistulae.

Thrombotic RiskPatient x value

DH	+	61	
BF	+	58	died at 2m
JG	+	40	
WV	+	24	
KH	+	21	died at 3m
AD	+	14.9	
MH	+	14	
BJ	+	13	<u>thrombosis at 3m</u>
JM	+	12.8	
GS	+	11.7	
DM	+	8.4	
AS	+	7.86	

Probable SuccessPatient x value

EM	-	50.7	
WB	-	50	
DJ	-	22	died at 2m
HL	-	20	died at 3m
BP	-	18	
EW	-	17.3	
TB	-	9	
DB	-	4.9	
EB	-	2.8	
TB	-	2.8	
MB	+	0.27	
HA	+	1.6	
SK	+	2.8	

in vessel size in the thrombotic and non-thrombotic group, absolute flow might still be significantly different. It would be important to know whether low flow at operation led to failure, or might later develop adequately. The results of this study are described in Chapter 7.

CHAPTER 7

CHAPTER 7

ARTERIO-VEINUS FISTULAE FOR DIALYSIS : BLOOD FLOW, VISCOSITY AND LONG-TERM PATENCY

INTRODUCTION

For long-lasting vascular access as many patients as possible should be considered for a wrist fistula (see Chapter Two). It is therefore important to know the fate of fistulae constructed from small veins. An established flow of about 300 ml/min is necessary for successful haemodialysis but it is not known whether in fistulae with a low initial flow the rate will later increase to this level. The reported successful development of fistulae after microvascular anastomosis (Mansfield & Cooke, 1978) would suggest that technique may be more significant than absolute flow. If this were confirmed more patients could benefit from a wrist fistula and other access sites could be saved for the future.

There are several published studies of the direct or indirect measurement of blood flow through dialysis fistulae (Gothlin et al., 1977; Wallace & Jamison, 1978; Anderson et al., 1977) but no prospective studies of the importance of flow rates at the time of operation in determining subsequent success or failure of the fistula. The aims of the present study were (i) to investigate the determinants of blood flow at the time of operation (ii) to study prospectively the relationship between operative blood flow and later fistula performance.

MATERIALS AND METHODS

Patients

During the period September 1980 to June 1981, 29 patients underwent 33 operations for the construction of an arteriovenous fistula at the wrist. There were 9 females of mean age 38 years (26-46 years) and 20 males of mean age 43 years (16-61 years). There were 19 first operations in new patients of which 17 were side-to-side and 2 end-to-side anastomoses. The remaining 14 operations were performed on 12 patients undergoing secondary access procedures. These were 6 side-to-side, 3 end-to-side anastomoses and 5 end-to-side conversions of established side-to-side fistulae which had developed a short segment of venous stenosis. These last 5 fistulae constructed with already arterialised vein were analysed together with and separately from the 28 new fistula operations. Mean follow-up time was 7.0 months (2.0-12.0). Nineteen of the 33 fistulae were used for dialysis, 9 have not yet been used, 4 were never used because of thrombosis or failure to develop adequately, and 1 patient died before use of the fistula. In total, there were 9 incidents of thrombosis, stenosis or failure to develop.

Methods

All operations were performed using local infiltration of 1% lignocaine, 10mgs of intravenous diazepam, and 5000 units of heparin intravenously before application of vascular clamps. Side-to-side fistulae were constructed by the method of Brescia et al (1966) with the following modifications: a sigmoid incision to facilitate vessel mobilisation, an anastomosis diameter of 1.0 - 1.5 cms (mean diameter

Before construction of the fistula systolic blood pressure was measured in the fistula arm using a sphygmomanometer. Pre-operatively blood was taken for viscosity measurements. A 2ml venous blood sample anticoagulated with 2mgs of K_2EDTA was kept at room temperature and viscosity estimated within one hour of venesection. Viscosity measurements were performed at 5 shear rates from $11.5s^{-1}$ to $230s^{-1}$ at $37^{\circ}C$ using a Wells-Brookfield microviscometer. Haematocrit was measured using the Hawksley microhaematocrit centrifuge. All fistula operations were performed by the same individual.

Statistical analysis was performed using linear regression analysis for correlating paired normally distributed values. Different populations were compared using the Wilcoxon rank sum test if sample size was small and the two sample 't' test if the sample size was large enough to determine normality. Mean values for groups are given $\pm$ standard deviation.

RESULTS

1. Blood Flow and Subsequent Patency

The mean total flow through the fistula for the whole group was 222 ± 171 ml/min (Figure 7.3). For the 28 new fistulae the flow was 178 ± 99 ml/min and for the 5 end-to-side conversions of established fistulae (with already arterialised veins) the flow was 470 ml/min (range 100-750). Mean flow for the 9 fistulae developing venous stenotic or thrombotic problems, including the two failing to develop sufficiently for dialysis was 221 ± 189 ml/min. Six of these were new fistulae with a mean flow of 166 ml/min (range 42-335),

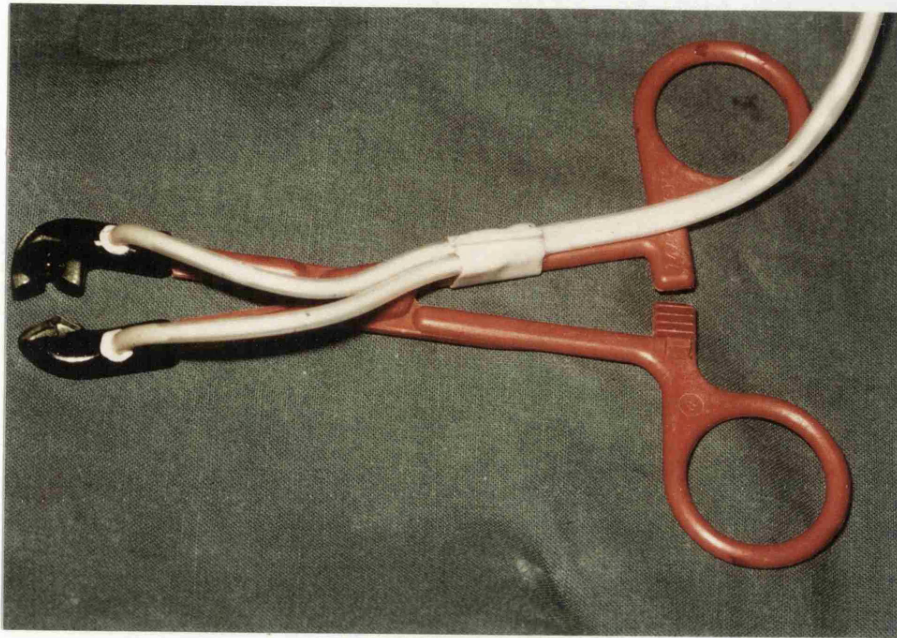


FIG. 7.1 Forceps-mounted electromagnetic flow probe.

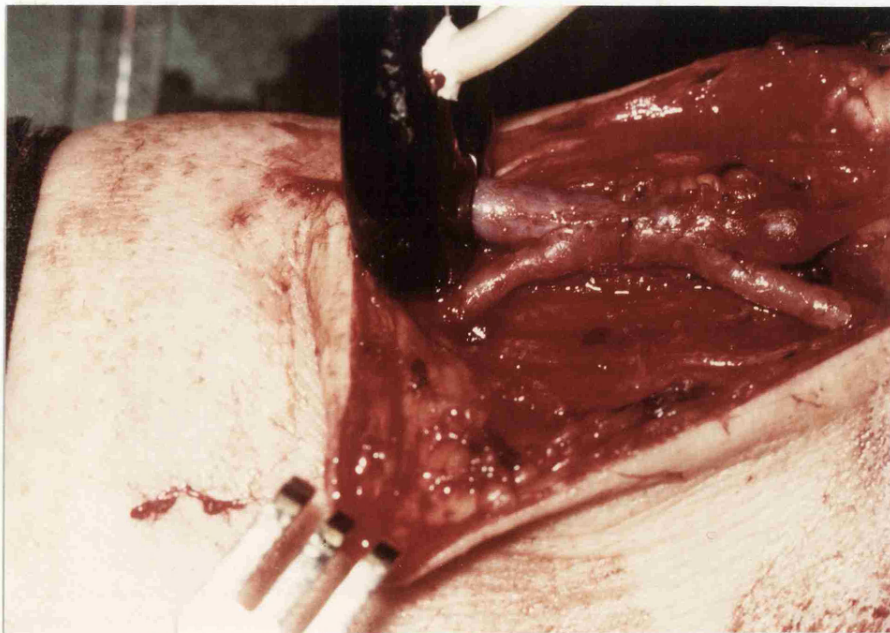


FIG. 7.2 Application of flow probe constricting the proximal vein slightly to ensure good electrical contact. Note valve in distal vein.

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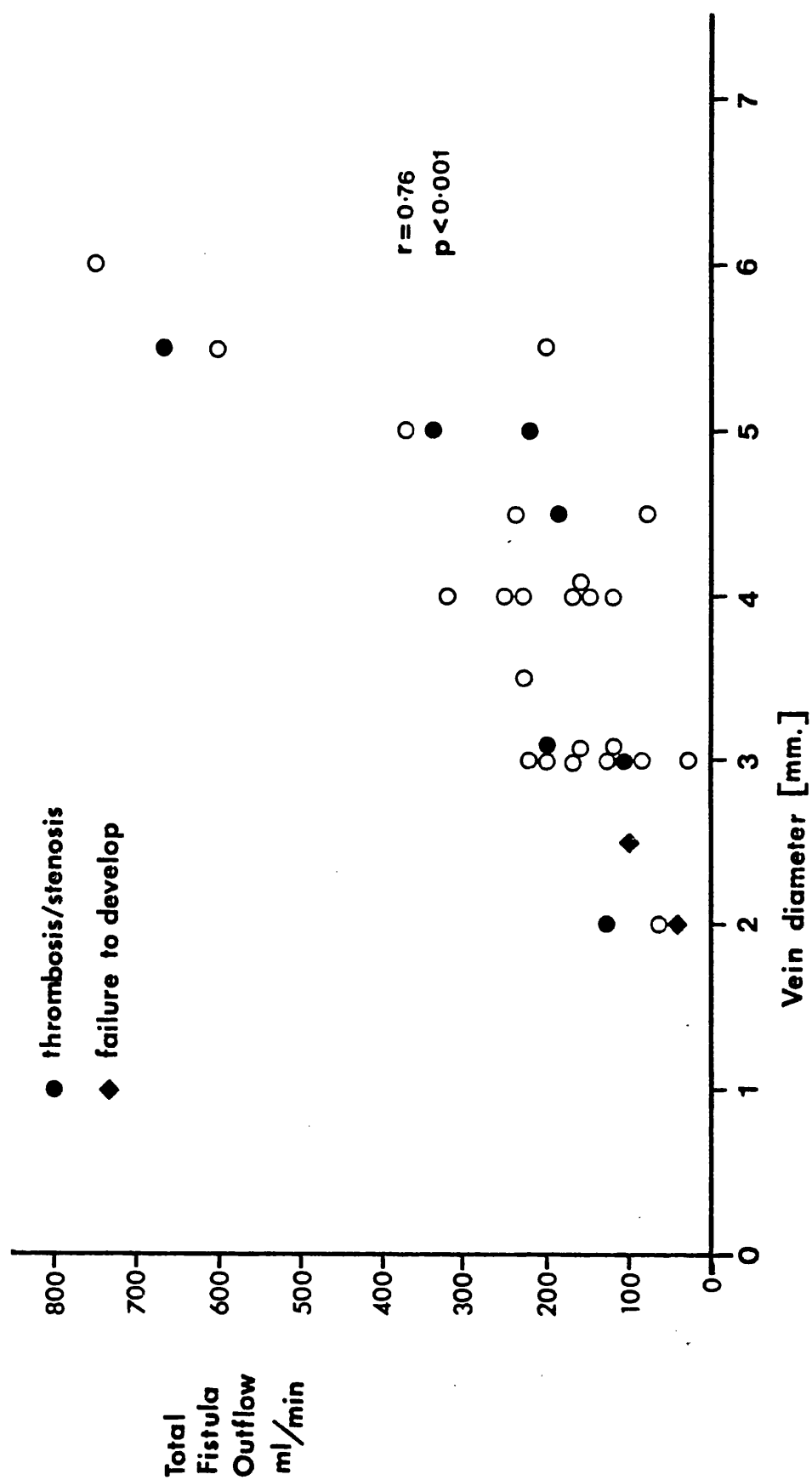


FIGURE 7.3

Relationship of total fistula outflow to vein diameter.

and 3 were end-to-side conversions of established fistulae, with a mean flow of 330 mls/min.

