# BLOOD LOSS AND THROMBUS FORMATION

## DURING HAEMODIALYSIS

A thesis submitted for the degree of

Doctor of Medicine

by

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TABLE OF CONTENTS

# LIST OF TABLES

## LIST OF FIGURES

LIST OF FIGURES   (Cont'd)

Page No.

## PREFACE

The objectives of this study are to emphasise the importance of extraneous blood loss to the patient receiving maintenance haemodialysis; to demonstrate the source and magnitude of this blood loss; to define the blood loss caused by some commonly used haemodialysers; and to explore the reasons why blood should remain trapped within them.

The work commenced in 1969 when I, in conjunction with Dr. J. F. Davidson (Department of Haematology, Glasgow Royal Infirmary), developed a technique for measuring dialyser blood loss with precision. The subsequent studies determined that thrombus formation on the dialysis membranes of certain artificial kidneys was an important factor in producing excessive dialyser blood losses. It was important, therefore, to study the mechanism of such thrombus formation in order to attempt its effective reduction. In January 1971 I attained the position of a Research Fellow (Ab Gambro Grant, University of Glasgow) and commenced work in the Coagulation Laboratory (University Department of Medicine, Glasgow Royal Informary) under the guidance of Dr. G.P. McNicol (now Professor McNicol, University of Leeds). Here I received initial training in aspects of platelet, coagulation and fibrinolytic studies and achieved competence in the appropriate laboratory methods and assays. The knowledge I gained was then applied to the study of my original objectives; a task which has occupied my attention to the present time. This work will now be presented.

2.

# PRESENTATION

As most of the studies have already been published I have chosen to present them in their original form. Without exception these papers are the work of several authors. I can affirm, however, that in all studies I have played a major part. A statement detailing the reasons why the particular study was undertaken and the extent of my personal contribution accompanies each paper. I have followed these papers with critical comments on the significance of the findings in relation to the other papers in this thesis and to the work of other authors. In some cases an account of additional work is included.

The thesis is divided into seven chapters.

Chapers 1 and 2 form an Introduction to the Experimental Work which is composed of Chapers 3, 4, 5 and 6. Chapter 7 summarises and concludes the thesis. An Appendix, which contains a brief and general description of haemodialysers and their membranes is added for the reader who may be unfamiliar with clinical dialysis.

# ACKNOWLEDGEMENTS

# INTRODUCTION

# CHAPTER 1.

# REGULAR DIALYSIS THERAPY

The term 'dialysis' was first coined by Thomas Graham in the late 19th century when he described the separation of crystalloids and colloids by a vegetable parchment 'membrane'; the crystalloids moving across the parchment along a concentration gradient leaving the non-permeable colloid behind (cited by Kennedy, 1973). Initially Graham demonstrated the separation of cane sugar and gum arabic but later he used urine which over a period of time gave its crystalloid contents to the external water. He found that evaporation of this external water "yielded a white saline mass which on extraction with alcohol gave pure urea in crystalline tufts". With unbelievable foresight he suggested that this phenomenon might have a future medical application. The principle of dialysis was first put into practice with the conception of the artificial kidney by Abel, Rowntree and Turner (1914). They devised an ingenious mechanism by which an animal's blood could be subjected to dialysis outside the body and then returned to the animal under sterile conditions. This was achieved by fashioning a system of celloidin tubes into a manifold-type apparatus; blood entered the manifold at one end, reversed on itself and flowed out at the same end. Around the outside of the celloidin tubes circulated a rinsing solution of 0.6% sodium chloride. The anticoagulant Hirudin, extracted from leeches, was used to prevent clotting within the apparatus. Using this system Abel and his colleagues demonstrated that poisons both exogenous (e.g. salicylate) and endogenous (non-protein nitrogen) could be removed from the blood by dialysis and coined the term "atrificial kidney". From this time many workers became envolved in attempts to design artificial kidneys that could be used on humans but were discouraged by two major difficulties. These were (a) the lack of a dependable, reproducible membrane and (b) the lack of a safe and reliable anticoagulant.

Thus progress was considerably delayed until early in the 1930's when the anticoagulant Heparin was purified and a cellulose based membrane called Cellophane was obtainable. Thalhimer (1938) was the first to use these materials in the dialysis of azotaemic dogs but it was not until 1943 that Kolff and Berk (1944) developed the first clinically successful haemodialyser. With this machine, ten patients underwent haemodialysis and showed a reduction in blood urea

nitrogen, creatinine and uric acid. The authors also stated that the dialyser could be used to remove certain poisons from the blood including salicylates. The apparatus was a rotating-drum type artificial kidney and was brought to the United States from Holland by Dr. Kolff in 1947. He soon published a more complete account of the structure, technique of application and clinical value of this dialyser, together with his experience of additional methods for treating uraemia (Kolff, 1947). From this time onwards there has been, and will continue to be, enormous research into dialyser design. It is not the purpose of this thesis to describe this in any detail other than to indicate some milestones in the history of the artificial kidney as follows :-

1948 :  Skeggs and Leonards introduced the first parallel
        flow dialyser (Skeggs and Leonards, 1948).

1956 :  Kolff and Watschinger introduced the disposable
        "Kolff twin coil". (Kolff and Watschinger, 1956).

1960 :  Introduction of the Kiil parallel flow dialyser
        designed by Frederick Kiil and modified by Cole
        et al (1963).

1967 :  Presterilised disposable parallel flow dialysers
        become available. Examples of these are the
        Rhone-Poulenc (Funch-Bretano et al, 1969) and
        the Gambro-Alwell (Malchesky et al, 1971)
        dialysers.

1967 :  In this year the first human haemodialysis was
        conducted using the hollow fibre (capillary)
        artificial kidney after some three to four years
        of animal experimentation. The initial human
        results were described by Stewart et al (1968).

For further descriptive details of the various types of artificial kidney the reader is referred to the Appendix (page 79 ) and to texts such as that by Bailey (1972).

In the 1950's there was considerable growth of units using artificial kidneys for the treatment of potentially reversible acute renal failure but it was not until 1960 that the development of the teflon-silastic shunt (Quinton et al, 1960) made it possible to use the artificial kidney for the long-term repetitive treatment of patients with irreversible renal failure (Scribner et al, 1960). During the ensuing 12 - 13 years enormous technical advances have occurred in dialysis equipment, and regular dialysis therapy has evolved from an expensive, research-type procedure, through a routine form of treatment in hospital dialysis centres (Pendras and Erickson, 1966), to the point where treatment can be carried out overnight in the home, with both the patient and his family sleeping undisturbed (Blagg et al, 1970). At the same time there has been a corresponding progress in the development of renal transplantation which, also, can no longer be regarded as an experimental procedure. Thus the physician is now able to regard the management of many patients with chronic renal failure as a therapeutic continuum beginning with the diagnosis of progressive irreversible renal failure and continuing through two phases. The first or conservative phase is characterised by efforts to preserve the patient's remaining kidney function and health, and by preparation for the second or definitive phase. The latter consists of the integration of regular dialysis therapy and renal homotransplantation, so that the patient can remain well, be able to work and live a life of reasonable quality.

In the early stages of regular dialysis therapy there were, naturally, intense arguments in the medical press about the ethics and the question of financial support of this form of therapy but gradually it became apparent that it could sustain life long after renal function had totally ceased and by 1965 the Central Health Department in this country expressed the view that there should be a controlled development of regular dialysis. A national network of dialysis centres on the basis of at least one per region was established and there are now approximately forty major units in the United Kingdom with considerably over 1,000 patients with terminal kidney disease under treatment. Similar developments

have occurred elsewhere in the world; for example the most recent report of the European Dialysis and Transplant Association indicates that there are now 568 centres providing dialysis and transplantation for over 18,000 patients in Europe (Gurland et al, 1973). Although these numbers seem large it is most unlikely that nephrologists are, as yet, treating all the patients with terminal renal failure who are likely to benefit from such therapy will continue for some years.

The individual patients on regular dialysis can expect a reasonable chance of survival; the latest figures from the European Dialysis and Transplant Association show that approximately 70% of home dialysis and 50% of hospital dialysis patients survive 6 years (Gurland et al, 1973). Furthermore, the quality of life that these patients experience is, in most cases, fairly satisfactory. The treatment, at leats, renders the patient fit enough to return to work, again the European Dialysis and Transplant Association figures indicate that nearly 90% of home dialysis patients and 70% of hospital dialysis patients are able to return to work within six months of the start of therapy and that only 3.8% of hospital dialysis patients are unable to care for themselves by that time (Gurland et al, 1973). However, the morbidity rate among patients is still high and medical complications such as hypertension, myocardial ischaemia, bone disease, neuropathy, pericarditis and viral hepatitis may occur in some individual patients (Curtis et al, 1969; Scribner and Blagg, 1972). One complication, namely anaemia, is almost universal amongst regular dialysis patients (Curtis et al, 1969; Scribner and Blagg, 1972 ) and it is a purpose of this thesis to consider this problem in more detail with a view to improving the wellbeing of these patients.

CHAPTER 2.

BLOOD LOSS AND THE ANAEMIA OF
THE REGULAR DIALYSIS PATIENT

The anaemia of chronic renal failure is seldom completely corrected by regular dialysis treatment. This anaemia is usually associated with a normochromic, normocytic blood film and is basically due to a decreased rate of erythropoiesis associated with the incomplete correction of the uraemic syndrome afforded by haemodialysis. The anaemia may be augmented and complicated by the development of folic acid deficiency, iron deficiency and considerable blood losses. Androgen therapy has been advocated as a means of increasing the rate of erythropoiesis (Richardson and Weinstein, 1970; Shaldon et al, 1971; De Palma et al, 1972; Ferrier et al, 1972) but results have not been universally successful (Mayer and Robinson, 1971) and side effects may occur (Richardson and Weinstein, 1970; Shaldon et al, 1971; Ferrier et al 1972). Beneficial effects of cobalt in the management of the anaemia of chronic renal disease have been reported (Gardner, 1953; Kasanen et al, 1963; and Geill, 1969) but this treatment has not been widely used because of the high incidence of gastro-intestinal side effects (Gardner, 1953). Because of these side effects enteric coated cobaltous chloride has been given to regular dialysis patients with some success (Edwards and Curtis, 1971). The mode of action of cobalt in these circumstances is not clear but is presumed to increase the rate of erythropoiesis. Folic acid deficiency, resulting from losses across the dialyser, is the simplest of the aetiological factors to deal with (Hampers et al, 1967) and most clinicians are satisfied with replacement therapy using oral folic acid supplements. Achievement of proper iron balance on regular dialysis is a more complex problem. Patients with chronic renal failure not on dialysis have been shown to have both decreased absorption of oral iron into erythrocytes (Dubach et al, 1948; Boddy et al, 1970). Thus, patients commencing regular dialysis treatment do so with clearly demonstrable abnormalities in iron metabolism. According to Lawson and his colleagues (1971) these abnormalities are not improved by regular dialysis treatment although Comty et al, (1968) have claimed that increased oral iron absorption may take place after the commencement of dialysis in patients who are iron deficient, and Eschbach et al (1967) noted an improvement in the ability to utilise

iron with increasing time after beginning regular dialysis treatment. Once on the dialysis programme the patient has a rate of loss of iron from the body significantly greater than normal controls and non-dialysed patients with chronic renal failure (Lawson et al, 1971) and this has been attributed to the volumes of blood lost during haemodialysis (Will et al, 1970; Hocken and Marwah, 1971; Evans et al, 1967; Wright et al, 1968).  Such iron loss is sufficient to cause a fall in the haemoglobin and it would appear to be at least partially remediable; Hocken and Marwah (1971), for example, were able to raise the mean haemoglobin of a group of patients from 5 g to 9 g/100 ml. with the use of intravenous iron. The work of Lawson et al (1971) however, would suggest that the administration of either oral or parenteral iron is unlikely to be wholly successful because they clearly demonstrated in their patients, using tracer doses of $^{59}$Fe, that the absorption of oral iron remained low and the incorporation of the radioactive iron into the erythrocytes remained sub-normal. It is certainly the experience of the Renal Unit (Glasgow Royal Informary) that the use of intravenous iron does not help the majority of patients. It may, indeed, be detrimental by causing haemosiderosis (Curtis et al, 1969).

The policy of maintaining haemoglobin levels by giving repeated blood transfusions in amounts varying from less than one unit of packed cells to four units per month (Eschbach et al, 1967) is no longer acceptable in regular dialysis patients because of the risks of hepatitis (Jones et al, 1967; Drukker et al, 1968; Brunner et al, 1972; Rosenheim Report, 1972; Gurland et al 1973) and because of the possible development of leucocyte antibodies which could jeopardise subsequent renal transplantation (Brunner et al, 1972).

Considering these factors, it is obvious that if we hope to maintain a reasonable haemoglobin level more attention must be paid to sources of blood loss  for there is no doubt that haemodialysis causes considerable volumes of blood to be lost over  periods of time. Hocken and Marwah (1971) found that a minimum of 1.57 litres of blood were lost per annum because of the residual blood volume in

the dialyser (standard two layer Kiil system) together with the
blood taken from the patient for laboratory investigations and that
this figure could rise to 4.62 litres per annum if the high volume
of blood sampling during the period of establishment on dialysis
were continued. In this thesis I wish to consider the sources of
what is equivalent to chronic haemorrhage during dialysis and to
discuss the mechanisms responsible so that attempts may be made
to minimise them. In this connection one may recollect that a
blood loss in excess of 2 - 4 ml. per day (730 to 1,460 ml per year)
will lead to iron deficiency anaemia in an otherwise normal
individual unless iron absorption from the gut is extremely
efficient (Moore, 1958).

SOURCES OF BLOOD LOSS DURING HAEMODIALYSIS

These may be enumerated as follows :

(1)     Blood loss from the arteriovenous shunt or fistula during
        connection and disconnection of the patient to the dialyser.

(2)     Blood samples taken for haematological and biochemical
        investigations, together with those taken for various
        research projects.

(3)     Blood loss should a dialyser leak or rupture during use.

(4)      The residual blood volume in the dialyser and its blood
        lines, not returned to the patient, after each dialysis.

(5)     Coincidental blood loss (e.g. gastro-intestinal and menorrhagia).

These sources may be considered in a little more detail :

(1)  Shunt and fistula blood loss
     The use of both the arteriovenous shunt and fistula may be
associated with the loss of considerable volumes of blood. This

problem is fully considered in Chapter 3 (Paper 3).

(2)   Blood sampling.

Hocken and Marwah (1971) state that "biochemical and haematological investigations are a potent source of iron deficiency in patients with chronic renal failure". They estimated that patients undergoing stabilisation on their regular dialysis programme in hospital had blood samples removed for such investigations amounting to well over 3 litres per annum. This figure does not take into account research work that many dialysis units may carry out. It can well be argued that there is little need for routine haematological and biochemical estimations once the patient is stabilised on dialysis. In fact home dialysis patients need usually have blood sample taken only once or twice a year. There also may be a case to be made out for rationalising our research so that not all units are investigating all aspects of regular dialysis.

(3)   Dialyser rupture

Dialyser leakage or bursting is regrettably still a relatively common occurence. I feel that the responsibility for this problem rests mainly with the manufacturer who must strive to make his disposable dialyser "burst-proof"; coil users will probably encounter a burst rate varying between 2 and 7% (Muir et al, 1970; Burton et al, 1972).   However, a good pre-dialysis pressure testing method in the dialysis unit should help to minimise leakages occurring during dialysis. It is difficult to  estimate the volume of blood that is lost when a dialyser ruptures in use. It could amount to a total volume of the dialyser and be in excess of 200 ml. but is usually less as a saline wash-back will recover some of this blood. To measure, with precision, the volume of blood so lost would involve whole-body monitoring of a patient whose red cells were labelled with either $^{59}$Fe or $^{51}$Cr before and after such a rupture had occurred. There would be   serious ethical problems  with such a study as one would have to keep a group of patients labelled continuously and await a chance burst. Assuming that 200 ml of blood were lost every time a coil dialyser burst this would amount to

each patient losing 600 ml. of blood per annum if he was dialysed twice weekly on a coil with a 3% burst rate.


(4)    Dialyser blood loss

At the end of each dialysis it is inevitable that some blood remains trapped in the dialyser and its blood lines and is not washed back to the patient. Lawson et al (1968), Will et al (1970) and Hocken and Marwah (1971) have suggested that the blood loss in the dialyser is a major source of iron loss to the dialysis patient. While blood requirements for biochemical and other investigations can easily be minimised blood losses in the dialyser are more difficult to reduce. The majority of this thesis is now devoted to the consideration of the factors responsible for blood remaining trapped in the dialyser and the stage is set for consideration of the Experimental Work.

## EXPERIMENTAL   WORK

# CHAPTER 3

## BLOOD LOSS DURING HAEMODIALYSIS

In this Chapter 3 papers are presented. They describe a technique for the measurement of the residual blood in dialysers. This method is used to estimate, with precision, the blood losses obtained with some commercially available dialysers and then is adapted to measure the blood losses encountered with the routine use of arteriovenous shunts and fistulae. A discussion of the factors responsible for dialyser blood loss is given.

Paper 1.  The measurement of dialyser blood loss (1973)

Clinical Nephrology 1, 1, 24 - 28.  R. M. Linsday, J.A. Burton, P. King, J. F. Davidson, K. Boddy and A. C. Kennedy.

## Purpose of investigation

One major potential source of blood loss to the regular dialysis patient is blood trapped within the dialyser and not returned to the patient after use. Different dialysers tend to trap differing amounts of blood and it is now accepted that measurements of blood loss should be included in evaluations of new dialysers. It is important, therefore, that accurate methods for these measurements should be available.

The standard method for the estimation of dialyser blood loss, as used by other groups, involved the estimation of the haemoglobin concentration or the haematocrit found in large known volumes of fluid washed through a dialyser  after use and then the calculation of the blood loss from the patient's own haemoglobin or haematocrit value. This method, which had never been evaluated, seemed to me to be subject to inaccuracies. Thus, in 1969, Dr. J. F. Davidson and I developed a technique involving the $^{51}$Cr labelling of patients red blood cells and the counting of dismantled dialysers in a large well scintillation counter to estimate dialyser blood loss. This we considered to be accurate.  At the same time Dr G. Will (Western Infirmary, Glasgow)  in collaboration with Dr. K. Boddy (Scottish Universities Reactor Centre) also developed an isotopic technique utilising a whole body monitor to measure dialyser blood loss which, likewise, was claimed to be highly accurate. Surprisingly, there were discrepancies in dialyser blood loss values as obtained by our and Dr Will's method. Thus, this study was undertaken to ascertain the accuracy of these isotopic methods and of the method involving haemoglobinometry in order to establish a method for practical use.

## Personal Contribution

The idea of the study was mine and it was planned in collaboration with Dr. K. Boddy. Dr Burton and Dr. J. F. Davidson helped me with the experiments while Dr P. King and Dr Boddy did the counting in the Merlin shadow shield whole body monitor. I wrote the paper with advice from Professor A. C. Kennedy,

# The measurement of dialyzer blood loss

R. M. Lindsay, J. A. Burton, P. King, J. F. Davidson, K. Boddy and A. C. Kennedy

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*The measurment of dialyzer blood loss.* The measurement of blood loss is important in dialyzer evaluation and accurate methods should be available. The commonly used technique involving hemoglobinometry on the saline wash-through volume was evaluated and found to be grossly inaccurate. Techniques using ^{51}Cr labelled red blood cells have been developed by two groups and claimed to be accurate and yet, surprisingly, these groups quote widely different figures for the blood loss caused by the same dialyzer. These methods were each used to estimate a known blood volume in 13 Ultra-Flo 100 (Travenol) coils. Both methods were found to be in agreement and highly accurate. It is concluded that the difference in the blood losses reported by the separate groups was not a factor of errors in the methodology of the estimations but rather related to differences in dialysis running and wash-back techniques.

*Mesure de la déperdition sanguine dans les dialyseurs.* La mesure du volume sanguin résiduel après dialyse est importante dans l'évaluation d'un dialyseur et des méthodes de mesure précises doivent être utilisées. La technique habituellement employée qui fait appel au dosage d'hémoglobine pratiqué sur le soluté salin utilisé pour le rinçage des dialyseurs a été trouvée très peu précise. Des techniques utilisant des globules rouges marqués au Cr[51] ont été mises au point par deux équipes et considérées comme exactes mais, paradoxalement, ces deux équipes obtiennent des valeurs très différentes de spoliation sanguine pour le même type de dialyseur. Ces techniques ont été utilisées pour l'estimation d'un volume connu de sang dans 13 bobines U.F. 100 (Travenol). Les deux méthodes se sont révélées fournir des résultats concordants très précis. On peut en conclure que les différences rapportées par les deux groupes dans l'appréciation des spoliations sanguines ne résultent pas d'une erreur dans la méthodologie de la mesure des pertes mais sont dues à des différences dans les techniques de dialyse et de restitution du sang.

*Das Messen von Blutverlusten in Dialysiergeräte.* Das Messen von Blutverlusten ist für die Bewertung eines Dialysiergerätes wichtig und genaue Methoden sollten zur Verfügung stehen. Die übliche Technik mittels Hämoglobinmessung im Waschwasser wurde nachgeprüft und für sehr ungenau befunden. Techniken mittels ^{51}Cr-markierten Erythrozyten wurden von zwei Forschergruppen entwickelt und als genau angegeben, jedoch führen diese Gruppen überraschenderweise für den durch das gleiche Dialysiergerät hervorgerufenen Blutverlust weit differierende Zahlen an. Jede dieser Methoden wurde angewendet, um ein bekanntes Blutvolumen in 13 Ultra-Flo 100 Spulen (Travenol) zu be-
stimmen. Beide Methoden ergaben übereinstimmende Resultate und erwiesen sich als sehr exakt. Es wird daraus geschlossen, daß die differierenden Messungen beider Arbeitsgruppen nicht durch die Meßtechnik an sich, sondern durch Unterschiede in Dialysiertechnik und Rücktransfusion bedingt sind.

The regular dialysis patient remains anemic in spite of efficient hemodialysis. In the past hemoglobin levels were maintained by giving repeated blood transfusions in amounts varying from under one unit of packed cells to four units per patient month (*Eschbach et al.* 1967). Most dialysis units now only transfuse in emergency situations because of the known risks of hepatitis (*Jones et al.* 1967, *Drukker et al.* 1968, *Brunner et al.* 1972). If routine blood transfusion is to be avoided then all iatrogenic sources of blood loss must be minimised. One major potential source is blood is trapped within the dialyzer which is not returned to the patient. Different dialyzers tend to trap differing amounts of blood and it is now accepted that measurements of blood loss should be included in evaluations of new dialyzers. It is important, therefore, that accurate methods for these measurements should be available. Most of the measurements in the past have been made by estimating the hemoglobin concentration or the hematocrit found in large known volumes of fluid washed through the dialyzer after use and then computing the blood loss from the patient's own hemoglobin or hematocrit value. Several workers have reported blood losses in both coil and Kiil artificial kidneys using this method (*Evans et al.* 1967, *Patel et al.* 1967, *Nidus et al.* 1969, *Muth* 1969, *Hocken* and *Marwah* 1971, *von Hartitzsch et al.* 1972, *von Hartitzsch* and *Hoenich* 1972). *Will et al.* (1970) considered this method to be inaccurate and so developed an isotopic technique using either ^{51}Cr or ^{59}Fe labelled red blood cells and a Merlin shadow shield whole body monitor (*Boddy* 1967) to measure the blood loss encountered in coil dialyzers. *Muir et al.* (1970) also employed ^{51}Cr labelling of their patient's red cells and a technique involving the counting of dismantled dialyzers in a large well scintillation counter to estimate the blood loss in a coil and in a disposable parallel flow dialyzer. Both these groups of workers chaimed that their isotopic techniques were highly accurate and yet, surprisingly,

they quoted widely differing figures for the blood loss caused by the same dialyzer. *Will et al.* (1970) estimated mean whole blood losses of over 30 ml per dialysis with the Ultra-Flo 100 (Travenol) coil while *Muir et al.* (1970) obtained a significantly lower (P < 0.001) mean blood loss of 6.6 ml for this dialyzer. This study was undertaken to assess the accurary of the above isotopic methods and also of the method involving hemoglobinometry.

## Methods

### I. Blood loss estimation by Hemoglobinometry

The method usually employed is to circulate through the dialyzer, after the usual wash-back procedure, a known volume of fluid, usually 1 litre of 0.9% saline, for an arbitrary period of time to ensure even mixing of the residual blood within the fluid. Hemoglobinometry is then undertaken on samples of the recirculated fluid and the patient's own hemoglobin value is measured. The blood volume in the dialyzer is then calculated as follows:

$$\text{Dialyzer blood volume} = \frac{F}{P} \times \text{volume of re-circulate (ml)}$$

F = hemoglobin concentration in the recirculating fluid (g/100 ml)

and

P = patient's hemoglobin concentration (g/100 ml)

We simplified our study by estimating as accurately as possible known volumes of blood in 1 litre of 0.9% saline. Volumes ranging from 0.5 ml to 25 ml of whole blood of known hemoglobin concentration were introduced into 10 cans each containing 1 litre 0.9% saline. The fluid was mixed thoroughly and 3 samples, each of 10 ml, were taken from each can and carefully coded. The hemoglobin concentration (g%) of each coded sample was then measured in a blind fashion by one of us (J. F. D.), using a Coulter-S counter. Using the above formula 3 results for the estimated blood volume in each can were calculated. Thus, 30 separate estimations of "dialyzer blood loss" were considered.

### II. Blood loss estimation by $^{51}$Cr-R. B. C. methods

Twenty ml of fresh blood were taken from a volunteer subject and labelled according to the method of *Dacie* and *Lewis* (1963) using 120 μCi $^{51}$Cr. Two ml of the final red cell suspension in 0.9% saline were added to

500 ml of out-dated AB0 homologous blood in acid citrate dextrose. After thorough mixing accurately measured volumes of blood (ranging from 3 to 50 ml) were introduced into 13 Ultra-Flo 100 (Travenol) coils which had previously been primed with 0.9% saline. Introduction was achieved by injection into the arterial line of each coil and washing the bolus of blood into the coil with a further small volume of 0.9% saline. The lines of each coil were then clamped and the blood volume within the coil was estimated separately by the two methods described below. The measured volumes of blood were introduced separately by one of the authors (J. F. D.), who coded each coil, and only after the estimations of the blood volume were carried out was the code broken and the accuracy of the methods checked.

### Method 1:

Radio-activity within the coils was first counted in the Merlin shadow shield whole body monitor (*Boddy* 1967). After counting, the coils were washed out with 0.9% saline and then recounted. The washings were collected in standard cans, made up to a standard volume of 4 litres with tap water, and radio-activity again determined. To obtain a standard 10 ml of the labelled blood was diluted to 4 litres with tap water and was counted in the same geometry as the washings. By comparing the radio-activity of the "standard" with that of the "washings" it was possible to measure accurately the amount of blood washed out of the coil after its initial counting. This volume therefore represented the difference between the first and second coil counts from which the total coil blood volume could then be calculated.

### Method 2:

Having been counted by Method 1 each coil was completely dismantled and cut into small pieces. The fragments were added to the can containing the washings previously obtained and counting was carried out with the can placed in a scintillation counter end-on to a sodium iodide crystal 7 cm in diameter × 8 cm in depth and surrounded by a lead shield 5 cm thick. The method of counting is identical to that used by *Watson* and *Dickson* (1964) for the estimation of fecal blood loss. The "standard can" containing 10 ml of blood in 4 litres of 0.9% saline used in Method 1 was also counted by Method 2. The volume of blood in the coil was then computed by a comparison with the standard.
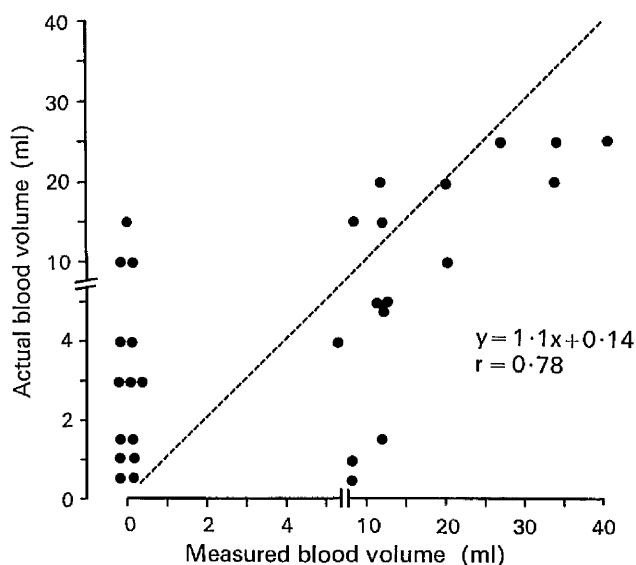
*Fig. 1*

Estimation of dialyzer blood loss by hemoglobinometry: There are 3 separate estimations for each of the 10 volumes giving a total of 30 estimations for the measured blood volumes.

