BLOOD-MEMBRANE INTERACTION AND TREATMENT OF HAEMODIALYSIS PATIENTS.
A STUDY OF VARIOUS FACTORS.

Lennart Lundberg
Umeå 1994
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ABSTRACT

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Blood-membrane contact during haemodialysis activates the alternative pathway of the complement system and may induce acute and chronic side-effects. This study was performed to elucidate the blood-membrane interaction due to both the membrane material and other factors.

Twenty-two patients were dialysed with either a cellulose acetate or a Hemophan membrane. Ten of the patients were dialysed against bicarbonate and twelve against acetate as dialysate. The data favoured the Hemophan membrane because the reduction in leukocytes and the increase in granulocyte elastase were greater during the use of cellulose acetate. In the bicarbonate group, the increment in C3d and fall in leukocytes were greater than in the acetate group, indicating favourable effects of acetate in this regard.

The interaction when whole blood was filtered through 4 different leukocyte-removal filters was studied. C3d did not increase, but haemolysis occurred due to a polyester filter and platelet activation occurred due to a filter made of cellulose acetate. This indicated that the membrane material, the filter composition and design could influence the blood components in different ways.

The influence on C3d was studied in two plate dialysers, one made of cuprophan and the other made of polycarbonate. Two hollow-fibre dialysers of cuprophan (one steam- and the other ethylene oxide [ETO] -sterilized) were also studied. The plate cuprophan dialyser increased C3d more than the other membranes. Two dialysate groups were compared: in the bicarbonate group (n=7), C3d increased more by the ETO-sterilized hollow-fibre dialyser and the polycarbonate plate dialyser than in the acetate group (n=5). In the bicarbonate group the steam-sterilized hollow-fibre cuprophan dialyser increased C3d less than the ETO-sterilized and less than the cuprophan plate.

Signs of backdiffusion were obtained by reduction in plasma protein and haematocrit in eight patients dialysed with two hollow-fibre dialysers when the ultrafiltration was "set at zero" and bicarbonate was used as dialysate. C3d increased significantly more at 15 min in the bicarbonate group as compared with 8 other patients dialysed with acetate and a transmembrane pressure (TMP) of 100 mmHg. This indicates that the bicarbonate dialysate or the backdiffusion per se may stimulate complement activation during blood-membrane interaction.

A study in 8 haemodialysis patients showed that a low initial TMP of 50 mmHg during the first 60 min of bicarbonate haemodialysis induced less complement activation measured as C3d, while increased TMP seemed to potentiate the differences between the membranes.

To conclude, complement activation is not only influenced by the reactivity of the membrane material but also by the dialysate composition, the membrane configuration and the sterilization method. These data favour steam sterilization, a low TMP of 50 mmHg during the early phase of dialysis and a hollow-fibre configuration.

KEY WORDS: Haemodialysis, cellulose acetate, Hemophan®, cuprophan, complement activation, C3d, granulocyte elastase, ß-thromboglobulin, backdiffusion, leukocyte-removal filter, dialysate, acetate, bicarbonate, hollow-fibre dialyser, plate dialyser, transmembrane pressure
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABBREVIATIONS</td>
<td>4</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>5</td>
</tr>
<tr>
<td>ORIGINAL PAPERS</td>
<td>6</td>
</tr>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td>7</td>
</tr>
<tr>
<td>Biocompatibility</td>
<td>8</td>
</tr>
<tr>
<td>Membrane factors and the complement system</td>
<td>8</td>
</tr>
<tr>
<td>Complement split product C3d</td>
<td>11</td>
</tr>
<tr>
<td>Activated blood cells</td>
<td>13</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>14</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>15</td>
</tr>
<tr>
<td>Platelets</td>
<td>15</td>
</tr>
<tr>
<td>Monocytes-macrophages</td>
<td>16</td>
</tr>
<tr>
<td>Factors that affect haemodialysis</td>
<td>17</td>
</tr>
<tr>
<td>Membrane configuration</td>
<td>17</td>
</tr>
<tr>
<td>Protein adsorption</td>
<td>18</td>
</tr>
<tr>
<td>Sterilization methods</td>
<td>19</td>
</tr>
<tr>
<td>Backdiffusion</td>
<td>20</td>
</tr>
<tr>
<td>Dialysate</td>
<td>21</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>21</td>
</tr>
<tr>
<td>Clinical aspects of biocompatibility</td>
<td>22</td>
</tr>
<tr>
<td>Catabolism</td>
<td>23</td>
</tr>
<tr>
<td>Dialysis-related amyloidosis</td>
<td>23</td>
</tr>
<tr>
<td>Infections</td>
<td>24</td>
</tr>
<tr>
<td><strong>AIMS OF THE STUDY</strong></td>
<td>25</td>
</tr>
<tr>
<td><strong>SUBJECTS AND METHODS</strong></td>
<td>26</td>
</tr>
<tr>
<td>Papers I-V</td>
<td>32</td>
</tr>
<tr>
<td>Statistics</td>
<td>32</td>
</tr>
<tr>
<td><strong>RESULTS</strong></td>
<td>34</td>
</tr>
<tr>
<td><strong>DISCUSSION</strong></td>
<td>40</td>
</tr>
<tr>
<td><strong>GENERAL CONCLUSIONS</strong></td>
<td>46</td>
</tr>
<tr>
<td><strong>ACKNOWLEDGEMENTS</strong></td>
<td>47</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>49</td>
</tr>
<tr>
<td>PAPERS I-V</td>
<td>3</td>
</tr>
</tbody>
</table>
ABBREVIATIONS

C3d  Split product of activated C3 complement
C3a, C5a  Anaphylatoxins (complement factors)
ß2-M  ß2-microglobulin
E- α1-Pl  Granulocyte elastase α1-protein inhibitor complex
ELISA  Enzyme-linked immunosorbent assay
EDTA  Ethylenediaminetetraacetic acid
RIA  Radioimmuno assay
HLA  Human lymphocyte antigen
CPD  Citrate-phosphate-dextrose
ß-TG  ß-thromboglobulin
CA  Cellulose acetate
UF  Ultrafiltration
TMP  Transmembrane pressure
ETO  Ethylene oxide
Hf-CuE  Hollow fibre dialyser with cuprophan membranes (ETO)
Hf-CuS  Hollow fibre dialyser with cuprophan membranes (steam)
P-Cu  Plate dialyser with cuprophan membranes (ETO)
P-Pc  Plate dialyser with polycarbonate membranes (ETO)
LAL-RM  Limulus-amebocyte-lysate-reactive material
IU  International Unit
PAF  Platelet activating factor
ABSTRACT

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This thesis is based on the following publications referred to in the text by their Roman numerals:

**ORIGINAL PAPERS**


III. Lundberg L, Johansson G, Karlsson L, Stegmayr BG. Complement activation is influenced by the membrane material, design of the dialyser, sterilizing method and type of dialysate. Nephrol Dial Transplant 1994, in press.


V. Lundberg L, Johansson G, Stegmayr BG. Complement activation may be reduced by a low initial transmembrane pressure during hemodialysis. Submitted for publication.
INTRODUCTION

The first haemodialysis to remove uremic toxins in a human with renal failure was performed in 1924 [Haas, 1925]. That dialysis was short and not successful due to lack of a non-toxic anticoagulant [Haas, 1928]. Besides removal of toxins, haemodialysis eliminates retained fluid (ultrafiltration) and corrects the hydrogen buffer capacity. In Sweden, the first dialyser with controllable ultrafiltration was developed by Alwall et al. [Alwall et al. 1949]. The principle for ultrafiltration was controlled by the application of a negative hydrostatic pressure. With time the technique has developed so as to use smaller blood volumes and yet have increased efficiency and improved safety control of the treatment.

During chronic haemodialysis the blood access usually comes from an arteriovenous fistula, first developed by Brescia et al. in 1966 [Brescia et al. 1966], or from a central venous dialysis catheter. The blood is pumped from the access at a rate of about 200-300 ml/min through plastic tubes, into the dialyser (the artificial kidney) and then returns back to the patient during a treatment procedure which lasts about 3-4 hours. To reduce clotting within the dialyser, heparin infusion after a bolus injection is frequently used. Low-molecular weight heparin has also been increasingly adopted for this purpose. Within the dialyser, a diffusion of various toxins and substances takes place, mainly from the blood, across the membrane, and into the dialysis fluid. The dialysis fluid passes through the dialyser in the opposite direction to the blood at a rate of about 500 ml/min. The dialysate is prepared by mixing of a concentrated solution and water within the dialysis machine. The water is usually purified by reversed osmosis. Uremic patients tend to develop metabolic acidosis and this is corrected by using a dialysis fluid with an excess of bicarbonate, a buffer source of bicarbonate, or acetate to restore the buffer capacity of the body fluids.

The numbers of patients and dialysis treatments have increased during the last decades due to economic and technical development. The treatment is now more tolerable and efficient for the patients, and serious adverse side-effects are extensively reduced. The necessary amount of haemodialysis has become easier to estimate due to kinetic modeling. An individualized therapy is possible by the use of various membranes with different properties such as high- or low-flux with altered permeability for certain sized molecules. Different profiles of ultrafiltration and modified profiles of sodium and/or bicarbonate are new aspects used to optimize the treatment. Further improvements have been made by the use of solutions as in haemo(dia)filtration, altered dialysate compositions of ions such as calcium, the use of intravenous vitamin D₃, in addition to erythropoietin, and intravenous iron. The complexity of haemodialysis
treatment today demands an increasing knowledge and control in order to individualize the therapy for each patient.

**Biocompatibility**

During evolution several different defense mechanisms against "foreign" substances and materials have developed. These mechanisms occur when blood comes in contact with "artificial" surfaces. Initially there was little concern about what occurred during haemodialysis as long as the patients survived. A dialysis-induced neutropenia was shown in 1968 by Kaplow & Goffinet [Kaplow & Goffinet 1968]. This was later explained by a complement-dependent interaction between blood and the membrane; the interaction caused a leukopenia and pulmonary leukostasis which were followed by secondary hypoxia [Craddock et al. 1977a, 1977b]. The amount of activated complement (anaphylatoxins C3a or C5a) which affects the patients is influenced by factors such as the membrane material, the transport of anaphylatoxins into the dialysate, and the adsorption onto the membrane. Other factors that determine levels of C3a and C5a are the generation rate and clearance by the individual patient, and the rate and extent of binding of these bioactive molecules to specific receptors on the granulocytes [Cheung et al. 1986a].

This knowledge about blood-membrane interaction is increasing and is now referred to as biocompatibility [Henderson & Chenoweth 1987; Basile & Drüeke 1989; Vanholder & Ringoir 1989; Cheung, 1990] and is defined by Hakim as the sum of specific interactions between blood and the artificial materials of the haemodialysis circuit [Hakim, 1993]. These interactions may cause side-effects with both acute and chronic consequences which may reduce the patients' quality of life. Such interactions are always more or less present and depend upon the type of reactions and their intensity. The aim is to minimize these events and thereby reduce the amount of side-effects which are often seen in chronic renal replacement therapy.