Although the mean flow for the group developing problems was lower than for the other new fistulae this was not significant. There were 3 complications amongst 15 fistulae with flow below the group mean of 178 ml/min, and 3 complications in the 13 with flow above 178 ml/min (Table 7.1). There were 3 complications within 6 months (2 thromboses, 1 failure to develop) amongst the 5 operations on established fistulae, with operative flow rates of 670, 220 and 100 ml/min. There was no definable upper limit of flow above which success of the fistulae was invariable, and no lower limit of flow below which failure was inevitable (Figure 7.3).

Pattern of Fistula Outflow

In the side-to-side fistulae ($n = 23$) proximal vein flow exceeded distal vein flow in 21. In 10 of these there was distal vein pulsation but no flow, presumably due to a valve. In the other 11 distal vein flow varied from 3 - 60 mls/min. In 2 patients distal vein flow exceeded proximal flow : one of these is dialysing satisfactorily; the other fistula developed proximal vein thrombosis leading to hyperaemia of the hand, requiring an end-to-side conversion operation.

Distal arterial flow was estimated indirectly by subtracting proximal vein flow with the distal artery clamped from proximal vein flow with the distal artery patent. Flow was found to be retrograde; the contribution made to proximal vein flow was expressed as a percentage (Table 7.2) and varied from 0 - 50%; for the 28 new fistulae

TABLE 7.1

OPERATIVE BLOOD FLOW AND INCIDENCE OF SUBSEQUENT COMPLICATIONS.

Group 1 = new fistulae with flow below 178 ml/min

Group 2 = new fistulae with flow above 178 ml/min

New Fistulae	No.	Mean Flow (ml/min)	End-to-Side	Side-to-Side	Thrombosis	Stenosis	Failed to Develop
Used for Dialysis							
Group 1	7	121	-	7	-	1	-
Group 2	8	238	2	6	-	1	-
Not yet used							
Group 1	4	117	1	3	-	-	-
Group 2	3	305	-	3	-	-	-
Never used							
Group 1	3	84	1	2	1	-	1
Group 2	2	270	-	2	2	-	-
Lost to follow-up	1	60	1	-	-	-	-
'Salvage' operations	5	470	5	-	2	-	1
	33	262	10	23	5	2	2
							146

the mean was 19%. In the 5 conversion operations on established fistulae the distal artery contributed only 6% of proximal vein flow (0 - 13%). From Table 7.2 it is also apparent that in most patients the contribution made by the distal artery rises considerably on clamping the proximal artery, from a mean of 25 mls/min to 64 mls/min.

2. Influences on Fistula Blood Flow

Blood Pressure

Mean blood pressure for the problem group (n = 9) was 153 mmHg compared with a mean of 158 mmHg for the group with no complications. No correlation between blood pressure and fistula outflow was observed unless the 5 established fistulae were excluded. Mean blood pressure for the new fistula group only was 158 ± 23 mmHg and flow correlated positively with fistula outflow ($r = 0.48$, $p < 0.05$) (Figure 7.4).

Vessel Dimensions

There was a good correlation between vein diameter and fistula flow (Figure 7.3); the wider the vein the greater the flow ($r = 0.76$, $p < 0.001$). However, failure was not confined to patients with small veins (Figure 7.3). There was also a significant correlation between arterial diameter and fistula outflow ($r = 0.57$, $p < 0.001$). The diameter of the anastomosis failed to show any correlation with fistula outflow.

Whole Blood Viscosity

Whole blood viscosity was measured before construction in 28 fistulae; the mean viscosity at $230s^{-1}$ was 3.12 ± 0.7 cps. This

TABLE 7.2

CONTRIBUTION OF DISTAL RADIAL ARTERY FLOW TO FISTULA OUTFLOW.

Fistula Type	No.	Distal Artery (A) flow ml/min	Proximal Vein (PV) flow ml/min	DA Flow PV Flow %	DA Flow With Proximal Artery Clamped
Side-to-side	23	25	142	19 (0-50)	64
End-to-side	5	19	127	19 (0-38)	64
'Salvage' operations	5	43	470	6 (0-13)	178

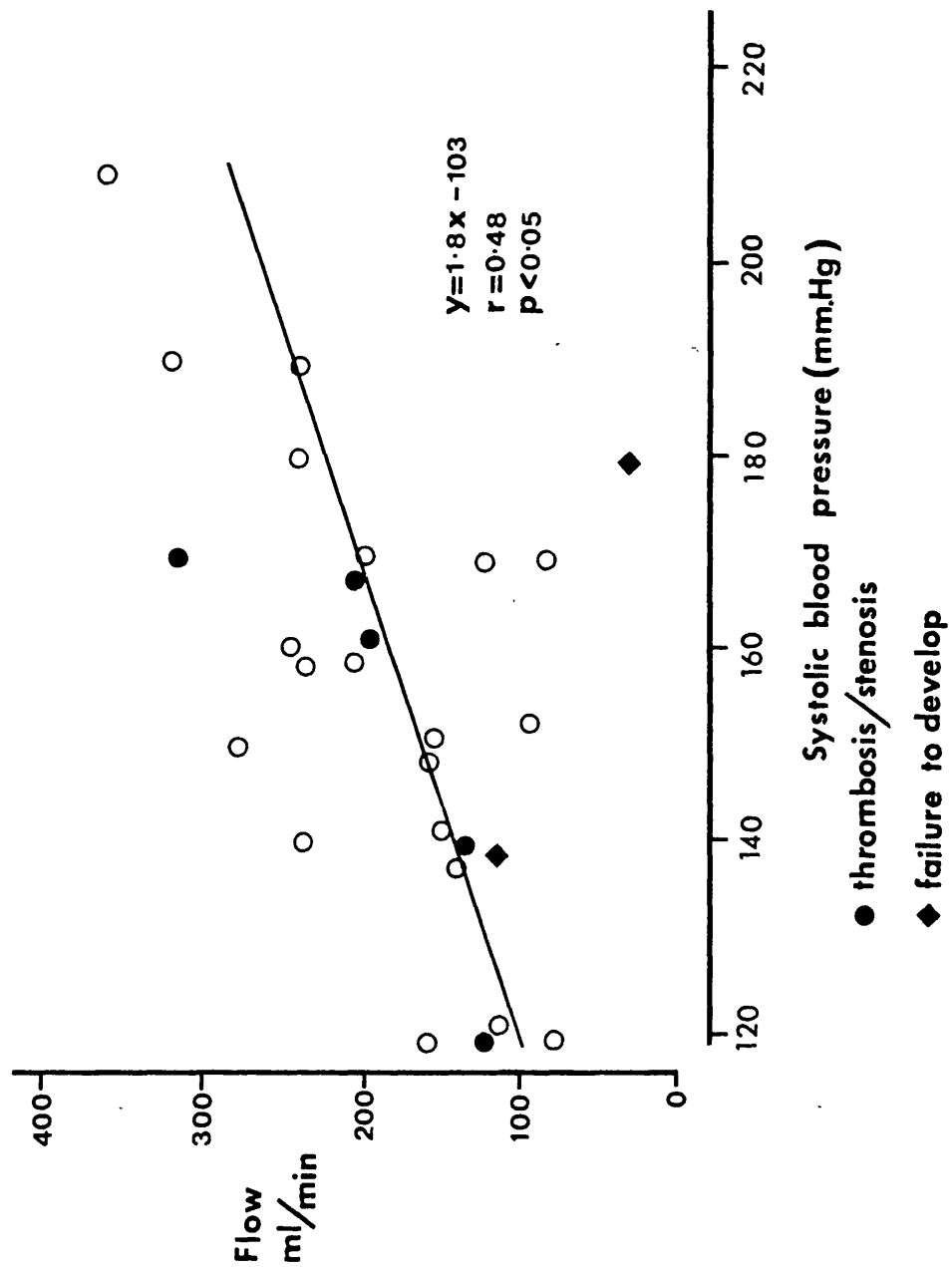


FIGURE 7.4

Relationship of total fistula outflow to systolic blood pressure in fistula arm.

reflected the low haematocrit of these patients with renal failure (mean PVC = $28.6 \pm 7\%$). For comparison, the mean whole blood viscosity at 230s^{-1} for 22 normal controls was 4.1 ± 0.5 cps at a haematocrit of $43.4 \pm 4\%$; extrapolated to the haematocrit of the renal patient this would adjust to a value of 3.0 cps, not significantly different. Flow correlated negatively with viscosity at 230s^{-1} ($r = 0.54$, $p < 0.05$) only for the 18 patients with veins 4mm in diameter or less (Figure 7.5) but not for larger vessels. Correlation was not significant at low shear rates.

Viscosity at 230s^{-1} for the 9 fistulae developing problems was 3.53 ± 0.85 cps (biased by 2 patients with viscosity of over 4 cps, operated on twice) compared with a viscosity of 2.95 ± 0.54 for the 17 fistulae without problems. This difference was not significant (Wilcoxon rank sum test).