## Results

### I. Blood loss estimation by Hemoglobinometry

The 30 blood volumes as measured by hemoglobinometry are shown plotted against the actual volumes in Figure 1. Although there is a significant linear relationship between the actual and measured volumes ($r = 0.78$) the percentage inaccurary of the measured value is high (fig. 2). There is also considerable variation in the results of the 3 estimations for each actual blood volume (fig. 1).

### II. Blood loss estimation by ^{51}Cr-R. B. C. methods

The actual blood volume in each coil together with the values as estimated separately by Methods 1 and 2 are shown in figure 3. The results indicate that, over a range of 3 to 50 ml, dialyzer blood loss can be estimated within ± 0.6 ml and ± 0.8 ml (68% confidence limits) using Methods 1 and 2 respectively. The percentage accuracy of Method 2 is shown in figure 2.

## Discussion

A knowledge of how much blood a patient will lose per hemodialysis is important because of the evidence that these patients may become iron deficient which will aggravate their pre-existing normochromic

anemia (*Evans et al.* 1965, *Wright et al.* 1968, *Will et al.* 1970, *Hocken* and *Marwah* 1971 and *Lawson et al.* 1971). Measurement of the potential blood loss to the patient should, therefore, be included in any evaluation of new dialysers. The most commonly used method involves hemoglobinometry on the volumes of fluid washed through the dialyzer.

There are three major sources of error in this technique. Firstly, it assumes that all blood remaining in the dialyzer is washed into solution which is unlikely should blood be trapped in the dialyzer as part of thrombus. Secondly, it assumes that it is easy to ensure even mixing of a few ml of red blood cells in 1 litre of washout fluid and then to obtain a representative sample for measurement; our results clearly show the lack of agreement among the triplicate results obtained for each actual blood volume (fig. 1). Thirdly, there is a lack of precision in the estimation, by standard hematological techniques, of hemoglobin values of 0.25 g% (equivalent to 25 ml whole blood with a hemoglobin concentration of 10 g% diluted in 1 litre 0.9% saline) and less. Considering all these factors it is not surprising that the accuracy of this method is in the order of ± 100–500% when attempting to measure blood volumes less than 5 ml and even when volumes of 25 ml are measured the accuracy is no better than ± 30% (fig. 2).
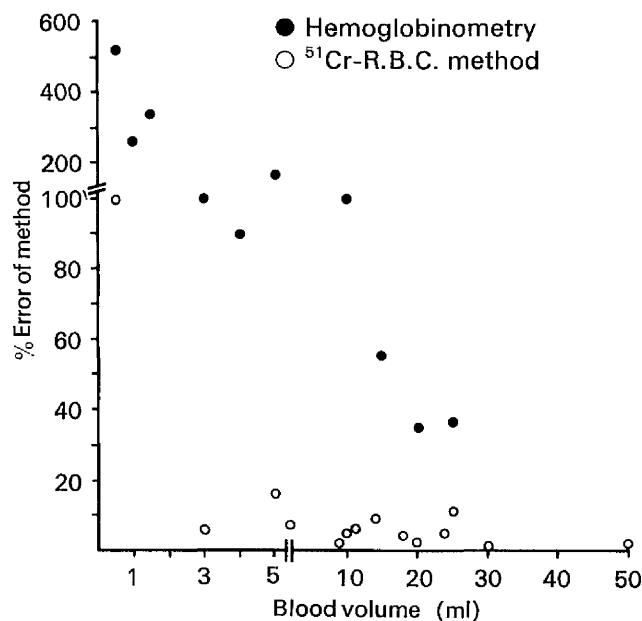


*Fig. 2*

Percentage error of blood loss estimation methods with varying blood volumes. Each plot for the hemoglobinometry method represents the mean error of the 3 estimations shown in figure 1. The plots for the error of the ^{51}Cr-R.B.C. method are those obtained using Method 2.

On the other hand this study demonstrates that the use of ^{51}Cr labelled red cells and a suitable counting method will allow the stimation of dialyzer blood loss with a high degree of accuracy (fig. 2 and 3). Red cell labelling with ^{51}Cr is a simple process taking little time and has an advantage over ^{59}Fe in that it can, in vivo, be carried out on the day of dialysis whereas some two weeks must be allowed for the incorporation of ^{59}Fe into the red cell. There is a potential source of error with the chromium technique in that chromium might become eluted from the red cells following the labelling process (*Mollison* 1967) and it is known that free ^{51}Cr may become adherent to the dialysis membranes (*Maher et al.* 1965). However, it is unlikely that this will cause a significant error as *Will* and his colleagues (1970) demonstrated similar results for coil blood loss estimations using ^{51}Cr and [59]Fe. The latter isotope is, of course, incoporated into the red cells excluding any error due to elution or to a membrane effect. The only disadvantages from the use of such isotopic techniques are the necessity for good counting equipment and that radioisotopes must be given to patients. We would submit, therefore, that such techniques should be limited to workers engaged in comparative evaluation studies.

A further object of this study was to assess the isotopic methods used in two separate studies (*Will et al.* 1970, *Muir et al.* 1970) of dialyzer blood loss which reported widely differing values for the same coil dialyzer. Our results indicate that there is excellent agreement between the two methods and, furthermore, that both methods are highly accurate. It is concluded that the difference in the blood losses reported by the separate groups was not a factor of errors in methodology but rather related to differences in dialysis running and wash-back techniques.

Fig. 3
Estimation of dialyzer blood loss by [51]Chromium labelled red ' blood cell methods. Method 1: whole body monitor technique as described by *Will et al.* (1970). Method 2: large well scintillation counter technique as described by *Muir et al.* (1970).

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## COMMENT

As indicated in this paper, several groups of workers have reported blood losses in both coil and Kiil artificial kidneys by measuring the haematocrit or haemoglobin concentration found in large volume of fluid washed through those dialysers after use (Evans et al, 1967; Patel et al, 1967; Muth and Wells, 1969; von Hartitzsch et al, 1972). The accuracy of such a technique is open to question. The technique obviously assumes that all blood remaining in the dialyser is washed into solution and even should this be the case it cannot be easy to mix completely a few ml of red cells in one litre of wash-out fluid and then to obtain representative samples for measurement. Will and his colleagues (1970) discussed this technique and felt that results so obtained were "so inaccurate as to be meaningless". Nevertheless it is still not uncommon to see blood loss figures derived in this way quoted to two decimal places; for example von Hartitzsch et al (1972) quote the mean blood loss in the Meltec Multipoint dialyser to be 0.85 ml. The results of our studies show that the accuracy of the technique of haemoglobinometry is in the order of $\pm$ 100 - 500% when attempting to measure blood volumes less than 5 ml (figure 2). The two isotopic techniques studies, however, were found to be highly accurate and in good agreement with each other. (figures 2 and 3). The accuracy of these techniques is such that losses of over 3 ml. can be estimated with an error not more than $\pm$ 16% (figure 2). As our method was the simpler of the two isotopic techniques it was chosen for the next study (Paper 2) in which the blood losses of different dialysers are estimated.

To my knowledge this is the only published work on the evaluation of methods of estimating dialyser blood loss. Such estimations are important in the evaluation of new dialysers (Kerr, 1969; Gotch et al, 1972). It is my view, therefore, that units engaged in comparative evaluation studies should use an isotopic method even though this will require expensive counting equipment and the necessity of giving radioisotopes to patients.

Paper 2.    <u>Dialyser blood loss (1973)</u>

## Purpose of Investigation

The regular dialysis patient remains anaemic in spite of efficient haemodialysis.  A major aetiological factor in the anaemia is blood losses of which the residual blood volume of the dialyser is an extremely important part.  The blood loss characteristics of each dialyser should therefore be included in evaluation studies. The purpose of this investigation was, therefore, to estimate, with precision, the blood loss values of some well known commercially available dialysers and to try to ascertain the factors responsible for blood remaining trapped in the dialyers.

## Personal Contribution

The study was conceived by me. The labelling of patients' red blood cells and the blood loss estimations were carried out mainly by myself and Dr. J. A. Burton.  We were helped by Dr. Dargie and Dr. Edward with the supervision of the haemodialyses and the dismantling of used dialysers.  I wrote the paper with advice from Professor A. C. Kennedy.

# clinical
# nephrology

# Dialyzer blood loss

R. M. Lindsay, J. A. Burton, N. Edward, H. J. Dargie, C. R. M. Prentice
and A. C. Kennedy

# Dialyzer blood loss

R. M. Lindsay, J. A. Burton, N. Edward, H. J. Dargie, C. R. M. Prentice
and A. C. Kennedy

*University Department of Medicine, Glasgow Royal Infirmary*

*Dialyzer blood loss.* The regular dialysis patient remains anemic in spite of efficient hemodialysis. A major etiological feature of the anemia is blood losses of which the residual blood volume of the dialyzer is an extremely important part. The blood loss characteristics of each dialyzer should therefore be included in evaluation studies. Using an accurate technique, involving ^{51}Cr labelled red blood cells, the blood loss values of 12 dialyzers were studied. In the majority the blood loss was under 10 ml. The Gambro-Alwall and the Cordis Dow hollow fibre dialyzers had excessive blood losses while the Gambro-Lundia and the Extracorporeal EX-03 coil had low losses. The roles of the two factors responsible for dialyzer blood loss namely machenical hold up of anticoagulated blood by manifold designs and thrombus formation within the dialyzer are analysed and discussed.

*Déperdition sanguine dans les Dialyseurs.* Le patient traité par dialyse itérative reste anémique en dépit d'une hémodialyse efficace. Un des facteurs étiologiques principaux de cette anémie est représenté par des spoliations sanguines, dont un élément très important est la quantité de sang résiduelle dans le dialyseur. L'évaluation des caractéristiques d'un dialyseur devrait ainsi comprendre la détermination du volume sanguin résiduel après dialyse. Une telle mesure a été effectuée pour 12 types de dialyseurs à l'aide d'une méthode précise faisant appel à des globules rouges marqués au [51]Cr. Pour la majorité des dialyseurs le volume sanguin résiduel fut trouvé inférieur à 10 ml. Les dialyseurs Alwall-Gambro et le dialyseurs à fibres capillaires Dow-Cordis ont des volumes sanguines résiduels excessifs, les dialyseurs Gambro-Lundia et Extracorporeal EX 03 ont des volumes sanguins résiduels particulièrement faibles. Les rôles respectifs des deux facteurs responsables de ces spoliations sanguines dans les dialyseurs (à savoir la séquestration de sang non coagulé en fonction des différents types d'appareils et la constitution de thrombi à l'intérieur du dialyseur) sont analysés et discutés.

*Blutverlust in das Dialysesystem.* Der chronisch dialysierte Patient bleibt trotz effektiver Dialyse anämisch. Einer der wesentlichen ätiologischen Faktoren dafür sind Blutverluste, wobei das Residualblutvolumen des Dialysierapparates eine äußerst wichtige Rolle spielt. Die charakteristischen Eigenschaften hinsichtlich des Blutverlustes bei jedem Dialysiergerät sollten deswegen beim Erproben mit erfaßt werden. Mittels einer exakten Technik unter Verwendung von ^{51}Cr-markierten Erythrozyten wurden die Blutverluste bei 12 Dialysiergeräten bestimmt. Bei der Mehrzahl der Geräte lag der Blutverlust unter 10 ml. Die »Gambro-Alwall« und die Cordis Dow Hohlfaser-Niere wiesen exzessive Blutver-luste auf, während die »Gambro-Lundia«- und die »Extracorporeal EX-03« niedrige Blutverluste zeigten. Die beiden Hauptursachen für den Blutverlust in das Dialysesystem, nämlich mechanisches Zurückbleiben von nicht koaguliertem Blut sowie Thrombus-bildung in der Dialyseapparatur werden analysiert und diskutiert.

The anemia of chronic renal fialure is usually associated with a normochronic normocytic blood film and is due, basically, to impaired erythro-poiesis. The anemia is seldom completely corrected by regular dialysis treatment (R. D. T.); indeed R.D.T., per se, may complicate the anemia by caus-ing folic acid deficiency, iron deficiency and blood losses. Folic acid deficiency is readily amenable to oral replacement therapy (*Hampers et al.* 1967) but the correction of iron deficiency is more difficult for *Lawson et al.* (1971) have demonstrated that an impairment both in the absorption of oral iron and the incorporation of iron into the red cells persists even after the institution of R. D. T. Furthermore, once on the dialysis programme, the patient has a greater loss of iron than normal controls or non-dialyzed patients with chronic renal failure (*Lawson et al.* 1971) because of the volumes of blood lost during hemodialysis (*Evans et al.* 1967, *Wright et al.* 1968, *Will et al.* 1970, *Hocken* and *Marwah* 1971). Androgen therapy has been advocated as a means of stimulating erythropoiesis (*Richardson* and *Weinstein* 1970, *Shaldon et al.* 1971, *De Palma et al.* 1972, *Ferrier et al.* 1972), but the results have not been universally successful (*Mayer* and *Robinson* 1971), and unpleasant side effects such as priapism may occur (*Richardson* and *Weinstein* 1970, *Shaldon et al.* 1971, *Ferrier et al.* 1972). In the past hemoglobin levels were maintain-ed by giving repeated blood transfusions, but this is contra-indicated by the risks of hepatitis (*Jones et al.* 1967, *Drukker et al.* 1968, *Brunner et al.* 1972), and the increased changes of early transplant rejection (*Brunner et al.* 1972).

There is no doubt that hemodialysis causes considerable volumes of blood to be lost over periods of time. *Hocken* and *Marwah* (1971) found, for example, that their patients could lose 1.57 liters to 4.62 liters of blood per annum because of the dialyzer

residual blood volume plus blood taken for laboratory investigations. As dialyzer blood loss is so important a factor in the anemia of the R.D.T. patient the precise blood loss characteristics of each type of dialyzer should be known.

Elsewhere in this issue (*Lindsay et al.* 1973a) we emphasise the inaccuracies of determining dialyzer blood loss by the commonly used technique of estimating hemoglobin concentration or hematocrit value in wash through fluid, and we demonstrate the accuracy of radio-isotope techniques (*Will et al.* 1970, *Muir et al.* 1970). Using a ^{51}Cr method we have measured with precision the blood losses of 12 well-known dialyzers.

## Methods

Blood loss was measured in 5 coil dialyzers, 6 parallel flow dialyzers and in the Cordis Dow hollow fibre artificial kidney. All the dialyzers studied were as supplied for use by the manufacturers, with the exception of the Gambro-Alwall with PT 250 membranes, which was specially prepared for us by Ab Gambro (Lund, Sweden). In general, the manufacturers' instructions for use were followed as closely as possible, but minor adjustments were made to heparinisation dosage and wash-back techniques in the light of previous experience, where this was thought to improve the blood loss characteristics.

With the exception of the Watson-Marlow Kiil dialyzer which was studied by one of us (N.E.) in another unit, all the dialyses were carried out in the same unit, by the same staff. Details of the individual dialyzers studied, their numbers, heparinisation regime and wash-back techniques are given in table 1.

## Wash-back technique

The wash-back technique varied with the dialyzer used, but was standard for each type of dialyzer. The basic method was as follows: At the end of dialysis the arterial line was disconnected and the blood in the line allowed to enter the dialyzer. The blood was then pushed through the dialyzer with 0.9% saline at a blood flow rate of 200 ml/minute, with the minimum venous back pressure possible. No changes were made to the dialysate negative pressure.

During the wash-back the coils were removed from the inflatable cuffs or plastic containers, and shaken to facilitate drainage of blood. The wash-back was terminated when the venous line no longer contained visible blood.

Wash-back of parallel flow dialyzers was conducted with the venous end of the dialyzer below the arterial end. The Ab Gambro dialyzers were originally positioned at a 45° angle for 30 minutes before the end of dialysis, and latterly were kept in the vertical position. All other parallel flow dialyzers were placed in the vertical position before was-back. The Rhone Poulenc dialyzer was allowed to drain by gravity only, no saline entering the circuit. The Gambro-Lundia was washed through with 300 ml 0.9% saline, followed by air, pumped at the rate of 200 ml/minute. A 400 ml

Table 1

Details of dialyzers, anticoagulation regimes, was-back techniques and blood loss values.

| Dialyzer | | Membrane | Number | Wash-back | | Heparin (units)[1] | | Blood loss |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | | | Start | Maintenance per hour | (ml±S.D.) |
| Parallel | Watson-Marlow Kiil | PT 150 | 18 | 400 ml 5% dextrose | | 5,000 | 1,500–2,000 | 11±7 |
| Flow | Cobe Mini Kiil | PT 150 | 6 | 400 ml 0.9% saline | | 2,000 | 2,000 | 6±2 |
| | Rhone Poulenc | PT 150 | 10 | Gravity return | | 2,000 | 2,000 | 6±3 |
| | Gambro-Alwall | PT 325 | 18 | 1,000 ml 0.9% saline | | 4,000 | 4,000 | 36±4 |
| | Gambro-Alwall[2] | PT 250 | 8 | 1,000 ml 0.9% saline | | 4,000 | 4,000 | 16±6 |
| | Gambro-Lundia | PT 250 | 6 | 300 ml 0.9% saline + air | | 10,000 | 2,000 (after 3rd hour) | 4±1 |
| Coils | Ultra-Flo 100 | PT 150 | 5 | 0.9% saline | | 2,000 | 1,500–2,000 | 7±1 |
| | Ultra-Flo 100 | PT 300 | 10 | 0.9% saline | Approx | 2,000 | 1,500–2,000 | 7±1 |
| | Ultra-Flo 145 | PT 300 | 6 | 0.9% saline | 400 mls | 2,000 | 1,500–2,000 | 5±2 |
| | Avon R70 | PT 150 | 5 | 0.9% saline | | 2,000 | 1,500–2,000 | 7±2 |
| | EX-03 | PT 150 | 9 | 0.9% saline 300–400 ml | | 2,000 | 1,500–2,000 | 4±2 |
| Hollow Fibre | Cordis Dow | Regenerated cellulose fibres | 7 | 0.9% saline 500 ml | | 10,000 | 2,000 (after 3rd hour) | 20±10 |

[1] Weddel Pharmaceuticals Ltd. 100 units is equivalent to 1 mg.    [2] Not commercially available with PT 250 membranes.
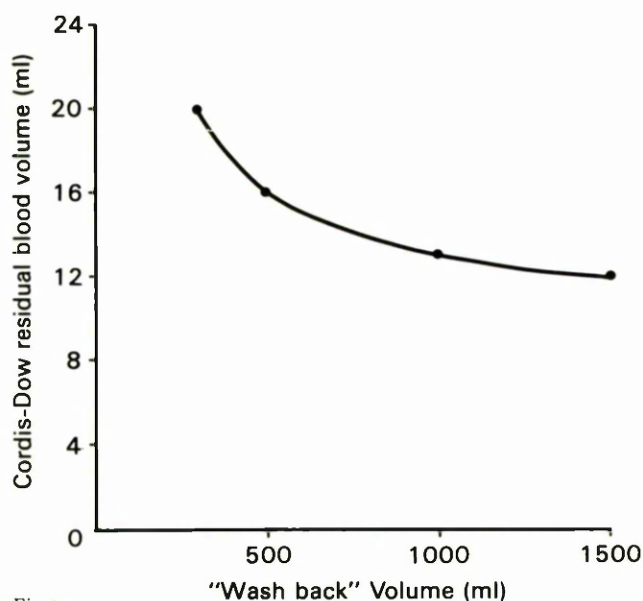
*Fig. 1*

Relationship between the residual blood volume of the Cordis Dow hollow fibre dialyser and the wash-back volume of 0.9% saline: plots are mean values from 4 dialysers in which thrombus formation has occurred.

volume of 5% dextrose was used for the Watson-Marlow Kiil which was placed at an angle of 60°, and the procedure terminated whether or not blood was visible in the venous line.

Wash-back of the Cordis Dow hollow fibre kidney was terminated after rinsing with 300 ml 0.9% saline. On four occasions further volumes of 0.9% saline were flushed through a dialyzer after disconnection of the venous line from the patient, the effluent being collected in 4 liter cans, in order to assess the influence of the wash-back volume upon the dialyzer blood loss.

### $^{51}Cr$ technique for blood loss measurement

Details of the technique and of its accuracy have been given elsewhere (*Muir et al.* 1970, *Lindsay et al.* 1973a).

### Other procedures

Each dialyzer was examined after use for the presence of thrombus and scanning electronmicrographs were made of membrane surfaces.

### Results

The blood loss values for the dialyzers tested are given in table 1. The relationship between the blood loss of the Cordis Dow hollow fibre dialyzer and the

wash-back volume is shown in figure 1 and thrombus formation within many of the fibres of the dialyzer in figure 2. Figure 3 shows the considerable thrombus formation upon the Cuprophan PT 325 membranes of a Gambro-Alwall dialyzer after use, figure 4 is a scanning electronmicrograph demonstrating fibrin strands trapping red cells on such PT 325 membranes, and figure 5 shows the lessened thrombus formation upon Cuprophan PT 250 membranes from a Gambro-Alwall dialyzer.

### Discussion

Examination of the results shows that in the majority of dialyzers the blood loss per dialysis is under 10 ml. This figure, however, still represents an annual blood loss to the patient in excess of 1 liter on the basis of 2 dialyses per week. It would also appear that coil dialyzers on the whole tend to be associated with slightly lower blood losses than parallel flow dialyzers. Two commercially available disposable dialyzers are seen to have what we would term excessively high blood losses. These are the original Gambro-Alwall dialyzer (Ab Gambro, Lund, Sweden), now obsolescent, and the Cordis Dow hollow fibre kidney. The regular use of either of these dialyzers will result in annual blood losses of over $3\frac{1}{2}$ and 2 liters respectively assuming only twice weekly dialyses.

Such variation in the blood loss characteristics of dialyzers leads to a consideration of the factors responsible for blood trapping within them. Firstly, manifold designs may cause mechanical hold-up of anticoagulated blood. Each dialyzer design will have its own particular problems which may be largely
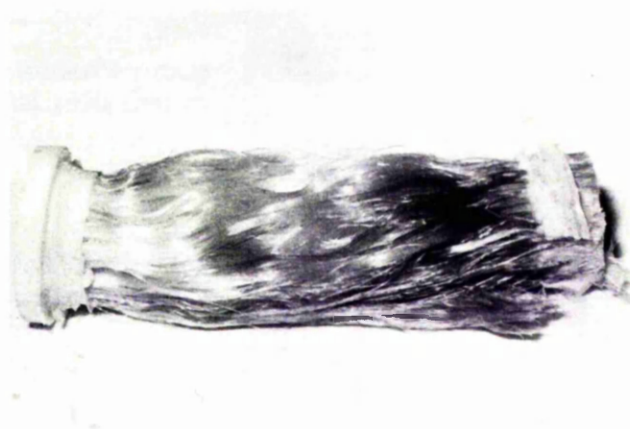


*Fig. 2*

A dismantled Cordis Dow hollow fibre dialyzer after use. Thrombus is seen within many of the fibres.

Considerable thrombus formation upon the Cuprophan PT 325 membranes of a Gambro-Alwall dialyzer after use.

overcome by an efficient wash-back technique. *Cole et al.* (1962) found that they were able to reduce considerably the blood loss in the standard Kiil from over 100 ml per dialysis by conducting the wash-back with the dialyzer vertical and venous end lowermost; attempting to wash-back with the dialyzer horizontal was associated with a return of plasma while most of the red cells remained in the dialyzer. These workers also found that this reduction in d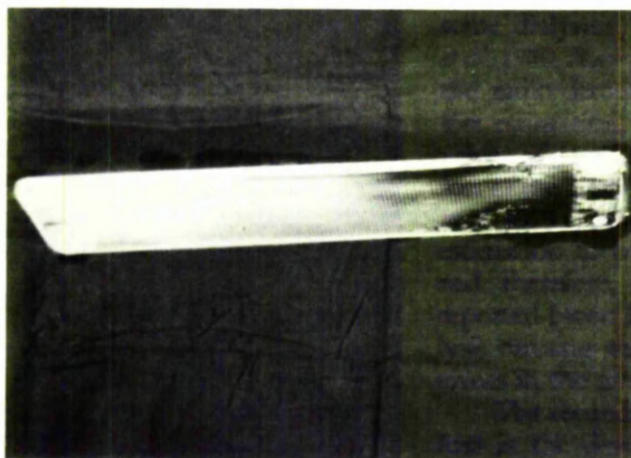ialyzer blood loss resulted in a fall of transfusion requirements from 5 to 2.5 units per patient per month. Using a complex double wash-back technique *Cole* and his colleagues (1963) were later able to reduce the blood loss of the Kiil to 35 ml. *Evans et al.* (1967) found the blood loss of this dialyzer to be 12.6 ml when the dialyzer was run in the vertical position for the last half hour of dialysis and kept so during the wash-back procedure. They considered that one factor in this further lowering of the blood loss was the improved quality of manufacture of Kiil dialyzers. The figure quoted by *Evans et al.* (1967) is in agreement with our results (Table 1). *Will et al.* (1970) found that blood loss could be reduced in the Ultra-Flo 100 (Travenol) coil by removing the coil from its container and placing it on its side during the wash-back procedure. They also noted that a further reduction in blood loss could be obtained by using 5% dextrose as wash-back fluid in place of 0.9% saline. They were unable, however, to offer any explanation for this latter finding. *Ghavamian et al.* (1972) confirmed that the blood loss in coil dialyzers could be reduced by running the coil in the horizontal position while *Nidus et al.* (1969) reduced their coil blood losses to under 5 ml by using a combined saline and air blow out technique. There is no doubt that different operational and wash-back procedures will influence the volume of blood left in

some dialyzers. For example, the two groups (*Will et al.* 1970, *Muir et al.* 1970) who separately developed the radio-chromium techniques for dialyzer blood loss estimation, surprisingly quoted widely differing values of 31 ml and 6.6 ml respectively for the blood loss of the Ultra-Flo 100 (Travenol) coil. We (*Lindsay et al.* 1973a) have found both methods of blood loss estimation to be in agreement and highly accurate and, therefore, conclude that the difference in the reported blood losses is a factor of differences in dialysis running and wash-back techniques rather than errors in the methods of estimation.

The second factor responsible for dialyzer blood loss is the development of thrombus on the dialysis membranes. If the Gambro-Alwall dialyzer is dismantled after use variable amounts of thrombus are found partially adherent to the dialysis membranes (Fig. 3). The use of a scanning electron microscope demonstrates fibrin-like strands trapping red blood cells (Fig. 4). Further studies have confirmed that these strands are fibrin and that this thrombus formation is associated with changes in the hemostatic status of the patient over the course of a dialysis (*Lindsay et al.* 1972). *Muir et al.* (1970) have already attributed the high blood loss of this dialyzer to thrombus formation occurring on the dialysis membranes. In our experience a variable percentage of the hollow fibres of the Cordis Dow artificial kidney, which is of novel design, may also contain a considerable quantity of thrombus (Fig. 2) and, we (Table 1) and others (*Mohring et al.* 1972, *Bosch et al.* 1972, *von Hartitzsch et al.* 1972) have found, in consequence, a rather variable but undesirably high blood loss with its use. The other commercially
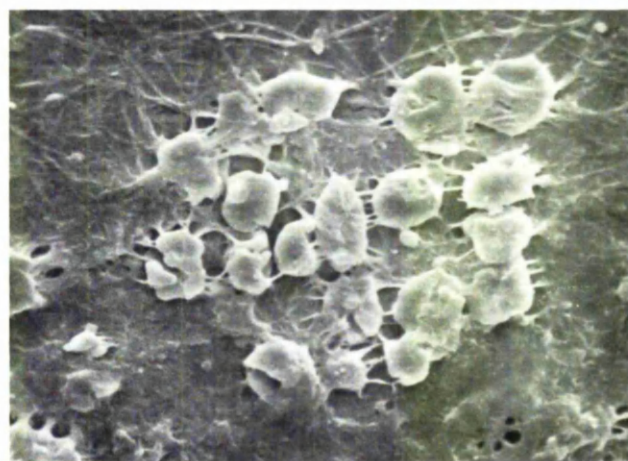
Scanning electronmicrograph (magnification × 2,000) demonstrating fibrin strands trapping red blood cells on the surface of Cuprophan PT 325 membranes of a Gambro-Alwall dialyzer after use.

available dialyzers which were studied, did not have any significant amounts of thrombus on their membranes after use and were associated with much lower blood losses (Table 1). If much thrombus formation has taken place within the dialyzer our experience with the Cordis Dow (Fig. 1) would suggest the dialyzer blood loss is unlikely to be influenced to any extent by either the technique or the volume of the wash-back.