**Membrane factors and the complement system**

The activated complement system induces a number of events such as leukocyte and platelet aggregation and release of constituents due to activation of platelets, neutrophils, basophils and monocytes. These events contribute both to the acute and the chronic side-effects seen in haemodialysis patients. The contact between blood and biomaterials triggers the complement cascade [Chenoweth, 1988], via the alternative pathway. The cascade is initiated by
adsorption of C3, and in particular C3b, to hydroxyls and other nucleophilic groups on the membrane. Subsequent binding of factors B and D generates C3 convertase activity and increases further deposition of C3b to the surface [Perrin et al. 1975; Cheung et al. 1989]. Activation of C3 also leads to the formation of C5 convertase. Further on, the released anaphylatoxins, C3a and C5a, are rapidly converted by serum carboxypeptidase [Chenoweth, 1986] to the stable peptides, C3a(desArg) and C5a(desArg) [Basile & Drüeke 1989]. C3a/C3a(desArg) and C5a/C5a(desArg) first bind to receptors on granulocytes and monocytes, and the peptides are then internalized and degraded. Membrane-associated C3b is degraded into iC3b by factor I; iC3b is further cleaved into equal molar amounts of the inactive forms, C3dg and C3c, and the former is further degraded into C3d and C3g. C3d is washed away from the dialyser during clinical dialysis [Cheung et al. 1989].

The formation of the bioactive peptides, C5a, C3a, and C5a(desArg), induces pulmonary artery hypertension [Craddock et al. 1977a; Cheung et al. 1986b] and a reduction in leukocytes [Cheung et al. 1989]. These bioactive peptides bind to neutrophil receptors and cause release of granulocyte proteases [Cheung et al. 1991]. This may induce pulmonary vascular changes, early during the first 10-20 min of routine haemodialysis [Henderson & Chenoweth 1987] together with pulmonary artery hypertension [Craddock et al. 1977a]. C5a is also a potent promotor of smooth muscle contraction, causes increased vascular permeability, and induces production of interleukin-1.

Complement activation via the alternative pathway may be stimulated by the binding of factors B or D (promotors) or inhibited by endogenous inhibitory factors [Hakim et al. 1984]. Factor H binds to C3b and thereby inhibits further binding of factor B, but factor H also acts as a cofactor in the enzymatic degradation of C3b to iC3b together with factor I [Cheung et al. 1989]. This net effect determines the degree of C3 conversion. The haemolytic factor D, a serine protease, is filtered through the glomerulus and catabolized by tubular cells in patients with normal renal function. The excess in factor D, as seen in haemodialysis patients due to impaired renal clearance, upregulates the alternative pathway of the complement system [Miyata et al. 1992].

Cellulose membranes activate the alternative pathway of the complement system (Fig. 1) more than synthetic membranes do because of the greater amount of reactive surface hydroxyl groups [Johnson et al. 1990]. The cuprophan membrane is made of repetitive polysaccharide units and thereby mimics the lipopolysaccharide structure of the bacterial wall [Basile & Drüeke 1989]. However, cuprophan membranes are frequently used due to their reliability, comparatively low costs, diffusive and convective properties. The cuprophan membrane binds C3b, C3b is degraded into iC3b, and iC3b acts as a specific receptor [Cheung et al. 1991], with which leukocytes adhere to the
membrane surface. Cuprophan causes complement activation more than does a modified cellulose membrane such as cellulose acetate or Hemophan [Henderson & Chenoweth 1987; Woffindin et al. 1992].

The modification of surface hydroxyl groups on regenerated cellulose reduces C3 and C5 convertase activity, probably by favouring factor H and thereby limiting complement activation [Johnson et al. 1990]. However, there is not a linear correlation between number of replaced hydroxyl groups and the

Figure 1. A simplified schematic diagram of the alternative and classical pathways of the complement system.

The modification of surface hydroxyl groups on regenerated cellulose reduces C3 and C5 convertase activity, probably by favouring factor H and thereby limiting complement activation [Johnson et al. 1990]. However, there is not a linear correlation between number of replaced hydroxyl groups and the
membrane reactivity [Woffindin et al. 1992], indicating that not all hydroxyl groups behave in a similar way. In the Hemophan membrane the substitution of hydroxyl groups with diethyl aminoethyl (DHAE) is less than 1% as compared with cellulose acetate where more than 80% of the reactive sites are replaced with acetate groups [Woffindin et al. 1992]. This is supposed to make Hemophan more biocompatible than cellulose acetate [Pengloan et al. 1991; Paper I] because it generates less complement activation and less reduction in leukocytes and platelets as compared with cuprophan or cellulose acetate. This beneficial effect is supposed to be due to the reduction in hydroxyl groups on the cellulose membrane [Bosch et al. 1986, 1987].

The reactivity of the membrane is not only influenced by the number of reactive groups and the membrane area but also by the surface potential. The surface potential influences the adsorption capacity on the surface [Cheung, 1990], as seen in the hydrophobic membranes, i.e., polyacrylonitrile (PAN) and polysulfone [Hakim, 1993]. Adsorption of the haemolytic factor D in PAN dialysers [Pascual & Schifferli 1993] or binding of factor H, in contrast to binding of factor B [Cheung, 1990; Cheung, 1993], have inhibitory effects on the alternative complement pathway.

C5a is a more potent anaphylatoxin than C3a. The rapid and irreversible binding of C5a desArg to specific granulocyte receptors and monocytes and the short C5a half-life (T1/2) of less than 2 min in the circulation [Hakim et al. 1984] makes measurement of C5a difficult. Such problems may explain why Woffindin et al. found only an increase in C5a during bicarbonate dialysis with cuprophan, by sampling from the venous side. No changes in C5a were found by other cellulose-based membranes [Woffindin et al. 1992]. However, another study has shown significant increases in venous C5a in more biocompatible membranes such as polycarbonate and polymethylmethacrylate but no changes in arterial C5a [Martin-Malo et al. 1991] using acetate as dialysate. Knudsen found an increase in arterial C5a/C5a desArg only for cuprophan while the venous samples showed an elevation for all membranes except cellulose acetate [Knudsen, 1987]. The increase in C5a/C5a desArg resembles the generation rate of C3d. Therefore, analysis of C3d serves as a suitable cumulative marker of activation events [Knudsen, 1987]. We therefore preferred to analyse the activation of C3 which includes both the classical and alternative pathway of complement by the derived catabolite C3d (Fig. 2).

Complement split product C3d

Complement activation and membrane biocompatibility during haemodialysis may be measured by arterial changes in the C3-derived catabolite, C3d, (including C3dg) [Svehag, 1991]. Membrane-associated C3b is degraded into iC3b, which is further cleaved into equal molar amounts of
inactive forms, C3dg and C3c; C3dg is further degraded into C3d and C3g by proteases. A modest amount of C3d is associated with the membrane, probably covalently bound, though it is washed away from the dialyser during clinical dialysis [Cheung et al. 1989]. C3d is a suitable cumulative marker for activation events [Knudsen, 1987].

Figure 2. A schematic diagram of the alternative pathway's amplification of C3 and generation of complement split products as C3d.

C3d is also generated in the dialyser "resembling the formation" of C5a/C5a<sub>des</sub>-Arg [Knudsen, 1987]. The maximal generation rate of C3d coincides
with the nadir of leukopenia at the beginning of haemodialysis, and the generation rate then declines until 120 min of the haemodialysis. Thus, plasma levels of C3d, sampled from the arterial side, increase until the end of treatment and reflect the biocompatibility of the membrane [Knudsen, 1987]. By analysing C3d from the arterial side the changes present in the patient and not only the process in the dialyser are seen.

Normally, C3d declines after an acute complement activation with a mean $T_{1/2}$ of 4 h with normal renal function. However, $T_{1/2}$ is 6 h in dialysis patients [Knudsen et al. 1985b] and C3d declines to pre-dialysis values after 24 h. In addition to the advantages in sampling C3d due to its kinetics, in comparison with C3a or C5a, the samples are stable in EDTA tubes [Perrin et al. 1975]. The samples can be stored up to 3 h at room temperature before centrifugation without changes [Parkin & Pinching 1984], and after centrifugation if stored at 4°C for 48 h there is only a marginal rise in C3d [Sinosich et al. 1982].

Perrin et al. initially described a two-step procedure for quantification of C3d: after precipitation of native C3 and C3b by polyethylene glycol, C3d was determined in the supernatant by radial immunodiffusion [Perrin et al. 1975]. The method was simplified into a one-step determination by using a double-decker immunoelectrophoresis [Brandslund et al. 1981]. Now there are also different ELISA methods [Mollnes, 1985b; Holmskov-Nielsen et al. 1986] which also have a close correlation with the double-decker immunoelectrophoresis method [Mollnes, 1985a].

C3d has been studied in association with other events such as the intense adverse drug reaction to penicillin which shows elevated levels for more than 15 days [Brandslund et al. 1983], whereas an anaphylactoid reaction to transfusion with leukocyte-depleted blood elevates C3d for about 4 days [Teisner et al. 1983]. The baseline level of C3d is also influenced by the cause of renal disease. Perrin et al. showed that about 68% of patients with SLE, 87% with membranoproliferative glomerulonephritis and 62% with other hypocomplementemic nephrits had elevated plasma levels of C3d fragment. The highest values in the SLE group were associated with the lowest concentration of C3 [Perrin et al. 1975] as an expression for consumption of C3.

The kinetics of C3d, its stability in vitro, and the analysis methods, convinced us to use C3d for measurement of blood-membrane interaction in these studies.

**Activated blood cells**

During the exposure of blood to membranes several homeostatic events are induced. Moreover, activation of different cellular mechanisms, such as cell adhesion, aggregation, release of constituents and induction of cytokines occur [Cheung, 1990]. Noxious humoral substances such as superoxide and
other free radicals, leukotrienes, thromboxane and other prostaglandins may be released [Vanholder & Ringoir 1989]. These mechanisms are both humorally dependent and complement-mediated but also complement-independent and activated by the exposure of the cells to the membrane.

**Neutrophils**

The neutropenia of haemodialysis is a consequence of C5a-induced neutrophil aggregation [Craddock et al. 1977b]. The degree of neutropenia varies not only due to different membranes but also among the patients [Pengloan et al. 1991].

The activation of neutrophils during blood-membrane interaction up-regulates adhesion receptors [Arnout et al. 1985]. C3b is deposited on the cuprophan membrane and degraded into iC3b, which mediates the neutrophil adherence via interaction with complement receptor 3 (CR3 or Mac-1) [Cheung et al. 1991]. The adherence of the neutrophil to the membrane, mediated by iC3b, can be inhibited by an antibody directed against the alpha-chain of the complement receptor 3 [Cheung et al. 1991]. Factor iC3b binds to the complement receptor 3 and this receptor belongs to a complex of surface glucoproteins, which is essential for chemotaxis and adhesion of neutrophils [Anderson & Springer 1987]. These leukocyte adhesion glycoproteins, so-called integrin receptors, such as Mac-1, p150,95 and LFA-1, may be upregulated on both granulocytes and monocytes by different mediators [Miller et al. 1987]. Cheung et al. showed that these integrin (CD11/CD18) receptors are required for neutrophil degranulation [Cheung et al. 1993], a mechanism that may also be noncomplement-mediated [Cain et al. 1987]. The expression of Mac-1 (receptor for complement factor iC3b) on granulocytes occurs already after 5 min, correlates with the degree of neutropenia [Lundahl et al. 1992], and is accompanied by an increase in plasma IL-1ß later on during haemodialysis [Thylén et al. 1992]. While Mac-1 is upregulated during dialysis with a cellulose membrane, the LAM-1 is down-regulated. The latter is a cell adhesion molecule from the family known as selectins or LEC-CAMS [Springer 1990, 1994]. These reciprocal changes in granulocyte adhesion molecules coincide with decreased adhesion to endothelial cell monolayers [Himmelfarb et al. 1992].