DISCUSSION

In 38 end-to-side wrist fistulae Anderson et al (1977) reported a mean flow rate of 242 ml/min at the time of operation. This is higher than the mean flow rate of 178 ml/min for 28 new fistulae reported here, and probably reflects the unit policy of attempting a wrist fistula in all patients without previous access surgery, despite apparently poor veins. Thus 14 of this series were patients with a vein diameter at the wrist of 2 - 3mm, of which 9 had no surgical complications. As expected there was greater flow in patients with larger veins, but this did not prevent later occurrence

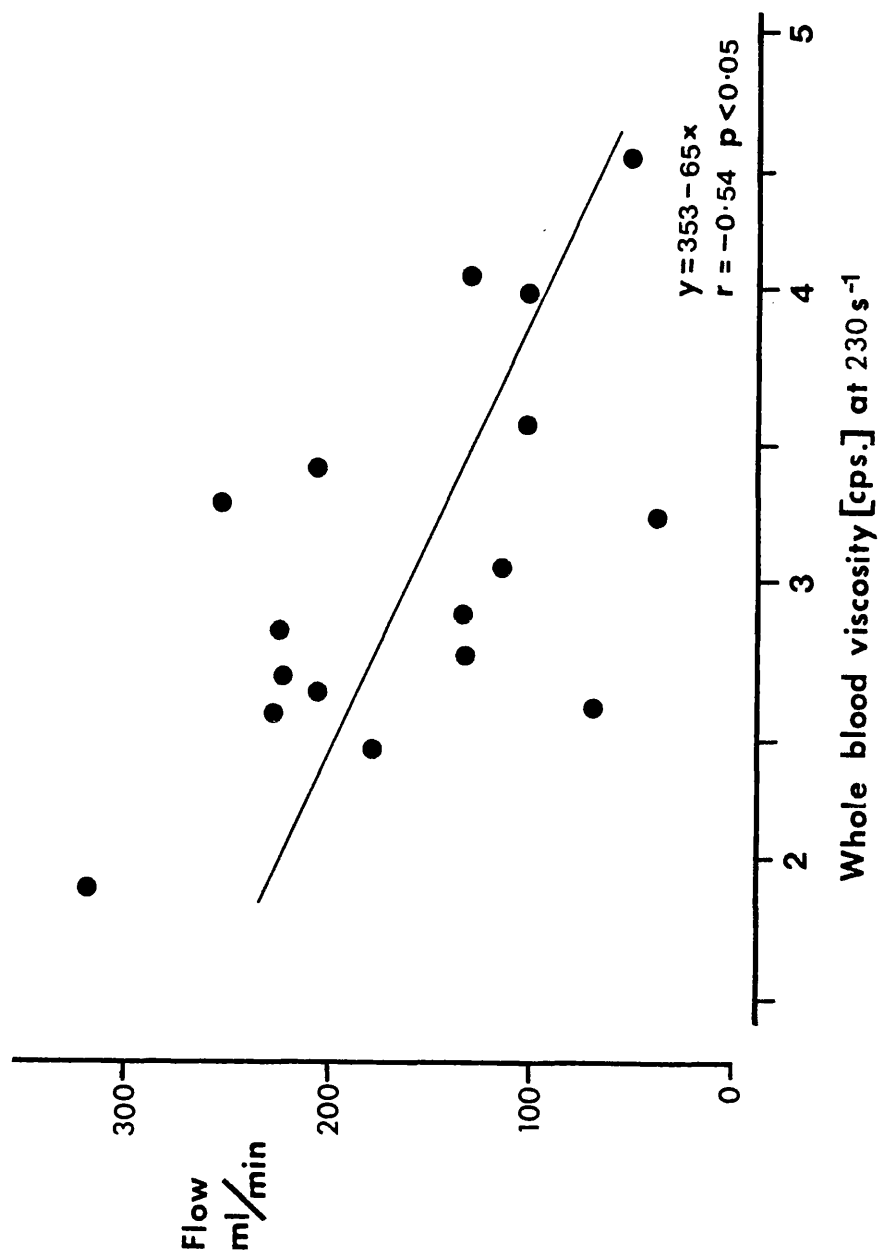


FIGURE 7.5

Relationship of total fistula outflow to whole blood viscosity in patients with a vein diameter of 4 mm or less.

of thrombosis, particularly if the operation was a conversion of an established fistula which had developed proximal vein stenosis or thrombosis.

The contribution of the distal radial artery to flow described by Anderson et al (1977) is confirmed and thus end-to-side or side-to-side fistulae are likely to be superior in flow to end-to-end fistulae. This may be the explanation for Kinnaert's finding that end-to-side fistulae performed better than end-to-end, although he ascribed it to a 'better vascular bed' (Kinnaert et al., 1977).

Local policy is to use side-to-side fistulae as the procedure of choice because many patients on home dialysis find the distal segment of the cephalic vein invaluable as a needling site. The side-to-side configuration has sometimes been criticised because of the risk of venous hypertension of the hand. In the present study only in two of the 23 side-to-side fistulae did flow into the distal vein exceed flow up the proximal vein; in one of these patients there was high flow in both directions, and in the other there was relatively low flow. In the latter proximal venous pulsation disappeared three months after operation and swelling of the hand occurred. A revision operation with a more proximal end-to-side anastomosis was then performed but despite high flow further thrombosis occurred three months later. It may be that early venous hypertension is an indication of pre-existing stenosis in the proximal vein, just as hyperaemia may develop late as a result of gradual stenosis of the proximal vein.

No attempt was made in Anderson's study to define whether critical flow rates exist below which success of fistulae is unlikely. In this study no such critical levels were found; even when the maximum flow achieved at operation was less than 150 ml/min, 9/13 patients developed adequate fistulae, and 4 of the 15 fistulae with a flow of 200 ml/min or more developed subsequent problems. This suggests that in the thrombosis of fistulae, absolute flow is not the most important of Virchow's triad, if careful attention is paid to technique. Despite the correlation of higher viscosity with lower flow it has not been shown that higher whole blood viscosity contributed significantly to fistula failure and other influences must be sought. A positive correlation between blood pressure and fistula flow has been shown in this study and it has been suggested that hypertensive patients should have fewer thrombotic problems with their fistulae (Hammill et al., 1980). On the numbers in the present series, it was not possible to detect any differences in mean blood pressure in the failure and success groups and it seems unlikely that blood pressure should exert a major influence. Vessel diameter, as discussed above, was also not crucial to success, which suggests that the possibility of a relatively hypercoagulable state should be considered. In addition more information is required about the maturation of fistulae, perhaps using the newer Doppler imaging devices for non-invasive measurement of both vessel diameter and blood velocity.

CHAPTER 8

CHAPTER 8

CONCLUSIONS

This study had two main aims; the first was to improve the provision of vascular access, and the second was to investigate the hypothesis that failure of arteriovenous fistulae was caused by a relative 'hypercoagulability' of the blood. The problem was approached by (1) a critical analysis of the complications of fistula operations (2) showing that absolute flow rates through the fistula did not dictate success or failure (3) looking for differences in rheological and haemostatic parameters between thrombotic and non-thrombotic patients, in both a retrospective and a prospective study.

Clinical Considerations

In the review of the 145 fistula patients the important points discovered were (1) a low rate of attempted salvage of fistulae in both early and late thrombosis, (2) the failure of fistulae if hyperaemia of the hand was treated only by ligation of the distal vein, (3) the acceptable success rate of brachial fistulae and hence the rare necessity for loop grafts. As a result of these studies more active measures were taken to salvage fistulae with poor flow. Angiography of every fistula giving trouble is now undertaken using the technique of Anderson (1979), in which a blood pressure cuff is inflated briefly to 250 mmHg; both artery and vein are visualised, allowing accurate delineation of the problem. This has shown that hyperaemia of the hand

usually indicates proximal vein stenosis, so that the fistula can be salvaged by an end-to-side conversion operation. It has also allowed detection of arterial stenosis, multiple vein stenoses, and shown suitable collateral veins to use.

These improvements in management will increase the lifespan of a fistula, but do nothing to prevent problems; there is definitely a sub-group of patients who suffer recurrent thrombotic problems despite apparently adequate vessels. Some failures are perhaps unavoidable, for instance, if associated with severe intercurrent illness, and some failures will be due to technical inadequacy; the prospective study with peroperative flow measurement gave a reminder that pulsation was not equivalent to flow, and on two occasions correction of technical problems due to tethering or twisting of the vein was possible. It has also been shown that small vessel fistulae are perfectly feasible and can develop adequately for dialysis; this has been useful when this has been the third or fourth access operation, perhaps after a previous brachial fistula or loop graft.

Laboratory Findings

In order to test the hypothesis that patients who suffered thrombosis of their fistula had a thrombotic tendency compared with patients without problems, a range of rheological and haemostatic factors was evaluated in a control population and then applied to patients with renal failure. Significant differences between thrombotic and non-thrombotic patients emerged in both the prospective and retrospective studies. In the prospective study it was apparent that platelets were significantly involved in that a higher platelet count

and β -thromboglobulin level correlated with later failure, although platelet counts were never in the 'thrombocythaemic' range. This is in line with current theories of arterial thrombosis which give platelet deposition a greater weight than alteration in coagulation factors, although of course the two are heavily interlinked. Similarly, in the patients on dialysis β -thromboglobulin levels were higher in the thrombotic patients. The higher viscosity at low shear rates in the thrombotic group is difficult to explain, as it should correlate with a higher fibrinogen level causing red cell aggregation. Either the finding is accidental, or other proteins are involved. In the prospective study, the low plasma viscosity may again be accidental; another possibility is that in fact the fibrinogen level was high, but was accompanied by raised fibrinogen degradation products, which could give a lower assay value for fibrinogen, and also by a solubilising effect possibly a lower plasma viscosity. These factors deserve further study; the fact remains that it was possible to separate out the thrombotic patients without overlap on the basis of blood tests, whereas it was not possible on the basis of such variables as age, sex, vessel size, and other variables, as described in the fistula review (Chapter 2). The final proof of the findings would be the successful prediction of thrombotic episodes in new patients.

Other Factors Studied : considerations for further studies

Whole Blood Viscosity has been shown to correlate with reduced flow in vessels less than 4mm in diameter, but to have no predictive value for future thrombosis. As a screening test, viscosity measurement may

have its uses, but does not contribute any profound insights. Whole blood viscosity at high shear rates is dictated by haematocrit, and at low shear rates by the degree of red cell aggregation, i.e. the fibrinogen level. As a model for the complex fluid dynamics of the circulation it is probably far too crude to measure the result of shearing blood between two metal surfaces.

Red Cell Deformability: red cell filterability was shown to be reduced in renal failure, confirming the findings of Forman (1973). Filtration was slightly but not significantly improved in dialysed patients. In trying to assess the relationship between deformability and filterability the insuperable obstacle was red cell aggregation under the influence of fibrinogen : a strong negative correlation was found between filterability and both fibrinogen and plasma viscosity. It was interesting that the patients who later thrombosed their fistulae had both low plasma viscosity and lower red cell filtration, but the filterability was not a good discriminator. In order to attempt to assess the differential effects of plasma proteins and red cell deformability per se on filtration it would be necessary to run two filtration tests in parallel, one with washed red cells in saline and one with red cells in their own plasma; this still would not completely solve the problem as to whether adsorbed proteins increased flexibility, or delayed filtration due to aggregation. It is possible that it could be a screening test like the ESR but it is unlikely to be of any great prospective use. The range of filtration index in normals, 0.3 - 0.7, allows detection of definite abnormalities: for example, patients with spherocytosis had an index of about 0.2, but renal failure patients heavily overlapped with the normal

range. In patients with intermittent claudication also, there was a large overlap with normals, and claudication distance showed no correlation with red cell filterability (Reilly, unpublished data).

Fibrinolytic Activity: a normal level of fibrinolytic activity in patients with renal failure was found, with dialysis patients showing a higher activity than normals; this would correlate with increased synthesis of fibrinogen as part of the continuing haematological stress of dialysis. No evidence for lowered fibrinolytic activity was found in patients suffering thrombosis; this could either reflect obscurement of a true difference by the variability of the test system, or could reflect the likelihood that the fibrinolytic capacity of an individual correlates more with venous than arterial thrombosis, and flow in an arteriovenous fistula is more like that in an arterial system. It would be worth pursuing this approach and refining further the test in view of a recent report that fibrinolytic capacity affects subsequent patency of femoro-popliteal grafts (Wu, 1981).