We have discussed the causes of thrombus formation on dialysis membranes elsewhere (*Lindsay* and *Kennedy* 1972). In brief, we have found that interaction between platelets and the dialysis membrane is an important early step in a reaction which may proceed to thrombus formation even in the presence of heparin. An in-vitro method to study the reaction between platelets and dialysis membranes has been developed and this may also be used to compare directly the platelet retaining properties of different dialysis membranes (*Lindsay et al.* 1973 b). Using this technique we found that fewer platelets adhere to Cuprophan (J. P. Bemberg) PT 250 membranes than to PT 325 membranes (*Lindsay et al.* 1972, unpublished results). With the co-operation of Ab Gambro (Lund, Sweden) Gambro-Alwall dialyzers containing PT 250 membranes were prepared and these were found to have less thrombus formation (Fig. 5) than those containing PT 325 membranes (Fig. 3) and consequently a lower blood loss (Table 1).

The linear velocity of blood travelling across the membrane surface may also influence thrombus formation upon that surface. Muir (personal communication) found that thrombus formation, in spite of heparinisation, would occur on cellulose-based membranes within the Ross-Muir dialyzer (*Muir* 1971) if the linear blood velocity remained below 5 cm/sec. for any period of time. He and his colleagues (1970) suggested that a low linear blood velocity was the cause of the thrombus formation occurring within the Gambro-Alwall dialyzer. We now doubt this for the linear velocity of blood passing through other parallel-flow dialyzers, e.g. the Kiil, Rhone-Poulenc, Cobe and Gambro-Lundia, is also under 5 cm/sec. during clinical use and these dialyzers do not have a problem with in vivo thrombus formation. However, the linear blood velocity may be of secondary importance in a situation where the dialysis membranes are retaining many platelets, a slow velocity encouraging thrombus formation, as in the Gambro-Alwall dialyzer, while a high velocity is protective, as with coil dialyzers.

In conclusion, we must again stress that blood loss is important to the regular dialysis patient and that the contribution of dialyzer blood loss is often not appreciated. Regular use of a high blood loss



*Fig. 5*
Reduction in thrombus formation when Cuprophan PT 250 membranes are used in a Gambro-Alwall dialyser.

dialyzer may lead to annual blood losses of over $3^{1}/_{2}$ litres per annum while the use of low blood loss dialyzers can reduce this loss to under 0.5 litres. We suggest that clinicians and manufacturers should bear the potential blood loss in mind; the former by developing optimum wash-back techniques and the latter by considering the thrombogenicity of materials used in dialyzers and also the design of the dialyzer manifolding which should allow efficient wash-back with a minimum volume of fluid. Finally, we suggest that any clinical unit doing basic evaluation work on dialyzers should, in addition to determining clearance and ultrafiltration characteristics, measure the blood loss accurately and, if found to be high, try to establish the reasons.

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ADDENDA TO PAPER 2

(1) It should be appreciated that the blood loss values given in this investigation represent a red cell loss equivalent to the whole blood volume in the patient. It is likely that with thrombus formation a mass of red cells will be left on the dialysis membrane while much of the plasma has been returned to the patient.

(2) Figure 3 (page 6) has been reproduced upside-down.

COMMENT

This study demonstrates that the majority of dialysers cause
a blood loss of under 10 ml per dialysis. This figure, per se, seems
small but it must be appreciated that the regular dialysis patient will
be receiving at least two and more probably three dialyses per week.
Thus, the figure may represent an annual blood loss of 1.5 L. The
study also demonstrates that some dialysers may cause an even
greater blood loss e.g. the use of the Gambro-Alwall three times a
week would lead to a mean annual blood loss of over 10 L; a loss
which would surely render the most healthy subject anaemic. The
investigation is one of two published works devoted entirely to the
blood loss characteristics of various dialysers using an accurate
methodology. The other paper is that of Enger and Halvorsen (1972)
who examined four dialysers in vitro using $^{51}$Cr labelled red cells
in bank blood. Their isotope counting technique was virtually
identical to ours and was developed after our initial publication on
the blood loss of the Gambro-Alwall dialyser (Muir et al, 1970) and
after personal communication between Dr Enger and myself. Dr.
Enger and his colleague did not, however, carry out any in vivo
studies and, thus, were not able to consider the factors responsible
for dialyser blood loss. Muir (1971) and Muir and Martin (1971) have
tabulated blood loss values for various dialysers in two general
articles on aritificial kidneys; their sources of information were
varied (and included private communications from myself) as were
the methods of blood loss estimation.

This paper is, to my belief, the only one which has considered the
two factors responsible for blood trapping in dialysers, namely (1)
mechanical hold-up of anticoagulated blood and (2) thrombus
formation upon dialysis membranes. With regard to the latter factor,
the Discussion section of this paper refers to work that will be
presented in detail later in this thesis.

Paper 2                    Additional Study

The dialyser blood loss values presented in paper 2 did not include the volume of blood left in the various arterial and venous blood lines. During the course of some of these estimations the blood lines were also taken after use, cut into small pieces, and placed into cans containg 4 L tap water and sealed. These cans were identical to those in which the dismantled dialysers were placed and to those containing the appropriate standards. Counting was carried out as previously and the mean blood line blood losses estimated. These are presented in Table 1 and show that the lines cause a further 2 - 3 ml blood loss to the patient each dialysis.

Table 1

RESIDUAL BLOOD VOLUME IN DIALYSER BLOOD LINES (ARTERIAL + VENOUS) AFTER USE

| Dialysis System | No. Studied | Blood Loss ML * (M + S.D.) |
|---|---|---|
| Rhone - Poulenc | 5 | $2 \pm 0.5$ |
| Gambro | 6 | $2 \pm 0.5$ |
| Travenol | 5 | $3 \pm 1.0$ |
| Extra-Corporeal | 5 | $3 \pm 0.5$ |

* Mean values are given to nearest whole number

S.D. values are given to nearest 0.5 ml

Paper 3   <u>Blood loss from cannulation sites during haemodialysis</u>

<div align="right">(1972)</div>

Scottish Medical Journal, 17:  266 - 269.  R. M.  Lindsay & J. A. Burton

## <u>Purpose of investigation</u>

During the course of our studies on dialyser blood loss it occurred to me that we could apply our radio-chromium red cell technique to the measurement of the blood losses occurring from Scribner shunts and arteriovenous fistulae during routine dialysis as this was obviously another source of blood loss to our patients.

## <u>Personal Contribution</u>

The study was undertaken at my suggestion. The work and the writing of the paper was shared with Dr J. A. Burton.

# BLOOD LOSS FROM CANNULATION SITES DURING HAEMODIALYSIS

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**Summary.** *The importance of reducing blood loss from any source in patients on regular dialysis treatment is generally recognised. This minimises blood transfusion requirements, and the consequent risks of serum hepatitis. Blood loss occurring from Scribner shunts and arteriovenous fistulae during routine dialysis has been measured. The blood loss from this source has been estimated as 1 to 2 litres per annum, and this may exceed the loss due to residual blood trapped in coil dialysers. The risks to the patients and staff due to this source of blood loss are emphasised.*

I T is generally recognised that all external sources of blood loss in patients undergoing regular dialysis treatment should be minimised. Such losses may aggravate the pre-existing normochromic anaemia, which persists in spite of efficient haemodialysis, by producing iron deficiency (Shaldon, 1966; Lawson, *et al.*, 1968; Will *et al.*, 1970; Hocken & Marwah, 1971). Furthermore, blood transfusion is undesirable in this group of patients because of the risks of hepatitis (Drukker *et al.*, 1968). The causes of blood loss during dialysis can be listed as follows; that occurring during patient connection and disconnection to the dialyser, by arteriovenous shunt or fistula; that volume of blood removed for biochemical and haematological investigations; that volume trapped in the dialyser and not returned to the patient during 'wash-back'; and that lost by technical mishaps such as dialyser rupture. Most renal units are aware of the residual blood volume of dialysers and the need for a careful 'wash-back' technique. We ourselves have recently completed a study in which we measured the residual blood volume of a number of different dialysers (Lindsay *et al.*, 1972a). Will *et al.* (1970) and Hocken and Marwah (1971) separately refer to blood removal for investigations as being a major source of blood loss. A ruptured dialyser can cause a considerable degree of blood loss to the patient but this may often be avoided by careful pressure testing before the dialysis is commenced. The role of the arteriovenous shunt or fistula in causing blood loss has not been fully considered. Oozing of blood from the exit sites of newly created shunts has been noted (Shaldon, 1966; Salaman, 1971) and the occasional accidental shunt separation can lead to a considerable blood loss (Kisken *et al.*, 1968). Bleeding from the cannulation sites of an arteriovenous fistula has been occasionally encountered both during dialysis (Eisinger *et al.*, 1969) and after dialysis (Patel *et al.*, 1968). The exact volume of blood routinely lost during uncomplicated dialysis using an arteriovenous shunt or fistula has not, however, been recorded, although Shaldon (1971) included losses from cannulation sites when calculating total blood loss from children on home dialysis. This we studied as we had the impression that the cannulation of arteriovenous fistulae might lead to greater blood loss than the use of shunts.

## PATIENTS AND METHODS

Nine regular dialysis patients were studied, 7 of whom had subcutaneous Cimino-Brescia arteriovenous fistulae (Fig. 1) and 2 had external Quinton-Scribner shunts (Saf-T, Extracorporeal Medical Specialties Inc.) (Fig. 2). The fistulae were cannulated under local anaesthesia using 14 gauge plastic cannulae (Bardic) for the dialyser 'venous' return and 14 guage stainless steel hollow needles (Baxter) for the 'arterial' supply. The cannulations were performed by the medical staff, while shunt connection and disconnection was carried out by the nursing staff according to the routine methods used in our unit. Dialyses were performed for 10 hours using coil dialysers (Ultra-Flo 100, Travenol, or EX-03, Extracorporeal Medical Specialties Inc.) and anticoagulation was achieved by the intermittent administration of 2,000 units of heparin (Weddel Pharmaceuticals Ltd.) per hour. None of the patients was on oral anticoagulants and protamine sulphate was not given at the end of dialysis. The blood lost during the course of dialysis was measured as follows:

Table I. Mean blood loss in ml. (± standard deviation) from shunts and fistulae during routine dialysis.

| Period of study | Blood loss (ml) | |
| --- | --- | --- |
| | Shunt | Fistula |
| Dialysis commencement | 4.3±2.5 (n=6) | 3.6±1.7 (n=6) |
| Duration of dialysis + termination procedure | 4.3±2.9 (n=6) | 3.7±1.8 (n=6) |
| Total | 8.6±4.1 | 7.3±4.2 |

1969). The arteriovenous fistula eliminates the need for an external prothesis and allows the patient greater freedom of activity (Shaldon, 1968). Accordingly, the majority of the patients in our unit are dialysed using arteriovenous fistulae. One complication common to both shunts and fistulae, which has received very little attention, is the loss of blood incurred by their use during dialysis. Accidents can lead to high blood losses (Kisken et al., 1968) but even in uncomplicated dialysis there is usually some spillage of blood from the shunt during patient connection and disconnection or oozing round the shunt skin exit sites or the fistula puncture sites. Furthermore, on removing the cannulae from the fistula at the end of dialysis bleeding usually occurs to a variable degree in spite of firm local pressure.

The results of this study emphasise 2 facts. Firstly, the amount of blood lost to the patient by the use of a shunt or fistula may be as high as that volume trapped in the dialyser. Using the same method for the estimation of blood loss we have found that our patients lose a mean of 6.6 ml. or 3.5 ml. whole blood per dialysis using the Ultra-Flo 100 coil (Travenol) or the EX-03 coil (Extracorporeal Medical Specialties Inc.) respectively (Muir et al., 1970; Burton et al., 1972). The blood loss from cannulation sites will amount to over 1.2 litre of whole blood per annum assuming an 8 ml. loss per dialysis and thrice weekly dialysis.

Hocken and Marwah (1971) rightly have emphasised the need to minimise blood loss in the regular dialysis patient and have calculated the annual losses to their patients due to blood sampling and to blood trapping within the dialyser. They did not, however, consider blood cannulation losses which would raise the blood lost to each home dialysis patient from 1.57 litres to 2.77 litres per annum. The second fact to emerge from the study is that there is no increase in blood loss resulting from the use of an arteriovenous fistula as compared with the use of the shunt. It had been our clinical impression that the reverse was true. In addition, there was no difference in the volumes of blood lost during the different periods of dialysis studied with either shunts or fistulae. It must be stressed that these dialyses were uncomplicated. Occasionally considerable bleeding can occur during dialysis from newly inserted shunts (Shaldon, 1966; Salaman, 1971) or from fistulae puncture sites (Eisinger et al., 1969) and such occurrences will enhance the annual blood loss figures. We would stress the need for meticulous care in the use of external shunts and in the cannulation of arteriovenous fistulae to minimise blood loss since this not only aggravates the anaemia of chronic renal failure but may facilitate the spread of hepatitis in an infected dialysis unit.

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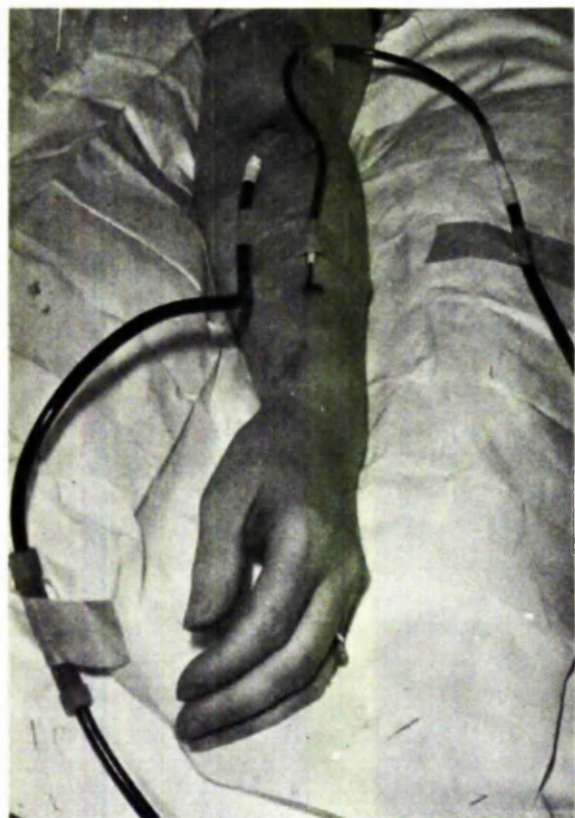
Fig. 1. The Cimino-Brescia arteriovenous fistula during dialysis. A drop of blood is seen at the arterial puncture site.
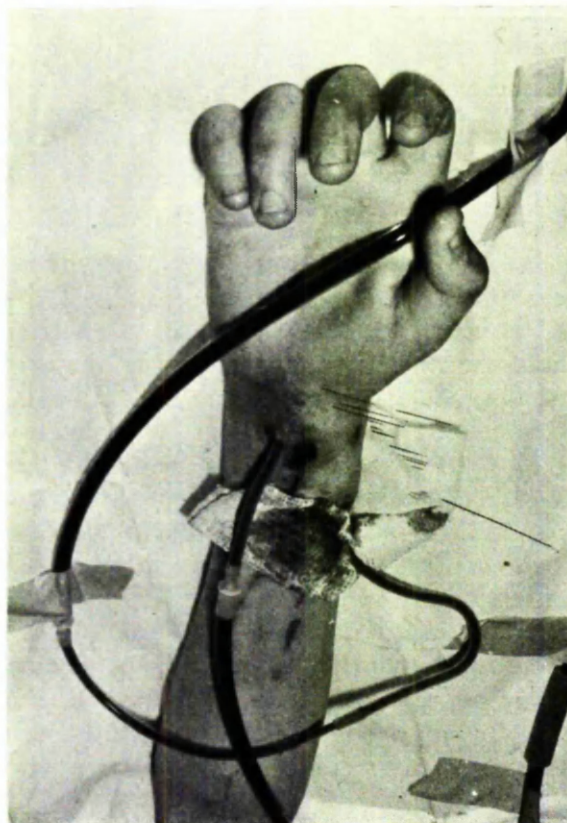


Fig. 2. The Quinton-Scribner silastic-teflon shunt in use. Bleeding from the skin exit site is clearly seen.

each patient had the red blood cells from 20 ml. whole blood labelled with 120 $\mu$Ci $^{51}$Cr, as for the technique of red cell survival (Dacie, 1963). At the commencement of each dialysis all spilled blood was carefully wiped up using sterile gauze swabs which were placed in identical cans (4 litre volume), and these were filled with tap water and sealed. All blood oozing from either the exit sites of the shunts or the fistulae puncture sites during dialysis was carefully collected on swabs. At the termination of dialysis either the blood spilled during shunt disconnection or the blood leaking from the fistulae venepuncture sites was similarly collected. Where fistulae were used the standard procedure was that each patient applied firm pressure, with sterile swabs, to the venepuncture sites for ten minutes and these swabs were kept. All swabs collected during dialysis, together with those from the termination procedure, were placed in a second 4 litre can. During each dialysis an accurately measured whole blood sample (5 to 10 ml.) was taken from each patient and placed in a third can in an identical fashion. This provided a standard for each measurement. Counting was carried out with each can placed in a large well scintillation counter end on to a sodium iodide crystal, 7 cm. in diameter $\times$8 cm. in depth, and surrounded by a lead shield 5cm. thick. This method is identical to that used by Watson (1964) for the estimation of faecal blood loss and by Muir et al. (1970) for the estimation of dialyser blood loss. This method is of proven accuracy (Lindsay et al., 1972b).

## RESULTS

The mean total blood losses caused by leakage from shunts and fistulae are shown in the table. Also shown are the separate estimates for the blood losses encountered at the commencement of dialysis and that lost thereafter. There was no significant difference (p $>$0.05) between the blood losses encountered by the use of shunts or fistulae either at the commencement of dialysis or thereafter Furthermore, the total blood losses were similar.

## DISCUSSION

Several authors have compared the advantages of the Cimino-Brescia arteriovenous fistula (1966) over the Quinton-Scribner shunt (1961) (Menno et al., 1967; Cohen et al., 1968; Byrne et al., 1971; Nolph, 1971). The major disadvantages of shunts are the frequent episodes of local infection and clotting (Conn et al., 1968; McIntosh et al., 1969) and these complications are much less common with fistulae (Cohen et al., 1968; Conn et al., 1968). Attempts to declot shunts may be hazardous (Gaan et al.,

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## COMMENT

Paper 3 confirmed my suspicion that blood losses from cannulation sites are significant. We estimated that the source of blood loss amounted to 1 to 2 litres per annum, a volume which may exceed the loss due to the residual blood volume of coil dialysis.

I believe this to be the only published study of its kind.

## Chapter 3 - Summary - Blood Loss During Haemodialysis

In the Introduction to this thesis, (Chapter 2, Page 13) I stated that there were the following sources of blood loss to the patient during haemodialysis:

(1)     Blood loss from fistula cannulation sites and from arteriovenous shunts during connection and disconnection of the patient to the dialyser.

(2)     Blood sampling

(3)     Blood loss from dialyser rupture.

(4)     The residual blood volume in the dialyser and its blood lines after each dialysis

(5)     Coincidental blood losses (e.g. gastro-intestinal and menorrhagia)

In this chapter of the thesis I have demonstrated that it is possible to estimate with precision (Paper 1) the blood losses occurring from the dialyser (Paper 2) and from arteriovenous shunts and fistulae (Paper 3). The estimation of blood losses from dialyser rupture has not been carried out for the reasons stated in the Introduction (page 14 ). If we make estimations for the possible blood volumes lost because of blood sampling and dialyser rupture we can tabulate (Table II) the annual losses of blood that a patient might sustain assuming thrice weekly dialyses using, for example, the Extracorporeal EX03 coil with a burst rate in clinical use as experienced by our unit of 2% (unpublished observation) and the Gambro-Alwall dialyser which has never leaked in our experience of over 100 dialyses. (unpublished observation). Table II shows that the probable annual losses encountered are staggering; even the use of a dialyser with a low intrinsic blood loss such as the EX03 coil may cause blood losses of over 3 L per annum while the use of a dialyser with a high blood loss raises this to over 8 L per annum. It is little wonder that our dialysis patients remain so anaemic.

It is important, therefore, that both the clinician and the
manufacturer should give greater consideration to blood loss
in these patients. The clinician should minimise his investigations;
take scrupulous care with the use of arteriovenous shunts and
fistulae; carry out thorough pre-dialysis pressure testing to
eliminate rupture during dialysis; and develop the optimum wash-
back technique for the dialyser he is using. The manufacturer must
design the manifolding of the dialyser so as to allow an efficient
wash-back with the minimum volume of fluid and, in the case of
disposable dialysers, that they should have the lowest possible burst
rate. In addition both clinician and manufacturer must consider the
occurrence of thrombus formation taking place on the dialysis
membranes for I have shown (Paper 2) that this is the factor which
differentiates dialysers with high and low blood loss value. Because
this is of importance with regard to patient blood losses, and also
because of the interesting fact that this thrombus formation takes
place in spite of adequate heparinisation, I decided to investigate
the mechanism of its occurrence. The results of these studies are
given in Chapter 4.

TABLE II

PROBABLE ANNUAL BLOOD LOSSES ENCOUNTERED BY R.D.T. PATIENT UNDERGOING

THRICE-WEEKLY DIALYSES.

| Dialysis System | Annual Losses From (ml) | | | | | TOTAL |
| | Shunts & Fistulae at 8 ml/dialysis | Blood Samples at 5 ml/dialysis | Dialyser Rupture at 200 ml/rupture | Dialyser Blood Loss | Vol. of blood in blood lines | |
| Extra-Corporeal EXO3 COIL | (Paper 3) 1248 | 780 | 600 | (Paper 2) 624 | (Paper 2 - additional study) 312 | 3,564 |
| Gambro-Alwall Dialyser | 1248 | 780 | 0 | 5,616 | 468 | 8,112 |

# CHAPTER 4

## THROMBUS FORMATION DURING HAEMODIALYSIS

In this chapter two papers are presented. They describe the changes which take place in platelet count, coagulation factors and the fibrinolytic system during haemodialyses. They show that these haemostatic changes are associated with the retention of platelets on the dialysis membranes and may be associated with the formation of platelet-fibrin thrombus. It is of pathogenetic significance that this thrombus formation may be diminished by pre-treating patients with anti-platelet agents.

Paper 4    Haemostatic changes during dialysis associated
           with thrombus formation on dialysis membranes (1972)

British Medical Journal **4**,  454-458  R. M. Lindsay,
C. R. M. Prentice, J. F. Davidson, J. A. Burton and G. P. McNicol.

## Purpose of Investigation

Thrombus formation may take place on the dialysis membranes
of the artificial kidney during haemodialysis, in spite of efficient
anticoagulation, and may be associated with a high patient blood
loss (Paper 2).  It was important, therefore, to study the cause
and extent of thrombus formation occurring on dialysis membranes
in the presence of heparin.  Accordingly, haemostatic studies were
carried out on regular dialysis patients before and after dialysis and
dialysis membranes were analysed after use.

## Personal Contribution

The study was undertaken at my suggestion.  The haemostatic
studies commenced early in 1971 at which time I had just completed
a period of training in the necessary laboratory techniques under the
guidance of Dr. G. P. McNicol (now Professor McNicol) and Dr.
C. R. M. Prentice and it was they who suggested which investigations
should be performed.  The majority of the platelet, coagulation and
fibrinolytic studies were carried out personally with some technical
help from Mrs A. Sandiford and Miss J. Grant. The dialysies were
supervised by myself and Dr. J. A. Burton. The technique of
labelling platelets with radiochromium was developed, with help
from Dr. J. F. Davidson, after a suggestion by Dr. G. P. McNicol.
The design of the radiochromium platelet study, thereafter, was
mine and the work was carried out personally with some technical
assistance from Mr I. Howe. The immunofluorescent studies were
carried out in collaboration with Dr. J. F. Davidson. The scanning
electronmicrographs were prepared by Miss R. Wilkinson
(Bioengineering Unit, University of Strathclyde) after membrane
preparation by myself and Dr. Burton. The paper was written by me.

**454**

# Haemostatic Changes during Dialysis Associated with Thrombus Formation on Dialysis Membranes

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## Summary

Platelet counts, coagulation factors, and the fibrinolytic system were studied in seven regular dialysis patients during the course of haemodialysis by parallel flow (Gambro-Alwall) and coil (Travenol Ultra-Flo 100) dialysers. Significant falls in the patients' platelet counts and rises in their factor V levels were found with both dialysis systems. The changes were more pronounced over the course of a Gambro-Alwall dialysis, when significant falls in the partial thromboplastin clotting time and in the plasminogen levels were also noted. These haemostatic changes were associated with the retention of platelets on the dialysis membranes and, in the case of the Gambro-Alwall dialyser, with the formation of platelet-fibrin thrombus. This thrombus formation may take place in spite of efficient heparin anticoagulation and may cause excessive blood loss to the regular dialysis patient.

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## Introduction

Haemodialysis with some types of parallel flow dialyser may be associated with a higher patient blood loss than with a coil dialyser of the same surface area. This is owing to thrombus formation on the cuprophane membranes of the dialyser (Muir et al., 1970; Lindsay et al., 1972). Such iatrogenic sources of blood loss may aggravate the anaemia of a regular dialysis patient by causing iron deficiency (Lawson et al., 1968; Hocken and Marwah, 1971) and should be avoided as blood transfusion is undesirable in these patients because of the risk of hepatitis (Brunner et al., 1972). It was important, therefore, to study the cause and extent of thrombus formation occurring on dialysis membranes in the presence of heparin. Accordingly, we have made haemostatic studies on regular dialysis patients before and after dialysis with parallel flow or coil dialysers and have analysed the thrombotic material which accumulates on the membranes during haemodialysis.

## Patients and Methods

Seven regular dialysis patients (four males and three females) were studied with their agreement. Each patient was dialysed by both a parallel flow and a coil system for six to eight hours. During dialysis all patients were heparinized with an initial dose of 2,000 units and then 2,000 units per hour of dialysis to maintain the clotting time at 37°C in excess of 30 minutes. Each dialysis was terminated with a standard "wash-back" procedure as described previously (Muir et al., 1970; Lindsay et al., 1972) during which 100 mg protamine sulphate (Weddel Pharmaceuticals Ltd.) was given into the dialyser "venous" line. At no time did protamine sulphate, an agent known to precipitate fibrinogen (Mylon et al., 1942), come into contact with the dialyser.

DIALYSERS

The 11-layered 1-m² disposable parallel flow Gambro-Alwall dialyser (Ab Gambro, Lund, Sweden) contains PT 325 cuprophane membranes (J. P. Bemberg), and is shown diagrammatically in Fig. 1. The 1-m² Ultra-Flo 100 (Travenol) coil has PT 300 cuprophane membranes.

After use each dialyser was dismantled and the site and macroscopic appearance of residual blood was noted. One dialyser of each type was filled with buffered glutaraldehyde immediately after the "wash-back" procedure, and after one hour membrane specimens were taken for scanning electronmicroscopy as described by Muir et al. (1970). Other 2-cm² membrane specimens were taken immediately after dialysis for immunofluorescent studies. The membrane was gently washed in 0·1 M phosphate buffer, mounted on glass microscope slides, and flooded with 0·5 ml fluorescein isothiocyanate conjugated rabbit antihuman fibrinogen (Hoechst Pharmaceuticals Ltd.), and incubated at room temperature in a moist Petri dish for 30 minutes. Unfixed antisera was then removed by washing in 0·1 M phosphate buffer, and the specimen was mounted in glycerol saline under a glass coverslip. Specimens were examined by phase contrast, incident fluorescence, and combined phase fluorescence in a Leitz ortholux microscope.

HAEMOSTATIC INVESTIGATIONS

Venous blood was taken in plastic syringes, with the minimum of venous occlusion, at the start of dialysis and again at five minutes after protamine sulphate had been given at the end of dialysis. Blood was mixed with one-tenth of the total volume 3·8% sodium citrate. It was kept at 4°C for coagulation, and at 20°C for platelet studies. A 5-ml sample was allowed to clot in a tube containing glass beads and 1 mg tranexamic acid for assay of fibrin-fibrinogen degradation products.

Platelet counts (Dacie and Lewis, 1970a) were carried out on citrated whole blood and on platelet-rich plasma prepared by centrifugation at 400 g for five minutes at room temperature. Platelet factor III availability was estimated by the method of Hardisty and Hutton (1965). The coagulation tests performed were thrombin clotting time (McNicol and Douglas, 1964), one-stage prothrombin time (Douglas, 1964), kaolin-cephalin clotting time (Proctor and Rapaport, 1961), and the partial thromboplastin time (Langdell et al., 1963). The plasma recalcification time was measured in a plastic tube by mixing 0·1 ml of 0·15 M saline and 0·1 ml of fresh non-contacted plasma at 37°C, adding 0·1 ml of 0·025 M calcium chloride, and recording the clotting time. Assays were carried out of factor V (Shanberge et al., 1967), factor VIII (Breckenridge and Ratnoff, 1962), and fibrinogen (Ratnoff and Menzie, 1964). Tests of fibrinolysis were plasminogen (Remmert and Conen, 1949), euglobulin lysis-time (Nilsson and Olow, 1962), and assay of serum fibrin-fibrinogen degradation products (Merskey et al., 1966). The urokinase-sensitivity test was performed as described by McNicol et al., (1965).