Neutrophil function is also influenced during dialysis: oxidative metabolism is reduced by the cuprophan membrane as compared with PAN [Descamps-Latscha et al. 1991], an effect that is suppressed between the dialysis sessions [Ritchey et al. 1981]. In association with dialysis the neutrophils release granulocyte components such as lactoferrin [Hällgren et al. 1981], myeloperoxidase, and elastase as measured by granulocyte elastase α1-protein inhibitor complex [Hörl et al. 1987]. Recurrent dialysis with a cuprophan membrane increases the pre-dialysis neutropenia [Hakim et al. 1984]. This
process is associated with an impairment of phagocytic function not seen in non-complement activating membranes [Vanholder et al. 1991]. This indicates that the neutrophil dysfunction is correlated with the degree of bioincompatibility and complement activation.

**Lymphocytes**

Information about the effects of haemodialysis on lymphocyte function is gradually accumulating [Cheung, 1990]. The cuprophan membrane may increase the surface expression of MHC class I antigen and may stimulate synthesis of β2-microglobulin by lymphocytes both in the presence and absence of complement [Jahn et al. 1991]. Moreover, dialysis with new cellulose membranes increases the baseline expression of the two subunits of the IL-2 receptors and reduces their reactivity to stimulation with phytohemagglutinin [Zaoui et al. 1991]. This effect is reversed by the use of a more biocompatible membrane. The similar modulation of the IL-2 receptor, as has been shown with the cuprophan membrane, is also associated with other diseases known for impaired immune responses [Zaoui et al. 1991]. Therefore, the blood-membrane interaction may induce a lymphocyte dysfunction. Neither of the lymphocyte products, IL-2 nor IFN-γ, can be detected in either first-time or long-term dialysis patients [Herbelin et al. 1990].

**Platelets**

The activation of platelets and the contact phase of blood coagulation is initiated during the blood-membrane interaction [Knudsen et al. 1985a]. Clotting problems are perhaps the most common manifestation of a foreign surface, and clotting consists of a complex of events, including activated platelets [Basile & Drüeke 1989]. This platelet interaction is dependent upon the dialysis membrane [Schulman, 1993]. The cellulose acetate membrane causes a decrease in platelet count with an increase in 6-keto-PGF1α (a prostanoid) after 15 min of dialysis. Besides this interaction there is also a decrease in predialysis β-thromboglobulin after 14 days of dialysis with cellulose acetate which is not seen by Hemophan or by some other membranes [Verbeelen et al. 1991]. Activation of platelets may be determined by the platelet reduction, platelet factor 4, β-thromboglobulin [Dawes et al. 1978], release of tromboxane [Schulman & Hakim 1991], and fibrin degradation products. Perhaps the combination of these mechanisms may induce microembolisation in the patients. Initiation of dialysis increases both β-thromboglobulin and tromboxane B2 which immediately rise to plateau levels. The increase is less for platelet-derived thrombospondin, probably due to storage pool deficiency [Gawaz et al. 1991]. Tromboxane may, in a sheep model, induce pulmonary hypertension, while addition of a cyclo-oxygenase inhibitor (prostacyclin), diminishes these pulmonary effects [Schulman & Hakim 1991].
Another activation event is the increased expression of GMP-140 (a 140-kD glycoprotein located in the secretory α-granulae of platelets) on the surface of platelets after haemodialysis [Deguchi et al. 1991]. This activation may mediate adhesion of stimulated platelets to neutrophils. Platelet activating factor (PAF) is generated by various cells besides the platelets and is a mediator of inflammation and shock. PAF is released by blood-membrane interaction during haemodialysis due to complement-dependent or -independent mechanisms [Tetta et al. 1993]. The sum of these activation events may contribute to the chronic side-effects seen in treatment of end-stage renal failure.

Monocytes-macrophages

The dialyser membrane polymer, microbial products in the dialysate exposed to the blood compartment, and the mechanical pumping of blood in the haemodialysis circuit all stimulate monocytes to release cytokines [Cheung, 1990]. Cytokines act in a complex network by interaction between each other and by synergistic or antagonistic actions on various cell functions. [Balkwill & Burke 1989]. Cytokines are mainly produced by monocytes/macrophages and are associated with some of the side-effects seen in haemodialysis patients. More than 20 cytokines are produced by the circulating mononuclear cells, such as interleukin-1 (IL-1), IL-2, interferon α and γ, IL-6 and TNF (tumor necrosis factor). Especially these cytokines have been analysed in relation to haemodialysis and reviewed by Dinarello [Dinarello, 1991]. TNF and IL-1 induce pyrogen fever, induction of PGE$_2$ synthesis, activation of endothelial cells, bone resorption, neutrophil activation and leukocyte adherence [Dinarello, 1988]. The elevated circulating levels of IL-6 seen in uremic patients are not different when comparing long-term haemodialysis patients with patients not yet started on dialysis [Herbelin et al. 1991].

Haemodialysis exerts a chronic effect on the monocytes and stimulates their production of TNFα and IL-1β [Cheung, 1990]. During a single dialysis session, a significant increase in IL-1, but not in TNFα, occurs regardless of the membrane used. Intracellular IL-1 levels may increase within 2 h of haemodialysis using cuprophan membranes, and the levels appear to correlate with the increase in plasma C3adesArg [Haeffner-Cavaillon et al. 1989]. However, in not yet dialysed uremic patients, there are no differences in IL-1 as compared with controls [Herbelin et al. 1990]. Davenport et al. found no change in TNF when using acetate as dialysate in healthy, well-nourished patients [Davenport et al. 1991]. Another study showed that a membrane made of Hemophan, but not of cuprophan, increased the circulating level of TNFα [Mege et al. 1991]. Instead, the cuprophan membrane caused a greater endotoxin-stimulated production of TNFα, probably by primed monocytes through activation of the complement pathway [Mege et al. 1991].
C5a-stimulation of the monocyte occurs by activation of the alternative pathway of the complement system [Henderson & Chenoweth 1987; Cheung, 1990]. There is an important synergism between C5a and endotoxins in the generation of cytokines [Morin et al. 1991]. C5a triggers the transcription of IL-1ß mRNA when using cuprophan, a mechanism which is inhibited by a monoclonal antibody against C5a [Schindler et al. 1993].

Endotoxin exposure during haemodialysis with low-flux membranes as a reason for monocyte activation is controversial since the membranes do not allow passage of endotoxin fragments above 2000 D [Port & Bernick 1983]. A passage of endotoxins is supported by the increased levels of endotoxin antibodies in patients treated with high-flux membranes. No differences were found between those using low-flux membranes and the controls [Taniguchi et al. 1990; Yamagami et al. 1990]. In vitro studies show a passage of low-molecular-weight lipopolysaccharide (LPS) subunits across cuprophan membranes [Lonnemann et al. 1989]. Urena et al. also found evidence for permeation of low-molecular-weight LPS subunits across cellulose and non-cellulose membranes. [Urena et al. 1992]. The importance of reducing the endotoxin levels is shown when using ultrapure dialysate in patients haemodialysed with cuprophane or cellulose acetate. The risk for haemodialysis-related amyloidosis is reduced [Baz et al. 1991]. This beneficial effect is suggested to be due to reduced monocyte activation.

According to Dinarello, future improvements in haemodialysis treatment will include a greater attention to removal of cytokine-inducing factors in the dialysate, an improvement of the haemodialysis membranes, and the therapeutic use of cytokine antagonists [Dinarello, 1991]. The results from the use of natural antagonists such as soluble TNF receptor and IL-1 receptor antagonists in animal studies are encouraging and may, in the future, alter the dialysis-related symptoms for the patients [Dinarello, 1992].

Factors that affect haemodialysis.

Membrane configuration

Factors associated with clotting and which are related to the surface structure include membrane composition, charge and roughness. Non-surface-related factors of importance may be the shear rate at the wall and the axial streaming of platelets which are related to the geometry and architecture of the artificial organs [Vanholder & Ringoir 1989]. Within a plate dialyser there is greater cell adhesion to the outer membranes than the ones deeper in, indicating that the blood flow is unevenly distributed due to the geometry of the blood channels.
[Kjellstrand et al. 1991]. The shape of the dialyser influences the blood flow resistance and the drop in blood pressure inside the fibre. The risk for backfiltration in hollow-fibre dialysers is dependent upon the filter length, the cross sectional area [Ronco, 1990], and the membrane architecture. The consequences of backfiltration also depend upon the ultrafiltration coefficient as in high-flux membranes where the endotoxin exposure is increased [Vanholder et al. 1992]

**Protein adsorption**

Several factors together determine the degree of complement activation, one such is the adsorption of proteins to the membrane. This adsorption is favoured by a more hydrophobic character of the polymer [Mandelius & Ljunggren 1991]. The protein adsorption occurs within seconds when blood comes into contact with an artificial surface and is considered as a two-layer process. There is one tightly and another more loosely bound layer. The pattern of proteins adsorbed varies among different membranes, when studied by elutions from heparinized plasma. The highest amount of protein is eluted from PAN and Hemophan, probably due to a more porous structure, followed by the cuprophan membrane, cellulose acetate and less by polymethylmethacrylate fibres [Francoise Gachon et al. 1991; Cornelius & Brash 1993].

A protein layer may be the reason why reused cellulose-based membranes have improved biocompatibility. The protein-coat in reused membranes may include C3b-like molecules which prevent further deposition of active C3b [Basile & Drüeke 1989]. Hakim and Lowrie showed that the neutropenia in patients exposed to reused cellulose membranes was only 40% as compared with 90% for new, first used, membranes [Hakim & Lowrie 1980]. Moreover, reused membranes seem to prevent the reduction in lung function as compared with new membranes [Shusterman et al. 1989]. In reused membranes, a protein layer is seen by ultrastructural studies, whereas this layer is not seen when using sodium hypochlorite at a concentration of more than 4%. This indicates that the use of sodium hypochlorite "scrubs" away the protective protein layer [Shusterman et al. 1989] and the membrane surface thereby retains its reactivity, as in new membranes [Gagnon & Kaye 1984].

The polyacrylonitrile membrane (PAN) is a synthetic, hydrophobic and apolar membrane known to adsorb proteins; that adsorption improves the biocompatibility of the membrane [Hakim, 1993]. Moore et al. [Moore et al. 1989] studied the effect by connecting a cuprophan membrane followed by a PAN membrane in a circuit. This showed that the PAN membrane adsorbed C3a, which had been released by the cuprophan membrane. PAN also adsors β2-microglobulin [Goldman et al. 1987], IL-1 [Lonnemann et al. 1988b] and
factor D. The latter is a specific serine protease which is elevated in haemodialysis patients and is an essential enzyme in the activation of the alternative pathway of complement [Pascual & Schifferli 1993]. The adsorption of factor D, in addition to C3a, further reduces the generation of C3 convertase. These adsorptive properties of dialysis membranes as in PAN encourage further development of new biomaterials with such abilities.

Sterilization methods

Limulus-amebocyte-lysate (LAL) is a commercial product prepared as a lysate from the only circulating cell, the amebocyte, in the haemolymph of the horseshoe crab. This preparation contains a series of clotting proteins, similar to the clotting cascade in humans, and clots in the presence of picograms of endotoxin and also in high amounts of a few other substances [Pearson & Weary 1980]. The latter clotting-inducing substances are called Limulus-amebocyte-lysate-reactive material (LAL-RM) and are found in strikingly larger amounts in the lumen of hollow-fibres than in the blood path of plate dialysers. This is supposed to be due to cellulose-derived leachables from the membrane with a range in molecular weight between 5 and 70 kD [Henderson & Chenoweth 1987; Pearson, 1987]. Leaching is the slow dissolution of various factors out from the solid phase into the surrounding liquid. Examples of leachables are silicon particles, polyvinylchloride, phthalates, low molecular weight contaminants and plasticizers [Vanholder & Ringoir 1989], the latters may influence the red cells' survival [Aubuchion et al. 1988]. The amount of LAL-RM is also influenced by the reactive sites on the membrane. The baselevel of LAL-RM and the increement at 15 min is higher when using hollow-fibre membranes than when using plate dialysers [Pearson, 1987].