The 'Hypercoagulable' State: It has been relatively easy in the past to categorise a number of 'hypocoagulable' states such as von Willebrand's disease, marked by a prolonged bleeding time. It is less easy to define a marker for a hypercoagulable state; for example, it is difficult to detect a shorter than normal bleeding time. As yet there are no reliable tests of hyperfunction; it may be that a standard whole blood clotting time, measured accurately by changes in electrical impedance (Ur, 1977) may be a useful screening test, as found in the prediction of deep vein thromboses after surgery (Janvrin, 1980), although this tells nothing of mechanisms. It is likely that as many different types of thrombotic tendency occur as types of bleeding tendency, and in some, platelets will have a dominant

role, and in others perhaps defective fibrinolysis or increased activation of clotting factors; all these systems will be involved but the balance may shift.

Future Studies

These would be both clinical and laboratory-orientated using more sophisticated parameters of platelet activity. The indications are that thrombosis of arteriovenous fistulae is platelet dependent, despite the initial impression that with dilute blood and a bleeding tendency thrombosis should be a rarity. Even if the activity of the platelet is a reflection of some plasma factor, therapy directed towards inhibiting platelet function should have a beneficial effect on both thrombosis and venous stenosis, which is likely to be an accelerated analogue of atheroma. A trial of antiplatelet medication in fistula patients is now justified, and could be confined to the patients at risk, if further studies confirm the predictive value of the tests used here.

The next problem would be the choice of agent. There are no very effective platelet inhibitors available yet, although there are promising developments. The choice of approaches can be seen from consideration of the pathways of platelet aggregation (Fig. 1.2, p 27). Drugs are known which block cyclo-oxygenase (aspirin and other non-steroidal anti-inflammatory drugs) and which increase cyclic AMP levels and so reduce phospholipase activity (dipyridamole); if used together aspirin and dipyridamole may be synergistic - the disadvantage is that endothelial cyclo-oxygenase may also be blocked by aspirin, so that the disaggregatory prostaglandin Pgl_2 is not produced.

There have been a number of attempts to find a dose of aspirin that maximally inhibits platelets and minimally inhibits endothelium, but whether such a dose exists is still unknown. A recent study showed that aspirin at varying doses did not prevent platelet incorporation into cardiac thrombi (Ezekowitz, 1981); a number of explanations were put forward, including the possibility that the damaged myocardial area produced no prostacyclin, or that the accretion of platelets was thrombin dependent, since thrombin can bypass the thromboxane pathway. This raises the question as to which pathway is the most important in platelet activation; this is still unknown but may be resolved eventually by clinical trials of agents affecting different pathways. It is likely that the thromboxane synthetase inhibitors currently being developed would be more effective than aspirin, in that they would be selective in effect, and leave prostacyclin production untouched (Vermylen, 1981).

On the other hand, if Born's hypothesis were correct, of thrombogenesis due to platelet aggregation induced by ADP from sheared red cells, then chlorpromazine, which stabilises red cells, would be a good bet (Born, 1979). Other approaches include prostacyclin analogues and dietary modifications to increase the ratio of eicosa-pentaenoic acid (EPA) to arachidonic acid in the blood : EPA is metabolised to an inactive thromboxane, A_3 , but is converted by the vessel wall to a potent disaggregatory prostaglandin PgI_3 . Such modification has so far been achieved only by a large daily mackerel intake (Siess, 1980). It seems likely that more than one solution is possible; what is being looked for is a diminution in platelet activity, rather than an abolition which would lead to haemorrhage.

However, any present trial would have to consider aspirin and dipyridamole until other drugs become more generally available; at a dose of 150 - 300 mgs aspirin alt.day and 75mgs dipyridamole t.d.s. gastro-intestinal problems should be minimised. The thrombin pathway to platelet activation cannot be adequately inhibited at present : heparin certainly does not prevent platelet and fibrin deposition on dialyser membranes (Lindsay, 1972) and may cause platelet aggregation in normals (McLean, 1981). It will be interesting to see whether the use of prostacyclin by Weston and his colleagues rather than heparin for dialysis (Turney et al., 1980) leads to fewer thrombotic problems in renal patients, by decreasing return of activated platelets to the circulation.

APPENDICES

APPENDIX 1WHOLE BLOOD VISCOSITY

A 1ml sample of blood anticoagulated with EDTA was placed in the viscometer cup at 37°C. Two measurements were made at each shear rate (11.5, 23, 46, 115, 230 sec⁻¹) when stable reading, and the mean taken. Microhaematocrit was estimated at the same time using the Hawksley centrifuge method, the blood being spun in 3 capillary tubes for 5 minutes and the mean taken.

For each shear rate, a multiplier was applied to the viscometer dial reading, and this gave the whole blood viscosity in centipoises (see diagram).

Example of Viscometer Reading

RPM	READING 1	READING 2	MEAN	MULTIPLIER	VISCOSITY cps	SHEAR RATE s ⁻¹
60	43.7	44.0	43.85	0.1	4.38	230
30	25.0	24.5	24.75	0.2	4.95	115
12	12.4	12.1	12.25	0.5	6.13	46
6	7.1	7.3	7.2	1	7.2	23
3	4.9	4.6	4.75	2	9.5	11.5

Haematocrit (1)	43	)	
		)	
(2)	43.5	)	mean = 43
		)	
(3)	43	)	

APPENDIX II

RED CELL FILTRATION TEST

A 10ml venous blood sample was obtained and anticoagulated with 0.1ml of a 15% solution of K_3 EDTA. The sample was centrifuged at 3000 rpm (1500g) for 10 minutes, and the supernatant plasma pipetted off and filtered through a 1.2 μ type RA millipore filter. The buffy coat containing white cells and platelets was discarded. 0.2mls of packed cells was resuspended in 3.8ml of plasma to give an approximately 5% suspension. Filtration was performed at 22°C within two hours of venesection. The filtration apparatus consisted of a Nucleopore 'pop-top' filter-holder modified by having:

1. a well cored out at the centre
2. the exit port bevelled to reduce drop size (Fig.II.1)

5 μ pore size polycarbonate filters from the same high grade batch were used (Nucleopore, Sterilin Ltd). 0.5ml of suspension was kept as a standard and three successive 1ml samples of suspension were allowed to filter for 45 seconds under their own hydrostatic pressure (Fig.II.2). The filter was not pre-wetted. The filter was changed each time, and the suspension remixed by inversion before each 1ml aliquot was withdrawn. The 0.5ml standard was lysed with 2ml of distilled water containing Zap.o.Globin (Coulter Electronics Ltd); the filtrates were made up to 5ml with distilled water. A 1ml aliquot from each was further diluted with 2ml distilled water before measuring the absorption at 540nm in a spectrophotometer. The mean absorption of the three samples was taken and divided by the absorption of the standard. The mean coefficient of variation for

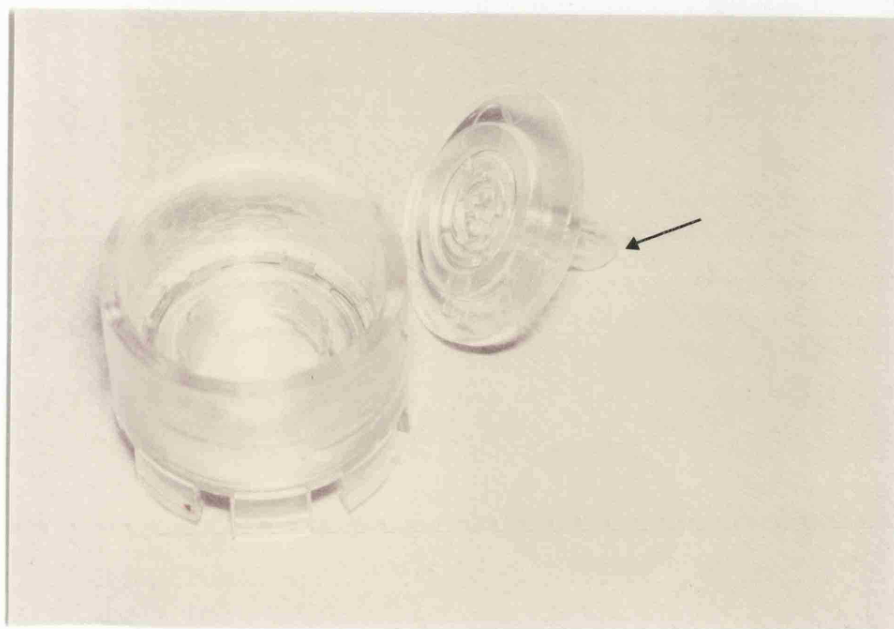


FIG. II.1 Nucleopore 'pop-top' filter holder showing central well and bevelled exit port (arrowed). 2 polycarbonate filters are just visible alongside.

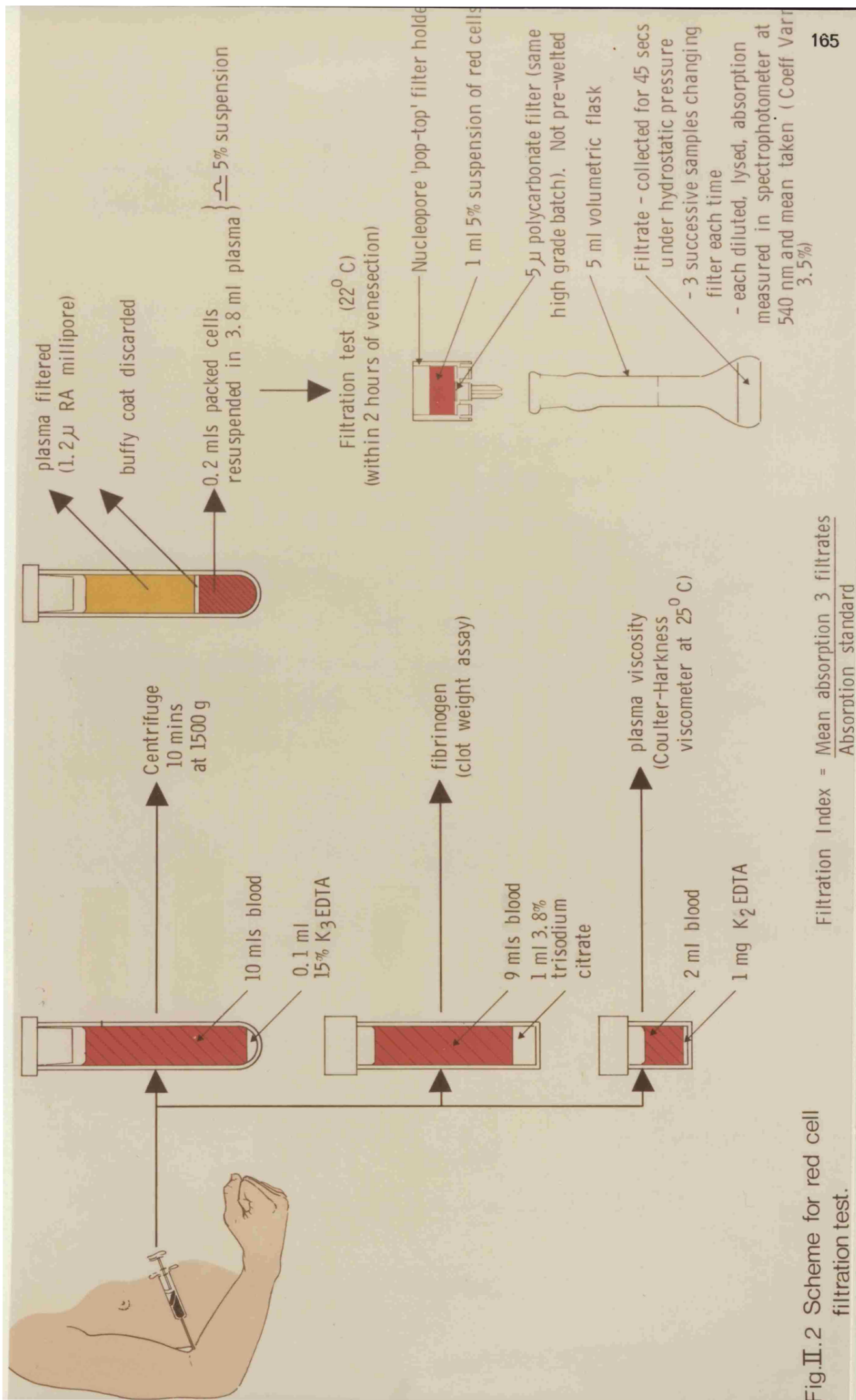


Fig.II.2 Scheme for red cell filtration test.