RADIOCHROMIUM PLATELET STUDY

In this separate study each of five regular dialysis patients underwent plasmaphoresis, and the platelets from 430 ml whole blood were obtained and labelled with radiochromium using 200 μCi $^{51}$Cr by the method of Dacie and Lewis (1970b). The labelled platelets were returned to each patient who two days later underwent an eight-hour dialysis using a Gambro-Alwall dialyser. At the start of dialysis a 100-ml whole blood sample was taken. A 20-ml subsample of this was divided by centrifugation at 450 g for five minutes and then at 1,500 g for 30 minutes at 18°C into platelet-rich plasma, platelet-poor plasma, and packed red cells. The latter were washed and then made up to a 10-ml volume using 0·15 M saline. The platelet-rich and platelet-poor plasma samples were also made up to 10-ml volumes using 0·15 M saline. The three samples were then counted in identical geometries using a scintillation counter to study the distribution of the isotope. On each occasion over 90% of the radioactivity was present in the platelet-rich plasma. After each dialysis the dialyser was dismantled and the membranes were placed in 4-litre cans which were filled with tap-water, sealed, and their radioactivity was counted in a large well scintillation counter. A standard was prepared on each occasion by taking the platelet-rich plasma from the remaining 80 ml of whole blood and estimating the total number of platelets in the sample. This standard was then placed in a 4-litre can, which was handled identically to the can containing the dialysis membranes. By comparing the radioactivity of each can an estimate of the numbers of platelets retained by the membranes was made. In doing this it was assumed that the $^{51}$Cr-labelled platelets behaved identically to unlabelled platelets. This method is similar to that used for the estimation of the dialyser residual blood volume (Muir et al., 1970; Lindsay et al., 1972). The estimated number of platelets on the dialysis membranes was compared with the expected platelet loss, assuming that platelets would be lost only according to their concentration in whole blood. To do this the predialysis platelet count was estimated, and the blood loss per dialysis was assumed to be 36 ml—a figure based on previous experiments (Lindsay et al., 1972).

## Results

*Dialyser Appearance and Membrane Studies.*—On dismantling each Gambro-Alwall dialyser variable amounts of blood were seen, especially towards the "venous" and "outlet" end of each membrane compartment (Fig. 1). The material had the macroscopic appearance of thrombus and was fairly adherent to the dialysis membrane. Scanning electronmicroscopy showed platelets and fibrin-like strands trapping red blood cells (Fig. 2) on the membrane. Immunofluorescent studies with antihuman

fibrinogen showed fibrin-positive material to be present on the membrane in the form of strands (Fig. 3). When each coil dialyser was dismantled small amounts of blood were found mainly at the junctions of the blood lines and the membrane tubing. This blood was easily washed away and did not resemble thrombus. Scanning electronmicroscopy showed platelets and few fibrin-like strands but these were much less evident than those seen on the membranes of the Gambro-Alwall dialyser. By immunofluorescence a few strands could be shown to react with antifibrinogen serum.

*Platelet Studies.*—The platelet counts before and after dialysis are shown in Fig. 4. A constant and significant fall in platelet count over the dialysis period was shown for both dialysers (Travenol P <0·02; Gambro P <0·01). The mean percentage fall of platelets after dialysis with the Gambro dialyser was
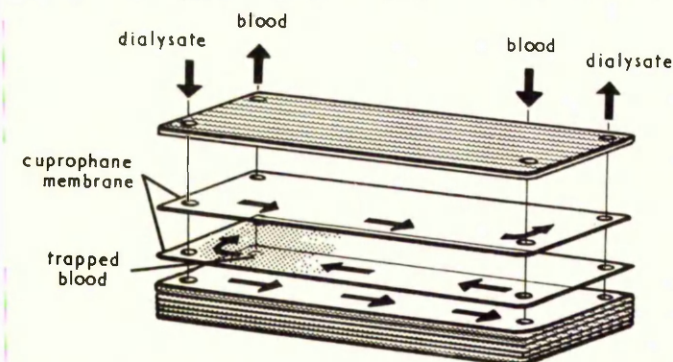


FIG. 1—Exploded diagram of the 11-layered Gambro-Alwall dialyser. Stippled area indicates the site of maximum thrombus formation.
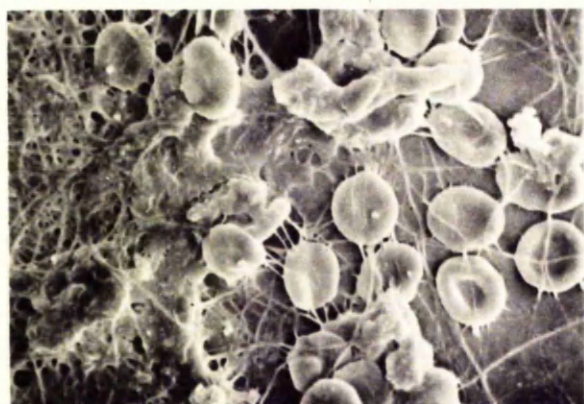


FIG. 2—Scanning electronmicrograph (× 1,000) of deposit found on PT 325 cuprophane membranes of Gambro-Alwall dialyser after use. Fibrin-like strands are seen trapping red blood cells; platelets are also in evidence.
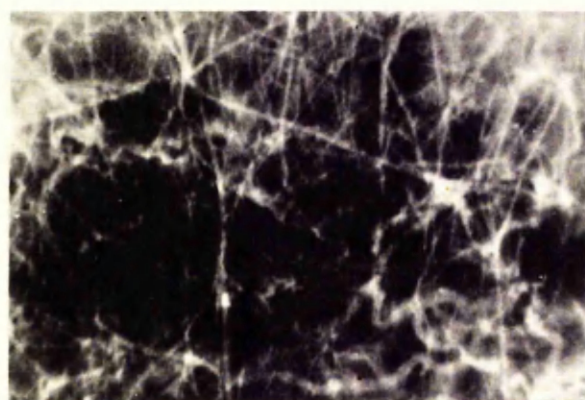


FIG. 3—Incident fluorescence seen when examining used PT 325 cuprophane membranes (from a Gambro-Alwall dialyser) after incubation with fluorescein isothiocyanate conjugated rabbit antihuman fibrinogen. The fluorescence suggests that the strands seen in Figure 2 are fibrin. (× 500.)
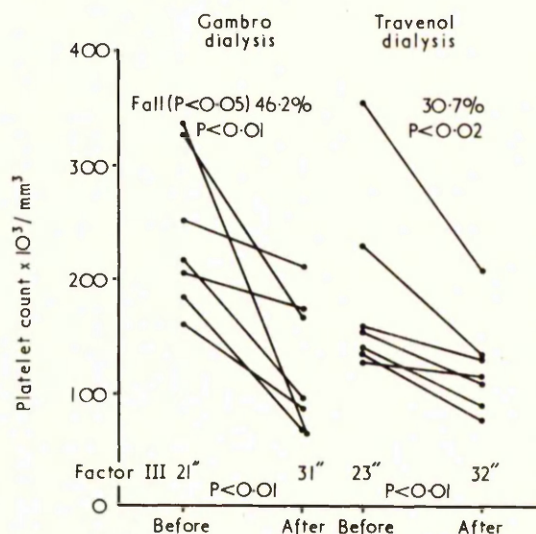


FIG. 4—Platelet counts and platelet factor III clotting times before and after dialysis from Gambro-Alwall and Travenol coil dialyses.

46%, compared to 31% after the coil dialyser (P <0·05). The platelet-rich plasma platelet counts followed the pattern of the whole blood platelet counts. As shown in Fig. 4 there was a significant decrease in the mean platelet factor III activity after dialysis by both systems (P <0·01); this was probably due to the decrease in platelet count.

*Coagulation Studies.*—In all instances the postdialysis thrombin clotting times were restored to the predialysis values, indicating that circulating heparin had been neutralized by the protamine sulphate. After Gambro-Alwall dialyses a shortening of the partial thromboplastin time from an initial mean value of 77 seconds to 65 seconds was noted (P <0·05). This did not occur after Travenol dialysis. There were no changes in the one-stage prothrombin time, the kaolin-cephalin clotting time, or the plasma recalcification time after dialysis. There was a significant increase in factor V activity after both forms of dialysis. The mean pre-Travenol value of 128% rose to 167% (P <0·05) and the mean pre-Gambro value rose from 125% to 177% (P <0·02). There were variable and non-significant changes in the factor VIII and fibrinogen levels of individual patients over the course of dialysis.

*Fibrinolytic Studies.*—As seen in Fig. 5 there was a significant fall in plasminogen after Gambro-Alwall dialysis (P <0·02) but not after a Travenol dialysis. There were no significant changes in the euglobulin lysis-time, urokinase sensitivity test, or in the levels of fibrin-fibrinogen degradation products after either form of dialysis.



FIG. 5—Plasminogen levels before and after dialysis from Gambro-Alwall and Travenol coil dialyses.

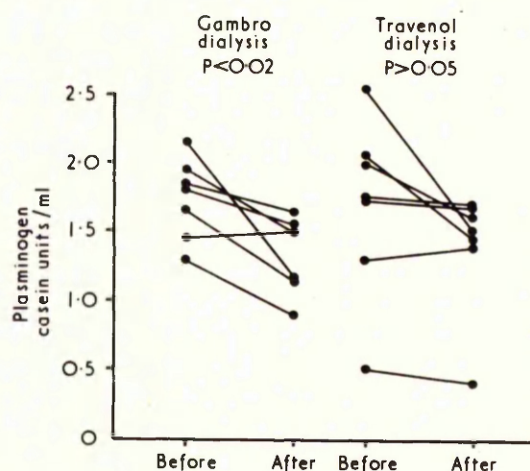*Radiochromium Platelet Study.*—The measured numbers of platelets on the dialysis membranes are shown in the Table where they are compared with the expected platelet loss as based on a dialyser blood loss of 36 ml. The number of platelets lost is greatly in excess of that attributable to the whole blood residual volume, suggesting that the platelets are retained preferentially by dialysis membranes.

*Comparison of the Number of Platelets Actually Retained on the Gambro-Alwall Dialysis Membranes After Use, Estimated by using $^{51}Cr$-labelled Platelets, and the Number of Platelets Assumed to Lie in the Dialyser as Part of a 36-ml Whole Blood Loss*

| Study No. | Platelets Retained on Gambro-Alwall Membranes | |
|---|---|---|
| | No. Measured by $^{51}Cr$ Technique | No. Assumed on Basis of 36-ml Blood loss |
| 1 .. .. | $184 \times 10^9$ | $3.9 \times 10^9$ |
| 2 .. .. | $42.4 \times 10^9$ | $6.4 \times 10^9$ |
| 3 .. .. | $43.4 \times 10^9$ | $6.7 \times 10^9$ |
| 4 .. .. | $59.9 \times 10^9$ | $7.1 \times 10^9$ |
| 5 .. .. | $52.7 \times 10^9$ | $8.4 \times 10^9$ |

## Discussion

The most striking change observed in these studies was the large fall in platelet count, which occurred after dialysis by both systems (Fig. 4). This postdialysis fall in platelet count does not appear to have been previously recorded, indeed Larsson (1971) found no change in the platelet count after Gambro-Alwall dialyses and Hesse *et al.*, (1970) similarly observed no change after Travenol dialyses. It must be noted, however, that in these studies the second specimens were taken at least 18 hours after dialysis. Furthermore, Anderson and De Palma (1966) found no drop in the platelet count of patients after 16 hours of Kiil dialysis. A large drop in platelet count was, however, noted by Mason *et al.*, (1972) when fresh blood ran over dialysis membranes using an ex-vivo test cell system. The platelet count fall was significantly greater over the course of a Gambro-Alwall dialysis than over a Travenol Ultra-Flo 100 coil dialysis. The $^{51}Cr$-labelled platelet studies (see Table) suggest that the drop in platelet count is secondary to platelet retention in the dialysis membranes.

The evidence provided by the appearance of the dialyser, scanning electronmicrographs, and by fluorescein-labelled anti-fibrinogen serum (Fig. 3), suggests that platelet-fibrin thrombus is deposited on the membranes of the Gambro-Alwall dialyser. In contrast, this process occurs only to a minor extent when the coil dialyser is used, even though the platelet count is reduced by 30% after this type of dialysis. It appears that platelets adhere to the dialysis membranes and that, in certain circumstances, this process may be accompanied by fibrin formation despite the presence of circulating heparin. Other workers have studied thrombus formation on materials used in cardiovascular surgery—for example, Teflon and Silastic—and also suggest that platelet retention to these materials is an important step in thrombus formation (Lyman *et al.*, 1968; Lyman *et al.*, 1969; Rodman and Mason, 1970a, 1970b). Indeed, Salzman (1971) stated "It is now customary to view surface-induced thrombosis as chiefly, if not exclusively, a platelet problem." It is likely that secondary factors such as the blood linear velocity are important in allowing the platelet reaction to proceed to fibrin formation. W. M. Muir (personal communication) noted that when heparinized blood flowing over cellulose-based membranes attains a linear velocity below 5 cm/sec thrombus formation is likely to occur. During normal clinical usage the linear velocity of blood flowing through the Gambro-Alwall dialyser is always below this level, whereas higher linear velocities (5-10 cm/sec) occur with the Travenol coil. It is possible that the high linear blood velocity through the coil prevents fibrin formation on its membranes. The difference in the linear blood velocities has already been suggested as an explanation for the different blood losses caused by these two dialysers (Muir *et al.*, 1970).

It was of interest to find that platelet and fibrin deposition could occur on dialysis membranes even in the presence of sufficient heparin to prolong the whole blood clotting to more than 30 minutes. This concentration of heparin would be expected to inhibit the action of thrombin on fibrinogen. Platelets are known to have clot-promoting effects on the intrinsic coagulation pathways as well as possessing a component which inhibits heparin, known as platelet factor IV (Godal, 1962). It is possible that sufficient thrombin is generated within the platelet micro-environment to clot fibrinogen. Alternatively, the fibrin strands may have been formed as a result of enzymes other than thrombin or even by non-enzymetic means. The fact that fibrin-like material can be deposited in the vicinity of platelets even in the presence of heparin has important implications in the study of arterial thrombosis where the conventional anticoagulants do not play a large part in prevention or treatment.

The postdialysis rise in factor V activity, together with a shortening of the partial thromboplastin time, may indicate activation of the patient's coagulation mechanism. Similar findings have also been recorded by Mason *et al.*, (1972) who observed a fall in the partial thromboplastin time when blood came in contact with dialysis membranes in an ex-vivo test cell system and by Larsson (1971) who noted a rise in factor V activity together with increases in factor VIII and fibrinogen levels the day after a Gambro-Alwall dialysis. Larsson (1971), however, could not find increased fibrinolytic activity after dialysis, indeed he found an increase in the level of urokinase inhibitors on the day after dialysis. Our results, however, show a significant fall in the plasminogen level immediately after a Gambro-Alwall dialysis (Fig. 5), which may reflect increased plasminogen activation secondary to the fibrin formation occurring in the dialyser.

The haemostatic changes during dialysis and the thrombus deposition on the dialysis membranes which we have observed are similar in many respects to the changes occurring during cardiopulmonary bypass surgery. A decrease in platelet count and plasminogen level has been observed among other changes in the blood coagulation factors of patients undergoing such surgery (Salzman and Britten, 1965), and fibrin formation within the extracorporeal circuit of the cardiopulmonary bypass is common.

In conclusion, we have observed haemostatic changes—namely, falls in the platelet count and plasminogen levels and a rise in factor V levels—in patients undergoing dialysis by the Gambro-Alwall dialyser, which would appear to be associated with the formation of thrombus on the membranes of that dialyser. These changes occur in spite of adequate heparin anticoagulation and may lead to an undesirably high blood loss for the regular dialysis patient. Our study suggests that platelet retention by the dialysis membranes may be an important early step in the reaction which proceeds to fibrin formation. Further studies on the effect of antiplatelet agents and different types of dialysis membranes in reducing dialyser thrombus formations are in progress.

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## ADDENDUM TO PAPER 4

There is an error in the Table shown on page 457 of Paper 4.
The number of platelets retained on the Gambro-Alwall membranes
should be multiplied by $10^9$ and not 109 as is indicated

Comment

This study confirmed that thrombus formation takes place on
the dialysis membranes of the Gambro-Alwall dialyser. The evidence
for this statement is based upon the macroscopic appearance of the
membranes, the scanning electronmicrographs, the incident
fluorescence seen after incubation of the membranes with fluorescein
isothiocyanate conjugated rabbit antihuman fibrogen, and upon the
demonstrable changes in platelet counts, coagulation tests and
plasminogen levels over the duration of a dialysis. The most striking
change observed in the haemostatic studies was the large fall in
platelet count found immediately after dialysis which was probably
due to platelet retention by the dialysis membranes (as suggested by
the $^{51}$Cr - labelled platelet study). These observations, which had
not been previously reported (see Discussion), suggest that this
platelet retention by the membranes may be an important early step
in the reaction which proceeds to fibrin formation. It was immediately
obvious to us that we could test this hypothesis by observing the
extent of thrombus formation, and the changes in the haemostatic
mechanism, that occurred when patients, whose platelet function had
been inhibited by drugs, were dialysed. This is the subject of Paper
5.

While the observations described in Paper 4 had not been previously
reported I must draw attention to the excellent studies of Larsson
(University of Lund, Sweden) who has studied the coagulation and
fibrinolytic status of convervatively treated chronic uraemics
(Larsson et al, 1971a), of patients with acute renal failure (Larsson
et al, 1971b), and of uraemic patients on maintenance haemodialysis
(Larsson, 1971). He did not, however, examine the acute changes
in the haemostatic system found immediately after dialysis and he was
not interested in the mechanism of thrombus formation within
extracorporeal circuits. The relationship between his and our work
is considered in the Discussion. Mention of a post-dialysis fall in
platelet count with the Gambro-Alwall, Travenol Ultra Flo 100, and
two other dialysers is made by Muir and Martin (1971) and Muir (1971)

in two separate review articles on haemodialysers. The data they
quote was from preliminary work carried out by myself and made
available to them. This data was, incorrectly, referenced in
these papers as that of 'Muir and Lindsay (unpublished results)'.

As the fall in platelet count was greater over the course of a
Gambro-Alwall dialysis than the fall over a Travenol dialysis I
wondered if the degree of platelet drop was, directly, related to
the formation of thrombus on dialysis membranes and, hence, to
the dialyser blood loss. This led to the Additional Study (Inter-
relationship of some factors in blood retention in dialysis systems
page 36) in which I also considered the possible role of the blood
velocity through the dialyser.

Finally, one last general comment is worthwhile repeating.
The fact that fibrin formation (or, at least, the formation of
fibrin-like material) can take place in the vicinity of platelets
in the presence of heparin has important implications in the study
of clinical thrombosis and its management.

Paper 4 - Additional Study

## Inter-relationship of some factors in blood retention
## in dialysis systems

### Methods

Platelet counts on regular dialysis patients were carried out by the method of Dacie and Lewis (1970) before and immediately after dialysis by the following dialysers :-

the Gambro-Alwall, the Gambro-Lundia, the Travenol Ultra-Flo 100, the Cordis Dow Hollow Fibre Artificial Kidney (H.F.A.K.) Model 3, the Rhone-Poulenc, and the Extra Corporeal EXO3.

Any post-dialysis fall in platelet count was expressed as a percentage of the pre-dialysis count. The dialysis membranes, the type of which was noted, were examined macroscopically and by scanning electron-microscopy for the presence of thrombus which was graded simply as being present (+) or absent (-). The dialyses were conducted with a blood flow rate of 220 ml/min, and from this, and the appropriate blood compartment cross-sectional areas, the linear velocity of the blood through each dialyser was calculated. The values for the blood compartment cross-sectional areas were estimated from the wet priming volume of the dialyser's blood compartment, as measured at atmospheric pressure, and from the dimensions of that blood compartment. The blood linear velocities were expressed as high ($>$ 5 cm/sec.) or low ($<$ 5 cm/sec.). The mean blood loss figures for each dialyser were taken from previous results. (paper 2).

### Results
These are shown in Table III.

### Comment

The results suggest that a large fall in the patient's platelet count is associated with thrombus formation on the dialysis membranes and a higher dilayser blood loss should there be a low blood linear

velocity through that dialyser.

It is possible, therefore, that the linear velocity of blood travelling across the membrane surface may influence thrombus formation upon that surface. Muir (personal communication) has carried out experiments using the Ross-Muir dialyser and found that thrombus formation occurred on cellulose-based dialysis membranes if the average linear blood velocity fell below 5 cm/sec. and remained so for five hours. These experiments were carried out in vivo with fully heparinised circulating blood. We (Dr. Muir, Dr. Davidson and myself) originally suggested that a low blood linear velocity was the major factor in the high blood loss of the Gambro-Alwall dialyser (Muir et al, 1970). I now doubt that this is the case for the linear velocity of blood passing through other parallel-flow dialysers, e.g. Rhone-Poulenc, Kiil, and Gambro-Lundia is under 5 cm/sec. and these dialyers do not have a problem with in vivo thrombus formation. However, the linear blood velocity may be of secondary importance in a situation where the dialysis membranes cause much retention of platelets, a low velocity encouraging thrombus formation while a high velocity is protective. For example, the Travenol Ultra-Flo 100 coil with PT 300 membranes causes a large drop in the platelet count yet, unlike the Gambro-Alwall situation, no significant thrombus formation takes place. (Table III). On the other hand the same dialyser with the thinner PT 150 membranes and the EXO3 dialyser, which also has PT 150 membranes, have the same blood linear velocity and do not cause any significant fall in platelet count after dialysis. These factors suggest that the nature of the membrane surface is of major importance in platelet retention and perhaps, therefore, in thrombus formation and this is discussed fully in Paper 7.

TABLE III

Inter-relationship of some factors in blood retention in dialyser systems

| System | No. of Observations | Mean Platelet Fall % | Dialysis Membrane | Blood Velocity | Thrombus | Mean Blood Loss * ml |
|---|---|---|---|---|---|---|
| Gambro-Alwall | 7 | 46 ' | Cuprophan PT 325 | Low | + | 36 |
| Gambro-Lundia | 5 | 21 | Cuprophan PT 250 | Low | – | 4 |
| Travenol-Ultra Flo 100 | 7 | 31 ' | Cuprophan PT 300 | High | – | 7 |
| Travenol-Ultra Flo 100 | 5 | 10 | Cuprophan PT 150 | High | – | 7 |
| H.F.A.K. (Cordis-Dow 3) | 5 | 56 | Regenerated Cellulose Fibres | Low | + | 20 |
| Rhone-Poulenc | 4 | 13 | Cuprophan PT 150 | Low | – | 6 |
| Extra-Corporeal EXO3 | 5 | 10 | Cuprophan PT 150 | High | – | 4 |

\* Data from Paper 2.

' Data from Paper 4.

Paper 5. <u>Reduction of thrombus formation on dialyser</u>
<u>membranes by aspirin and RA.233</u> (1972)

Lancet <u>ii</u>, 1287-1290. R.M. Lindsay, C.R.M. Prentice,
D. Ferguson, J.A. Burton and G.P. McNicol.

## Purpose of Investigation

In Paper 4 we had observed a fall in platelet counts of patients
undergoing Gambro-Alwall dialyses. We believed this fall to be
secondary to the retention of platelets by the dialysis membranes and
had formed the hypothesis that this platelet retention may be an
important early step in a reaction leading to subsequent thrombus
formation. This present study was designed to test whether
inhibition. of platelet function might lessen platelet retention and
thrombus formation within the dilayser.

## Personal Contribution

The study was instigated and planned jointly by myself, Dr.
Prentice and Dr. McNicol. The dialyses were supervised by myself
and Dr. Burton. I was helped in the experimental work by Dr.
Burton (who carried out most of the blood loss estimations), by
Mr. Ferguson (a medical student who helped with the estimation of
platelet adhesiveness) and by our technicians (Miss Martin, Miss
Grant and Miss Smith) who helped with the various coagulation tests.
I was responsible for the interpretation of the results, the
statistical analyses and the writing of the paper.

# REDUCTION OF THROMBUS FORMATION ON DIALYSER MEMBRANES BY ASPIRIN AND RA 233

R. M. Lindsay          C. R. M. Prentice
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**Summary**      Thrombus formation, despite efficient heparin anticoagulation, takes place on the dialysis membranes of the Gambro-Alwall dialyser, causing a high blood-loss to the patient. This thrombus formation is associated with a fall in the patient's platelet-count over the course of dialysis. To test the hypothesis that platelet retention on these membranes is an early step in the reaction leading to thrombus formation, a double-blind trial of anti-platelet agents (aspirin and a pyrimido-pyrimidine compound [RA 233]) was carried out. These agents significantly lowered platelet-adhesiveness, reduced the fall in platelet-count over the duration of dialysis, abolished the significant fall in plasminogen level seen during placebo therapy, and reduced the dialyser blood-loss. The results of the trial support the initial hypothesis and also suggest that these anti-platelet agents may be valuable in preventing thrombotic disease.

## Introduction

HÆMODIALYSIS using certain dialysers may be associated with a high patient blood-loss due to thrombus formation on the ' Cuprophan ' membranes of the dialyser.[1,2]   This blood-loss may aggravate the anæmia of the regular dialysis patient by causing iron deficiency [3,4] and should be avoided since blood-transfusion is undesirable in these patients because of the risks of hepatitis.[5] Thrombus formation upon the

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† We wish to point out that the Gambro-Alwall dialyser is now obsolete. Its successor, the Gambro-Lundia, is not associated with the occurrence of thrombus formation upon its membranes and has a low residual blood-volume.

membranes of the Gambro-Alwall dialyser † is associated with a fall in platelet-count in patients because of the retention of platelets within the dialysis membranes.[6] Since this thrombus formation takes place despite efficient heparin anticoagulation, it is possible that the platelet retention on the dialysis membrane is an important step in the reaction leading to thrombus formation. Accordingly, we have carried out a trial of anti-platelet agents on patients undergoing Gambro-Alwall dialyses to find out whether these compounds can reduce the degree of thrombus formation and retention of blood within the dialyser.

## Methods

Nine regular dialysis patients (seven males and two females) consented to the study. Each patient was dialysed by the Gambro-Alwall system in four distinct treatment weeks separated by rest intervals of 2 weeks. During each treatment week the patient received one of the following regimens: placebo medication; soluble aspirin (0·6 g. per day); RA 233, which is 2,6-bis(diethanolamino)-4-piperidinopyrimido-(5,4-d) pyrimidine (1 g. per day); both soluble aspirin and RA 233 together. The drugs and identical placebos were prepared by Boehringer Ingelheim, Isleworth, Middlesex, and were administered by the hospital pharmacist on a randomised double-blind basis. During each rest period the patients received no drugs and returned to the routine dialysis programme of our unit. During each treatment period the patients had two dialyses, the
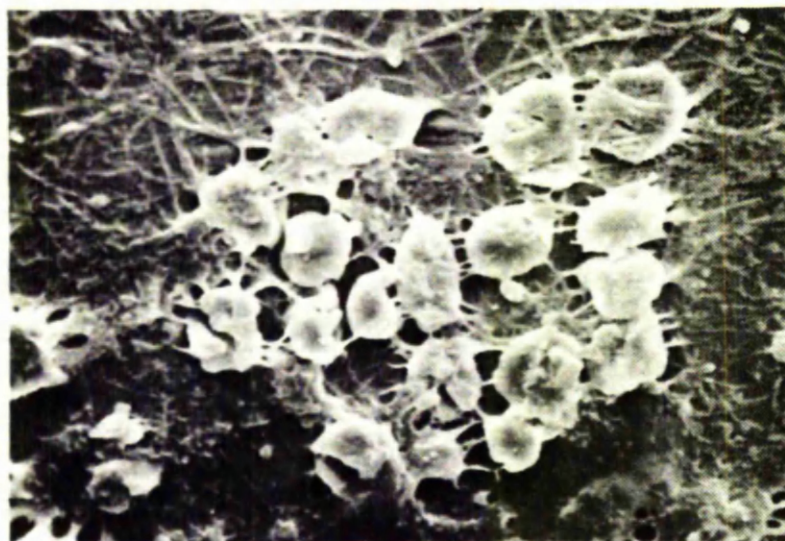


Fig. 1—Scanning electron micrograph of dialysis membrane from the Gambro-Alwall dialyser after use.

