CHAPTER 1

INTRODUCTION
group at high risk of systemic inflammatory response after cardiac surgery supplemental pentoxifylline treatment did not reduce mortality.\textsuperscript{16,17}

Ultrafiltration techniques are used to restore the intraoperative fluid balance and to reduce the inflammatory response. This approach is based on the idea that ultrafiltration removes factors that trigger the inflammatory response. Ultrafiltration has found a place mainly in paediatric cardiac surgery where it has been shown to reduce body water, and to increase the haematocrit.\textsuperscript{18} In adults however, the effects are less clear and in a recently published study comprising 3,988 patients who underwent coronary artery bypass grafting, it was suggested that ultrafiltration may have adverse effects on operative outcome.\textsuperscript{19}

Fat also contributes to postoperative tissue injury. Fat microemboli have been demonstrated in brain tissue after cardiopulmonary bypass.\textsuperscript{20} These microemboli were related to the retransfusion of cardiotomy suction blood,\textsuperscript{21} and were associated with postoperative neurocognitive dysfunction. In addition, the role of fat on tissue injury is underestimated, because fat microemboli have not only been demonstrated in brain tissue after cardiopulmonary bypass, but also in lung and renal tissue.\textsuperscript{22,23}

Cell savers are increasingly used to process cardiotomy suction blood, but these devices might be less than ideal for several reasons. First, fat is not completely removed by cell savers. Second, their use is expensive and requires attention and time to process. Third, processed cell saver blood contains increased levels of interleukin-I and activated leucocytes, which may aggravate the inflammatory reaction associated with cardiopulmonary bypass.\textsuperscript{24} Kaza et al. found that cell savers were not more effective than a filter after the cardiotomy reservoir for the elimination of small and large fat emboli.\textsuperscript{25}

Therefore leucocyte and fat depletion by means of a filter may offer a good and practical alternative to modify the postoperative inflammatory response in cardiac surgical patients. The aim of this thesis is to demonstrate that leucocyte and fat filtration, applied in the setting of cardiac surgery, has a beneficial effect on inflammatory markers and postoperative organ injury.

2. History of leucocyte filtration

Various aspects of leucocytes as markers of infection were already known in the beginning of the 20\textsuperscript{th} century. For example, Gibson, a surgeon, wrote in 1906 in the first clinical paper on leucocyte differential count that “... the differential blood count and its relation to the total leucocytosis is today the most valuable diagnostic and prognostic aid in acute surgical diseases that is furnished by any of the methods of blood examination.”\textsuperscript{26} In 1928, Fleming, a pathologist, was the first to use a cotton wool plug as a filter for the removal of leucocytes from blood.\textsuperscript{27} His apparatus is shown in figure 1, and consists of a bend glass tube with a constriction. Cotton wool was introduced in the constricted limb of the tube and pressed down as tightly as possible with a cork-borer. Blood was placed above the cotton wool and under pressure forced through the cotton wool with a teat. The aim of Fleming was not the modification of the patient’s inflammatory response, but he needed leucocyte depleted blood as a diluent for certain tests in connection with the antibacterial power of leucocytes. We demonstrate his apparatus, because the compressed cotton wool used in his filter resembles the structure of a modern depth filter and the pressure applied by the teat equals the pressure that may be generated to force blood through modern leucocyte depletion filters.
By the end of the second World War more was known about function and properties of leucocytes. It became clear that it was undesirable to have white blood cells in transfusion components. Thus, the history of leucocyte filtration has been closely related to the history of blood transfusion. From the beginning of transfusion medicine blood clots and debris were observed in transfusion bottles. This remained the case even after the introduction of citrated blood in 1914 by Hustin.\textsuperscript{28} It was therefore necessary to filter the blood just before transfusion. This was initially accomplished by pouring the blood over gauze swabs, which was not only a cumbersome and messy undertaking, but also resulted in contamination of the blood. However, in 1939 several types of filter were in routine use, all allowing sterile processing of the blood.\textsuperscript{29} These filters consisted of glass beads in a glass cylinder and were to be attached in line to the tubing of the transfusion system.

In the second World War stainless steel wire cloth incorporated in a glass cylinder was used.\textsuperscript{30} Although these filters were intended to be disposable, their cost was high. They were therefore often cleaned after use, which was of course not easy. From then on the filtering of the blood became rapidly more sophisticated and less expensive, and from 1950, 230\textmu m disposable filters were standard employed throughout the world.