An allergic reaction, "first-use reaction", can occur with different clinical symptoms such as hives, laryngeal oedema, chest pain and shock as seen in anaphylactiod reactions. This occurs within the first 20-30 min of dialysis and is more often seen with an ethylene oxide-sterilized hollow-fibre membrane with a large membrane area. It occurs more often during dialysis with regenerated cellulose than membranes made by cellulose acetate [Daugirdas & Ing 1985], which shows that the amount of reactive hydroxyl groups is also important. This indicates that the first-use syndrome includes both an activation of the classical and alternative pathways; the classical is activated by LAL-RM containing immune complexes and the alternative by membrane activation [Henderson & Chenoweth 1987]. In addition, often IgE-mediated reactions caused by ethylene oxide hypersensivity are involved. LAL-RM can be a carrier for ETO [Pearson, 1987] and this hapten is thereby presented to the imunesystem [Henderson & Chenoweth 1987]. This hypersensitivity can be
confirmed by a positive radioallergosorbent test against ETO [Vanholder & Ringoir 1989]. This together with environmental, toxicological and allergological aspects support the use of other sterilizing methods than ETO. Leachables and LAL-RM further emphasize the importance of thorough pre-rinsing of the dialyser; 800 ml of saline does not eliminate the residual leachables [Inagaki et al. 1987].

Backdiffusion

The interest in microbiological contamination of the dialysate is further increased by the use of bicarbonate, the use of high-flux membranes and the knowledge that backfiltration of dialysate occurs across the membrane, especially in hollow-fibre dialysers. The dialysate is neither sterile nor non-pyrogenic, and endotoxins or their fragments may be transferred to the blood compartment and stimulate cytokine formation. Penetration of endotoxins or subunits through low-flux membranes has been reported but is controversial [Lonnemann et al. 1989; Urena et al. 1992]. This has initiated the use of a filter to eliminate such toxins from the dialysate. The weight of evidence that favours passage of biologically active endotoxin fragments across dialysis membranes is supported by Dinarello [Dinarello, 1991]. An increase in LAL reactivity during haemodialysis but not during haemodiafiltration with a high-flux polysulfone membrane is reported, while no increase occurs in LAL during low-flux polysulfone dialysis [Vanholder et al. 1992]. This indicates the presence of backdiffusion during haemodialysis with high-permeable membranes such as polysulfone. Such exposition of dialysate may stimulate the monocyte to synthesize cytokines due to direct stimulation by endotoxins in addition to the stimulation by C5a [Cheung, 1990].

Backdiffusion occurs near the end of the hollow-fibre dialyser where the dialysis solution enters. This process is influenced by the filter and fibre geometry and by the water permeability of the membrane [Ronco, 1990]. No backfiltration is detected in low-flux dialysers using a net ultrafiltration of 10 ml/min [Leypoldt et al. 1991a, 1991b]. The fibre length, cross-sectional area, the haematocrit and total protein concentration are also important factors that influence the pressure drop inside the filter and thereby contribute to induce backdiffusion [Ronco, 1990].

The importance of reducing the contamination of dialysate is shown by the use of ultrapure water, defined as endotoxin less than 0.008 ng/ml and less than 1 bacteria/ml. Dialysis with low-flux membranes and ultrapure water may reduce the incidence of carpal tunnel syndrome [Baz et al. 1991], possibly due to less stimulation of the monocytes.
Factors that induce hypotensive events due to fluid depletion and volume shifts during haemodialysis are mainly, but not only, dependent upon the dialysate sodium concentration [De Vries et al. 1991]. Besides sodium concentration, also acetate dialysate [Jones et al. 1992], dialysis temperature and autonomic dysfunction are factors that contribute to the number of hypotensive events [Zuchelli, 1987]. By lowering the dialysate temperature it may improve cardiovascular stability [Sherman et al. 1984]. However, Kerr et al. found that decreased dialysate temperature had only a small benefit on symptoms reported during haemodialysis and that the patients felt cold [Kerr et al. 1989].

Presumably acetate has the capacity to induce interleukin-1 release [Bingel et al. 1987; Cheung, 1990]. Oxidation of acetate to carbon dioxide can contribute up to 40% of the total energy expenditure during acetate dialysis [Skutches et al. 1983]. This is also associated with a decrease in arterial oxygen tension during haemodialysis when using acetate as a base but not with bicarbonate [Jones et al. 1992]. This is probably due to the large loss of carbon dioxide in the acetate dialysate. Wiegmann et al. found no differences in initial reduction of leukocytes between acetate and bicarbonate [Wiegmann et al. 1988]. The bicarbonate diminished the hypoxemia, often associated with acetate dialysate, when cellulose acetate membranes were used [Wiegmann et al. 1988]. The use of acetate instead of bicarbonate induces more vascular instability [Jones et al. 1992], formation of ketone-bodies [Akanji & Sacks 1991] and less compensatory peripheral vasoconstriction [Vagge et al. 1988].

Even if bicarbonate has advantages as compared with acetate, the risk of contamination by endotoxins or bioactive fragments is more difficult to handle with bicarbonate than for acetate [Kumano et al. 1993]. Such contamination may reduce some of the benefits of bicarbonate, especially in high-flux membranes. Maiorca et al. showed differences between the dialysates: haemodialysis with bicarbonate had increased morbidity, measured by number of hospitalisations and days, despite risk-factors and other causes as compared with acetate [Maiorca et al. 1993]. This could indicate that bicarbonate contributes to some undesired side-effects of clinical importance.

Anticoagulants

Various drugs may interact with the complement system [Svehag, 1991]. Heparin not only blocks coagulation but also influences the complement system in different ways, such as by inhibition of the C3 convertase formation [Maillet et al. 1983]. This and other effects on the complement system are not related to the anticoagulant activity of heparin or other sulphated
polysaccharides but rather to the number and position of sulphate groups per disaccharide repeat unit [Jepsen et al. 1987].

Heparin coating of the surfaces in the oxygenator and tubes improves the biocompatibility during extracorporeal circulation [Videm et al. 1991]. This effect of heparin is separate from its anticoagulant effect since it is mediated by a different site on the molecule and is dependent upon the type of heparin binding to the surface [Svennevig et al. 1993]. An in vivo study shows a 45% reduction of complement activation by the use of heparin-coated membranes [Videm et al. 1991]. Activation of complement by membrane interaction may be reduced by the use of heparin or citrate as anticoagulant [Blumenstein et al. 1986]. Wiegmann et al. found a higher complement activation during the first 60 min, measured by C3a and C5a, when heparin was used as compared with citrate. This difference is suggested to be due to reduced ionized calcium caused by citrate [Wiegmann et al. 1988]. In the future, modification of the anticoagulant therapy may further reduce complement activation.

Clinical aspects of biocompatibility

The uremic syndrome is complex and affects different metabolic systems. This must be taken into account when the morbidity and mortality vs. biocompatibility are studied. The haemodialysis procedure is life-supporting but is also associated with acute side-effects and chronic complications that diminish the patient's quality of life. Adverse symptoms during the dialysis procedure such as chest pain, dyspnea and back pain are more common in the "first-use" of cellulose membranes with large surface areas [Daugirdas & Ing 1985] than in reused membranes [Hakim, 1993]. During haemodialysis with non-biocompatible membranes, the humoral immune system becomes activated. This activation induces leukopenia, pulmonary dysfunction and may induce pulmonary hypertension due to the bioactive peptides C3a, C5a and C5adesArg [Cheung et al. 1989]. The pulmonary hypertension may also involve formation of prostaglandin activation products such as tromboxane [Schulman & Hakim 1991].

When using a rat model where acute renal failure is induced, the recovery in renal function is considerably less and slower for animals dialysed to cellulose membranes as compared with more biocompatible membranes. A clinical study has shown similar, preliminary results when patients were randomly dialysed with either cuprophan or a low-flux PMMA membrane; the results suggest a significant improvement in both mortality and earlier recovery of renal function in the patients dialysed with a more biocompatible membrane [Hakim et al. 1992]. However, when measuring the acute intradialysis well-being of the
patient during the dialysis with cuprophan or polysulfone, no differences are seen between the membranes when acetate is used as dialysate [Bergamo Collaborative Dialysis Study Group 1991]. This may indicate that the dialysate base is of more importance for the patient’s well-being.

**Catabolism**

The induction of cytokine formation by haemodialysis may contribute to the catabolic state with muscle wasting which is seen in dialysis patients. The cuprophan membrane induces more protein catabolism than more biocompatible membranes [Guitierrez et al. 1992], and a net degradation of approximately 15 to 20 g of muscle protein occurs due to a 150-min exposure to a Cuprophan membrane [Guitierrez et al. 1990]. Release of proteolytic enzymes from the neutrophils may also contribute to the catabolic state [Hörl & Heidland 1984] and probably also participates in the pathogenesis of carpal tunnel syndrome [Yoshida et al. 1989]. Therefore, besides the catabolic effect due to renal failure, the repetitive blood exposure to a non-biocompatible membrane further diminishes the muscle mass of these patients.

**Dialysis-related amyloidosis**

β2-microglobulin (β2-M) is expressed on the surface of all nucleated cells and is also released from intragranular stores by degranulation. β2-M amyloidosis has been identified in uremic patients undergoing long-term haemodialysis. After 8-10 years of haemodialysis, the amyloid is deposited in articular and periarticular structures. This amyloid is often associated with the carpal tunnel syndrome, pathologic fractures, tendinitis and chronic arthropathies that may lead to complete joint destruction. β2-M amyloidosis is increased in patients dialysed with cuprophan membranes as compared with patients dialysed with PAN membranes [Basile & Drueke 1989]. In addition, the synthesis rate of β2-M is 25% higher during the use of a cuprophan dialyser than in patients dialysed with a more biocompatible membrane [Floege et al. 1991]. The cuprophan membrane may increase surface expression of MHC class-I antigen [Jahn et al. 1991] and stimulate synthesis of β2-M both in the presence and absence of complement. These two mechanisms might work synergistically to increase synthesis of β2-M [Schoels et al. 1993]. In addition, activation of neutrophils by membrane materials leads to release of proteases which may further enhance the polymerization of the amyloid [Hakim, 1993].
Infections

Bacterial infections and sepsis are important factors that contribute to the morbidity and mortality in end-stage renal disease. Many studies have shown that infections stand for about 20%, and up to 36%, of the mortality in long-term haemodialysis patients [Mailloux et al. 1991]. Several factors influence the patients' defense against infections. Dialysis with non-biocompatible membranes induces leukopenia associated with functional alterations of the remaining leukocytes. This has been shown by a more pronounced pre-dialysis neutropenia and a faster endogenous clearance of C3adesArg, after a month of dialysis with a cuprophan membrane as compared with two more biocompatible membranes [Hakim et al. 1984]. The neutrophil dysfunction as degranulation, diminished oxidative metabolism, abnormal chemotaxis [Cheung et al. 1993] and reduced phagocytic function [Descamps-Latscha et al. 1991] impair the defense against infections. In addition to the uremia, the cuprophan membrane material also reduces the phagocytic capacity by about 60% [Vanholder et al. 1991] and this effect is reversible by changing from cuprophan to a more biocompatible membrane [Vanholder et al. 1991]. A similar reduction in monocyte function is also seen during chronic exposure to a cellulose membrane but not when a more biocompatible membrane is used [Zaoui et al. 1991]. In addition, the enhanced protein catabolism [Guitierrez et al. 1992] and the increased release of proteases as granulocyte elastase [Hörl & Riegel 1984] during cuprophan dialysis further increase the catabolic effect and thereby reduce the host defense. Two studies have shown that the incidence of infections is decreased by approximately one-half by switching the haemodialyser from cellulose to a more biocompatible one [Lewin et al. 1991; Hornberger et al. 1993]. Two non-randomized studies have also shown that the use of more biocompatible membranes may reduce the degree of hospitalization [Chanard et al. 1982] and probably also the mortality (7% vs. 20%) when comparing high-flux polysulfone with cellulose membranes [Hornberger 1993]. The dialysate may also have an impact upon morbidity, measured as the amount of hospitalization, and notably favours acetate [Maiorca et al. 1993].
AIMS OF THE STUDY

The aim of this study was to investigate various factors which may be of importance in the interaction between blood and membranes during treatment of haemodialysis patients and thereby improve biocompatibility and reduce the patients' side-effects.