Filtration Index = $\frac{\text{Mean absorption 3 filtrates}}{\text{Absorption standard}}$

these three samples was 5%. The result was expressed as a 'filtration index'. The fact that an index is used corrects for minor variations of the haematocrit of the suspension.

Pitfalls in the technique included (1) air locks in the system, or air bubbles between the filter and the grid on which it rested. These were minimised by hydrating the plastic holder by soaking in water; the filter was always inspected after filtration and the measurement repeated if bubbles were seen. (2) failure of filtration due to electrostatic problems; this was prevented by discharging the filter with a cheap de-ioniser.

In the study three different observers measured red cell filtration in the control group. There were no significant differences between observers. One observer measured filtration in the renal failure group.

APPENDIX III

PLASMA VISCOSITY

Plasma from a 2ml blood sample anticoagulated with EDTA was placed in a Coulter-Harkness capillary viscometer at 25°C. The time taken for the plasma sample to pass between two points under a standard head of pressure is measured electronically. A conversion factor (using Poiseuille's equation) gives the viscosity in centipoises (cps). The plasma viscosity estimations were performed routinely by the haematology laboratory. The reference range for the laboratory was 1.50 - 1.72 cps with an accuracy of ± 0.02 cps.

APPENDIX IV

PLASMA FIBRINOGEN

Plasma from a 9ml blood sample, anticoagulated with 1ml of 3.8% trisodium citrate, was recalcified with an equal quantity of 0.025M calcium chloride and placed in a 37°C waterbath. After 15 minutes the clot was wound onto an orange-stick and the serum expressed and blotted. Then the clot was washed under running water for 5 minutes, followed by immersion in acetone for 10 minutes. The clot was then dried under a lamp for 30 minutes, weighed, and the concentration in plasma calculated. The fibrinogen assay was performed routinely by the haematology laboratory. The normal range for the laboratory was 2 - 4 g/L with an accuracy of ± 0.3 g/L.

APPENDIX V

FIBRINOLYTIC ASSAY

5mls of venous blood were withdrawn from a forearm vein into an EDTA tube after venous occlusion of less than 30secs; placed immediately in melting ice and assayed within four hours. A volume of 200 μ l of venous blood was mixed with 200 μ l of Tris NaCl buffer in a I^{125} fibrin coated polystyrene tube and incubated at 37°C for 30 minutes. The reaction was stopped with the addition of 400 μ l of cold Tris NaCl and an aliquot of the solution was counted in a gamma scintillation spectrometer. Knowing the specific activity of the fibrin used, the results were expressed as ng fibrin lysed/ml/hr. Each sample was assayed in quadruplicate and the mean taken.

The tubes were prepared by adding 100 μ l of fibrinogen solution to each polystyrene tube. The human I^{125} fibrinogen (Amersham) was dissolved in 0.015M phosphate buffer at pH 8; 100 μ l of this solution represented 10 μ g of I^{125} fibrinogen. The tubes were incubated at room temperature for 3 hours. The unbound fibrinogen was aspirated and the tubes washed three times with Tris NaCl buffer. Then 200 μ l of Tris NaCl containing 2 units of bovine thrombin (Sigma) were added and the tubes were incubated at 37°C for 15 minutes after which they were again washed with Tris NaCl buffer and stored at -20°C until required.

Since a proportion of the total radioactivity of the assay tube (1-2%) is released on incubation with Tris NaCl alone, the amount

of fibrin lysed in buffer blanks was subtracted from the results of tubes incubated with blood.

APPENDIX VI

PLATELET AGGREGATION

Sample Preparation

A 9ml venous blood sample was taken without stasis or frothing via a 19 gauge needle and anticoagulated with 1ml 3.8% trisodium citrate in a plastic tube. Samples are kept covered at room temperature and assays completed within 2 hours of venesection. The sample was centrifuged for 10 minutes at 1000rpm (200g) to obtain platelet rich plasma (PRP) which was pipetted off and the sample further centrifuged for 15 minutes at 3000rpm (1500g) to obtain platelet poor plasma (PPP). 0.5ml aliquots of PRP were placed in siliconised glass cuvettes, containing siliconised stainless steel stirrer bars, when ready to proceed with aggregometry.

Aggregometry

A cuvette containing PPP was placed in the light path of the aggregometer (Upchurch Ltd) (Fig.VI.1) and the output adjusted to read 90% on the chart recorder (Vitatron, Fisons Ltd) (Fig.VI.2). This was then replaced by a cuvette containing PRP and the zero set adjusted to read 10% light transmission (Fig.VI.2). These adjustments were repeated until stable readings were obtained. Successive samples of PRP were studied. For each sample a period of at least 1 minute recording was made until a stable base-line was established before adding the aggregating agent (ADP, Sigma).

ADP Dilutions

ADP dilutions were prepared as follows. A solution of ADP

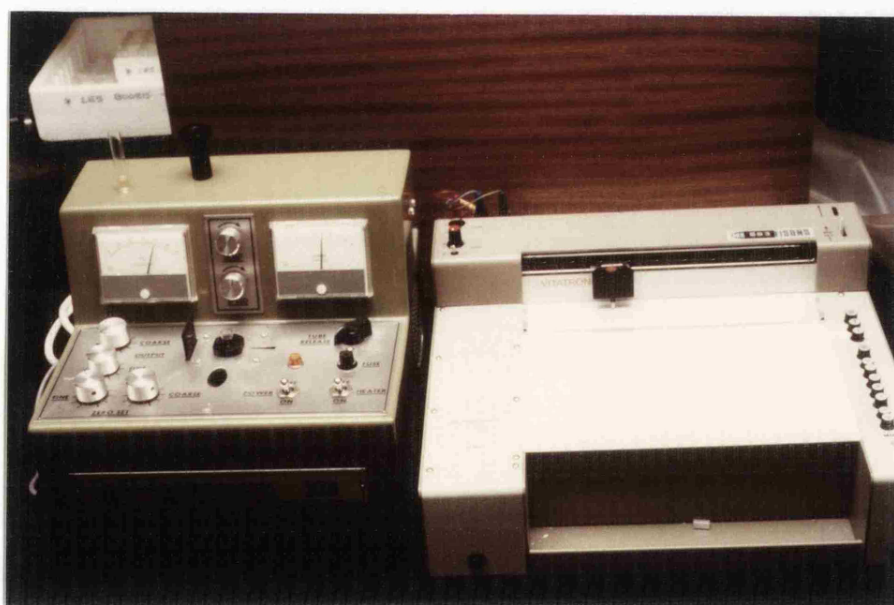
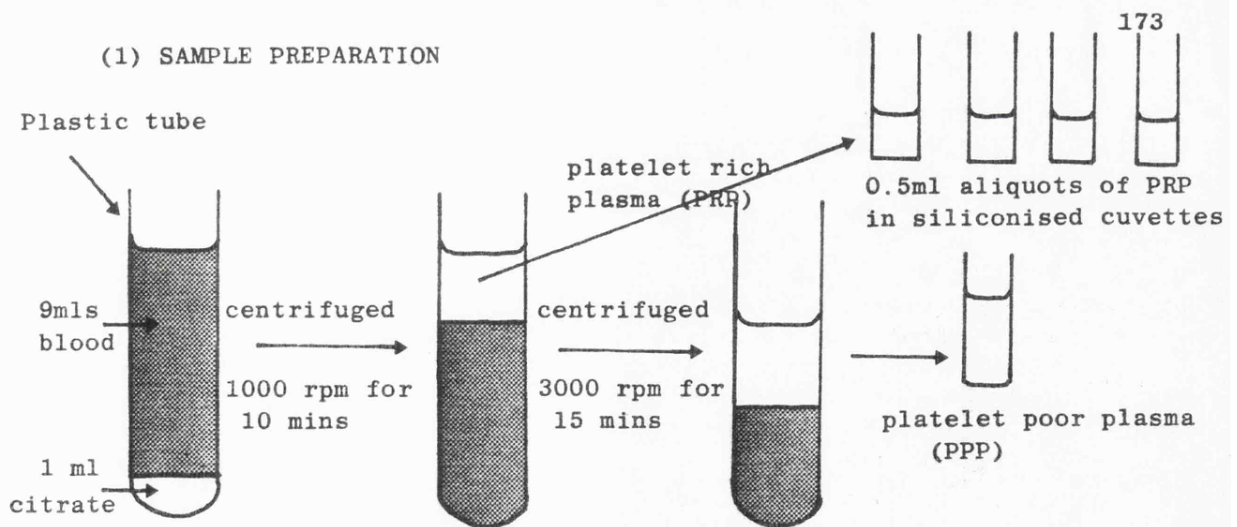


FIG. VI.1 'Aggregometer' coupled to chart recorder.