Red blood-cells and platelets are seen ensnared in fibrin strands. Reduced to a ½ of ×1000.

first of which was 48 hours after the start of the drugs. The dialyses were carried out with standard procedures as described previously.[1,2] All patients were heparinised during dialysis with 4000 units of heparin per hour to maintain the clotting-time at 37°C in excess of 45 minutes. At the end of each dialysis 100 mg. of protamine sulphate was given into the dialyser " venous line "; this was sufficient to return the thrombin clotting-time to normal. At no time did protamine sulphate, an agent known to precipitate fibrinogen,[7] come into contact with the dialyser.

Blood was drawn into plastic syringes at the start of dialysis and at 5 minutes after the protamine-sulphate administration at the end of dialysis. Blood was mixed with 3·8% sodium citrate, 9 parts to 1 by volume; it was kept at 4°C for coagulation and at 20°C for platelet studies. Platelet-counts were carried out by the method of Dacie and Lewis.[8] Platelet-adhesiveness was estimated using the membrane test cell described by Lindsay et al.[9] Factor V,[10] fibrinogen,[11] plasminogen,[12] and fibrin/fibrinogen degradation products (F.D.P.) in the serum [13] (determined by using blood clotted with 1 mg. tranexamic acid) were assayed.

The residual blood-volume of each dialyser was estimated using chromium-51 labelling of the patients' red blood-cells as described elsewhere.[1,2] The labelling of the patients' red blood-cells was carried out twice; initially on the morning of the first dialysis during the first treatment period and, again, 6 weeks later on the morning of the first dialysis of the third treatment period. On each occasion 120 μCi of chromium-51 was used.

With every investigation the values from each individual patient-treatment period was the mean value obtained from the two individual dialyses. Statistical analysis of the results are based on paired $t$ tests.

PRE-DIALYSIS AND POST-DIALYSIS FACTOR V AND PLASMINOGEN VALUES FOR EACH TREATMENT PERIOD

| Treatment | Factor V (mean ±s.d., units/100 ml.) | | Plasminogen (mg. per 100 ml., mean ±s.d.) | |
|---|---|---|---|---|
| | Pre-dialysis | Post-dialysis | Pre-dialysis | Post-dialysis |
| Placebo | 117±41 | 162±45 (P < 0·05) | 2·9±0·8 | 2·3±0·9 (P < 0·01) |
| Aspirin | 122±26 | 157±52 (N.S.) | 2·7±1·1 | 2·6±1·0 (N.S.) |
| RA 233 | 115±18 | 191±74 (P < 0·0025) | 2·4±0·8 | 2·4±0·8 (N.S.) |
| Aspirin and RA 233 | 103±69 | 150±42 (P < 0·005) | 2·3±0·6 | 2·2±0·9 (N.S.) |

The significance of any change is indicated by the P values in parentheses.
N.S. = Not significant.

## Results

The typical appearance of the Gambro-Alwall dialyser membrane after use is shown in fig. 1. In this photomicrograph, obtained with the scanning electron microscope, red blood-cells and smaller particles, which probably represent platelets, are seen bound together by fibrin-like strands.

In fig. 2 the mean ($+$ S.D.) values for platelet-adhesiveness are shown for each of the treatment periods. It can be seen that the anti-platelet agents, singly or in combination, significantly reduced platelet-adhesiveness. There was no significant difference in the platelet-adhesiveness values obtained during the different periods of active drug therapy.

The fall in the patients' platelet-count over the course of dialysis expressed as a percentage of the pre-dialysis count is shown in fig. 3 for each treatment period. The mean value during the placebo period was 31 %, which fell to 22% while on aspirin therapy; this fall was not statistically significant with the numbers of patients involved. RA 233 and combination therapy, however, did significantly reduce the fall in platelet-count compared to the placebo group.
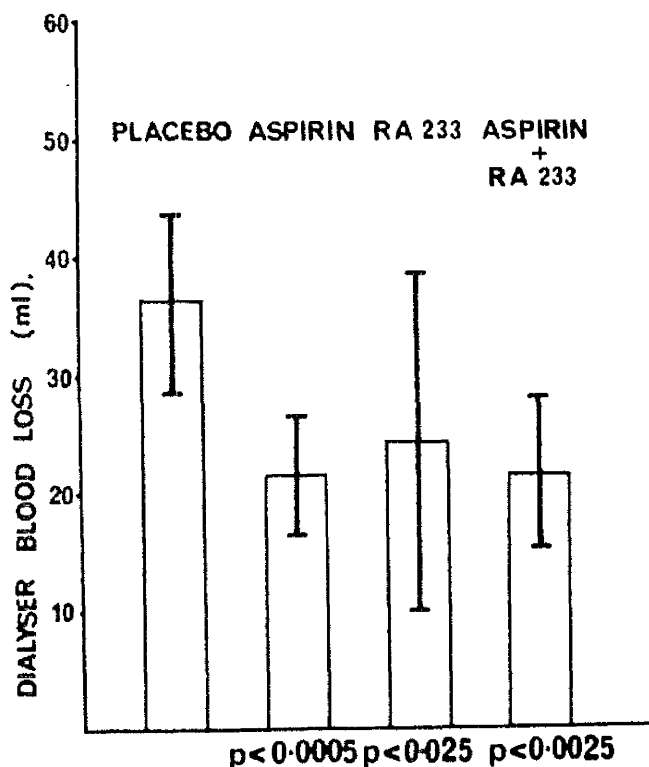


Fig. 4—Dialyser residual blood-volume (" blood-loss ") (mean $\pm$ S.D.) during treatment with placebo and anti-platelet agents.
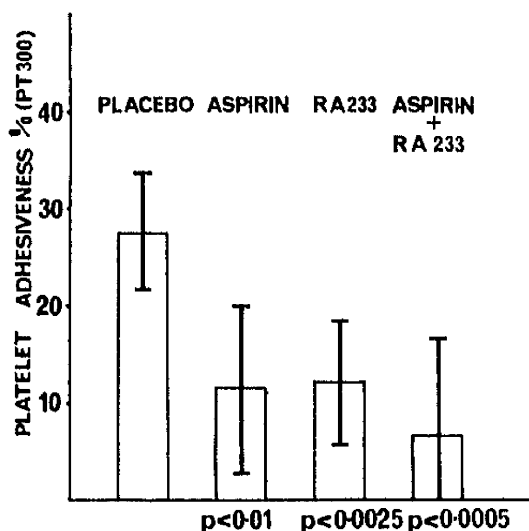
Fig. 2—Platelet-adhesiveness values (mean±S.D.) of regular dialysis patients during treatment with placebo and anti-platelet agents.

A significant increase in factor V over the course of dialysis was noted during the placebo, RA 233, and aspirin and RA 233 treatment periods (see accompanying table); the apparent increase during aspirin therapy was not significant. When the increments for each period were expressed as a percentage of the pre-dialysis value, it was found that there was no significant difference between the increment found during placebo therapy and that obtained during treatment with active agents. A significant fall in plasminogen level was noted over the course of dialysis during placebo therapy (see table); no significant changes occurred during aspirin, RA 233, or combination therapy.

No significant changes were noted in the patients' fibrinogen or F.D.P. levels over the course of dialysis during any of the treatment periods.

The mean (±S.D.) volume of blood remaining in the dialysers after use during each of the treatment periods is shown in fig. 4. The mean value for the dialyser blood-loss during the placebo period was 36 ml. This fell significantly to 22, 24, and 22 ml. with aspirin, RA 233, and combination treatment, respectively.

## Discussion

The use of the Gambro-Alwall dialyser is associated with a high blood-loss due to thrombus formation on the dialysis membranes (fig. 1), despite the fact that the
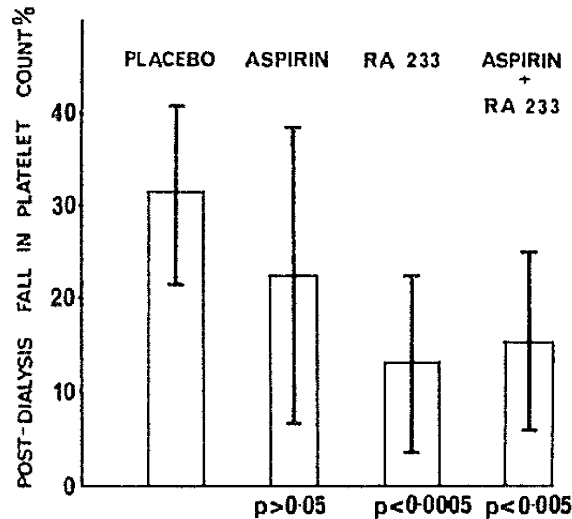
**Fig. 3—Fall in platelet-count (mean ± S.D.) over the duration of dialysis during treatment with placebo and anti-platelet agents.**

patient is adequately anticoagulated with heparin.[2] During the course of dialysis several changes in hæmostatic function were noted; namely, a fall in platelet-count, rise in factor-V level, and fall in the plasminogen level.[6] The fall in the platelet-count was secondary to the retention of platelets within the dialyser, suggesting that the platelet retention by the dialysis membranes may be an important early step leading to subsequent thrombus formation.[6] The present study was designed to test whether therapeutic inhibition of platelet function might lessen platelet retention and thrombus formation within the dialyser.

Salicylates are known to inhibit the platelet-release reaction triggered by adenosine diphosphate or collagen.[14][16] Aspirin, in doses as low as 0.15–0.3 g. daily, will reduce the platelet-aggregation induced by collagen[17,18] and lower platelet-adhesiveness.[18] Dosages of 0.6 g. daily also reduce platelet-adhesiveness.[9,19] RA 233 is a new pyrimido-pyrimidine compound which is a powerful inhibitor of platelet aggregation and platelet-adhesiveness.[20]

The results of the present study show that the ingestion of both soluble aspirin and RA 233 reduced platelet-adhesiveness as measured by the retention of platelets within a test cell lined by cuprophan dialysis membrane. The potency of both drugs in this system seemed the same and the use of combination therapy did not seem to produce a greater reduction in platelet-adhesiveness. The reduction in platelet-adhesiveness induced by aspirin and RA 233 was associated with a

reduction of the decrease in platelet-count usually seen after dialysis. This suggests that fewer platelets were retained within the membranes of the dialyser during these periods.

One advantage of using the membrane test cell is that platelet-adhesiveness seems to be almost independent of the hæmatocrit.[9] In contrast, in the glass-bead-column technique there is a strong correlation between platelet-adhesiveness and hæmatocrit.[21,22] Since almost all patients with chronic renal failure have low hæmatocrits, the finding that uræmic patients have lowered platelet-adhesiveness [23] must be interpreted with caution. The initial mean platelet-adhesiveness of these uræmic patients was $28\% \pm 6\%$ ($\pm$S.D.), which was not significantly different from the mean value of $31\% \pm 9\%$ ($\pm$S.D.) found in 52 healthy control subjects.[9]

Evidence that thrombus formation was reduced by the anti-platelet agents was given by the dialyser blood-loss values. During placebo ingestion the mean blood-loss was 36 ml. and this figure was reduced to just over 20 ml. per dialysis during active treatment periods. This supports our initial hypothesis that platelet retention by the dialysis membranes is an important early step in the reaction which proceeds to thrombus formation upon the membranes. Other workers have studied thrombus formation on materials used in cardiovascular surgery (e.g., ' Teflon ', ' Silastic ') and they also suggest that platelet retention to these materials is an important step in thrombus formation.[24-27] Salzman [28] has agreed that it is customary to view surface-induced thrombosis as chiefly, if not exclusively, a platelet problem.

Consideration of the plasminogen levels also suggests that the reduction in platelet retention by the dialysis membranes reduces thrombus formation. A fall in plasminogen, over the course of dialysis, only occurred during the placebo treatment period (see table). It is likely that the presence of fibrin within the extracorporeal circuit brought about the conversion of plasminogen to plasmin, with a consequent fall in the former. On the other hand, the increase in factor V was not influenced by anti-platelet preparations, suggesting that activation of this component took place independently of platelet activity.

Although no patient experienced any hæmorrhage or other side-effects as a result of the administration of the drugs in the present study, we would not

suggest that they be used therapeutically for the dialysis patient in view of the hæmorrhagic risk when anti-platelet agents and heparin are used concurrently. Rather, we recommend that studies of the thrombogenic properties of dialysis membranes be continued with special reference to platelet adhesion to different dialysis membranes. It has been shown that certain cellulose-based membranes attract more platelets than do others.[9] Additionally, if PT 250 membranes are substituted for the PT 325 membranes of the Gambro-Alwall dialyser, the in-vivo blood-loss is reduced to under 20 ml. per dialysis.[2]

The present study, nevertheless, confirms that anti-platelet agents may be of therapeutic value in the prevention of intravascular thrombus formation. Dipyridamole (' Persantin ') has been shown to inhibit thrombus formation at sites of vascular injury in laboratory animals,[29] and Sullivan et al.[30] reported a significant decrease of thromboembolic incidents in patients with prosthetic heart-valve replacement who were treated with combined dipyridamole and anti-coagulant therapy compared with those treated with anticoagulants alone. RA 233, a new analogue of dipyridamole, is likely to be of similar value. Finally, it may be that the extracorporeal circuit used in the present study might provide a suitable model for studies on intravascular thrombosis.

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Comment

This study confirmed our hypothesis that the thrombus
formation on the dialysis membranes was, at least, partly due to
platelet retention by these membranes. This is in keeping with the
views of most workers involved in the study of thrombus formation
upon non-biological surfaces (Salzman, 1971). It was now obvious
that my further studies should focus on the thrombogenic properties
of dialysis membranes with a special reference to platelet retention
by them. A preview of this work, which is dealt with in Chapter 5,
is mentioned in the Discussion of this paper.

The method for the measurement of platelet adhesiveness used
in this study is described in detail in Paper 6.

This investigation, again, had an additional more general
interest in that it suggests that anti-platelet agents may have a role
in the prevention of intravascular thrombus formation. This study
is, incidently, the first in vivo report of an anti-thrombus effect
by the analogue of dipyridamole RA 233. It mat be that extracorporeal
circuits, similar to the one used in this study, provide a suitable
model for studies on intravascular thrombosis and its modification
or prevention by drugs.

In Discussion brief mention is made of an interesting coincidental
finding; the platelet adhesiveness values of our regular dialysis
patients, while not on active drugs, were normal. This is in contrast
with the findings of Larsson (1971) who found that the decreased retention
of platelets in a glass bead column, occurring in non-dialysed
ueaemics, often persisted after regular dialysis had commenced.
As there is a strong correlation, with the glass-bead-column technique,
between platelet-adhesiveness and haematocrit (Hellem, 1960;
Hassanein, 1968; Eknoyan et al, 1969) and as almost all patients
with chronic renal failure, dialysed or conservatively treated, have
low haematoctits, the finding that uraemic patients have lowered
platelet adhesiveness must be treated with caution. It may be that

newer techniques in evaluating platelet function, such as the
membrane test cell system (Paper 6), will enable more
sophisticated studies of the platelet defect in uraemia.

## Chapter 4 - Summary - Thrombus Formation During Dialysis

In this chapter of the thesis I have set out the data confirming that thrombus formation may take place upon dialysis membranes in spite of adequate heparinisation (Paper 4) and that this is associated with demonstrable changes in the haemostatic status of the patient over the course of a dialysis (Paper 4). A considerable fall in the platelet count is seen, with an evaluation of factor V and a fall in the plasminogen level. The rise in factor V is associated with a fall in the partial thromboplastin time and indicates activation of the coagulation cascade mechanism, while the fall in plasminogen suggests that the fibrinolytic system has been activated by the presence of fibrin. It is interesting that post-dialysis falls in platelet count (Paper 4 - Additional Study) and plasminogen levels (Paper 4; Paper 5) may be indications of thrombus formation on the membranes which, in turn, is invariably associated with a high dialyser blood loss (Paper 2; Paper 4 - Additional Study). If much thrombus formation has taken place upon the dialysis membranes then the volume of blood remaining in the dialyser is unlikely to be influenced to any extent by either the technique or the volume of wash-back. I demonstrated this using volumes of saline in an attempt to reduce the blood loss caused by the Cordis Dow H.F.A.K. (Paper 2).

Blood circulating within the body has, effectively, an infinite clotting time. However, when blood comes in contact with a foreign surface, coagulation may occur within minutes. Furthermore these studies have shown that heparin anticoagulation does not necessarily prevent coagulation on foreign surfaces. In recent years there has been a search for artificial surfaces of low thrombogenicity for use in cardiovascular surgery but there has been comparitively little interest in the thrombogenicity of the materials used in the extracorporeal circuit of the artificial kidney or in the factors leading to thrombus formation on them.

So far, I have demonstrated that platelet retention by the dialysis membranes plays an important role in thrombus formation upon them (Paper 5) and have suggested that the linear velocity of blood travelling across the membrane surface may play a minor role (Paper 4 - Additional Study).

The form of anticoagulation used during haemodialysis obviously merits consideration. Long term oral anticoagulants, such as Warfarin, given in addition to heparinisation during dialysis may reduce thrombus formation by their action on the vitamin K dependent factors but there is dispute as to whether they have any significant antiplatelet effect (Hellem and Stormoken, 1969) which may be of greater importance. Furthermore the serious complications of long term anticoagulation are well known in dialysis units from the days when arteriovenous shunts were commonly used. Ancrod with its unique effect upon fibrinogen, has been used in place of heparin as an anticoagulant during dialysis and was observed to reduce the formation of fibrin on, and the retention of white blood cells by, the membranes of a Kiil dialyser (Hall et al, 1970). However, it would be impractical to use this drug routinely for dialysis as its administration is more complex than heparinisation and, as it is not easily neutralised, the patient would have a hypofibrinogenaemic clotting defect for some time after dialysis which might, inter alia, lead to a high blood loss from arteriovenous fistula cannulation sites. The use of antiplatelet preparations, such as soluble aspirin on a long term basis, can reduce dialyser blood loss (Paper 5) but the risk of side effects such as gastrointestinal bleeding is a contra-indication. I do not think, therefore, that any changes in the current policy of anti-coagulation during dialysis are indicated for, in the majority of cases, dialyser thrombus formation is minimal and, therefore, the use of heparin is satisfactory. It is more logical to identify and use less thrombogenic materials within dialysers than to attempt to reduce thrombus formation by drugs. Therefore, in Chapter 5 the nature of the dialysis membrane will be considered with regard to

in vit ro platelet retention and in vivo thrombogenicity and in
Chapter 6 the role of the configuration of the membrane surface
is similarly discussed.

# CHAPTER 5

## THE NATURE OF THE PLATELET - DIALYSIS MEMBRANE INTERACTION AND ITS ROLE IN THROMBUS FORMATION DURING HAEMODIALYSIS

Here, 2 papers are presented, the first of which describes a new method for the measurement of platelet adhesiveness by the use of dialysis membranes in a test cell. The second paper uses this method to assess, in vitro, the thrombogenicity of dialysis membranes.

Paper 6 -  A method for the measurement of platelet adhesiveness
by use of dialysis membranes in a test cell    (1973)


British Journal of Haemotology, 24  377 - 379.
R. M. Lindsay, C. R. M. Prentice, D. Ferguson, W. M. Muir,
and G. P. McNicol.


## Purpose of Investigation

This was to devise an in vitro system whereby the retention of
platelets by different dialysis membranes could be measured.


## Personal Contribution

The idea of the method was conceived jointly by myself, Dr.
Prentice and Dr. McNicol.  The test cell was constructed at the
University of Strathclyde, Bio-Engineering Department work-shop.
Its evaluation was carried out by myself, with help from Mr. D.
Ferguson ( a medical student in receipt of a Carnegie Vacation
Scholarship), with advice and encouragement from Dr. Prentice
and Dr. McNicol.  I was primarily responsible for the writing of
the paper but I received considerable help with this from  Dr.
Prentice.

COMMENT

This investigation was particularly rewarding to us. Not only had we developed a method that allowed us to quantitate the number of platelets retained by a particular dialysis membrane but, by using twin test cells simultaneously, we were now able to directly compare the platelet retaining properties of different membranes with considerable sensitivity. This was, obviously, the next step in the project and is the subject of Paper 7. Furthermore, this study gives some slight indication of the nature of the platelet-dialysis interaction in that the presence of adenosine diphosphate is necessary as are divalent cations ( the retention of platelets being blocked by the presence of ethylene diamine tetra-acetic acid) for platelet retention by the membranes.

Perhaps of even greater importance is the fact that we had developed a test for platelet adhesiveness which, in certain aspects, is more sensitive and reproducible than a glass bead column technique. One major advantage of our method over glass bead column techniques is that platelet adhesiveness values are virtually independent of haematocrit and thus further studies of platelet adhesiveness in anaemic states (for example, in uraemia) are now possible. Furthermore, the fact that retention of platelets occurs when platelet rich plasma is used suggests that the test cell system measures, in part, the initial adhesive stage of the platelet adhesion-aggregation reaction. In the glass bead column method it is platelet aggregates that are retained by the beads. Thus it is likely that the test cell measures a slightly different phenomenon that the standard glass bead techniques and, as such, may increase the armamentarium of the platelet investigator. To date, the method has been used very successfully in two published studies, not included in this thesis, which demonstrate the effect of prostaglandins (Howie et al, 1973) and heparin (Thomson et al, 1973) on platelet function.

At this stage in the evaluation of the membrane test cell

system I considered three problems. Firstly, our evidence for the
retention of platelets by the membranes was by inference from platelet
counts on blood before and after contact with membranes and not by
direct examination of the membranes. This problem is dealt with
briefly in Additional Study 1 (Page 49 ). Secondly, the membranes
we used in this study were not necessarily homogeneous. Cuprophan
PT 300 and PT 150 membranes were obtained from different sources
(e.g. from University of Strathclyde or from the cannibalisation of
commercially prepared haemodialysers) and hence were likely to
have been of different ages, and batches and some had even undergone
sterilisation by ethylene oxide gas. What effect this might have on the
reproductibility of results obtained by the test cell was uncertain.
However, the manufacturers J.P. Bemberg (Wuppertal, West Germany)
kindly provided us with a series of Cuprophan membranes of varying
dry weight per unit surface area but of the same age and chemical
composition and these have, subsequently, been exclusively used.
Finally, the 0.1 M phosphate buffer used in the test cell priming was
hyperosmolar to plasma  (500 m. osm/Kg) and I wondered what effect
this might have on the results. Owren's buffer is isomolar (300 m
osm/Kg) and thus the membrane test cell system was briefly
reassessed using this buffer and the new Cuprophan membranes.
(Additional Study 2, page 54 ).

Paper 6 - Additional Study 1

Purpose of Study :    To demonstrate visibly that platelets adhere to Cuprophan dialysis membranes

Methods

    Pieces of Cuprophan PT 325 dialysis membrane (30.5 Gm dry weight /$M^2$) (J.P. Bemberg, Wuppertal, West Germany) measuring 10 cm x 5 cm were used to line each of 2 plastic test tubes. 20 ml of heparinised blood (final heparin concentration 4 units per ml) was taken from a healthy volunteer and after centrifugation at 400 g for 5 minutes and then at 2000 g for 20 minutes 5 ml platelet rich and 5 ml platelet poor plasma was obtained and added to each of the membrane lined tubes. The tubes were sealed and rotated on their sides at room temperature for 30 minutes. Platelet counts were performed on the platelet rich plasma before and after contact with the membrane. After contact with the platelet rich and platelet poor plasmas the membranes were carefully removed from the tubes and soaked in a 2% buffered gluteraldehyde for 24 hours to allow fixing of any platelets to take place on the membranes. After this time several samples, 0.6 cm x 0.6 cm, were randomly taken from both pieces of membrane and these were coated, in vacuo, with approximately 35 nm of gold palladium and then examined by a scanning electron microscope. (Cambridge Stereoscan Mark 2A).

Results

    Platelet clumps together with fibrin-like strands were seen in abundance on the membrane samples after contact with platelet rich plasma (figures 1, 2 and 3). The associated fall in the platelet count of the platelet rich plasma was 30%. No such cellular clumps or strands were seen on the membranes contacted with platelet poor plasma.

## Comment

This study verified that the observed fall in platelet counts from the test cell studies was likely to be due entirely to the retention of platelets by the membrane.

## FIGURE 1
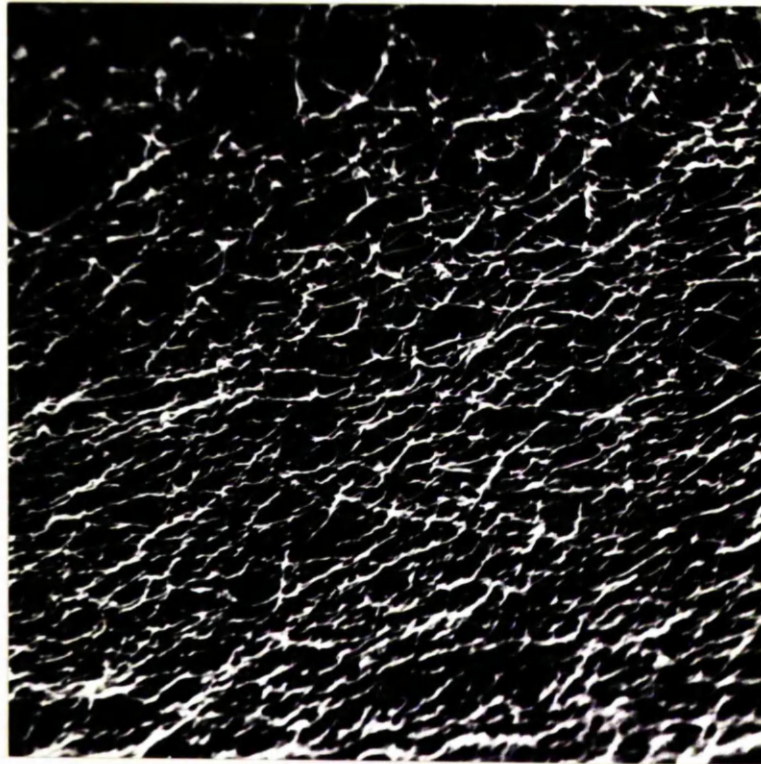


Scanning electronmicrograph (magnification x 530) of PT 325
Cuprophan dialysis membrane after contact with heparinised
platelet rich plasma. Platelet clumps with fibrin like strands
are seen on the membrane surface.

## FIGURE 2
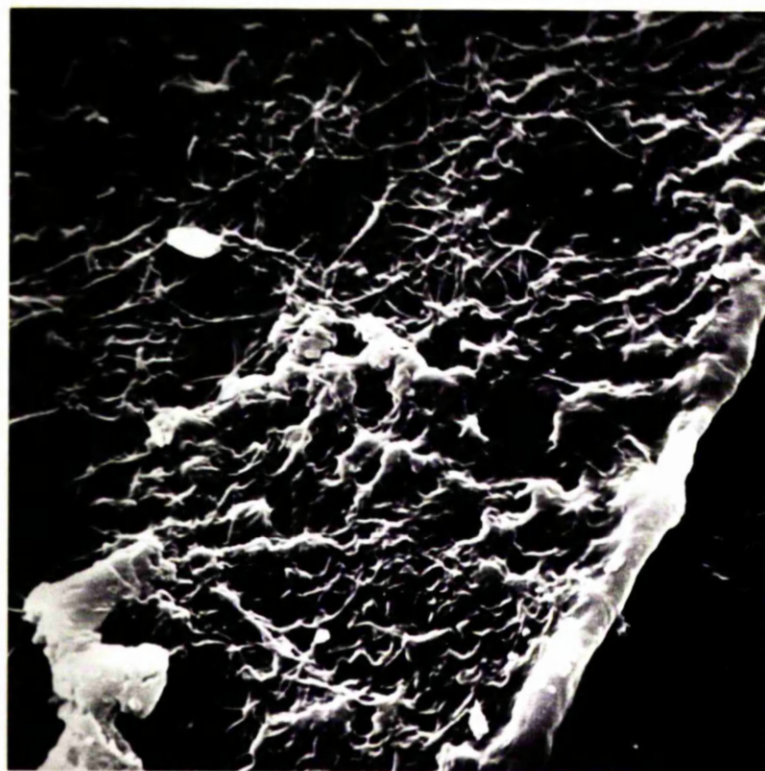


Scanning electronmicrograph (magnification x 1000) of PT 325
Cuprophan dialysis membrane after contact with heparinised
platelet rich plasma. Platelet clumps with fibrin like strands
are seen on the membrane surface.