This study was focused on:

I. the influence on blood cells and complement system in patients dialysed with either acetate or bicarbonate as a base and using hollow-fibre dialysers made of two different membrane materials.

II. the influence on blood cells and complement system by the use of whole blood filtered through 4 different leukocyte-removal filters.

III. blood-membrane interaction, measured by complement activation, and the influence of membrane design, sterilization methods and type of dialysate base.

IV. complement activation, measured by C3d, during haemodialysis with ultrafiltration "set at zero" as compared with a TMP of 100 mmHg and the use of bicarbonate or acetate as dialysate.

V. the influence upon complement activation, by the use of different transmembrane pressures of either 50, 100 or 200 mmHg during the first 60 minutes of haemodialysis and the use of bicarbonate as dialysate.
SUBJECTS AND METHODS

Paper I

This multi-center study contained 22 stable haemodialysis patients. They ranged in age between 35 and 79 years (mean 62 years) and had undergone maintenance haemodialysis for a mean of 29 months (range 6-98 months). Two hollow-fibre dialysers with membranes made of cellulose acetate or Hemophan were studied. Twelve patients were dialysed with acetate and 10 other patients with bicarbonate as dialysate base. Patients with diabetes mellitus, non-uremic disorders affecting leucocytes-, platelets-, and coagulation function, septicaemia or invasive infection were excluded. Informed consent was obtained from all patients.

Table 1. In vitro data about the dialysers, according to the manufacturers, used in paper I.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Hemophan®</th>
<th>Cellulose acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialyser</td>
<td>Bio-Nephross</td>
<td>C-DAK 4000 HP</td>
</tr>
<tr>
<td></td>
<td>Andante</td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td>0.8 m²</td>
<td>1.4 m²</td>
</tr>
<tr>
<td>Sterilizing</td>
<td>ETO</td>
<td>ETO</td>
</tr>
<tr>
<td>Clearance (ml/min):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>158</td>
<td>157</td>
</tr>
<tr>
<td>Creatinine</td>
<td>144</td>
<td>126</td>
</tr>
<tr>
<td>Phosphate</td>
<td>123</td>
<td>90</td>
</tr>
<tr>
<td>B 12</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>UF-coeff:</td>
<td>4.1</td>
<td>3.8</td>
</tr>
</tbody>
</table>

ETO=ethylene oxide; UF-coeff=ultrafiltration coefficient in ml · mm Hg⁻¹ · h⁻¹

The blood samples were drawn from the arterial side at 0, 15, 60 and 180 min to measure events occurring in the patient and not only within the dialyser. The decreases in leukocytes and platelets were used to quantify aggregation or activation. In addition, the release of granulocyte elastase α₁-protein inhibitor complex (ELISA-method, Diagnostica Merck, Copenhagen, Denmark) and β-thromboglobulin (RIA-method, Amersham, Copenhagen, Denmark) were used
as markers of leukocyte and platelet activation, respectively. Complement activation was measured by changes in C3d, a complement split product of activated C3. B2-microglobulin was analysed by an RIA-method (Pharmacia, Uppsala, Sweden) in serum and dialysate (only in patients dialysed with bicarbonate).

Samples for C3d were collected in tubes containing disodium-EDTA, which effectively prevents complement activation in vitro [Perrin et al. 1975]. The samples were immediately chilled on ice and kept so until centrifugation. After separation they were stored at -20°C until assayed. The determination of C3d was performed with an ELISA [Holmskov-Nielsen et al. 1986]. Low values of C3d were obtained in the acetatate group. To exclude interference by acetate in the assay, another ELISA method [Mollnes, 1985b] was also used in 10 samples, and it confirmed the results.

To reduce the inter-center error, the blood sampling at each center was done by the same trained nurse. All samples of C3d, granulocyte elastase and B2-thromboglobulin were analysed at the same laboratory, using the same kits for each individual. Plasma protein was analysed according to Lowry [Lowry et al. 1951] and used to calculate the changes in plasma volume and thereby used as a factor to correct for the influence of ultrafiltration. Without such correction changes, for instance, B2-microglobulin can be overestimated [Bergström & Wehle 1987]. The mean value for each parameter of three dialysis treatments was calculated and corrected by this factor.

A questionnaire (visual analogue scale) was used to register the patient’s symptoms and included 13 various symptoms (headache, dizziness, eye complaints, feeling of weakness, loss of appetite, indigestion, vomiting, chest pain or cramps, itching and others) by the use of a visual analogue scale. The scale was graded from 0 to 100 mm (0 = absence of symptoms; 100 = highest level of a symptom). Example:

Moderate chest pain registered using a visual analogue scale marked by the patient with a -x-

No pain at all                                      Extremely painful
0________________________x________________________100

Paper II

Haemodialysis patients are often exposed to dialysis membranes two or three times weekly and the additional use of leukocyte-filtered blood is frequent. The leukocyte-filtering may further potentiate these activation events. The aim was to evaluate the influence upon blood cells as activation or lysis by
filtration through different filters; the aim was not to evaluate the efficiency of leukocyte removal.

Whole blood from twelve healthy donors with addition of citrate-phosphate-dextrose (CPD) was filtered through four different leukocyte-removal filters. Prior to the experiment, the blood was allowed to reach room temperature and was maintained thoroughly mixed. Control (pre-filtration) samples were obtained and then the blood were filtered through each filter (in the same order). About 20 ml of whole blood (of a total of 110 ml through each filter) was filtered by gravity before post-filtration samples were taken for blood corpuscle count, plasma haemoglobin, serum albumin, C3d, calcium, granulocyte elastase α1-protein inhibitor complex (PMN-Elastase, IMAC, MERCK, Kebo Lab AB, Spånga, Sweden), and β-thromboglobulin (Asserachrom, β-TG, Amersham, Solna, Sweden). Serum albumin values were used to adjust for changes in haematocrit. The concentrations of blood cells (erythrocytes, leukocytes, platelets) were measured by an automated counter (Coulter Counter S-Plus, STKR, Kebo, Spånga, Sweden) with a lowest detection limit of 1 x 10^8/l ±2.5%. The Erypur b made of cellulose acetate was more time-consuming to handle due to the need for pre-rinsing with saline to eliminate acetic acid. Leucostop (Baxter, Bromma), PALL RC 100 (Mediwest, Askim) and Sepacell R-500 A (VingMed, Uppsala) were made of various polyester compositions, various filter designs and thicknesses of the filter.

The removal efficiency is crucial [Solheim et al. 1990] and accurate quantification of the leukocyte depletion is necessary [Kao & Scornik 1989; Wenz & Besso 1989]. The leukocyte removal efficiency is not only caused by a filtration effect but also by activation and adhesion of the leukocytes to the filter material [Gu et al. 1993]. This activation may be measured by granulocyte elastase α1-protein inhibitor complex, β-thromboglobulin and plasma haemoglobin were studied to relate the influence upon the cell components in comparison to the reduction in platelets and erythrocytes, respectively. C3d was measured by rocket immune electrophoresis [Brandslund et al. 1981].

Paper III

Twelve stable end-stage renal failure patients (mean age 60 years, range 25-82 years, see Table 2) were dialysed, in a cross-over design, three consecutive times on each of four different dialysers. The dialysers had approximately the same membrane area, ultrafiltration (UF)-coefficient and clearance (Table 3).

Three dialysers were made of cuprophan, a non-biocompatible membrane material and thereby used to determine differences in complement activation by factors such as configuration, sterilization-method or dialysate.
Table 2. Patient data.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Cause of uraemia</th>
<th>Dialysis (h· times/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>56</td>
<td>Glomerulonephritis</td>
<td>3x3</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>58</td>
<td>Nephrosclerosis</td>
<td>4x2</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>61</td>
<td>Interstitial nephritis</td>
<td>4x3</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>61</td>
<td>Polycystic kidney dis.</td>
<td>3x3</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>66</td>
<td>Nephrosclerosis</td>
<td>4x3</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>62</td>
<td>Micropolyarteritis</td>
<td>4x3</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>82</td>
<td>Goodpasture's syndrome</td>
<td>3x3</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>64</td>
<td>Glomerulonephritis</td>
<td>4x3</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>76</td>
<td>Interstitial nephritis</td>
<td>4x2</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>25</td>
<td>Glomerulonephritis</td>
<td>4x3</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>60</td>
<td>Glomerulonephritis</td>
<td>4x3</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>50</td>
<td>Pyelonephritis</td>
<td>4x3</td>
</tr>
</tbody>
</table>

Table 3. Dialyser data. * Ultrafiltration coefficient in ml·mm Hg$^{-1}$·h$^{-1}$, clearence in vitro ml/min (Q$_b$=200 ml/min and Q$_d$=500 ml/min).

<table>
<thead>
<tr>
<th>Dialyser</th>
<th>Material</th>
<th>Sterilizing</th>
<th>Type</th>
<th>Area/thickness m$^2$/µm</th>
<th>UF coeff*</th>
<th>Cclearance urea/crea</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFE12</td>
<td>Cupro</td>
<td>ETO</td>
<td>hollow</td>
<td>1.3 /8</td>
<td>5.8</td>
<td>177/151</td>
</tr>
<tr>
<td></td>
<td>(Hf-CuE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFS 12</td>
<td>Cupro</td>
<td>Steam</td>
<td>hollow</td>
<td>1.3 /8</td>
<td>5.8</td>
<td>177/151</td>
</tr>
<tr>
<td></td>
<td>(Hf-CuS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LUNDIA 5N</td>
<td>Cupro</td>
<td>ETO</td>
<td>plate</td>
<td>1.1/10</td>
<td>5.5</td>
<td>171/142</td>
</tr>
<tr>
<td></td>
<td>(P-Cu)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro 5</td>
<td>Polyc.</td>
<td>ETO</td>
<td>plate</td>
<td>1.1/16</td>
<td>6.1</td>
<td>165/150</td>
</tr>
<tr>
<td></td>
<td>(P-Pc)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ETO= ethylene oxide
The cuprophan dialysers were: a plate dialyser (P-Cu) and two hollow-fibre dialysers sterilized with either steam (Hf-CuS) or ethylene oxide (Hf-CuE). One plate dialyser was made of polycarbonate (P-Pc) which is considered to activate complement less than cuprophan [Martin-Malo et al. 1991; Knudsen, 1987] and was used for comparison. All were purchased from Gambro, Lund, Sweden. The dialysers were rinsed with 1.8 litre of 0.9 % saline before dialysis and were not reused.