(2) PRINCIPLES OF AGGREGOMETRY

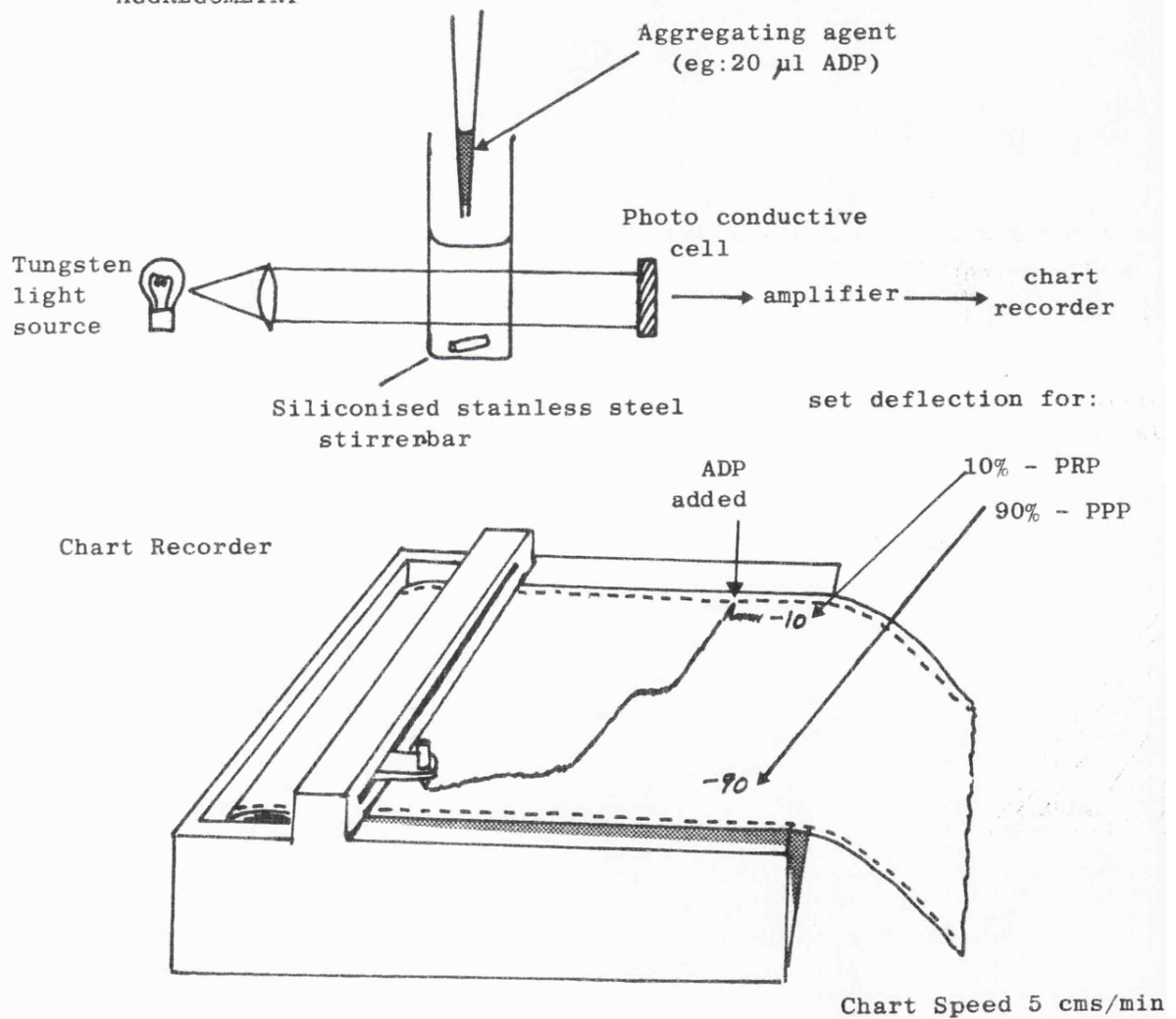


FIG. VI.2

Scheme for measurement of platelet aggregation in vitro.

in distilled water containing 10 mgs/ml was stored in 0.1 ml aliquots at -20°C . For each experiment further dilutions of one of these aliquots were made and the dilutions kept in an ice and water bath. 20 μL doses of ADP solution were added to the PRP, such that the final dilution for the standard ADP stimulus for provoking maximal response was 10 $\mu\text{mol/L}$ (using ADP of mol.wt 460).

Successive dilutions of ADP were added to successive PRP samples. Below the threshold level aggregation would be followed by disaggregation - the 'monophasic' response. Just above the threshold a second wave of aggregation would start after the first wave had formed a plateau; this is the 'biphasic' response. The ADP concentration just able to cause this response was recorded, as was the concentration just allowing disaggregation. These limits were usually determined to within 0.25 $\mu\text{mol/L}$ of each other and the mean taken to be the 'threshold'. In the controls the limits were wider, since thresholds were generally higher and could not be defined as accurately, because of the limiting factors of time and available volume of plasma. To define the ADP threshold usually required 4-6 samples of PRP and 20-30 minutes. Two measurements were made from this recording:

- (1) the extent of aggregation at 2 minutes from the addition of 10 $\mu\text{mol/L}$ ADP - measured as a percentage of the difference between the light transmission of PRP and PPP
- (2) the ADP dose for threshold response

APPENDIX VII

PLATELET ADHESION (Adeplat T test)

A 6ml venous blood sample obtained without stasis via a 19G needle was immediately (within 15 seconds) attached to the disposable glass bead column, after placing a 1ml sample in a tube marked 100% containing K_2EDTA for platelet count. The pump system (Adeplat, Immuno Ltd) was started (Fig.VII.1) and the blood passed at a standard rate through the column (containing beads of a known surface area). The third ml to pass through the system was collected into an EDTA tube marked X%.

Platelet counts were performed on both X% and 100% tubes; to determine the platelet adhesion percentage, if C_{100} was the number of platelets in the 100% tube, and C_X was the number in the X% tube, then:

$$\% \text{ platelet adhesion} = \frac{(C_{100} - C_X)}{C_{100}} \times 100$$

According to the manufacturers, platelet adhesiveness determined by the Adeplat T test on 55 volunteers, varied from 3% to 38% with a mean of 18.2% and a standard deviation of 9.2%. Since occasional values in normals were found in the range 27.4 - 38%, a value over 38% is deemed to reflect a hyperadhesive state.

Platelet Counting

50 μ l of EDTA anticoagulated blood, after mixing, was added to 950 μ l of platelet diluting fluid, and agitated gently. A drop was added to the Neubauer haemocytometer chamber and left to settle for at

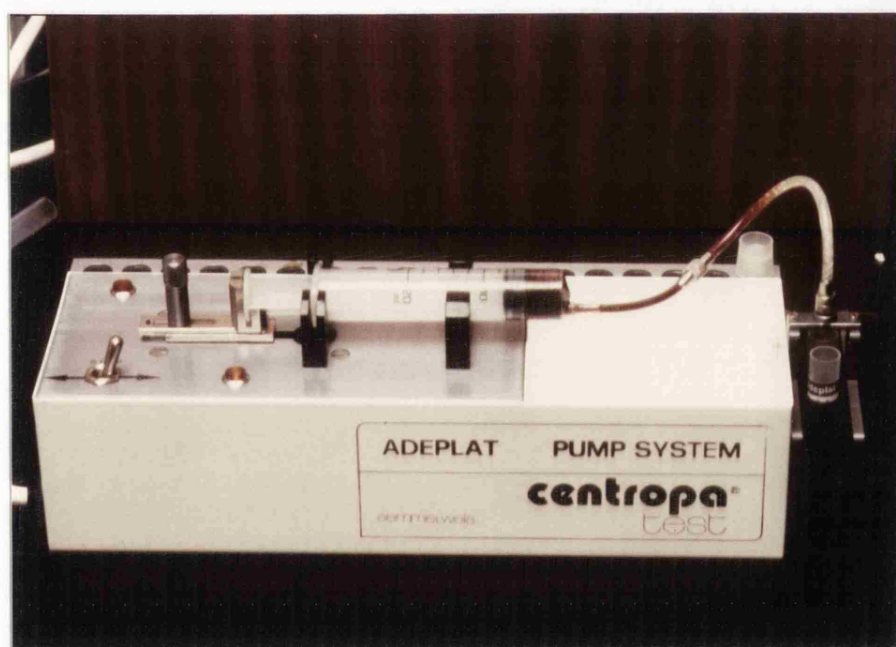


FIG. VII.1 Portable electric pump system for platelet adhesion estimation.

least 30 minutes in a humidified chamber. Platelets were then counted using a light microscope.

Platelet diluting fluid composition:

white saponin	...	0.25g
sodium citrate	...	3.52g
formaldehyde	...	5mls
distilled water	to 100mls	

This was mixed gently and filtered before use.

APPENDIX VIII

BETATHROMBOGLOBULIN ASSAY (Ludlam, 1975)

Blood Sampling

The critical stage in the method is the taking and preparation of the blood sample, since platelet release reaction occurs during collection of normal plasma.

2.5ml venous samples were taken without stasis through a 19 gauge needle and immediately placed in the pre-chilled tubes provided containing a platelet preservation mixture of EDTA and theophylline. The tubes were mixed gently and kept in an ice/water batch. Within 3 hours the samples were spun down in a refrigerated centrifuge at $2-4^{\circ}\text{C}$ for 30 minutes at 1500g. A 0.5ml aliquot was taken from the middle layer of the supernatant to avoid platelet contamination from the surface meniscus (Rasi, 1979). The sample was stored for 1-2 months at -20°C until the betathromboglobulin (β -TG) content was estimated by radioimmunoassay using the kit provided (β -TG-RIA, Radiochemical Centre, Amersham, England).

Principle of Radio-immunoassay

The assay depends on the competition between β -TG and ^{125}I -labelled β -TG for binding sites on a β -TG specific antibody. The amount of ^{125}I -labelled β -TG bound by the antibody will be inversely proportional to the concentration of unlabelled β -TG present in the platelet poor plasma samples. The ^{125}I -labelled β -TG bound by antibody can be separated by precipitation with ammonium sulphate; after centrifugation and decantation of the supernatant, the radioactivity of the precipitate is counted in a

gamma counter. A standard curve is constructed by plotting the radio-activity in the precipitate for a series of known concentrations of β -TG. The β -TG level for each unknown plasma can then be read off the curve.

Assay Technique

The standards, antiserum and ^{125}I -labelled β -TG were reconstituted with distilled water, and the plasma samples thawed. 50 μl aliquots of standards and unknowns were placed in duplicate polystyrene tubes. Each kit allowed estimation of 19 unknowns and 5 standards; 48 tubes were therefore required. 200 μl aliquots of ^{125}I -labelled β -TG solution were added to each tube, followed by 200 μl aliquots of antiserum. The tubes were vortex mixed and centrifuged at 1500g for 10 minutes. The supernatant was poured off without disturbing the sediment. The tubes were drained for 5 minutes, blotted and counted in a gamma counter for 60 seconds with energy window set at 18-56.

The means of the duplicate counts for the β -TG standards were plotted on linear graph paper (Fig. VIII.1) and a smooth curve drawn through the points. Using the means of the duplicate counts for the unknowns, their β -TG concentrations in ng/ml were read off from this curve.

The makers report a within assay coefficient of variation of less than 7.5% in all kits, for a control plasma containing 45 ng/ml β -TG. The range covered by the assay is 10-225 ng/ml β -TG.