In 1961 Swank accidentally made an important observation while studying blood viscosity in a model of small blood vessels. His observation served as a milestone for the rapid development of transfusion related filtration techniques, because it demonstrated the importance of leucocyte removal. He found, using a microfilter as model (figure 2), that very high pressures were necessary to force blood that was stored in acid-citrate-dextrose for 2 to 10 days through the filter. Microscopic examination of the filters revealed that many openings were occluded by debris and aggregates of platelets and leucocytes.\textsuperscript{31} Swank then passed the old blood through a glass wool filter and found that after this procedure the pressure to force the blood
through his experimental micro-filter was similar to that of fresh blood. Most of the aggregates in the glass wool filter were less than 50 μm in size on microscopic examination and he called them micro-aggregates. A second observation was that plasma, that had been spun free of white blood cells and platelets did not require high pressure to force it through the microfilter, even after storage for 10 days. On the other hand, plasma containing leucocytes and platelets exhibited the same filtration problems after storage for 10 days as shown by whole blood. Dacron and polyester filters in wool form also removed the micro-aggregates effectively. In the late 1970s specific filters for routine leucocyte removal in clinical practice have been developed.

3a Leucocyte depletion filters

The first generation of leucocyte depletion filters for routine use that became available in the 1970s were made of cellulose and had a leucocyte removal rate of about 98%. They were developed for blood bank use to obtain leucocyte depleted blood for specific purposes such as the prevention of non-haemolytic transfusion reactions and viral transmission. However, these filters appeared also to activate complement C3, which promotes vasoconstriction and increases capillary permeability. A second drawback was that the efficacy of leucocyte removal was determined by the flow over the filter. Filtration was therefore a slow process and took about 30 minutes for one unit of red blood cells.

Over the last years however, a new generation of filters has become available which combines rapid flow with an excellent leucocyte removal rate. These new filters remove 99,995% of the leucocytes from the blood, but for cardiopulmonary bypass perfusate this is somewhat lower with a 96.8% removal of leucocytes. The improved flow properties allow them to be used in settings with higher fluid requirements. For an explanation of these improved flow properties, some aspects of the biomaterials and design that are used will now be discussed.

The design of a leucocyte depletion filter is of course a compromise between several properties. At this moment depth and screen filters are used. In depth filters, the filter material has the form of compressed wool fibres. These filters are made of polyester or sometimes polyurethane, and promote adhesion of the leucocytes throughout the filter material. In contrast, screen filters consist of layers of woven polyester filter material. In this type of filter the leucocytes are bound to subsequent
layers of filter material. Most leucocytes are thus trapped at the outermost portion of the filter and this may increase the resistance over the filter.

The way leucocytes are trapped inside the filter influences filter efficacy and capacity. At least 4 active and passive mechanisms have been described (figure 3). The most important mechanism is adhesion. The negatively charged leucocytes are attached to the filter material by Van der Waals- and electrostatic forces. It is, therefore, an active process from the side of the leucocytes. The advantage is that a larger pore size is possible in the filter with subsequent higher flow rates. Passive mechanisms of leucocyte entrapment are blocking, bridging and interception. By blocking, the leucocyte is trapped in a pore between two fibres. By bridging, 2 or more leucocytes form an aggregate in a pore between two fibres. By interception, leucocytes are mechanically trapped in the dead space around the fibres. All these mechanisms may occur together.

Thus, properties of the filter material like surface charge and hydrophilicity greatly determine the efficacy of the filter. Therefore, coating of the filter material is often used to improve the filter efficacy. A coating of methacrylate creates a more positive surface charge that results in a stronger bond with the negatively charged leucocytes. Hydrophilicity is important for optimal contact between the leucocytes and the fibres and thus for the subsequent adhesion. This implies that optimal leucocyte depletion can only occur if the whole filter is exposed to blood, which means that de-airing before use must be carefully performed. Insufficient de-airing results in disturbances of optimal blood flow and thus in a reduction of filter efficacy.

Another effect of the physicochemical properties of the filter material is that leucocytes appear predominantly to stick to the crossing points of the filter fibres. Thus, more crossing points increase the efficacy of the filter. More crossing points require thinner fibres. However, thinner fibres also lead to an increase in resistance and thus to flow reduction.

The filter capacity depends on the construction of the filter, i.e. the available surface and the thickness of the filter. A simple rule is that the log of the leucocyte reduction in the filter relates to the thickness of the filter material. The current generation of leucocyte depletion filters may be pressurized up to 300 mmHg. This allows rapid transfusion in a clinical setting, but decreases the efficacy as it has been shown that a longer contact time of the leucocytes in the filter increases the filter efficacy.