Five of the patients were dialysed using acetate as a buffer (Gambrolys® 01; acetate 35 mmol/l) and seven using bicarbonate-based dialysis fluid (BiCart® 201; bicarbonate 34 mmol/l and acetate 3 mmol/l). The dialysates were handled according to the manufacturer's directions (Gambro, Lund, Sweden). The dialysis machines were AK-10 and AK-100 (Gambro, Lund, Sweden). The blood flow used was set at 200 ml/min (range 150-250 ml/min), and the dialysate flow was 500 ml/min. The temperature was kept at 37.5°C during the treatments. The patients received an initial loading dose of heparin, approximately 50 IU/kg body weight. A continuous infusion of heparin, based on data from activated prothrombin time of between 45 and 100 sec, was used. The infusion was stopped 30 min before the end of the dialysis. The same individual conditions (blood flow, heparin dose, and ultrafiltration) were used throughout the study period in each of the patients.

Side effects were scored by the patients before start and after 30, 60 and 180 min of dialysis. The subjective symptoms were scored using a visual analogue scale from 0 to 100 mm (0 = absence of symptoms, 100 = highest level of a symptom).

Samples
At 0 and 180 min of dialysis blood samples were drawn from the arterial line of the dialysis circuit for concentrations of urea, creatinine and C3d. In the bicarbonate group samples were also taken at 15 and 60 min to verify the C3d kinetics. Samples for C3d were collected in tubes containing disodium-EDTA to prevent in vitro complement activation. The plasma was separated by centrifugation of the blood samples and stored at -70°C until assay. The determination of C3d (U/l) was performed according to Brandslund et al. [Brandslund et al. 1981], using a rabbit antihuman C3d (DAKO A/S, Glostrup, Denmark) and a double-decker immunoelectrophoresis after precipitation of C3 with a rabbit anti-human C3c (DAKO A/S, Glostrup, Denmark). This assay excludes C3b, iC3b, and intact C3 and includes C3dg [Stegmayr & Tärnvik 1989]. As a standard, a pool of serum from healthy blood donors was used. The serum was incubated for 5 days at 37°C in the presence of azid (15 mmol/l NaN3) to allow conversion of all C3 and the content in this pool is arbitrarily set at 1000mU/l [Mlynek & Nilsson 1985]. The mean changes of 3 runs on each
filter and in each patient, were calculated between start and 180 min for creatinine, urea and C3d. The mean values for the changes in C3d were used for further calculation.
The same method for measurement of C3d [Brandslund et al. 1981] was used in papers II, III and V.

**Paper IV**

Sixteen patients, from two dialysis units, eight patients in each group, were included in the study. The mean age of the patients in group I was 57 years vs. 69 years in group II (range 38-66 and 58-78 years, respectively). The patients had been on dialysis for 6-98 months (mean 29 months). Renal diagnoses in both groups were similar. Patients were randomly selected for dialysis in a cross-over design with two different hollow-fibre dialysers, both of which were sterilized by ethylene oxide. The membranes of the dialyzers were either made of cellulose acetate (C-DAK 4000 HP, 1.4 m², C D Medical, New Jersey, USA) or Hemophan® (Bio-Nephross Andante, 0.8 m², AKZO, Organon Teknika, Boxtel, Netherlands). C-DAK 4000 HP was comparable with Bio-Nephross Andante in efficiency as well as ultrafiltration coefficient (see paper I).

During the first 60 min the ultrafiltration process was standardized for all patients in the two groups. In group I, eight patients were dialysed with a transmembrane pressure (TMP) of 100 mmHg and with acetate as a dialysate base. For eight other patients (group II), the ultrafiltration was "set at zero" during the first 60 min, using the ultrafiltration monitor FCM 10-1 (Gambro, Lund Sweden). Bicarbonate was used as the base. The dialysate base was not changed during the cross-over of the dialysers.

At 0, 15, 60 and 180 min, blood was sampled from the arterial side in order to measure the concentrations of complement fraction C3d, total plasma protein and haematocrit. Samples used for C3d measurement were drawn in EDTA tubes. After centrifugation the plasma samples were frozen to a minimum of -20°C pending analysis. C3d was analyzed at the same laboratory and by an ELISA-method described by Holmskov-Nielsen et al. [Holmskov-Nielsen et al. 1986]. Total protein in plasma was measured together with haematocrit changes in order to estimate fluctuations in the intravascular volume. The protein concentrations for each dialysis were adjusted, and a ratio was obtained by dividing the protein concentrations at 15, 60 and 180 minutes by the protein concentration obtained before the start of dialysis. C3d levels were corrected for this ratio to eliminate false values due to ultrafiltration. The analyses of haematocrit, total protein and C3d were performed at the same laboratory using the same kits for each individual. The protein adsorption to the filter was
estimated in vitro by recirculating an albumin solution for 30 minutes, using a Hemophan dialyser.

Paper V

The study included eight stable end-stage renal failure patients on regular haemodialysis for more than 6 months (5 men and 3 women, mean age 59 years, range 25-82). One patient was a black African and 7 were Caucasians. All patients were dialysed (3-4h) thrice weekly against bicarbonate (BiCart®201; bicarbonate 34 mM and acetate 3 mM). The dialysate buffer was handled according to the manufacturer’s directions (Gambro, Lund, Sweden). The dialysis monitors used were AK-100 (Gambro, Lund, Sweden). The same individual conditions (blood flow, heparin dose, and ultrafiltration) were used throughout the study period.

The study included the same four types of dialysers as in paper III (Table 3). The patients were dialysed three times using each of the four membranes, at three consecutive times. The transmembrane pressure on each occasion was either 50, 100 or 200 mmHg during the first 60 minutes of the treatment. After the first 60 minutes, the dialysis was converted from manual to automatic control to achieve each patient’s "ideal" weight at the end of the treatment. The patients registered their symptoms before start and at 30, 60 and 180 minutes of the treatment by the use of a visual analogue scale graded from 0 to 100 mm (0 = absence of symptoms; 100 = highest level of a symptom). At 0, 15, 60 and 180 min of dialysis blood samples were drawn from the arterial line of the dialysis circuit for concentrations of serum creatinine, urea, albumin and C3d. Samples for C3d were handled and analysed as in paper III. On each occasion for blood sampling, serum albumin was measured and a factor for the extent of ultrafiltration was obtained by dividing the albumin concentration at times 15, 60 and 180 minutes by the concentration at the start of dialysis. The factors were used to correct the C3d concentration due to influence of the ultrafiltration.

Statistical analyses were performed by Wilcoxon’s paired signed rank test. Student’s paired t-test was used when the mean values of the four dialysers were compared using different TMP.

Statistics

The data are presented as means ± SD, unless stated otherwise. A nonparametric Wilcoxon’s paired signed rank test or paired Student’s t-test was used for analysing the differences in various parameters for the same individuals.
Wilcoxon’s paired signed rank test was preferred to reduce the risk for interaction by more or less skewed distribution and when paired samples were 6 or more. Differences between groups were performed by unpaired (independent) Student’s t-test, as described in the individual papers.
RESULTS

Granulocyte elastase, β-thromboglobulin, and C3d during acetate or bicarbonate hemodialysis with Hemophan® or a cellulose acetate membrane. (paper I)

No differences in clearance efficiency were seen between the membranes. No significant changes were seen in serum for β2-microglobulin (β2-M); it was detected in the dialysate at the end of treatment with cellulose acetate but not with Hemophan dialysis. Despite correction for UF, serum β2-M was increased 10-20% in 5 patients (out of 10) after 180 min.

The pre-dialysis values of leukocytes, granulocyte elastase α1-protein inhibitor complex (E-α1-Pl) and C3d were higher in the bicarbonate group as compared with the acetate group. The difference in pre-dialysis values in C3d were maintained at 180 min by the use of Hemophan. C3d increased more at 15 min, irrespective of the filter used, for patients using bicarbonate than acetate (p=0.005). The cellulose acetate membranes reduced leukocytes more in patients using bicarbonate than acetate. This leukocyte reduction was not associated with an increase in E-α1-Pl. The pre-dialysis value of β-thromboglobulin (β-TG) was higher followed by a greater drop in platelets by the use of acetate, irrespective of the membrane used (P<0.038).

Table 4. Summary of differences between membranes and dialysates. (H=Hemophan, CA= cellulose acetate, Ac=acetate, Bic=bicarbonate)

<table>
<thead>
<tr>
<th>Dialyser</th>
<th>Leukocytes Ac / Bic</th>
<th>Platelets Ac / Bic</th>
<th>C3d Ac / Bic</th>
<th>E-α1-Pl Ac / Bic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophan</td>
<td>0 / 0</td>
<td>r * / 0</td>
<td>0 / i °</td>
<td>0 / i °°</td>
</tr>
<tr>
<td>Cellulose acetate</td>
<td>0 / r *</td>
<td>r ** / 0</td>
<td>0 / i *</td>
<td>0 / i °°</td>
</tr>
</tbody>
</table>

r=reduction; i= increment; * at 15 min ; ** at 60 min; ° pre-dialysis, 15 and 180 min; °° pre-dialysis.

The comparison between the filters showed a significant reduction in leukocytes at 15 min by both membranes, more pronounced by the cellulose acetate membrane (p<0.01), and the reduction in leukocytes was greater in the bicarbonate group. Despite the increased baselevel of C3d in the bicarbonate group, C3d increased more at 15 min of dialysis irrespective of the membrane used, as compared with acetate. The side-effects were higher in the acetate group when using cellulose acetate (p=0.021) but not when patients were dialysed with Hemophan.
A biochemical study of the influence upon blood cells by four different leukocyte-removal filters (paper II).

The effects caused by filtration of whole blood (stored with CPD) through 4 different leukocyte-removal filters are shown in Tables 5 and 6.

**Table 5.** Mean concentrations of β-thromboglobulin (β-TG), granulocyte elastase (E-α₁-Pl) and plasma haemoglobin (P-Hb), (n=12) pre-(control) and postfiltration. (±1SD is given in brackets.)

<table>
<thead>
<tr>
<th>Leukocyte-removal filter</th>
<th>β-TG IU/l</th>
<th>E-α₁-Pl μg/l</th>
<th>P-Hb mg/l</th>
<th>S-Ca mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>414 (±280)</td>
<td>23.6 (±12.9)</td>
<td>18.9 (±6.74)</td>
<td>1.9 (±0.1)</td>
</tr>
<tr>
<td>Erypur b</td>
<td>447 (±180)</td>
<td>22.0 (±8.9)</td>
<td>24.7 (±10.2)</td>
<td>1.8 (±0.2)</td>
</tr>
<tr>
<td>Leucostop</td>
<td>245 (±121)</td>
<td>22.2 (±6.7)</td>
<td>52.1 (±16.2)**</td>
<td>2.2 (±0.3)**</td>
</tr>
<tr>
<td>PALL</td>
<td>246 (±121)</td>
<td>26.4 (±8.0)*</td>
<td>17.4 (±5.61)</td>
<td>1.9 (±0.0)</td>
</tr>
<tr>
<td>Sepacell</td>
<td>196 (±84)</td>
<td>20.9 (±8.5)</td>
<td>63.6 (±120.3)**</td>
<td>1.9 (±0.0)</td>
</tr>
</tbody>
</table>

* increase as compared with Erypur b or Sepacell p<0.024.
** increased as compared with controls (p<0.01) and *** (p=0.001)

**Table 6.** Mean concentrations of platelets, leukocytes and erythrocytes in whole blood (n=12) pre-(control) and postfiltration (±1SD). Analysed by Coulter Counter S-Plus, with lowest detection limit 1 x 10^8/l ±2.5%.