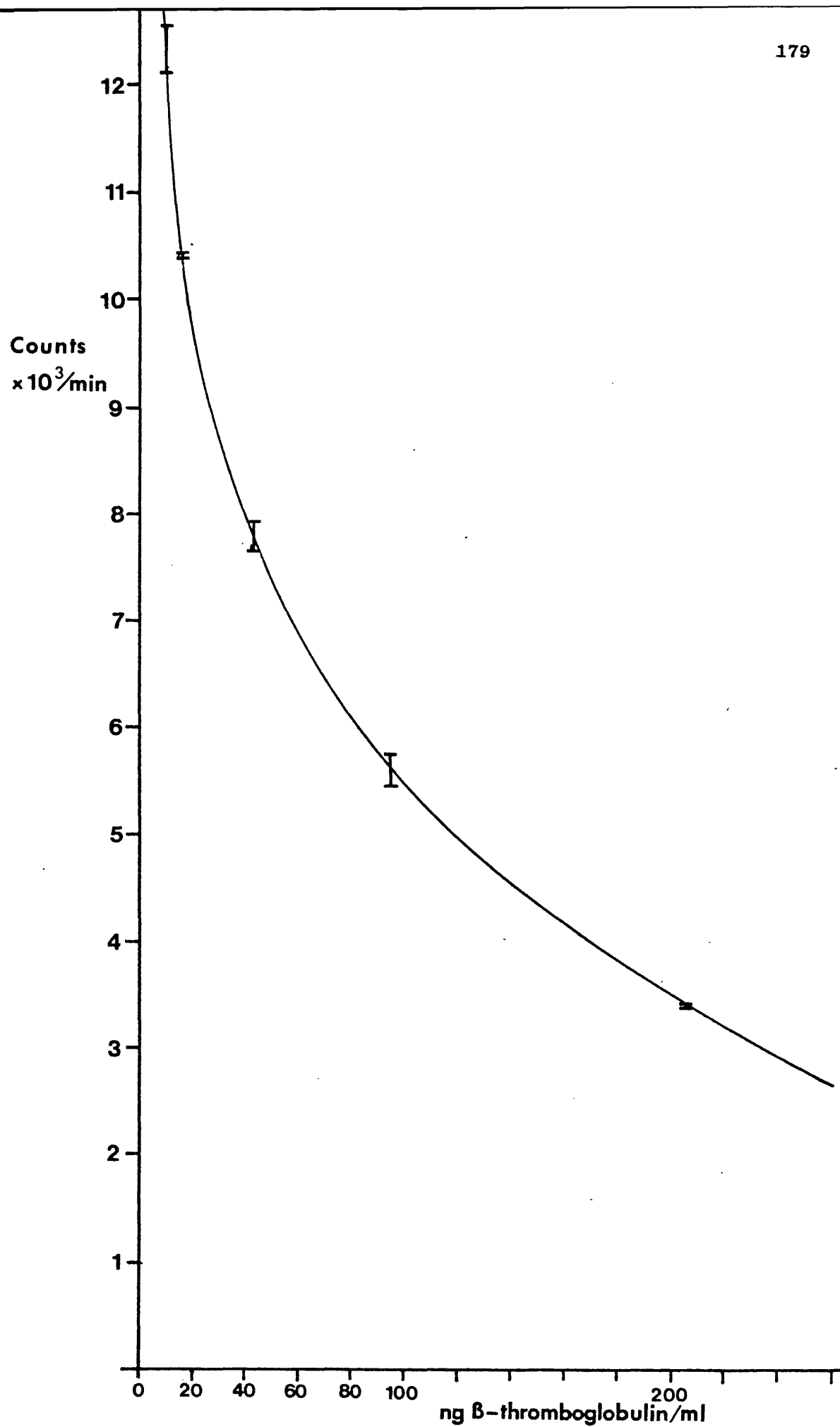


FIG. VIII.1

Standard curve for interpolation of β -thromboglobulin values.

Each point represents the mean of two assays.

APPENDIX IXTECHNIQUE FOR VENEPUNCTURE IN PATIENTS

Blood was taken from a forearm vein, with venous occlusion nil or less than 30 seconds, through a 19G needle. The first 2.5ml in a separate syringe was immediately placed in the pre-chilled collection tube for beta-thromboglobulin assay and mixed gently with the platelet preservative and anticoagulant. The next 6ml was immediately (within 15 seconds) attached to the glass bead column and the pump started for the adhesion test. In two other syringes blood was taken for platelet aggregation studies (9ml in a citrated tube), fibrinolysis (5ml in an EDTA tube), fibrinogen (9ml in a citrated tube), plasma viscosity (2ml in an EDTA tube), red cell deformability (10ml in an EDTA tube) and whole blood viscosity and haematocrit (2ml in an EDTA tube). This required 45.5ml of blood. These blood samples were all taken by the same observer.

APPENDIX X

FLOW MEASUREMENT

Method

The flowmeter used was a Cliniflow Model 601D (Carolina Medical Electronics) with three probe sizes of 6, 8 and 14mm internal circumference. The probes consist of electromagnetic coils and detectors on a Spencer-Wells type of forceps so that the probe can be accurately placed around the vessel (Fig. X.1). The principle of flow measurement is that the movement of the conducting plasma passing through the magnetic field stimulates an e.m.f. proportional to the velocity. This is detected by the probe and since the probes are calibrated and the cross-sectional area of the probe lumen is known the volume flow can be read on the meter. The critical factors in using the probe are (1) providing close apposition to the vessel for good electrical contact, (2) placing the probe far enough from the fistula to avoid turbulent flow, which causes aberrant readings, (3) the haematocrit should be known, as this affects calibration.

The degree to which the vessel is constricted does not matter within certain limits (since velocity increases and flow remains constant for a short segment stenosis until at least a 50% reduction in diameter).

At operation flow was measured in radial artery and cephalic vein after mobilisation, and after application of topical naftidrofuryl to eliminate spasm. After construction of the fistula, flow was measured in the 4 limbs of the fistula. This was repeated at 10 minutes, although often flow was already maximal after naftidrofuryl application.

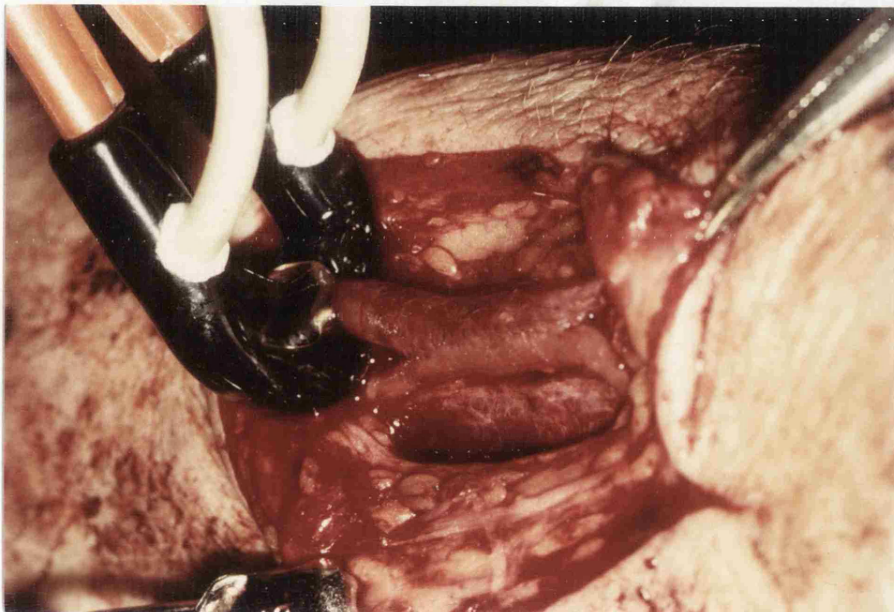


FIG. X.1 Electro-magnetic flow probe around proximal vein of a side-to-side fistula.

APPENDIX XI

OPERATIVE TECHNIQUE

In the 37 first-time fistulae studied there were 34 Brescia-Cimino side-to-side wrist fistulae and 3 end of vein to side of artery fistulae.

Preparation

The patient was sedated with diazepam 10mgs. The course of the cephalic vein and its tributaries was marked on the skin with an indelible pen. The arm was cleaned with chlorhexidine in alcohol and sterile drapes positioned. Anaesthesia was effected by local infiltration of 1% plain lignocaine.

Procedure

A sigmoid incision was made at the wrist to allow adequate exposure of the vessels (Fig.XI.1). The cephalic vein was mobilised and held with slings of 2/0 silk; small branches were tied with 4/0 plain catgut. The radial artery was mobilised and side branches were coagulated with diathermy and divided. Sufficient lengths of artery and vein were mobilised to allow anastomosis over 0.5 - 1.5cm without tension or angulation (Fig.XI.2). If the vessels were widely spaced, the distal cephalic vein was tied and divided, and an end-to-side anastomosis performed. 5000 units of heparin were injected intravenously before clamping the artery. Narrow veins were dilated with heparinised saline before anastomosis. Corresponding longitudinal incisions were made in artery and vein and anastomosis was performed using continuous polypropylene (Prolene) either 6/0 or 7/0 gauge, depending on vessel size.

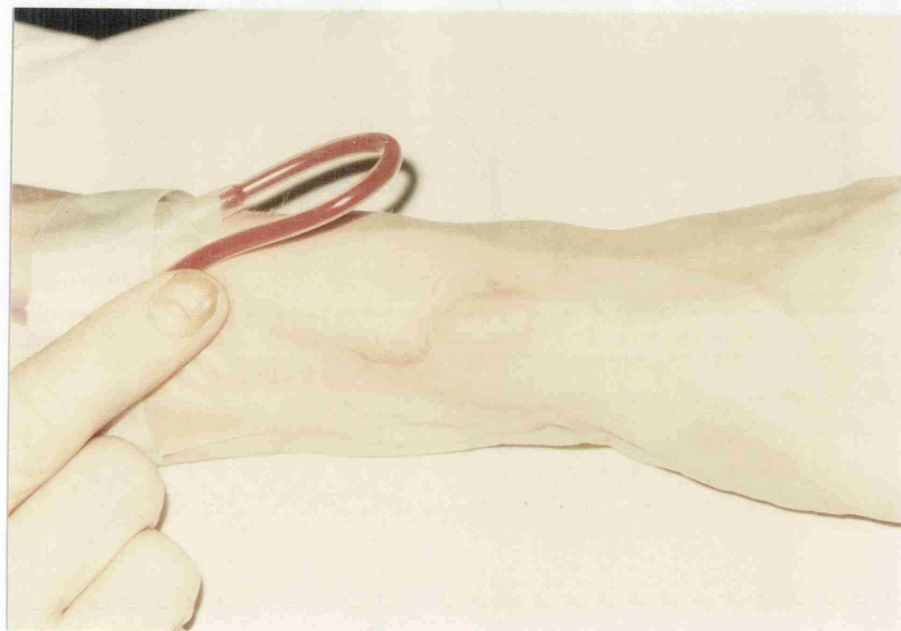
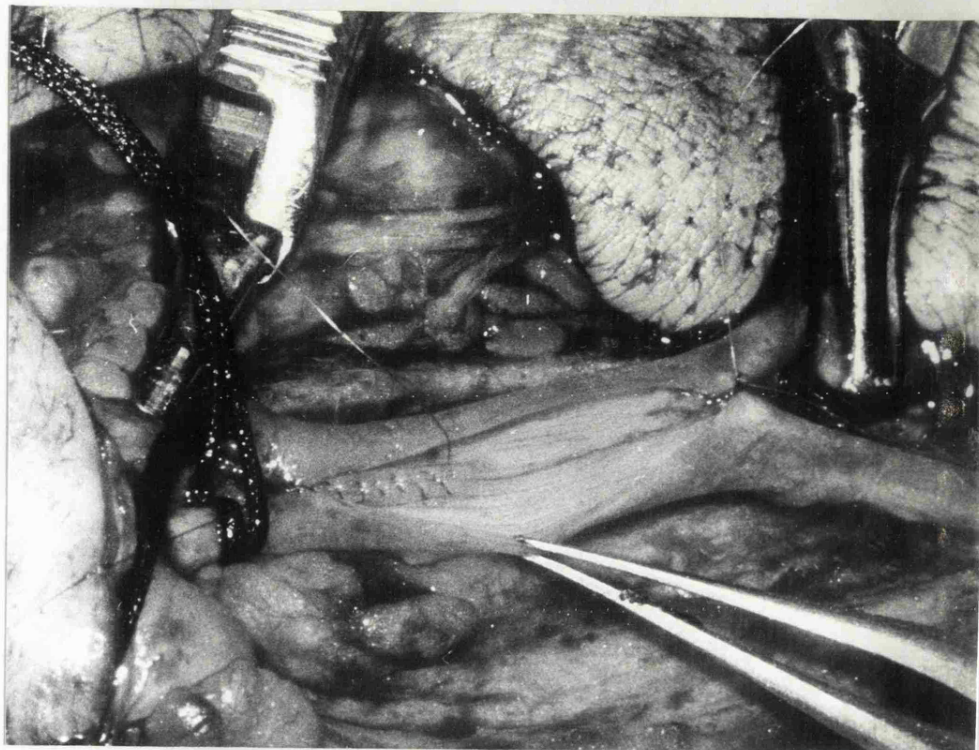


FIG. XI.1 Site of arteriovenous fistula construction at wrist.
Sigmoid incision allows maximum exposure for good
vessel mobilisation.