FIGURE  3



Scanning electronmicrograph (magnification x 2, 200) of PT 325
Cuprophan dialysis membrane after contact with heparinised
platelet rich plasma. Platelet clumps with fibrin like strands
are seen on the membrane surface.
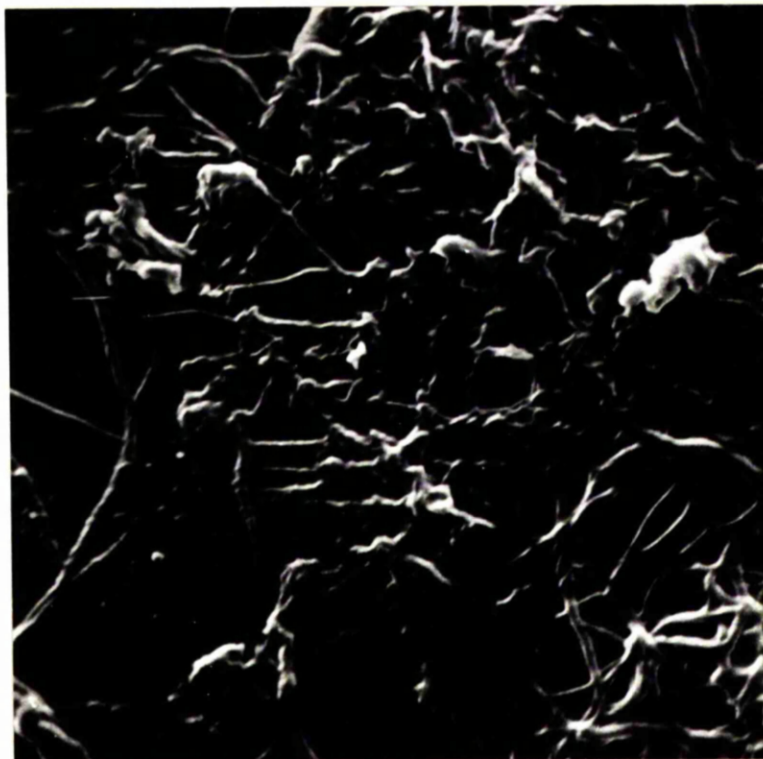
Paper 6 - Additional Study 2

## Purpose of Study

To obtain a 'normal' range of platelet adhesiveness values when the standard Cuprophan PT 325 membranes and Owen's buffer were used in the membrane test cell system.

## Methods

The range of platelet adhesiveness values was obtained for a further group of 40 healthy volunteers (age range 21 - 40 years; 29 M. 11 F.). No volunteer had taken any drug for a minimum of two weeks prior to the estimation. The measurement of platelet adhesiveness was carried out as described in Paper 6 with the following modifications :-

(1)     The dialysis membrane used was Cuprophan PT 325 (30.5 Gm dry weight/$M^2$).

(2)     The membranes were washed twice in distilled water before soaking in buffer.

(3)     Owen's buffer (ph 7.35; 300 m Osm/Kg.) was substituted for the 0.1 M phosphate buffer previously used for soaking the membranes and for priming the test cell.

(4)     The test cell r ocking was carried out for 10 minutes.

## Results and Comment

The mean value for the 40 estimations of platelet adhesiveness was 29 ± 7% (M ± S.D.). This value is not significantly different from the previous value (31 ± 9%) nor is the S.D. any less indicating that these changes had not necessarily increased the sensitivity of the test. Nevertheless, these changes have now been incorporated as standard for the reasons previously discussed.

Paper 7   -   <u>The role of the platelet - dialysis membrane</u>
<u>interaction in thrombus formation and blood</u>
<u>loss during haemodialysis.</u>   (1973)

Transactions American Society for Artificial
Internal Organs. Vol. XIX,   487 - 491.

R. M. Lindsay, C. R. M. Prentice, J. A. Burton,
D. Ferguson and A. C. Kennedy.

## Purpose of Investigation

Because of the apparent importance of platelet retention by
dialysis membranes in thrombus formation during haemodialysis an
in vitro method using a membrane test cell system was developed
which allowed the platelet retaining properties of different dialysis
membranes to be directly compared. This method is described in
Paper 6. Using this system I decided to examine platelet retention
to the Cuprophan (J. P. Bemberg, Wuppertal, West Germany) series
of membranes and to compare in vitro platelet retention with in vivo
thrombus formation.

## Personal Contribution

The study was initiated and planned by myself. Mr. D. Ferguson
(a medical student in receipt of a Carnegie Vacation Research
Fellowship) helped with the in vitro experimental work while
Dr. J. A. Burton helped with the setting up and supervision of the
dialyses and with theestimations of dialyser blood loss. I was
responsible for the statistical analyses. The paper was written by me
with advice from Professor A.C. Kennedy and Dr. C.R.M. Prentice.

# THE ROLE OF THE PLATELET-DIALYSIS MEMBRANE INTERACTION
## IN THROMBUS FORMATION AND BLOOD LOSS DURING HEMODIALYSIS

R. M. Lindsay, C. R. M. Prentice, J. A. Burton, D. Ferguson, and A. C. Kennedy

The regular dialysis patient remains anemic in spite of efficient hemodialysis. In the past hemoglobin levels were maintained by giving repeated blood transfusions in amounts varying from under one unit of packed cells to 4 units/patient month[1]. Most dialysis units now only transfuse in emergency situations because of the known risks of hepatitis[2-4]. If routine blood transfusion is to be avoided then all iatrogenic sources of blood loss must be minimized. One major potential source is blood trapped within the dialyzer which is not returned to the patient[5,6]. Two factors are responsible for dialyzer blood loss. Firstly, manifold designs may cause mechanical hold-up of anticoagulated blood and each type of dialyzer will have its own particular problems which may be largely overcome by an efficient wash-back technique at the termination of dialysis. Secondly, thrombus formation may take place on dialysis membranes, in spite of efficient heparinization, and cause undesirable high blood losses which are not influenced by either the technique or the volume of the wash-back[6]. The use of the Gambro-Alwall dialyzer* (Ab Gambro, Lund, Sweden) is complicated by a high blood loss due to thrombus formation on its membranes[6,7]. This thrombus formation is associated with a fall in the platelet count of patients undergoing dialysis by this system due to the retention of platelets within the dialysis membranes[8] suggesting that the platelet retention on the dialysis membrane is an important step in the reaction leading to thrombus formation. This hypothesis is supported by the fact that the administration of antiplatelet agents (aspirin and dipyridamole compounds) will reduce not only the retention of platelets on the dialysis membranes but also the degree of thrombus formation and patient blood loss[9]. Because of the apparent importance of platelet retention by dialysis membranes we have developed an in vitro method using a membrane test cell system whereby the platelet retaining properties of different dialysis membranes may be directly compared. Using this system we have examined platelet retension to cuprophane (J. P. Bemberg, Wuppertal, West Germany) membranes and have compared in vitro platelet retention with in vivo thrombus formation.

## METHODS

The membrane test cell and the measurement of platelet adhesiveness. The membrane test cell system is fully described elsewhere[10]. It consists of 2 perspex blocks so constructed as to allow the introduction of blood between 2 sheets of dialysis membrane by means of unplasticized polyvinylchloride entry ports and plastic (Kematal, I.C.I.) spreaders. The 2 spreaders and a plastic (low density polyethylene) gasket maintain a blood compartment height of 0.07 cm. The compartment which is 12.8 cm long and 10.5 cm wide has a volume of 9 ml. An exploded view of the cell is shown in Figure 1. To measure platelet retention (platelet adhesiveness) dialysis membrane sheets cut larger than the test cell are washed twice in distilled water and then soaked in Owren's buffer (pH 7.35, mOs/kg) for 5 min. They are then placed in a test cell the 2 blocks of which are clamped together. The cell is then primed with 9 ml Owren's buffer and emptied after 10 mins. For each test cell experiment 9 ml venous blood from a healthy volunteer is taken into a plastic syringe and mixed with 1 ml of 3.8% sodium citrate in a plastic tube; 0.2 ml of this blood is taken for platelet counting, the rest being introduced into the test cell by plastic syringe in a standard fashion. The blood ports are then clamped and the test cell rocked by hand, to an angle of 30° at 5 second intervals. The rocking is carried out using a simple seesaw device (Figure 2) which also allows 2 test cells to be used simultaneously. After 10 minutes the test cell is emptied by opening the ports and withdrawing the blood into a plastic syringe. The withdrawn blood is mixed gently in a plastic tube and a further 0.2 ml is taken for a second platelet count. The platelet counts are carried out in duplicate by the method of Dacie and Lewis[11] on coded samples so that the observer does not know whether the microscope chamber contains blood from the pre- or post-test cell sample. By comparing the pre- and post-test cell platelet count the number of platelets retained within the test cell is calculated. The drop in platelet count expressed as a percentage of the initial count gives a value termed platelet adhesiveness.

In vitro comparison of platelet retention by cuprophane membranes. Using twin test cells of identical construction and the rocking device (Figure 2) paired estimations for platelet adhesiveness can be made. If both test cells contain identical membranes an excellent correlation is obtained between the 2 sets of results[10]. A test membrane may then be inserted into one test cell and compared directly with a standard membrane by examination of paired results for platelet adhesiveness.

Cuprophane membranes of varying dry weight/unit surface area but of the same age and chemical composition were provided by the manufacturers (J. P. Bemberg, Wuppertal, West Germany). The membranes studied were PT 150 (16.5 Gm/M$^2$), PT 200 (19.6 Gm/M$^2$), PT 250 (25.5 Gm/M$^2$) and PT 325 (30.5 Gm/M$^2$). The PT 325 membrane was chosen as the initial standard against which the other membranes were compared and the following series of experiments were carried out. Pt 325 against PT 325 (7 observations); PT 325 against PT 250 (12 observations); PT 325 against PT 200 (9 observations); PT 325 against PT 150 (7 observations). Subsequently PT 250 and PT 200 membranes were used as standards and the following comparisons were made: - PT 250 against PT 200 (7 observations); PT 250 against PT 150 (7 observations); PT 200 against PT 150 (9 observations). For each of these experiments 18 ml whole blood was taken from healthy volunteer subjects, mixed with 2 ml of 3.8% sodium citrate and then divided. All platelet counts were performed blindly using coded counting chambers, the observer being unaware of which membranes were being studied.

In vivo dialyzer studies. Blood loss was measured in 18 standard Gambro-Alwall dialyzers (containing PT 325 membranes) and in 10 Gambro-Alwall dialyzers specially prepared for us by Ab Gambro (Lund, Sweden). These latter dialyzers were identical to the others with the exception that they contained PT 250 membranes. The blood losses were estimated with precision using 51 Cr-labelled red blood cells: the details of the techniques and of its accuracy have been given elsewhere[7, 12]. The dialyzers were used during the routine regular dialysis program of this unit and were conducted in a standard fashion and terminated using a standard "wash-back" procedure. Anticoagulation was achieved using 4000 units heparin (Weddel Pharmaceuticals Ltd; 100 units is equivalent to 1 mg) at the start of dialysis and repeated hourly; this was sufficient to maintain the whole blood clotting time at 37°C in excess of 40 min in all cases. Platelet counts[11] were carried out on each patient before and after each dialysis and the percentage fall in platelet count calculated.

## RESULTS

In vitro experiments. In Figure 3 the paired results for platelet adhesiveness obtained with both test cells containing PT 325 membranes are plotted; the values show good correlation (r = 0.92) indicating that there is little error in the method. The paired results for platelet adhesiveness obtained during the series of experiments where membranes PT 150, PT 200 and PT 250 were compared against PT 325 are plotted compositely in Figure 4 and indicated that fewer platelets were retained by these membranes than by PT 325 membranes. Using a series of paired Students' "t" tests these apparent differences were shown to be statistically significant: - PT 325 v. PT 250 (p < 0.025), PT 325 v. PT 200 (p < 0.0005), PT 325 v. PT 150 (p < 0.0005). The paired results for the experiments using PT 250 membranes as the standard showed that significantly fewer platelets were retained by PT 200 membranes (p < 0.0005) and PT 150 membranes (p < 0.0005). The results for the PT 200 and PT 250 experiments are shown plotted in Figure 5. Finally the paired results also showed that significantly fewer platelets were retained by PT 150 membranes than by PT 200 membranes (p < 0.025). Summarizing these results it is apparent that there is a direct relationship between the platelet retention and the thickness (or weight per unit surface area) of the cuprophane membrane.

In vivo experiments. The mean ($\pm$SD) values for dialyzer blood loss and the percentage fall in patients' platelet counts over the course of dialysis are shown in Table I. The substitution of PT 250 membranes for the standard PT 325 membranes in the Gambro-Alwall dialyzer is associated with significant reductions in the fall in platelet count over the duration of dialysis and in the dialyzer blood loss.

TABLE I

DIALYZER BLOOD LOSS AND FALL IN PATIENTS' PLATELET COUNT USING
GAMBRO-ALWALL DIALYZERS CONTAINING DIFFERENT DIALYSIS MEMBRANES

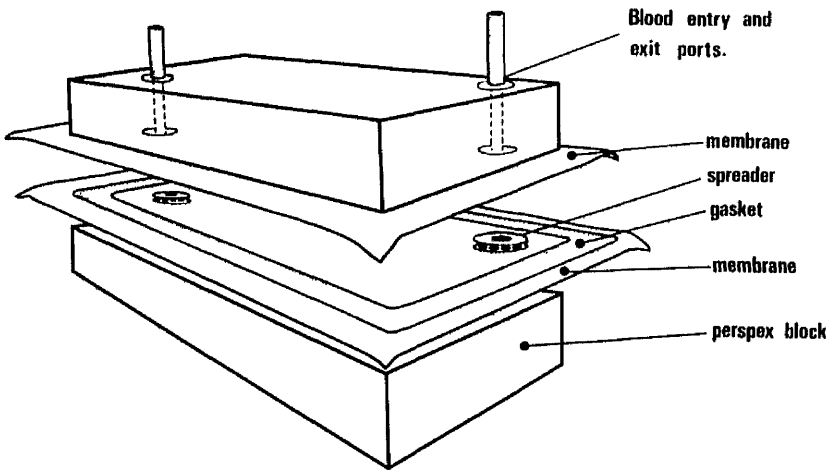| Dialysis Membrane | Number of Observations | Fall in Platelet Count (%) (M ± SD) | Blood Loss ML (M ± SD) |
|---|---|---|---|
| Cuprophane PT 325 | 18 | 31.2 ± 9.7 | 36 ± 4 |
| Cuprophane PT 250 | 10 | 21.6 ± 15.2 | 18 ± 8 |
| | | p < 0.05 | p < 0.0005 |

Figure 1. * Exploded view of the membrane test cell.



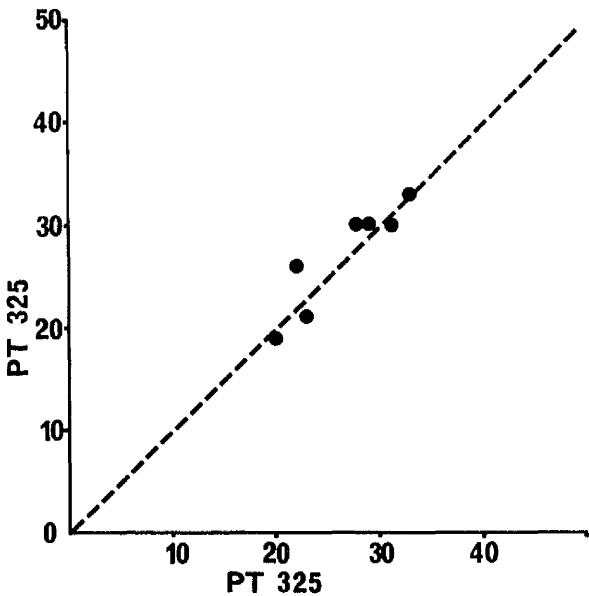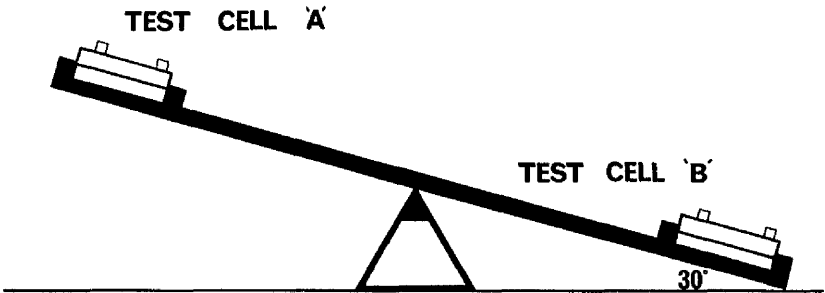Figure 2.* Rocking device for simultaneous use of 2 test cells.



Figure 3. Comparison of simultaneous estimations of platelet adhesiveness values (%) using PT 325 membranes and twin test cells. The broken line is at 450°.

*Figures 1 and 2 are reproduced by permission of the Editor, British Journal of Haematology.

489

## DISCUSSION

The use of the Gambro-Alwall dialyzer is associated with a high blood loss due to thrombus formation on the dialysis membranes in spite of the fact that the patient is adequately anticoagulated with heparin[6, 7]. During the course of dialysis several changes in hemostatic function occur; namely a fall in platelet count, rise in factor V level and fall in the plasminogen level[8]. Using $^{51}$Cr-labelled platelets it has been shown that the fall in platelet count is secondary to platelet retention within the dialyzer[8]. This platelet retention by the dialysis membranes is an important step in a reaction leading to subsequent thrombus formation for therapeutic inhibition of platelet function by aspirin and RA 233 (a dypiridamole compound) lessens both the platelet retention and the thrombus formation (and hence the blood loss) within the dialyzer[9]. Other workers have studied thrombus formation on materials used in cardiovascular surgery (e.g. teflon, silastic) and also suggest that platelet retention to these materials is an important step in thrombus formation[13-16]. Salzman[17] has agreed that it is customary to view surface induced thrombosis as chiefly, if not exclusively, a platelet problem.

The results of this study indicate that the nature of dialysis membrane itself influences the number of platelets that will be retained by it and that with cuprophane membranes of identical chemical composition, there is a direct relationship between the membrane thickness and the in vitro platelet retention. This suggests that the thicker cuprophane dialysis membranes may be the more thrombogenic in vivo. This hypothesis is supported by this and our previous study[6] demonstrating that the substitution of PT 250 membranes for PT 325 in the Gambro-Alwall dialyzer is associated with a lessening of both the post-dialysis drop in platelet count and the dialyzer blood loss. The latter is directly related to the degree of thrombus formation. We have attempted to use even thinner cuprophane membranes, e.g. PT 150 in the Gambro-Alwall dialyzer but were unable to continue the experiments because of dialyzer ruptures.

At present we are unable to state why there is variable retention by different membranes but the surface charge[18, 19] or the surface free energy[12] may be among important determinants.

Studies such as these suggest that the membrane test cell system described is of potential value in the evaluation of the potential thrombogenicity of different dialysis membranes. This would be in keeping with the recommendations of the Gotch report[20].

## SUMMARY

A major part of blood loss in some dialyzers is due to thrombus formation on the dialysis membrane surface. Studies using the Gambro-Alwall dialyzer have suggested that such thrombus formation is largely mediated by an interaction between platelets and the dialyzer membranes. Platelet retention to cuprophane dialysis membranes was studied, in vitro, using a test cell system and it was found that there was a direct relationship between platelet retention and the membrane thickness. A good correlation between platelet retention in vitro and the amount of thrombus formed in the Gambro-Alwall dialyzer in vivo was also obtained.

### ACKNOWLEDGMENT

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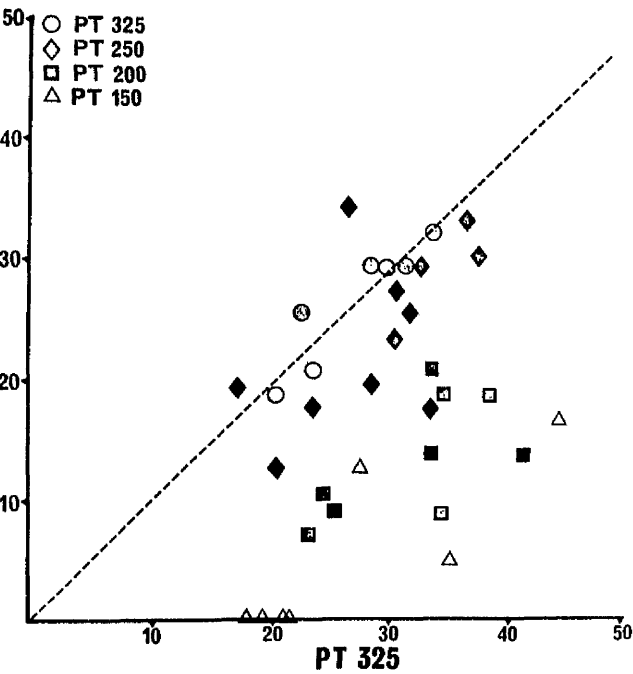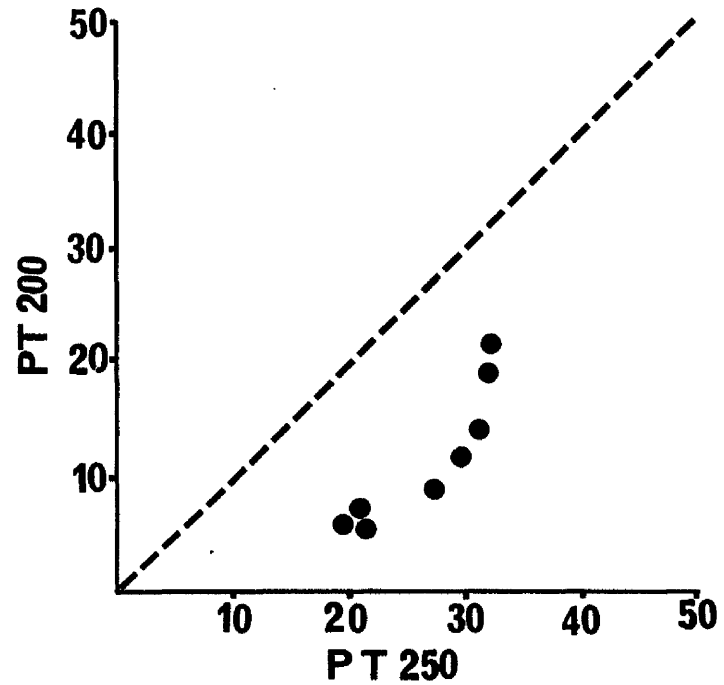
Figure 4.  Platelet adhesion to PT 325, PT 250, PT 200 and PT 150 membranes when each is compared simultaneously with platelet adhesion to PT 325 membranes.  The values shown are for platelet adhesiveness (%).

Figure 5.  The comparison of platelet adhesion to PT 250 and PT 200 membranes.  The values shown are for platelet adhesiveness (%).

Comment

This study was straightforward in its aim and in its result. It showed that the nature of the dialysis membrane itself influences the degree of platelet retention that occurs and that this, in turn, is directly proportional to its thrombogenicity. The unsolved question is, of course, what factors cause the variable retention of platelets by different membranes. At this time, neither I, nor my fellow workers, can answer this question.

Muir (1971) demonstrated visually, by scanning electronmicrographs of dialysis membranes after 4 hours of dialysis, that fewer platelets and red blood cells were deposited on acrylic copolymer membranes than on Cuprophan PT 150 membranes. In another study, he and his colleagues (Muir et al, 1971) showed that when unheparinised dog blood passed over such membranes in parallel Grimsrud-Babb test cells (Grimsrud and Babb, 1966) thrombus formation on the Cuprophan PT 150 membranes was more extensive than on the copolymer membrane. These experiments by the University of Strathclyde Bio-Engineering unit were, I believe, the first studies to demonstrate that certain types of dialysis membranes were likely to be more thrombogenic than others. Our study (paper 7) was the first to show that, with Cuprophan membranes, there is a direct relationship between thrombogenicity and the membrane thickness. In thrombogenicity studies, as part of the evaluation of future membranes for haemodialysis, I would suggest that both the membrane test cell system (Paper 6) and animal studies, such as those of the University of Strathclyde, may play important roles.

The results of this study appeared to me to have additional scientific interest in that the possession of membranes with different platelet retaining properties might be useful in platelet function studies. To study, for example, a drug which might reduce platelet adhesiveness it would be advisable to use a membrane with a high platelet retaining quality in the test cell. On the other hand, in the study of situations

possibly associated with enhanced platelet stickiness the use of
a membrane with low platelet retaining properties may be
advantageous. Because of this I examined the range of values for
platelet adhesiveness in normal subjects that had been obtained,
throughout these studies, using the different Cuprophan membranes.
These values are shown in the Additional Study which follows.

Paper 7 - Additional Study

Purpose of study :   To obtain a series of 'normal ranges' (that is, as found in healthy volunteers) for platelet adhesiveness using the membrane test cell system (Paper 6) with the different members of the Cuprophan series of dialysis membranes (J.P. Bemberg, Wuppertal, West Germany).

Method

The 'normal ranges' were derived from the results obtained during the study on the inter-relationship of platelet adhesiveness and the thickness of Cuprophan membranes (Paper 7).

Results

These are shown in Table IV

Comment

These values indicate that the use of the thinner Cuprophan membranes (e.g. PT 150) in the test cell if associated with a loss of sensitivity of the test, as indicated by a wide range, and this may limit the usefulness of the test in situations where enhanced platelet adhesiveness is sought.

Note :       PT .300 membranes, as used in the original study
             (Paper 6) are no longer produced by J.P. Bemberg
             and thus were not examined in the study described
             in Paper 7.

TABLE  IV

NORMAL RANGE FOR PLATELET ADHESIVENESS

USING CUPROPHAN MEMBRANES OF VARYING THICKNESS

| CUPROPHAN MEMBRANE | PLATELET ADHESIVENESS $\%$ M $\pm$ SD) | No. of OBSERVATIONS |
|---|---|---|
| PT 325 | $29 \pm 7$ | 40 |
| PT 250 | $29 \pm 8$ | 24 |
| PT 200 | $16 \pm 13$ | 26 |
| PT 150 | $10 \pm 9$ | 24 |

Chapter 5 - Summary - <u>The nature of the platelet-dialysis membrane</u>
<u>interaction and its role in thrombus formation</u>
<u>during haemodialysis</u>

In this Chapter I have demonstrated by scanning electronmicro-
scopy that platelets readily adhere to the cellulose-based Cuprophan
dialysis membranes in vitro (Paper 6, Additional Study 1) which
supports the in vivo study using radio-chromium labelled platelets
(Paper 4). This in vitro retention of platelets by the membranes can
be quantitated using a test cell system (Paper 6) which allows not only
the nature of platelet dialysis membrane interaction to be studied but
also the platelet retention by different types of membrane to be directly
compared (Paper 6; Paper 7). The studies indicate that adenosine
diphosphate and divalent cations are necessary for the retention of
platelets by the Cuprophan membranes (Paper 6) and that there is a
direct relationship between the thickness of the Cuprophan membrane
and the platelet retaining properties of that membrane (Paper 7).
Finally, I have demonstrated that the in vitro platelet retention by a
dialysis membrane as measured by the test cell system reflects both
the post dialysis fall in platelet count and the degree of thrombus
formation taking place on that membrane during clinical haemodialysis
(Paper 7). The studies of Chapter 4, which suggested that the degree
of platelet retention was important in thrombus formation upon
membranes during haemodialysis, are further supported by these
studies of Chapter 5.

In addition to the general theme of the thesis I have shown that
the membrane test cell system may have a place as a test of platelet
function (Paper 6).

CHAPTER 6

## THE ROLE OF THE MEMBRANE SURFACE GEOMETRY
## ON PLATELET RETENTION AND THROMBUS FORMATION

One paper is presented which describes the alteration of the membrane surface geometry by the membrane support system of various dialysers and discusses the importance of this in thrombus formation.

As only one paper is discussed no separate summary of Chapter 6 is given.

Paper 8    The membrane support system and thrombus
           formation on dialysis membranes    (1973)

           Proceeding of the European Dialysis and Transplant
           Association,    Volume X    306 - 313.  R. Wilkinson,
           R. M. Lindsay and J.A. Burton

## Purpose of Investigation

The surface geometry of a dialysis membrane during heamodialysis
will be influenced by the construction of the membrane support system
used in a particular dialyser.  This study was undertaken to examine
the influence of this upon platelet retention.

## Personal Contribution

During the course of the previous studies scanning electronmicro-
graphs were made of membranes obtained from various dialysers after
use (Papers 2, 4 and 5).  The scanning electronmicrographs were
kindly prepared by Dr. R. Wilkinson (Bio-engineering Unit,  University
of  Strathclyde).  She observed the patterns of cellular deposition upon
the various membranes and their relationship to the membrane support
systems. I realised the relevance of this observation to our studies on
the factors influencing platelet retention by dialysis membranes and
planned the continuation of the investigation.  The dialysers studied
were prepared by Dr. Burton and myself and we set-up and supervised
all the dialyses other than those involving Extracorporeal EX03 and
Travenol UF 100 coils which are in routine use in our unit. I wrote
the paper.