<table>
<thead>
<tr>
<th>Leukocyte-removal filter</th>
<th>Platelets 10⁹/l</th>
<th>B-leuko 10⁹/l</th>
<th>B-erythro 10¹²/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>221 (±52.7)</td>
<td>5.53 (±1.29)</td>
<td>4.02 (±0.44)</td>
</tr>
<tr>
<td>Erypur b</td>
<td>5.0 (±2.43)</td>
<td>0.19 (±0.12)</td>
<td>4.10 (±0.60)</td>
</tr>
<tr>
<td>Leucostop</td>
<td>4.6 (±2.93)</td>
<td>0.26 (±0.25)</td>
<td>3.88 (±0.39)</td>
</tr>
<tr>
<td>PALL</td>
<td>4.0 (±3.23)</td>
<td>0.19 (±0.15)</td>
<td>3.87 (±0.35)</td>
</tr>
<tr>
<td>Sepacell</td>
<td>2.3 (±1.38)*</td>
<td>0.19 (±0.16)</td>
<td>3.74 (±0.41)*</td>
</tr>
</tbody>
</table>

* Reduction of erythrocytes (p<0.05) and platelets (p<0.01) as compared with the other filters.
Complement activation is influenced by the membrane material, design of the dialyser, sterilizing method and type of dialysate (paper III).

Table 7. The mean concentrations of creatinine and urea levels before and after dialysis with the different dialysers (± 1SD).

<table>
<thead>
<tr>
<th>Dialyser</th>
<th>Creatinine (μmol/l)</th>
<th>Urea (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre dialysis</td>
<td>Post dialysis</td>
</tr>
<tr>
<td>GFS 12 (Hf-CuS)</td>
<td>777 (±222)</td>
<td>414 (±97)</td>
</tr>
<tr>
<td>GFE 12 (Hf-CuE)</td>
<td>783 (±201)</td>
<td>408 (±95)</td>
</tr>
<tr>
<td>Lundia 5N (P-Cu)</td>
<td>767 (±182)</td>
<td>397 (±74)</td>
</tr>
<tr>
<td>Pro 5 (P-Pc)</td>
<td>791 (±211)</td>
<td>411 (±87)</td>
</tr>
</tbody>
</table>

Table 8. The mean (±1SD) base-level of C3d (U/l) and the increase after 180 min (Δ 180 min) by different dialysers using acetate or bicarbonate.

<table>
<thead>
<tr>
<th>Dialyser</th>
<th>Acetate Baselevel</th>
<th>Bicarbonate Baselevel</th>
<th>Acetate Δ 180 min</th>
<th>Bicarbonate Δ 180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hf-CuE</td>
<td>32.5 (±11.2)</td>
<td>46.3 (±34.7)</td>
<td>13.6 (±9.1)</td>
<td>36.9 (±10.1)</td>
</tr>
<tr>
<td>Hf-CuS</td>
<td>29.9 (±8.9)</td>
<td>49.1 (±31.9)</td>
<td>21.8 (±6.9)</td>
<td>22.5 (±11.4)</td>
</tr>
<tr>
<td>P-Cu</td>
<td>34.3 (±15.2)</td>
<td>45.3 (±18.2)</td>
<td>31.6 (±5.7)</td>
<td>42.7 (±10.2)</td>
</tr>
<tr>
<td>P-Pc</td>
<td>35.2 (±11.2)</td>
<td>55.9 (±48.5)</td>
<td>9.8 (±2.7)</td>
<td>29.1 (±10.0)</td>
</tr>
</tbody>
</table>

For all patients analysed together, there was a significant increase in C3d during dialysis with P-Cu as compared with both hollow-fibre dialysers, Hf-
CuE (p=0.011) or Hf-CuS (p<0.001) and the polycarbonate plate dialyser, P-Pc (p<0.001). No other differences were present.

Patients dialysed with acetate as a base (n=5) had a greater increase in C3d during use of the dialyser P-Cu as compared with the other types of dialysers (p<0.024). Hf-CuS showed significantly more complement activation than P-Pc (p=0.006). No differences were seen between the P-Pc and Hf-CuE nor between the hollow-fibre dialysers, Hf-CuE and Hf-CuS.

During dialysis with bicarbonate as a base (n=7), C3d increased more during use of the P-Cu than the Hf-CuS (p=0.003) and the P-Pc (p=0.022). The Hf-CuE caused more complement activation than the Hf-CuS (p=0.013), while no differences were found between the P-Pc and Hf-CuE or Hf-CuS.

In the patients dialysed with acetate as a base (compared with the bicarbonate group), the complement activation was significantly less by the use of Hf-CuE (p=0.002) and P-Pc (p=0.002) while no significant differences were found for P-Cu or Hf-CuS.

The side-effects were significantly less for the three cuprophan dialysers when using bicarbonate than acetate (p<0.034), but no differences were seen for P-Pc.

In conclusion, this study showed that the cuprophan plate dialyser caused a greater complement activation, measured by C3d, as compared with the hollow-fibre cuprophan membranes and the polycarbonate plate. The steam-sterilized hollow-fibre membrane increased C3d less than the ETO-sterilized when using bicarbonate as dialysate, indicating that the type of dialysate buffer is of importance. The steam-sterilized hollow-fibre was comparable with polycarbonate when using bicarbonate, but this difference was not seen in the acetate group.
Backdiffusion or bicarbonate may stimulate complement activation during haemodialysis with low-flux membranes (paper IV).

C3d increased more in group II (UF="set at zero") at 15 min than for group I (TMP=100 mmHg, p<0.03), irrespective of the filter used (Fig. 1).

**Figure 1.** Change in C3d (U/l) during dialysis using TMP 100 mm Hg and UF "set at zero" (H=Hemophan and CA=cellulose acetate).

When using a stable TMP at 100 mmHg (group I) there was a linear increment in serum protein concentration during dialysis up to 60 min without any differences between the dialysers used.

During dialysis with ultrafiltration "set at zero" (see Fig. 2 in paper IV), there was a significant reduction in total protein concentration after 15 and 60 min (p<0.02) for Hemophan® and at 60 min (p<0.01) for the cellulose acetate membrane.

The reduction in plasma protein concentration from 66.1 to 61.6 g/l (6.8%) was associated with a decrease in haematocrit (see Figs. 3a+b in paper IV).
Complement activation may be reduced by a low initial transmembrane pressure during haemodialysis (paper V).

By comparing various TMPs, the urea clearance obtained by the plate cuprophan dialyser (P-Cu) was higher with an initial TMP of 200 as compared with 50 mmHg (p=0.025). A significantly greater urea clearance was also found for ETO-sterilized hollow-fibre dialyser (Hf-CuE) at 100 as compared with 50 mmHg (p=0.014). No other differences in clearance were seen.

Initial TMP 50 mm Hg, 100 mm Hg or 200 mm Hg

After using a TMP of 50 mmHg initially there were no differences in the change of C3d between the dialysers at 15, 60 or 180 minutes.

When an initial TMP of 100 mmHg was used, no changes were seen at 15 or 60 min. At 180 min the use of the steam-sterilized hollow-fibre cuprophan dialyser (Hf-CuS) caused less increase in C3d than the other types of dialysers (p <0.019).

After 15 min with a TMP of 200 mmHg there were no differences in C3d while at 60 min the Hf-CuS activated the complement system less than the plate dialysers P-Pc and P-Cu (p<0.032). After 180 min of haemodialysis, the Hf-CuS activated C3d less than P-Cu (p=0.006) and Hf-CuE (p=0.034). No other differences were seen (See Figs. 1 and 2 in paper V).

By comparing each dialyser during different transmembrane pressures at 15, 60 and 180 min, the only significant difference in C3d was seen for P-Pc where C3d was higher at 180 min with an initial TMP of 100 as compared with 50 mmHg (p=0.009).

When comparing the mean changes in C3d for all patients after 180 min for each dialyser (Fig. 3), C3d was lower after an initial TMP of 50 than after 100 or 200 mmHg (p<0.047).

Fig. 3. Mean change in C3d after 180 min by different dialyzers and TMP 50 (open square), 100 (filled square) and 200 (triangle) mmHg.
**Side effects**

There were no significant differences in side effects between the dialysers nor when comparing each dialyser using different transmembrane pressures.

**DISCUSSION**

**Paper I**

Two membranes were studied, each with modification of the surface hydroxyl groups. The substitutions had been performed with either diethyl aminoethyl (DEAE) in Hemophan, or by replacement of the hydroxyl groups with acetate in the cellulose acetate membrane. A comparison of haemodialysers summarized by Henderson and Chenoweth [Henderson & Chenoweth 1987] showed that C3a formation and leukopenia are both reduced by about 40% by Hemophan, whereas cellulose acetate reduces C3a by 33% and leukopenia by 57%, as compared with cuprophane. We found similar results in complement activation, as measured by C3d, but found more pronounced leukopenia with the cellulose acetate membrane. This latter effect could be explained by the greater area of the cellulose acetate membrane used in our study. Another study, with the same membranes and approximately same area, showed less differences between the membranes in leukocyte reduction and confirmed our results, by showing more complement activation (C3a) and change in granulocyte elastase with cellulose acetate as compared with Hemophan [Woffindin et al. 1992].

Differences were seen between the acetate and bicarbonate groups, both in pre-dialysis values and during the treatment. Besides a higher baseline level in C3d prior to the use of bicarbonate, also the increase in C3d at 15 min and the leukocyte drop were greater during bicarbonate dialysis than with acetate. The differences in C3d were also confirmed by another ELISA [Mollnes, 1985b]. The pre-dialysis level of β-TG and the drop in platelets were higher when using acetate as dialysate.

The alterations between the groups may have different explanations. There were no differences in pre-dialysis levels of plasma protein or haematocrit which would indicate differences in nourishment between the groups. Besides individual factors, possible reasons could be dialysis system-derived factors such as different qualities in water or dialysate. Another possibility is the influence upon the membrane surface by acetate, which could favour platelet activation but reduce the membrane reactivity by less binding of C3b or by enhancing the activity of inhibitory factors H or I (C3b inactivator) at the
surface. The lower pre-dialysis levels of C3d may be due to acetate oxidation to carbon dioxide and can contribute up to 40% of the total energy expenditure during acetate dialysis [Skutches et al. 1983]. An energy depletion, in addition to reduced arterial oxygen tension, showed by Jones et al. [Jones et al. 1992], may act to reduce the degree of complement activation and thereby contribute to the differences between the dialysates. It is noteworthy that the clinical side-effects were less evident during bicarbonate dialysis even though bicarbonate had a more negative influence upon the biocompatibility factors than acetate.

Paper II

Haemodialysis patients are exposed to membranes, often three times weekly, and transfusion of leukocyte-filtered blood may add activating events to these patients. This study was performed with the use of whole blood stored in CPD. Since CPD binds calcium it may contribute to reduce the activation events [Blumenstein et al. 1986]. Recalcification during the experiment would probably increase the complement activation and thereby cause greater differences between the filters, as has been studied in vitro with artificial kidneys [Cheung et al. 1986a]. However, when the blood enters the patient, the activation events by filtration may be amplified due to the recalcification. This is difficult to estimate in vivo, in addition to a possible transfusion reaction.