(a) Suturing the back wall with 7/0 Prolene.



(b) The completed anastomosis, approx. 1.2cms long.

FIG. XI.2 Side-to-side anastomosis of radial artery and cephalic vein (arrowed) at the wrist, with no tension and minimum angulation.

Topical naftidrofuryl (Praxilene) was applied to the vessels and, after flow measurements, the wound was closed with interrupted 4/0 silk. The wound was dressed with gauze and plastic spray.

Post-Operative Management

No anticoagulation was used. Frequent nursing observations were made on the fistula arm for the first 24 hours. The patient was allowed home at 48 hours. A one-week post-operative course of flucloxacillin 250mgs qds was given to prevent staphylococcal infection.

Brachial Fistula

Side-to-side fistulae were constructed similarly under local or regional anaesthesia, as shown in Fig. XI.3.

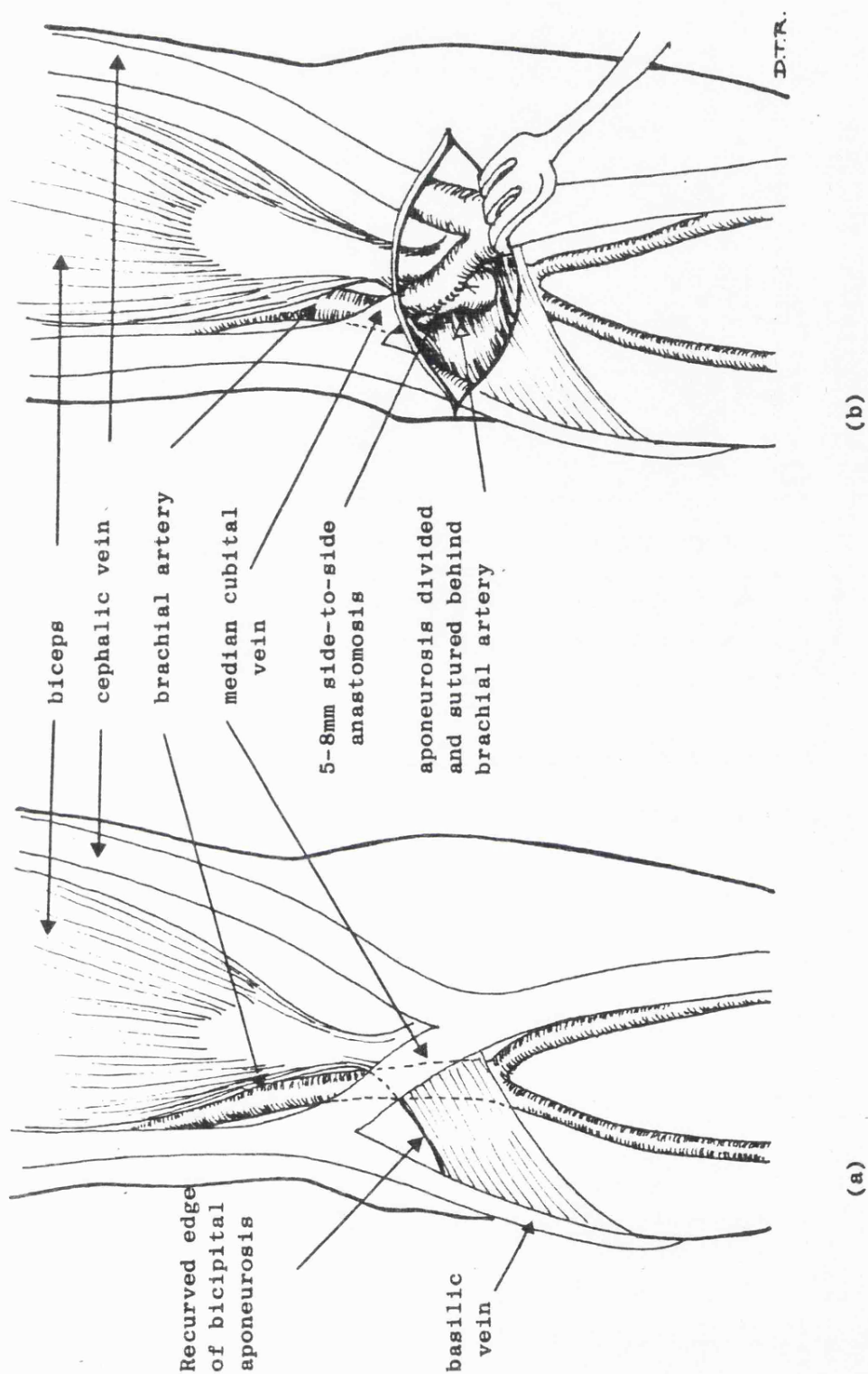


FIG. XI.3

Technique for construction of brachial side-to-side fistula.

APPENDIX XIICREATININE

This was measured routinely in the biochemistry laboratory on the serum from a 5ml specimen of venous blood. A Jaffé technique was used; the alkaline picrate reaction with creatinine was followed colorimetrically using an IL 919 analyzer. This gave a linear response up to creatinine levels of 1300 $\mu\text{mol/L}$: high levels were repeated with a 1:2 dilution. The error was of the order of 5 - 10%.

APPENDIX XIIIMATERIALSEQUIPMENTSUPPLIER

LKB 1280 Counter

LKB Instruments Limited,
232, Addington Road,
Selsdon, South Croydon,
Surrey. CR2 8YD.Aggregometer - manufactured by
H. Upchurch & Co. Leicester.Albert Browne,
Chancery House,
Abbey Gate, Leicester. LE4 0AA.

Vitatron Flat Bed Chart Recorder

Fisons/MSE Scientific Instruments,
Manor Royal,
Crawley, Sussex. RH10 2QQ.

Centrifuges: MSE Multex

as above

MSE Chilspin

Micro Haematocrit Centrifuge

Gelman Hawksley Limited,
12 Peter Road, Lancing,
Sussex. BN15 8TN.Automatic Pipettes - Gilson
Pipettman.Anachem Limited,
15 Power Court, Luton,
Beds. LU1 3JJ.

LP3 Polystyrene tubes

Luckham Limited,
Labro Works, Victoria Gardens,
Burgess Hill,
West Sussex. RH15 9QN.

EQUIPMENTSUPPLIER

Nucleopore Polycarbonate
Membranes 5 μ pore size, 13mm
diameter. Cat.No. 110413.
Specified for cell deformability
studies.

Sterilin Limited,
43 - 45 Broad Street,
Teddington,
Middlesex. TW118 Q2.

Adeplat T test (glass bead
column kits and syringe pump)

Immuno Limited,
Arctic House, Rye Lane,
Dunton Green, Nr. Sevenoaks,
Kent. TN14 5HB.

REAGENTS

Fibrinogen F3879(Type I) 60%
protein, 90% clottable, 15%
Na citrate, 25% NaCl

Sigma London Chemical Co. Ltd.,
Fancy Road,
Poole,
Dorset. BH17 7NH.

Thrombin T6634
Highly purified lyophilized
powder, 600NIH units/mg protein
Substantially free of Factors
II, V, VII, IX, X, plasmin and
activated factor X.

" "

Adenosine 5'-Diphosphate A8146 Grade
I 95-99% sodium salt, from equine
muscle. Anhydrous mol. wt 460, for
1.5 moles Na/mole ADP.

" "

REAGENTSSUPPLIER

Beta-thromboglobulin (β -TG)
RIA kit.

Amersham International Limited,
White Lion Road, Amersham,
Bucks.

125 I labelled Human fibrinogen
550 μ C (110 μ C/0.8mgs fibrinogen)
.1M53P

" "

Sodium chloride; trisodium citrate; BDH Chemicals Limited,
formaldehyde; $K_2HPO_4 \cdot 3H_2O$; KH_2PO_4 . Fourways, Carlyon Ind. Est.,
Atherstone,
Warwickshire. CV9 13Q.

BUFFER CONSTITUTIONTris NaCl

4.74g Tris HCl
17.53 NaCl

pH 7.4, with dilute NaOH
made up to 1.0dm³ with distilled water.

PHOSPHATE BUFFER0.05M

8.938g $K_2HPO_4 \cdot 3H_2O$
0.325g KH_2PO_4

pH 8.0
made up to 1.0dm³ with de-ionised water.

0.015M

pH 8.0

Dilute 0.05M buffer 3:10 with de-ionised water.

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BLOOD FLOW AND THROMBOSIS IN ARTERIOVENOUS FISTULAE

David Tempest Reilly

Abstract

An increasing number of patients are being maintained on long-term haemodialysis for end-stage renal failure, despite the renal transplantation programme and the introduction of peritoneal dialysis. Until prevention or cure of chronic renal failure becomes a possibility patients will continue to require long-lasting vascular access via surgically created arteriovenous fistulae. The Brescia-Cimino radiocephalic fistula at the wrist has remained the best procedure, and many patients use one satisfactorily for several years; even if later complications occur such as thrombosis or stenosis, the fistula can often be salvaged for further long-term use. In a group of patients, however, recurrent thrombotic problems occur, which can be life-threatening if access proves impossible. This study was aimed at identifying risk factors for thrombosis in these patients, and to improving clinical management of fistula complications.

Initially the size of the problem was assessed by studying over 150 patients admitted to the dialysis programme over five years, from which two main facts emerged. (1) Analysis of management showed that more fistulae could be salvaged than was previously thought. (2) Although more problems occurred in patients with small vessels, it was impossible to predict which fistulae would fail.

A prospective study of blood flow through the fistula at the time of operation showed that thrombosis occurred equally often in high and low flow fistulae, and fistulae with low initial flow could develop adequately for dialysis. This suggested that technical factors alone could not explain failure of fistulae. Patients maintained on dialysis with and without thrombotic problems were then studied, and significant differences in parameters of rheological and platelet function emerged. A prospective study was made of a group of patients undergoing fistula construction, who had blood taken pre-operatively for estimation of rheological and haemostatic factors, including platelet function. The later incidence of thrombosis after one year of follow-up was related to the pre-operative tests. It was possible on the basis of these tests to separate the thrombotic from the non-thrombotic patients, which has important implications for prophylaxis.