# THE MEMBRANE SUPPORT SYSTEM AND THROMBUS
# FORMATION ON DIALYSIS MEMBRANES

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&

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&

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Royal Informary, Glasgow, Scotland

## INTRODUCTION

Thrombus formation may take place on dialysis membranes, in spite of efficient heparinisation, and cause the regular dialysis patient to have undesirably high blood losses (Lindsay and Kennedy, 1972; Lindsay et al, 1973a). The use of dialysers, which are complicated by in-vivo thrombus formation, is associated with a fall in the platelet count of patients undergoing dialysis due to the retention of platelets within the dialysis membranes (Lindsay and Kennedy, 1972; Lindsay et al, 1972a), suggesting that the platelet retention on the dialysis membranes is an important step in the reaction leading to thrombus formation. This hypothesis is supported by the fact that the administration of antiplatelet agents will reduce not only the retention of platelets on the dialysis membranes but also the degree of thrombus formation and the patient blood loss (Lindsay et al, 1972b). Because of the effects of platelet retention by dialysis membranes it is important to study factors which may influence it. The nature of the dialysis membrane per se will influence the number of platelets retained by it; for example with Cuprophan membranes of identical chemical composition, there is a direct relationship between the membrane thickness and the in-vitro platelet retention (Lindsay et al, 1973b). Furthermore the thicker Cuprohpan membranes appear to be the more thrombogenic in vivo (Lindsay et al, 1973b). The surface geometry of the dialysis membrane will be influenced by the construction of the membrane support system and this study was undertaken to examine the influence of this upon platelet retention.

## METHODS

Five types of disposable dialyser were studied. They were the Gambro-Alwall, its successor the Gambro-Lundia, and the Rhone-Poulenc parallel flow dialysers, and the EX-03 (Extra-corporeal) and UF 100 (Travenol) coils. Three dialysers of each type were examined after use, the respective dialyses being performed under standard conditions and terminated by standard 'wash-back' procedures. To fix any cellular or proteinaceous deposit a 2% buffered glutaraldehyde solution was pumped slowly through each dialyser's blood manifolds immediately after the 'wash-back' procedure had been completed. Care was taken not to allow air to enter the dialyser between the saline 'wash-back' and the filling with glutaraldehyde. The blood ports were sealed and each dialyser was then left for a minimum of twenty-four hours to allow fixing to take place. The dialyser was then dismantled and membrane samples, 0.6 x 0.6 cm, were taken from the arterial and venous ends of the blood compartments and also at a point approximately mid-way along the compartment. With the parallel flow dialysers samples were also taken from the top, middle and bottom layers. Each sample was coated, in vacuo, with approximately 35 nm of gold-palladium and then examined by a scanning electon microscope (Cambridge Stereoscan Mark 2A).

## RESULTS

Under low power magnification (x20) cellular deposits were seen arranged in regular patterns which were obviously related to the geometry of the underlying dialysis membrane supporting system. This feature was constant for each dialyser and is demonstrated in figures 1 - 5. The deposits were oval in shape with the coil and Gambro (Alwall and Lundia) dialysers and the spacing between them indicated that they related to the intersections of the coil meshes

(figures 1 and 2) or to the 'high spots' of the Gambro moulded polystyrene support systems (figures 3 and 4). The deposits were linear with the Rhone-Poulenc dialyser which corresponded to the parallel ridges of its supporting system (figure 5). With higher power magnification (x 1000 ──➤ x 5000) the deposits were seen to consist of red and white blood cells and platelets (figure 6) with variable amounts of amorphous proteinaceous material and fibrin-like strands. The regular arrangement of these deposits was fairly uniform along the entire length of the coil dialysis membranes; fibrin formation, ensnaring red cells, only occurring at the junction of the membrane and the blood tubing at the venous or outlet end. Towards the venous end of the Gambro Alwall dialyser the regular pattern of deposit was lost and widespread thrombus formation was seen with fibrin strands trapping large numbers of red blood cells (figure 7). Very little fibrin formation was seen on the membranes of the Rhone-Poulenc dialyser. With the parallel flow dialysers there was no difference in the pattern of deposits or fibrin formation on the membranes taken from various layers of the dialyser.


DISCUSSION

We have already shown that platelets will .adhere to dialysis membranes both in vitro (Lindsay et al, 1973c) and in vivo (Lindsay et al, 1972a). The deposition of platelets on dialysis membranes during haemodialysis may be associated with fibrin formation in spite of heparin anticoagulation (Lindsay et al, 1972b) and thus studies of the interaction between platelets and dialysis membranes are important as membranes with low platelet retention may be less thrombogenci than membranes with a high retention (Lindsay et al, 1973c, 1973b). Other workers have studied thrombus formation on materials used in cardiovascular surgery

(e.g. Teflon, silastic) and also suggest that platelet retention by
these materials is an important step in thrombus formation
(Lyman et al, 1968, 1969; Rodman and Mason 1970a, 1970b).
Salzman (1971) has agreed that it is customary to view surface
induced thrombosis as chiefly, if not exclusively, a platelet
problem.

The nature of the platelet dialysis membrane interaction has
been partly studied. As far as the platelet is concerned we have
demonstrated that it has an 'intrinsic' ability to adhere to
cellulose based dialyser membranes but this reaction is dependent
upon the presence of adenosine diphosphate, possibly released from
the platelets themselves and from red blood cells, and upon
divalent cations (Lindsay et al, 1973c). Furthermore the use of
anti-platelet agents such as salicylate and dipyridamole compounds
will reduce platelet retention by these membranes (Lindsay et al,
1972b, 1973c). The nature of the dialysis membrane itself will
influence the interaction. The chemical composition of the membrane
may be important for fewer platelets will adhere to 'series 10'
(co-polymer of N-butyl methacrylate and acrylic acid) membranes
(BioEngineering Unit, University of Strathclyde) than to cellulose
based membranes (unpublished observations). With cellulose based
membranes of identical composition (Cuprophan, J.B. Bemberge,
West Germany) there is a direct relationship between platelet
retention and the membrane thickness (Lindsay et al, 1973b). It is
also likely that other membrane factors such as surface charge
(Sawyer and Pate, 1953; Sprinivasan and Sawyer, 1970) or the surface
free energy (Lyman et al, 1968) may also influence platelet retention.
In this study we have demonstrated that the membrane supporting
system influences the retention of platelets, together with red and
white cells, almost certainly by influencing the geometric and
hydrodynamic characteristics of the membrane surface. Cellular
deposits on membranes are seen in regular patterns which correspond
to the coil mesh intersection pattern or to the 'high spots' or ridges

of the support system in parallel flow dialysers (figures 1 - 5). With regard to the supporting meshes of the coils it must be appreciated that while there is no elevation at the points of intersection of the mesh strands (figures 1 and 2) a coil config- uration will cause opposing membrane surfaces to be supported by meshes with strands taking different directions. The spaces between the membrane faces directly over-lying the mesh strands are presumably narrower than the spaces between areas of unsupported membrane due to membrane distortion by positive pressure in the blood compartment. It is likely that alterations in blood flow characteristics at these points cause cellular deposition.

The importance of this cellular deposition is twofold. Firstly it is likely that the platelet adhesion - aggregation reaction is followed by the liberation of platelet factors which may lead to thrombus formation downstream should the local conditions encourage this. This is apparent in the case of the Gambro- Awlall dialyser (figure 7). Thrombus formation, in turn, may lead to excessive blood losses (Lindsay et al, 1973a). Secondly, these cellular aggregates may come off the membrane and form a source of microemboli to the patient. Bischel (1973) has demon- strated that microemboli $> 20$ $\mu$ diameter are present in the blood leaving the dialyser and suggested that these may cause pulmonary microembolism with demonstrable lung function changes. She also demonstrated that filtration of the blood leaving the dialyser by dacron wool or polyester urethane foam filters reduced these pulmonary effects. Examination of these filters after use by scan- ning electron microscopy showed that these microemboli consisted of platelet-leucocyte aggregates. A source of these cellular aggregates has now clearly been demonstrated.

We submit, therefore, that in the design of membrane supporting systems for dialysers consideration should be given to potential biocompatibility as well as to the efficiency of solute and water transport.

## ACKNOWLEDGEMENTS

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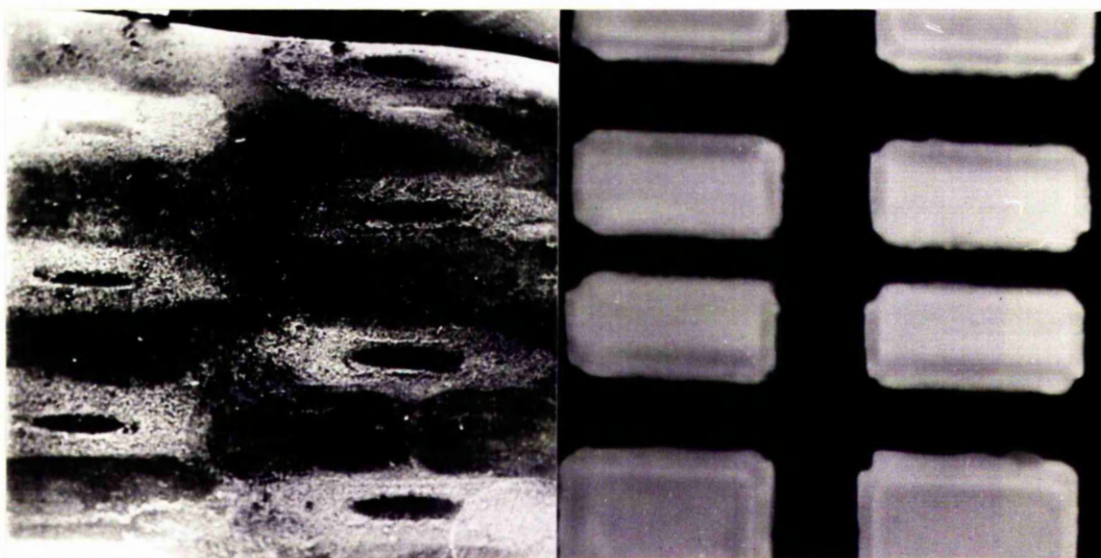    Journal of Colloid and Interface Science, 32, 3 : 456

FIGURE 1
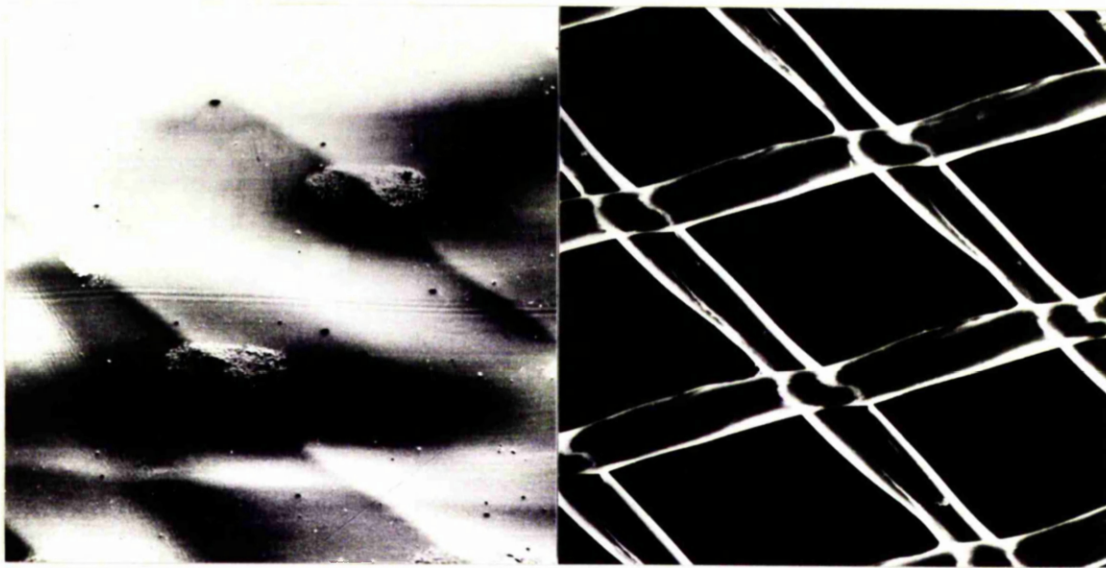


Composition photograph (magnification x 20) showing the cellular deposits on the Extracorporeal EX-03 Cuprophan membrane and also the mesh supporting this membrane
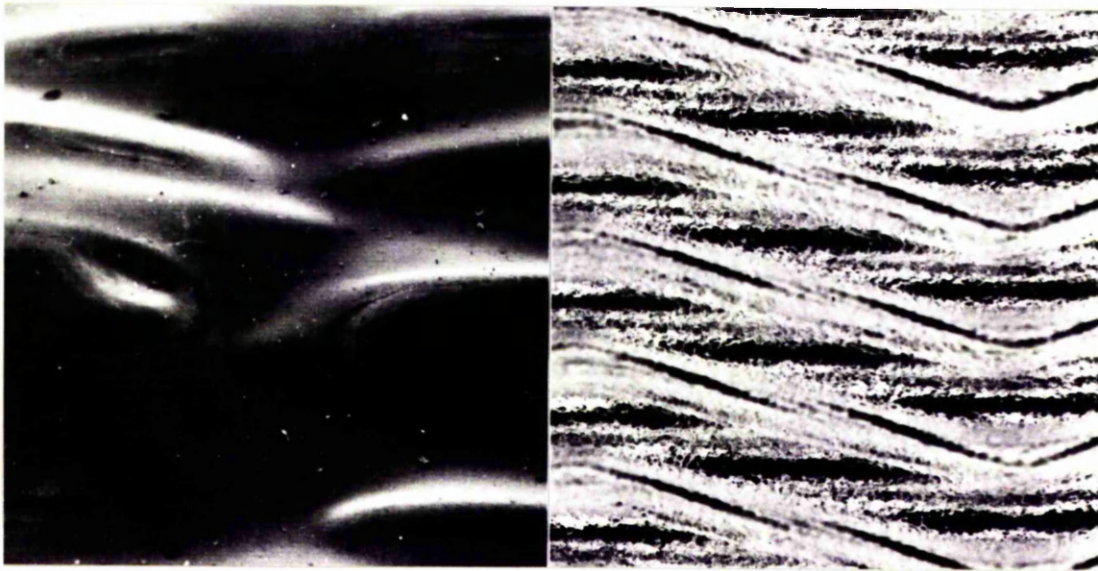
FIGURE 2
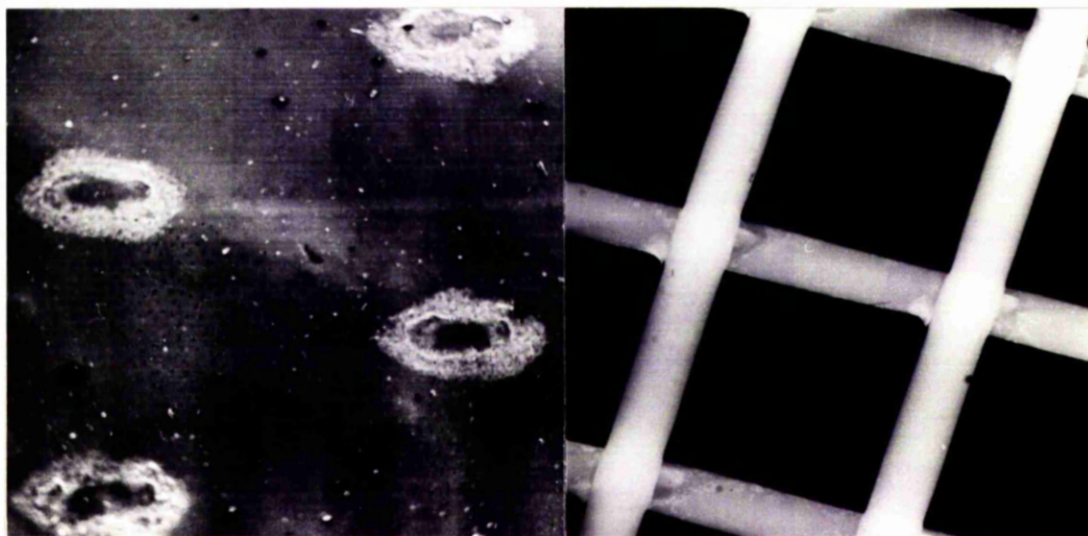


Composition photograph (magnification x 20) showing the cellular deposits on the Travenol Ultra Flo 100 Cuprophan membrane and also the mesh supporting this membrane.

FIGURE 3



Composition photograph (magnification x 20) showing the cellular deposits on the Gambro-Alwall Cuprophan membrane and also the pattern of the moulded polystyrene membrane support system.

FIGURE 4


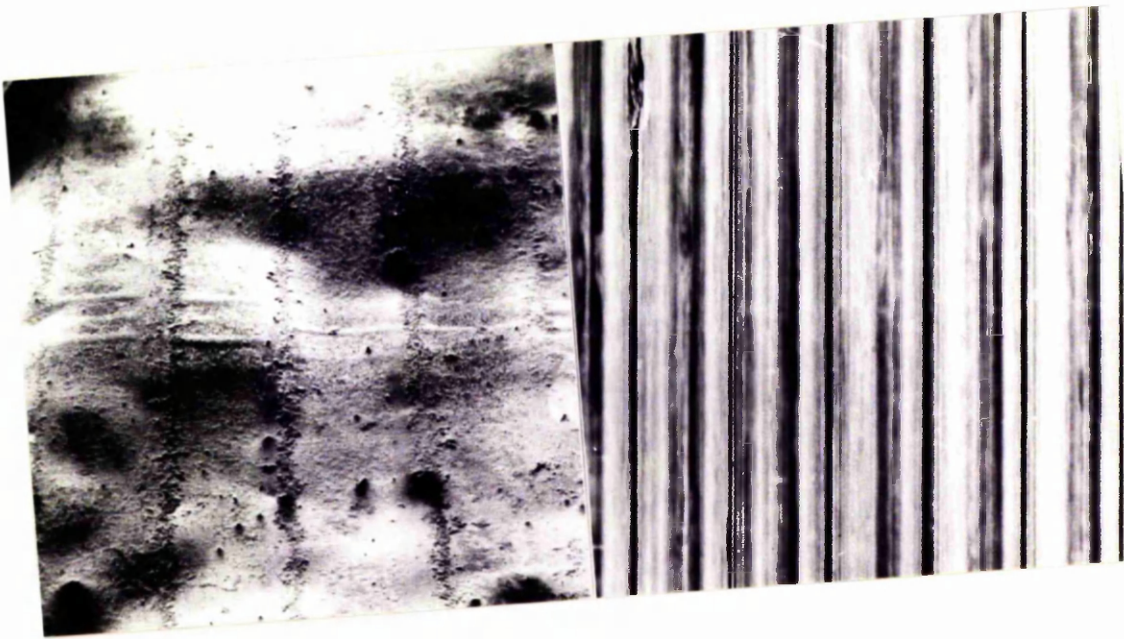
Composition photograph (magnification x 20) showing the cellular deposits on the Gambro-Lundia Cuprophan membrane and also the pattern of the moulded polystyrene membrane support system.

FIGURE 5



Composition photograph (magnification x 20) showing the cellular deposits on the Rhone-Poulenc Cuprophan membrane and also the grooved plastic membrane support system

FIGURE 6



Scanning Electronmicrograph (magnification x 2000) of the cellular deposit on the Cuprophan membranes of the Rhone Poulenc dialyser. An aggregation of platelets is seen with some red blood cells.

FIGURE 7
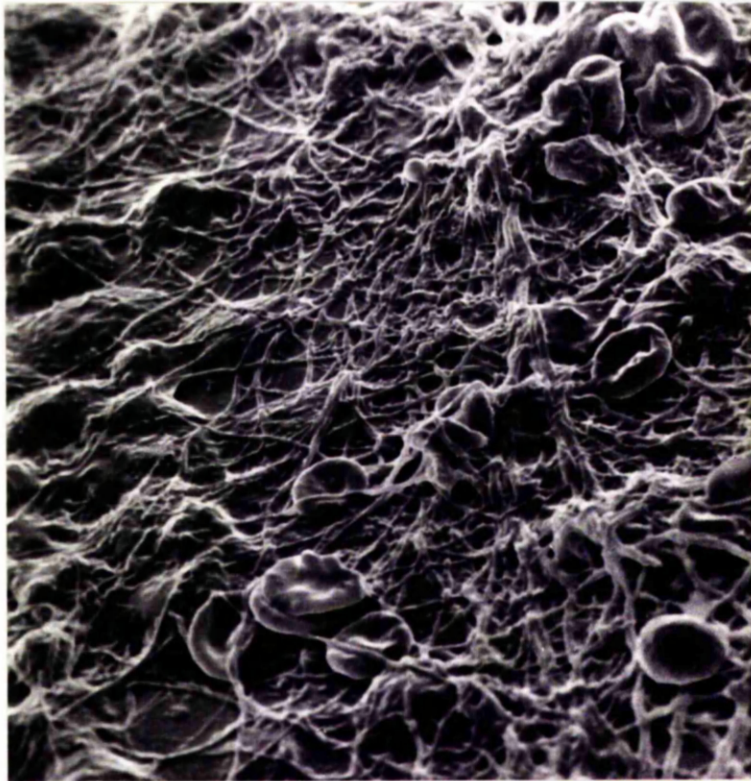


Scanning electronmicrograph (magnification x 1000) of the membrane surface from the venous end of the Gambro-Alwall dialyser. Red blood cells are seen trapped by fibrin strands.

Note :

Although this paper has been published, no reprints
were available at the time of submission.  Thus, the
paper has been retyped.

COMMENT

I have little to add to the Discussion in Paper 8. This study shows that the dialysis membrane surface takes on a configuration during dialysis, because of the presence of a positive pressure across the membranes, related to the underlying membrane supporting structure. Cellular deposition takes place in abundance where there are 'high-spots' or ridges on the membrane. If this effect should enhance the overall degree of platelet retention by the membranes then it is likely to have a bearing on downstream thrombus formation. This statement is, as yet, conjecture. The use of scanning electronmicroscopy, with models simulating 'intravascular' coagulation such as extra corporeal dialysis circuits, may in the future provide valuable information regarding mechanisms of thrombus formation and their prevention.

CHAPTER 7

SUMMARY AND CONCLUSIONS

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A detailed and linking discussion has been given with each
Paper and Chapter of the experimental work. I do not propose
to reiterate this detail but, rather, will enumerate the conclus-
ions that can be drawn from this study. Also I will list the
contributions to knowledge that have been gained and indicate
where further studies may be of value.

CONCLUSIONS

In this thesis I have attempted to stress the importance of
blood loss to the regular dialysis patient and to demonstrate the
sources and the magnitude of such losses ( Table II, page 30).
I have indicated that while some sources of blood loss, e. g.
blood sampling for biochemistry and research projects, can
easily be minimised by the clinician, others e. g. dialyser
blood loss, may be more difficult to minimise. There are two
reasons for blood remaining in a dialyser at the end of a dialysis.

(1)   The mechanical hold-up of anticoagulated blood due to
      the nature of the blood compartment. This source can
      be minimised by the development of the most efficient
      'wash-back' procedure for that particular dialyser.

(2)   The development of thrombus upon the dialysis mem-
      branes. The trapping of red blood cells in this way is
      uninfluenced by either the nature or the volume of the
      'wash-back' used.

The factors responsible for this thrombus formation, which occurs
in spite of adequate heparinisation, have been delineated as follows :-

(1)   Role of Platelets
      The observations made indicate that platelet retention by the
dialysis membrane is an important early step in a reaction which

may proceed to thrombus formation.

(2)   Nature of the dialysis membrane

Different dialysis membranes appear to attract different numbers of platelets and there is a direct relationship between the degree of platelet retention and the degree of thrombus formation during dialysis.  As far as the Cuprophan series of membranes is concerned there appears to be a direct relationship between the thickness of the membrane, its platelet retaining properties, and its thrombogenicity.

(3)   Configuration of the membrane surface

Paper 8 indicates that platelet deposition, together with red and white cell deposition, is likely to be enhanced if the membrane support system causes 'high spots' or ridges. It is suggested that any factor leading to platelet retention by dialysis membranes may, thus, enhance the probability of thrombus formation.

(4)   Other factors which have been discussed are (a)  the role of a low blood linear velocity and (b) the form of anticoagulation used.

In the summary of Chapter 3 (page  29  ) I indicated that both the clinician and the manufacturer should give greater consideration to blood losses.  I am now able to add that the manufacturer must not only produce dialysers with a negligible rupture rate but they should be so designed as to allow an efficient 'wash-back' using a minimum volume of fluid.  Furthermore special consideration should be given to the thrombogenic properties of the membranes and other materials incorporated in the artificial kidney.

During the pre-clinical evaluation of a new dialysis membrane I would recommend that the platelet retaining properties of this membrane be established using a membrane test cell system as this will rapidly give an indication of its potential thrombogenicity.

Furthermore, the manufacturer should consider what effects the membrane support system will have upon the membrane during dialysis. To do this scanning electronmicroscopy of the membranes following dialysis may yield valuable information.

The Gambro-Alwall dialyser has proved to be an ideal model to study. It so happened that this dialyser was constructed to have a low blood linear velocity, a complicated blood compartment manifolding which made 'wash-back' difficult, and contained Cuprophan PT 325 membranes (which were the thickest and most thrombogenic available) supported by a heavy moulded polythene supporting system which indented the membranes; all these features contributed to a high blood loss. I believe that the study of this dialyser has yielded information of importance that will help in the design of future artificial kidneys. Indeed, the lessons learnt from the studies reported in this thesis were contributory to the design of the Gambro-Lundia dialyser which has been fully evaluated (Lindsay et al, 1973) and has been found to be free from the problems of a high blood loss.

This type of study has been in keeping with the recommendations of the Gotch report (1972) on the evaluation of dialysers.

## CONTRIBUTIONS TO KNOWLEDGE

How these studies have contributed, and may continue to contribute, to knowledge can be listed as follows :-

1. With regard to blood loss studies :
   (a)   The technique of blood loss measurement as used in dialyser evaluation were, for the first time, fully evaluated and a relatively simple isotopic technique has been developed.

   (b)   Knowledge of the sources of and, in some cases, the magnitude of the blood losses experienced by the regular dialysis patient has been gained.

   (c)   The blood loss characteristics of some individual dialysers has been defined.

2. With regard to the occurrence of thrombus formation on dialysis membranes :
   (a)   These studies were the first to demonstrate that platelet retention by dialysis membranes is an important early stage in the reaction leading to thrombus formation.

   (b)   These studies were also the first to document the commonly observed post-dialysis fall in platelet count and to suggest the inter-relationships between this, thrombus formation within the extracorporeal circuit of the artificial kidney, and the linear blood velocity through the artificial kidney.

   (c)   A membrane test cell system has been developed which may well be of value in the evaluation of new dialysis membranes with regard to their potential thrombogenicity.

   (d)   Paper 7 is the only published study to have demonstrated the relationship between the thickness of Cuprophan membranes and their thrombogenicity.

3.  With regard to the general study of thrombus formation :

    (a)      A test cell system has been devised which,
             in some respects, is more sensitive than a
             glass bead column technique in the measurement
             of platelet adhesiveness. This system may be of
             value in future studies not only of platelet
             function but also of potential anti-platelet agents.

    (b)      The extra-corporeal circuit of the Gambro-Alwall
             dialyser provides a model for the study of intra-
             vascular thrombosis and its therapeutic inhibition.
             Paper 5 reports a study which demonstrates the
             reduction of thrombus formation by anti-platelet
             agents suggesting that such agents may have a place
             in the prevention of clinical thrombosis. It may be
             that such an extra-corporeal circuit will be of
             value in the study of new agents with an anti-
             thrombotic effect.

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APPENDIX


A DESCRIPTION OF HAEMODIALYSERS

AND THEIR MEMBRANES

A brief and very general description of haemodialysers and
their membranes is given here for the reader who may be un-
familiar with clinical dialysis.

## COIL HAEMODIALYSERS

A coil haemodialyser consists of a tubular membrane and a
flexible support wrapped around a rigid cylindrical core. The
coil membrane supports are constructed of woven screens or
unwoven lattices of plastic. Design simplicity, low supporting
material cost, and feasible methods for joining dry cellulose
membranes to blood conduits resulted in the coil designs becom-
ing the first prefabricated haemodialysers.

Examples of coil haemodialysers are the Travenol Ultra
Flow 100 (fig. 4); the Avon R 70 and the Extracorporeal EX 03
(fig. 5).