We used whole blood to study the influence upon the blood cells; the removal efficacy of the filters was not evaluated. In leukocyte-depleted blood, the number of immunogenic cells is reduced to about $1 \times 10^6$ unit, which is probably the threshold for immunisation [Vakkila & Myllylä 1987; Andreu et al. 1988; Saarinen et al. 1990]. Leukocyte-removal reduces the risk for transmission of leukocyte-born viruses, non-haemolytic febrile transfusion reactions and refractoriness to platelet transfusion [Sirchia et al. 1990; Solheim, 1990].

Our results showed that the removal filters influenced the different blood components to different extents such as measured by haemolysis, release of granulocyte elastase, release of β-thromboglobulin and by the loss of erythrocytes or platelets. A recent study of cardiopulmonary bypass perfusate showed differences in complement activation between different leukocyte-removal filter materials [Gu et al. 1993]. The polyester filters were more biocompatible but had less removal-efficacy as compared with cellulose acetate [Gu et al. 1993]. This emphasizes that the depletion capacity is related to the degree of activation. Steneker et al. studied the cells inside the leukocyte filter; they found both passive trapping and an activation which caused adhesion, aggregation or fragmentation of the cells, depending upon the filter composition [Steneker & Biewenga 1991]. These activated cells or cell
fragments, which pass through the filter and into the patient, may activate various cascade systems.

**Paper III**

In addition to the study of the 4 different types of dialysers, two groups of patients were compared during dialysis with either acetate or bicarbonate. By analysing C3d (including C3dg) [Brandslund et al. 1981] from the arterial side, we analysed changes that occurred also in the patient and not only in the dialyser. In contrast to C5a, arterial plasma C3d increases in concentration throughout the haemodialysis [Knudsen et al. 1984], as was also confirmed in our study, although the generation rate for C3d declines to baseline at 120 min of haemodialysis [Knudsen, 1987]. In addition of the accumulation of C3d, the C3d elimination T\(_{1/2}\) is 6 hours [Knudsen et al. 1985b], and the samples of C3d are quite stable [Sinosich et al. 1982; Parkin & Pinching 1984; Knudsen, 1987]. These together reduce bias in the data due to sampling errors.

Since the cuprophane membrane activates the complement system more than other membranes, it is suitable to display differences due to other factors such as sterilizing methods, membrane design or dialysate composition. These data showed that the cuprophan plate dialyser (P-Cu) induced complement activation more than the hollow-fibre membranes. This indicates that the design of the dialyser is important, thereby favouring the hollow-fibre dialysers. Various reasons for this difference may be the increased membrane thickness, the dialyser housing or the larger priming volume within the plate dialyser. Additionally, the blood flow present in the microchannels may be unevenly distributed within the plate dialyser [Kjellstrand et al. 1991]. In capillary fibres there is a laminar flow which may reduce cell-adhesion, in contrast to the wavelike blood flow within the plate dialyser. The latter, which may increase the turbulence, could favour activation events at the membrane. The leukocyte adhesion to the vessel wall consists of a rolling movement and three sequential steps of binding [Springer, 1994]. Perhaps the laminar flow in the hollow-fibre dialysers influences the cell rolling and thereby reduces cell adhesion and complement activation.

In this study the sterilization technique seemed to have little impact on the complement activation. The only difference was a significantly lower complement activation when using the Hf-CuS as compared with the Hf-CuE, favouring the steam-sterilization method. Note that it was only valid for patients using bicarbonate as base. However, this result is welcome since the steam-sterilizing technique is also preferable due to environmental, toxicological and allergic aspects.
The complement activation during bicarbonate dialysis did not differ between the steam-sterilized hollow-fibre dialyser made of cuprophan and the plate made of polycarbonate even though polycarbonate is considered to be a more biocompatible membrane [Henderson & Chenoweth 1987; Martin-Malo et al. 1991]. However, when acetate was used, polycarbonate showed less increase in C3d which suggests that the dialysate had an impact upon the membrane reactivity. Not only activation of complement but also adsorption to the membrane is strongly determined by the nature of the surface [Parzer et al. 1993] such as the charge on the polymer surface. The polycarbonate polymer has a more hydrophobic character than the cuprophan membrane [Mandelius & Ljunggren 1991] and this character may be influenced by the dialysate composition. Perhaps acetate, as compared with bicarbonate, alters the surface, and thereby influences the regulation of the alternative pathway of complement. An influence on stimulatory factors such as B or D, or on inhibitory factors such as H or I, may influences the C3 convertase activity. The importance of the dialysate is strengthened by the differences found in paper I.

Paper IV

Total protein and haematocrit before treatment were not different in group I (TMP at 100 mmHg and acetate) or group II (UF "set at zero" and bicarbonate). This does not support any differences in the nourishment between the groups nor any differences in the oncotic pressure that may influence the flux across the membrane. The reduction in protein could not be explained by binding to the membrane since adsorption of plasma protein to a Hemophan membrane during in vitro perfusion was less than 0.1 g in total. The reduction of total protein by about 7% was associated with a reduction in haematocrit during the first 60 min of dialysis in group II (bicarbonate base), and indicates a backdiffusion.

Data in this study showed differences between the study groups and indicated an increased complement activation due to backdiffusion. This activation could be due to, e.g., contaminated dialysate or the bicarbonate per se. Backdiffusion has been studied in membranes with large-pores (high-flux) and increased LAL reactivity [Vanholder et al. 1992], and increased levels of endotoxin antibodies are shown with these membranes [Yamagami et al. 1990]. However, in vitro studies have shown that small endotoxin fragments may pass through low-flux membranes in vitro [Lonnemann et al. 1989], causing interleukin-1 production [Lonnemann et al. 1988a,1988b]. Even if bicarbonate as a base has advantages as compared with acetate, it is more difficult to handle. Both the bicarbonate and the water quality can influence the complement
activation due to contamination by endotoxins and bioactive fragments [Kumano et al. 1993]. This could be one explanation for the increased complement activation found in patients on bicarbonate in this study.

This present study favours the use of a TMP during the first 60 min, avoiding backdiffusion, and acetate as dialysate to reduce the complement activation.

Paper V

The use of sequential ultrafiltration profiles mainly favours the use of high TMP at the start of dialysis to avoid a drop in blood pressure [Wehle et al. 1979], and bicarbonate reduces the reduction in blood pressure as compared with acetate [Vagge et al. 1988]. This study showed that the use of an initial low ultrafiltration, expressed as a TMP of 50 mmHg during the first 60 minutes of bicarbonate haemodialysis, caused less increase of the mean change in C3d for all patients for each dialyser at 180 min than was present with a TMP of 100 or 200 mmHg.

This favourable effect during TMP 50 may be due to a protein-coating effect or due to a reduced cell-adhesion caused by less turbulence in the laminar flow. A low initial TMP during haemodialysis may allow a better forming of this protein coat on the membrane due to the laminar blood flow in the hollow-fibre dialysers and thereby reduce the reactivity of the membrane. An axial streaming of platelets reduces clotting [Vanholder & Ringoir 1989]. The rolling movement of leukocytes, as seen in the blood vessels [Springer, 1994], may be influenced by a low initial TMP and an improved laminar flow.

In membranes such as cuprophan the surface potential is neutral and protein adsorption is less than with synthetic membranes [Cornelius & Brash 1993; Hakim, 1993]. High molecular-weight protein, mainly albumin, can be eluted from cuprophan membranes after dialysis [Françoise Gachon et al. 1991], and this protein coat may reduce the membrane reactivity. This coating could be similar to the effect seen in reused dialysers, where less complement activation is seen [Shusterrman et al. 1989], probably due to binding of C3b-like molecules [Basile & Drüeke 1989].

There were no differences between the membranes at 15, 60 or 180 min when using a TMP of 50 mmHg, even when using the synthetic membrane polycarbonate, which is known to be more biocompatible than cuprophan. [Henderson & Chenoweth 1987; Martin-Malo et al. 1991]. This illustrates the importance of the blood-membrane interaction by factors other than just the material. The greater complement activation with plate dialysers may be due to deformability of the membrane by increased transmembrane pressures. This
theory may be strengthened by the fact that when starting haemodialysis with 200 mmHg, C3d was significantly higher already after 60 min by both types of plate dialysers (cuprophan and polycarbonate) as compared with the steam-sterilized hollow-fibre cuprophan dialyser. However, the distribution of the blood flow probably does not reduce the membrane area, since urea clearance by the cuprophan plate at TMP 200 mmHg was higher than at TMP 50 mmHg. This may instead indicate an increased contact between blood and the membrane due to an increased TMP.

In conclusion, a low initial transmembrane pressure reduces complement activation measured as C3d, while increased TMP potentiates the differences between the membranes. A steam-sterilized hollow-fibre cuprophan dialyser seems preferable to the ETO-sterilized one of the same design and compared with plate dialysers made of either cuprophan and polycarbonate.

These data point to the different extents of complement activation depending on the initial TMP during dialysis. This aspect must be considered when individual ultrafiltration profiles are more frequently performed.
GENERAL CONCLUSIONS

These investigations were performed to study various factors which could affect the blood-membrane interaction in the treatment of haemodialysis patients. The aim was to detect factors which influence biocompatibility and thereby be able to reduce the patients side-effects.

The following conclusion can be drawn from the present studies:

The hollow-fibre cuprophan dialyser induced less complement activation, measured by C3d, than the plate dialyser made of the same material. This indicates the importance of the dialyser configuration and not only the membrane material. When comparing membrane material the data favoured the polycarbonate membrane in comparison with the cuprophan plate.

Patients dialysed with bicarbonate had increased baseline levels of complement split products. The leukocyte reduction and the increase in C3d were greater at 15 min during dialysis with bicarbonate as compared with acetate as dialysate base. The differences in C3d between the dialysates were confirmed using both different cuprophane dialysers and two hollow-fibre dialysers made of Hemophan and cellulose acetate. This supports the concept that the dialysate influences the degree of complement activation although the side-effects were greater in the acetate group. The sterilization methods also influenced the change in C3d, favouring steam as compared with ETO, but this was only seen when using bicarbonate as dialysate. A group of patients with signs of backdiffusion during bicarbonate dialysis had a greater complement activation than patients without backdiffusion and acetate as dialysate. The flux across the membrane may influence the complement activation. A low initial transmembrane pressure of 50 mmHg during the first 60 min of haemodialysis seemed to reduce complement activation as compared with a higher one. This could be explained by a protein coating effect or less cell adhesion to the membrane. Since the haemodialysis patients are exposed to dialysis several times a week the effects of additional blood-membrane interaction can accumulate over time. Such addition seems to occur by leukocyte-filtered blood. These filters consist of different materials and composition. The polyester filters influenced the blood cells in different ways. Even if no complement activation was found (probably due to citrate), activation events such as haemolysis and loss of both erythrocytes and platelets occurred inside the filters.
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REFERENCES


Floege J, Bartsch A, Schulze M, Shaldon S, Koch KM, Smeby LC. Turnover of $^{131}$I-$\beta_{2}$-m in hemodialyzed patients J Lab Clin Med 1991;118:153-165.

Gagnon RF, Kaye M. Hemodialysis neutropenia and dialyzer reuse: Role of cleansing agent. Uremia Invest 1984;8:17-23.


Mollnes TE. Early- and late-phase activation of complement evaluated by plasma levels of C3d,g and the terminal complement complex. Complement 1985a;2:156-64.