Overall they are usually not bigger than 20 cm. in height
and 15 cm. in diameter and contain tubular dialysis membranes
providing dialysis surface areas of approximately 0.7 sq. m. to
1.5 sq. m. depending on the make. To be used they are placed
in some form of machine which delivers dialysate ( a suitable
prepared electrolyte solution) to the coil and washes this over
the membranes. In fig.6 an Extracorporeal EX 03 coil is seen,
in situ, in a Travenol R.S.P. (Recirculation Single Pass)
machine.

## PARALLEL FLOW DIALYSERS

The parallel flow haemodialysers o flat plate dialysers are
constructed of two dialysing membranes sandwiched between two
rigid or semirigid supporting surfaces. The blood passes between
the two dialysing membranes with dialysate delivered from a
machine, flowing on the outside of the membrane in a counter-
current or crosswise direction. These modules can be stacked

in multiple layers to make a dialyser with variable dialysing surface area. Most parallel flow dialysers used for regular dialysis treatment have surface areas of about 1 sq. m. The most commonly used parallel flow dialyser is the Kiil which is composed of two layers, and is made of three rigid polypropylene boards with dialysing membranes laid between them. It is cumbersome, the outside measurements being approximately 110 x 33 x 12 cm. and it weighs about 30 Kg. A Kiil dialyser is shown in fig. 7. In recent years several presterilised disposable parallel flow dialysers have been available, these include the Rhone Poulenc (fig. 8) the Gambro-Alwall (fig.9) and the Gambro-Lundia (fig. 10). These are much smaller and more convenient to handle than the Kiil, the external dimensions of the Gambro-Lundia being 67 x 8 x 9 cm. and it's weights a mere 2. 8 Kg. A Gambro-Lundia dialyser, prepared for dialysis, is seen attached to a Travenol R. S. P. Machine (which will deliver dialysate to the haemodialyser) in fig. 11.

## HOLLOW FIBRE DIALYSIS

The hollow fibre or capillary artificial kidney is of novel concept and design and is the result of nearly 10 years research and development. In this dialyser regenerated cellulose triacetate capillaries are extruded and further treated. These capillaries have an inside diameter of approximately 200 $\mu$ m. and a wall thickness of 25 $\mu$m. Some 10, 000 such capillaries are jacketed in a plastic cylinder some 18 cm. in length and 6 cm. in diameter and weighing less than 1 Kg. Blood flows through the capillaries, which act as dialysing membranes, while their external surfaces are bathed in dialysate flowing within the plastic jacket. The overall dialysis membrane surface area is just over 1 sq. m. A Cordis Dow hollow fibre kidney is shown in fig 12.

The comparitive sizes of the various types of dialysers are shown in fig. 13.

## DIALYSIS MEMBRANES

Finally, mention must be made of the conventional dialysis membranes which are inserted in sheet or tubular form within dialysers. The membranes most often used are cellophane (cellulose regenerated by the viscose process) or Bemberg Cuprophan (cellulose regenerated by the cupric ammonium process). The cellophane membranes have an average dry wall thickness of about 20 - 30 $\mu$m while the Cuprophan membranes range from 13 - 25 $\mu$m. The Bemberg series of membranes are catelogued by a prefix PT followed by a number: PT 150 being the thinnest membrane and PT 325 the thickest (see Paper 7).

FIGURE 4.
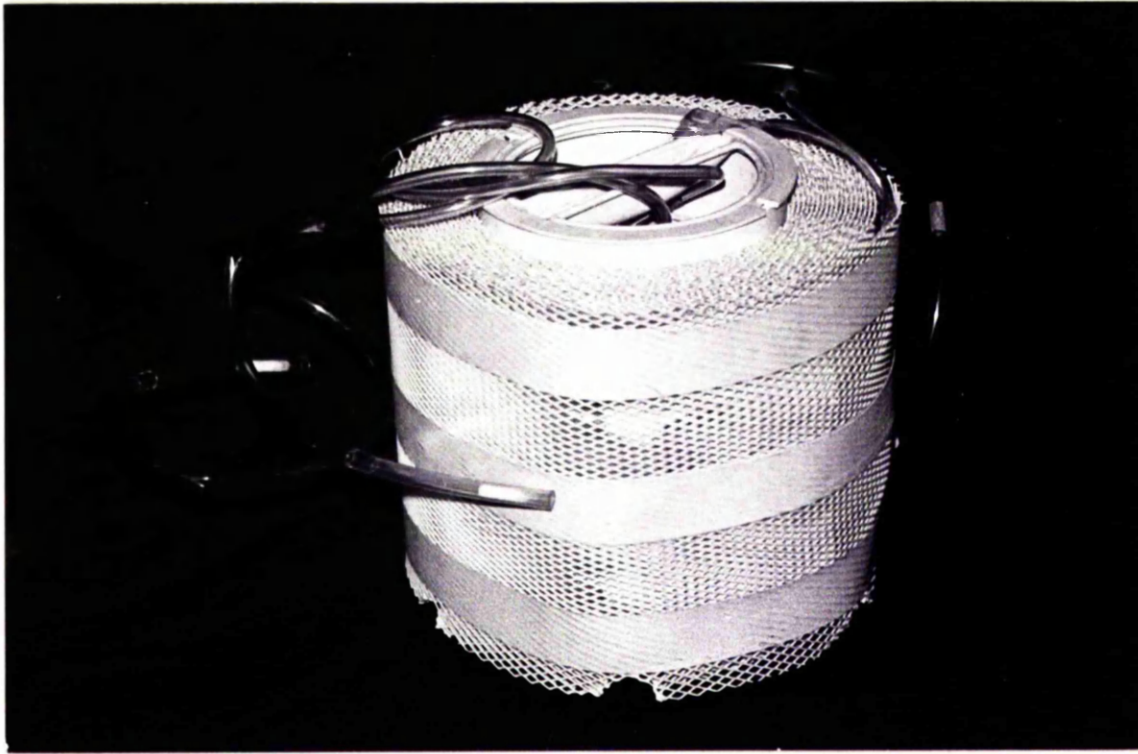


The Travenol Ultra-Flo 100 Coil. The coil is obtained presterilised
and it is disposable.

FIGURE 5.
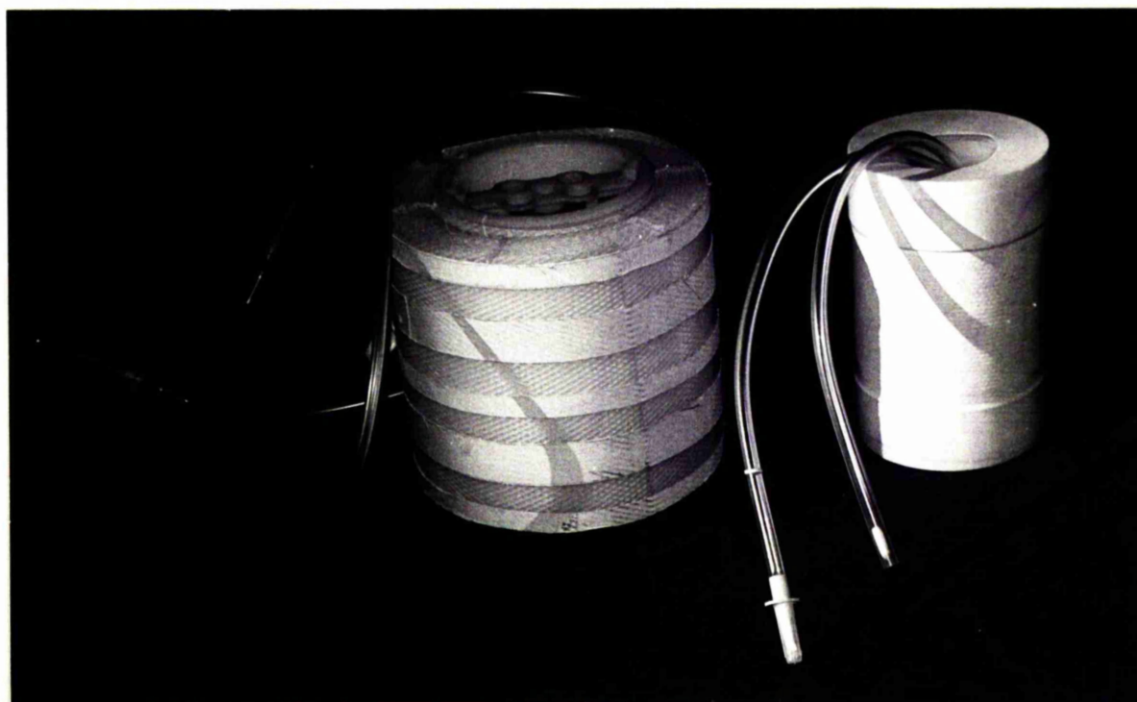


The R70 (left) and Extracorporeal EX 03 (right) Coils. The
coils are presterilised and disposable.

FIGURE 6.



An Extracorporeal EX 03 Coil is in situ. in a Travenol R.S.P. machine

FIGURE 7.



A Kiil parallel flow dialyser.

FIGURE 8.
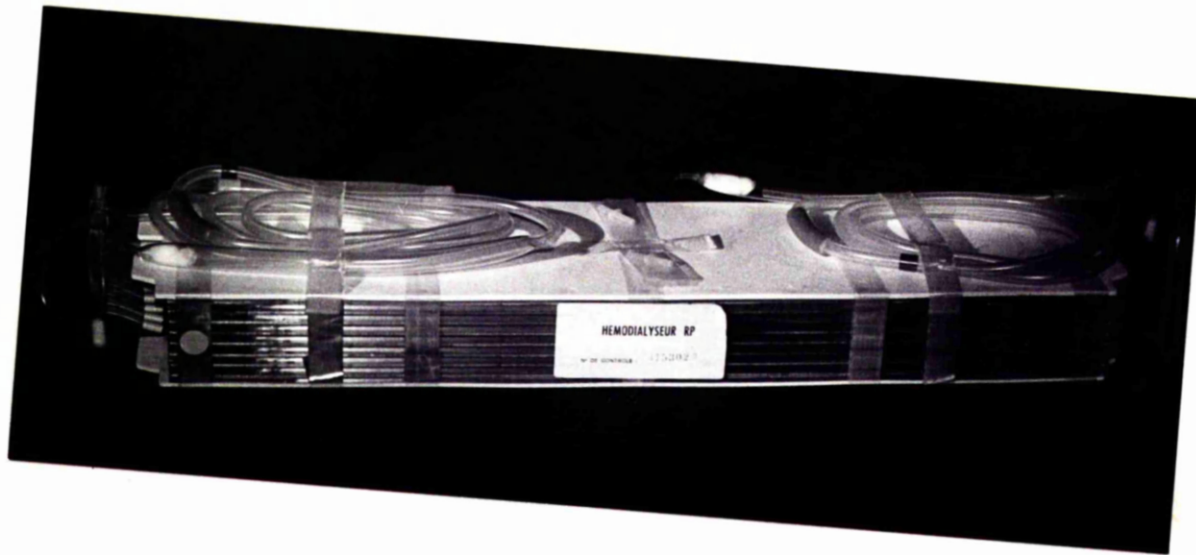


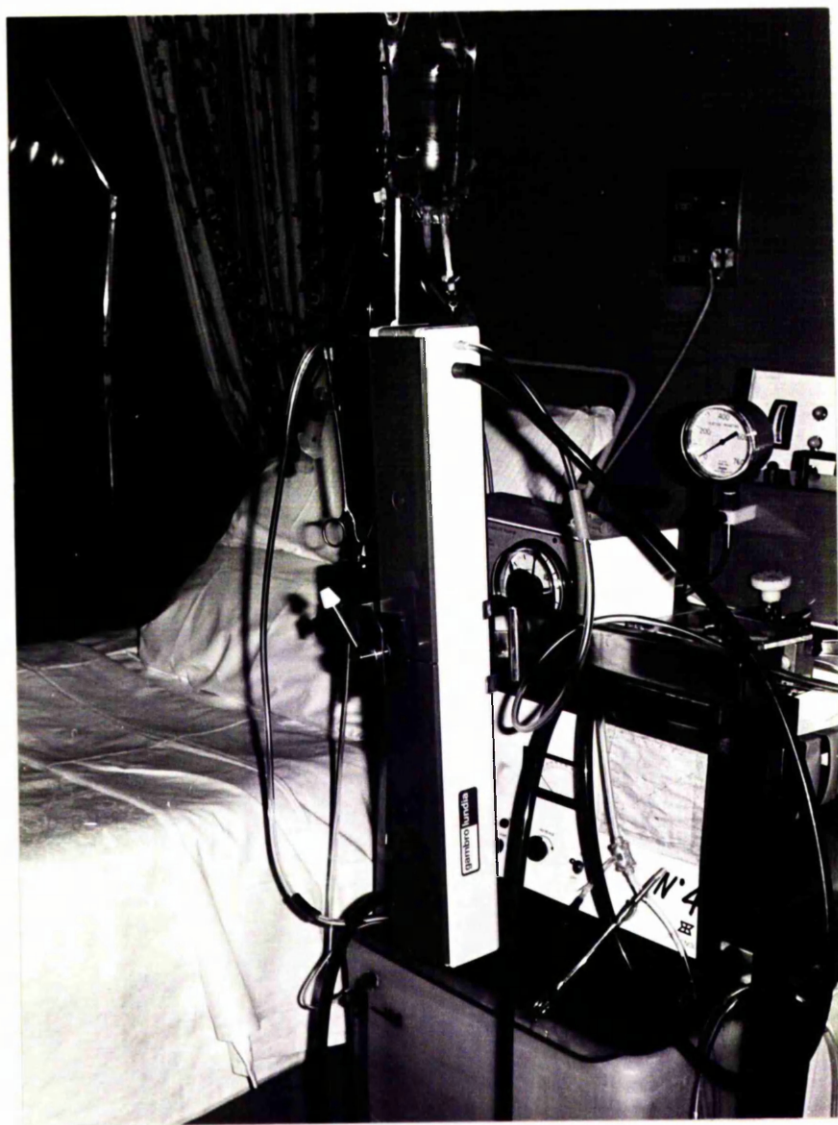The Rhone-Poulenc presterilised disposable parallel flow dialyser.

FIGURE 9
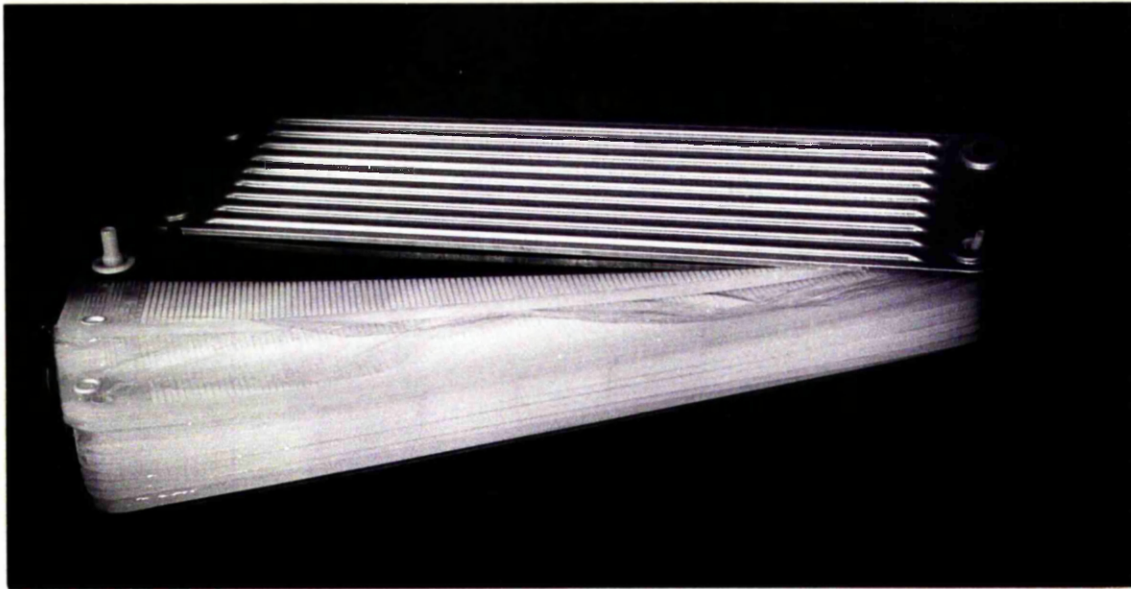


The Gambro-Alwall presterilised disposable dialyser. The dialyser
is opened up to show the multi-layered parallel flow arrangement.

FIGURE 10
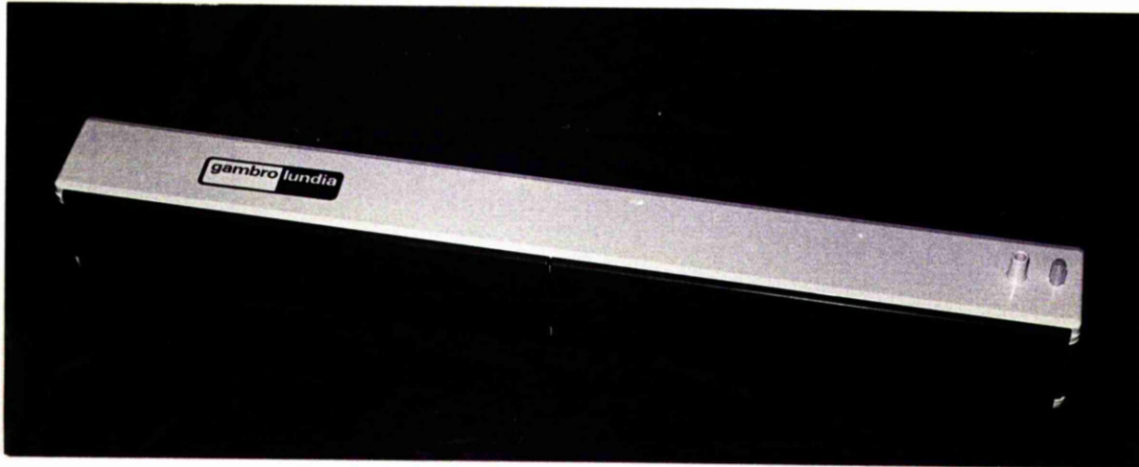


The Gambro-Lundia presterilised disposable parallel flow dialyser.

FIGURE 11



The Gambro-Lundia dialyser ready for use. Dialysate is obtained
from a Travenol R.S.P. machine.

FIGURE   12
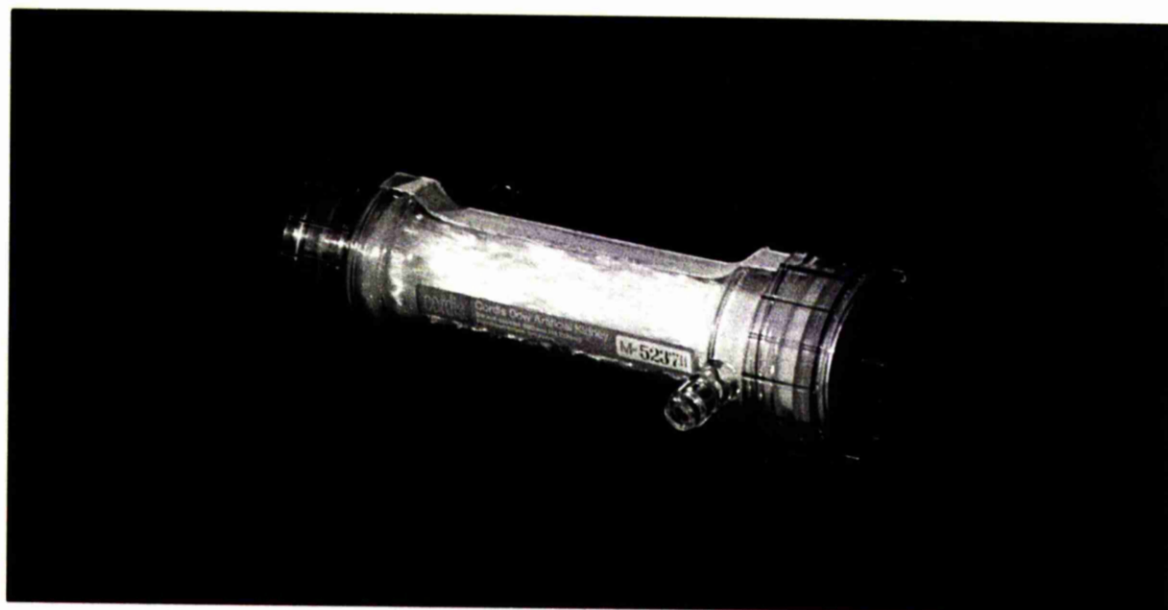


The Cordis Dow Hollow Fibre Kidney

FIGURE 13.
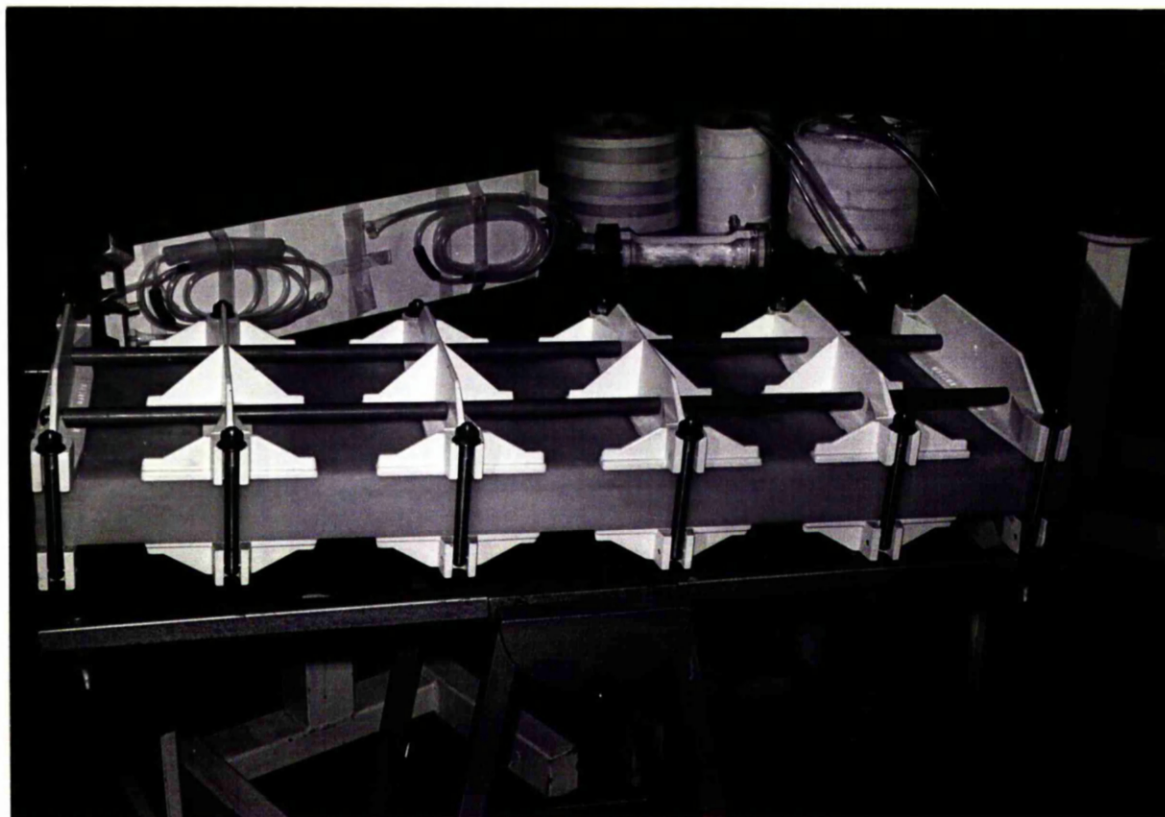


The comparitive size of the Kiil dialyser with the Rhone-Poulenc
the Cordis Dow, and the Avon R70, Extracorporeal EX 03 and
Travenol Ultra-Flo 100 coils is shown.

# BLOOD LOSS & THROMBUS FORMATION DURING HAEMODIALYSIS

BY

## ROBERT McGREGOR LINDSAY, M.B., Ch.B.

## SUMMARY

The objectives of this thesis are to emphasise the importance of extraneous blood loss to the patient receiving maintenance haemodialysis; to demonstrate the source and magnitude of this blood loss; to define the blood loss caused by some commonly used haemodialysers; and to explore the reasons why blood should remain trapped within them.

The anaemia of chronic renal failure is seldom corrected by regular dialysis treatment and may be augmented and complicated by the development of folic acid deficiency, iron deficiency, and considerable blood losses. This anaemia is usually associated with a normochromic, normocytic blood film and is basically due to a decreased rate of erythropoiesis associated with the incomplete correction of the uraemic syndrome afforded by haemodialysis. Androgen and cobalt therapy have been advocated as a means of increasing the rate of erythropoiesis but results have not been universally satisfactory. Folic acid deficiency is easily dealt with by oral supplements but the achievement of proper iron balance on regular dialysis is a complex problem as the patients tend to have a decreased ability both to absorb oral iron and to incorporate iron into erythrocytes. Furthermore, iron losses attributable to volumes of blood lost during haemodialysis, are high. In previous years haemoglobin levels were maintained by repeated blood transfusions but this policy is no longer acceptable because of the risks of hepatitis. Considering

these factors it is obvious that attention must be paid to
sources of blood loss.

The sources of blood loss during haemodialysis
may be enumerated as follows:   blood loss from the
arteriovenous shunt or fistula during connection and
disconnection of the patient to the dialyser;  blood samples
taken for investigations;  blood loss following dialyser
rupture;  the residual blood volume in the dialyser and
its blood lines after use;  and other co-incidental losses
such as menorrhagia.  It soon became apparent to the
author that much was unknown about the degree and relative
importance of these various potential sources of blood loss.
An early necessity was to establish a method for measuring
the dialyser blood loss.  This was developed and found to be
highly accurate.  Using this method the magnitude of the blood
losses from various commercially available dialysers and
their blood lines was estimated as were the volumes of blood
lost during routine use of the arteriovenous shunt and
fistula.  This data made it possible to consider the probable
volumes of blood that a patient undergoing thrice weekly
haemodialysis would lose per annum.  The volumes ranged
from 3 to 8 litres of blood depending upon the type of
dialyser used.

Special consideration was, therefore, given
to the reasons why blood would remain trapped in a
dialyser and not be returned to the patient.  Two factors
were found;  firstly, the mechanical hold-up of anticoagulated
blood due to the design of the blood compartment and secondly,
the development of thrombus upon the dialysis membranes.
The first source can be minimised by the development of
efficient 'wash-back' procedures but the trapping of red blood
cells by thrombus is uninfluenced by either the nature or the
volume of the 'wash-back' used.  Thrombus formation upon
dialysis membranes is the factor which differentiates dialysers
with high blood losses from those with acceptable losses.

The factors responsible for this thrombus
formation, which occurs in spite of adequate heparinisation,
have been delineated. A study of the changes in haemostatic
factors occurring in the blood of patients underoing
haemodialysis suggested that platelet retention by dialysis
membranes is an important early step in a reaction which
may proceed to thrombus formation. This hypothesis is
supported by a subsequent investigation in which it was
shown that the inhibition of platelet function by aspirin
and a dipyridamole compound was associated with a
reduction in the numbers of platelets retained by dialysis
membranes and in the amount of thrombus formed on the
membranes.

The nature of the platelet-dialysis membrane
interaction has been partly studied. Using a new membrane
test cell system it was concluded that the platelet has an
'intrinsic' ability to adhere to cellulosed based dialysis
membranes. This property is dependent upon the presence
of adenosine diphosphate, possibly released from the platelets
themselves and from red blood cells, and upon divalent
cations. The membrane test cell system may have an
additional role in that it was considered to be more sensitive
than a glass bead column technique as a test for platelet
adhesiveness. The nature of the dialysis membrane itself
will influence the interaction for different dialysis membranes
appear to attract different numbers of platelets. As far as
the Cuprophan (J.P. Bemberg, Wuppertal, West Germany)
series of membranes is concerned it was found that there was
a direct relationship between the thickness of the membrane,
its platelet retaining properties, and its thrombogenicity.
Finally, a study of the role of the configuration of the membrane
surface on thrombus formation indicated that platelet deposition
was enhanced if the membrane support system causes 'high
spots' or ridges and thus might increase the probability of
thrombus formation.

The information obtained from this study enables certain recommendations to be made to both the clinician and to the manufacturer regarding the minimisation of blood loss during haemodialysis. The clinician should limit his investigations; take scrupulous care with the use of shunts and fistulae; carry out thorough pre-dialysis pressure testing to eliminate ruptures during dialysis; and develop the optimum wash-back technique for the dialyster he is using. The manufacturer must design the manifolding of the dialyser so as to allow an efficient wash-back with the minimum volume of fluid and, in the case of disposable dialysers, they should have the lowest possible burst rate. Furthermore, he must give special consideration to the thrombogenic properties of the membranes and other materials incorporated in the artificial kidney.

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