![Figure 3. Diagram of proposed mechanisms of leucocyte retention in a depth filter according to Bruil. A, blocking; B, bridging; C, interception; D, adhesion. (Bruil A; Leucocyte filters. Thesis TU Twente; 1993)](image)
The efficiency of the filters decreases over time as the filter becomes saturated with cells and debris.\textsuperscript{37,38} For a blood cardioplegia filter this results in a pressure gradient of about 10mmHg at a mean flow of 300 ml.min\textsuperscript{-1}. In up to 2500 ml about 75\% of the leucocytes were removed.\textsuperscript{39}

One major drawback of the leucocyte depletion filters is the concomitant removal of platelets. Thus, application of leucocyte depletion filters may interfere with blood coagulation. This is a problem of all leucocyte depletion filters as generally 40\% of the platelets that pass through the filter are trapped.\textsuperscript{40} Allen et al. demonstrated a significant difference in platelet counts by drawing samples simultaneous up- and downstream of the filter.\textsuperscript{41} Nonetheless, a certain platelet deposition on the polyester fibres of the filter facilitates the adherence of the leucocytes.\textsuperscript{42} It appears that platelets have a higher affinity for the filter material than leucocytes,\textsuperscript{43} while platelets have active surface receptors so that they rapidly establish a strong bond with leucocytes.\textsuperscript{44} Another aspect of the removal of platelets is that platelets also are responsible for the release of different vasoactive substances. Therefore, removal of platelets could also lead to a reduction in thromboxane release and therefore to a reduction in vasoconstriction.\textsuperscript{45}

3b. Fat removal filters

Little is known about the clinical filtration of fat emboli. As a consequence, part of the technology for fat filtration is derived from the diary food industry and water cleaning processes. For example, hydrophobic cotton fibres, which are obtained by acylation of cellulose, have a high selective affinity for fat in an aqueous medium.\textsuperscript{46} They are used for water processing, but resemble the first generation of leucocyte depletion filters. Fat filtration appears to be technically difficult, because the process is temperature dependent. A fat removal filter removed 50\% of the fat load at 37°C which increased to 80\% at 10°C, due to the increased viscosity of the fat. However, at lower temperatures, haemolysis of the blood and clogging of the filter were observed.\textsuperscript{47}

A standard polyester arterial line filter with a 40 μm pore size does not seem to filter fat during cardiopulmonary bypass.\textsuperscript{48} However, passing cardiotomy suction blood through a standard 30 μm cardiotomy suction filter and subsequently through a 21 μm arterial line filter almost completely eliminated fat.\textsuperscript{25} These findings suggest that the filter pore size is a major determinant for fat filtration. In 1973, Arrants et al. used an ordinary blood filter with a pore size of 35-40 μm, but they could not demonstrate a clinical effect.\textsuperscript{23} Thus, a small pore size is necessary and supports the concept that fat globules are highly deformable.

Based on the work of Swank, Hill et al. used a dacron wool filter in the cardiotomy suction line.\textsuperscript{49} This resulted in a reduction in postoperative cerebral dysfunction. Clark et al. studied the fat filtration characteristics of a packed polyester wool filter.\textsuperscript{50} They found that significant quantities of solids, two thirds of which were fat, were removed by the filters. Their findings about the efficacy of polyester for the removal of fat are in line with a more recent study evaluating a specific fat removal filter.\textsuperscript{51} This filter is also made from polyester fibers, but contains less filter material to improve the flow properties than the leucocyte removal filter from which it was derived. However, a specific coating of the filter material should compensate for this. In a laboratory study with reconstituted outdated blood and soya oil, the filter removed fat, but was less effective than a leucocyte depletion filter.\textsuperscript{51}
This fat removal filter was also used in conjunction with a leucocyte removal filter for processed cell saver blood. This combination again emphasizes the small pore size that is needed for fat filtration. However, a small pore size reduces the flow properties of the filter. Better coating of the filter fibers may offer an alternative. Therefore, sorbent technology which is currently used in dialysis filters, may hold promise for the future as a result of improved coating techniques.

4. What is the evidence that leucocyte and fat depletion filters are beneficial during cardiac surgery?

In the 1980s attention was focussed on what happened during ischaemia and reperfusion in organs. Engler et al. demonstrated in dogs that the myocardial stunning which was observed after occlusion of the left anterior descending coronary artery up to 5 hours, resulted largely from reperfusion injury. They observed during reperfusion an incomplete restoration of the blood flow in the microvasculature of the heart. This so called no-reflow phenomenon was associated with capillary leucocyte plugging and endothelial cell protrusion and was based on an acute inflammatory response. This revealed a central role for leucocytes and Engler et al. hypothesized that leucocyte depletion might be beneficial in the setting of ischaemia and reperfusion. They tested this hypothesis in their dog model and found that reperfusion with leucocyte depleted blood almost completely prevented reperfusion injury. In addition, leucocyte depletion prevented the increases in tissue water content seen in control hearts and decreased the incidence of ventricular arrhythmias. Shortly after these observations, Kutsumi et al. applied leucocyte filtration clinically in the setting of ischaemia and reperfusion. After percutaneous transluminal coronary angioplasty (PTCA) they drew blood from the femoral vein which they passed through a leucocyte filter before it was injected in the coronary vessels. This procedure resulted in a significant reduction in reperfusion arrhythmias.

These findings on reperfusion injury stimulated research in the setting of heart and lung transplantation. Here, the beneficial effects of leucocyte depletion were quickly recognized and gradually found a clinical application during lung transplantation, and as an adjunct to blood cardioplegia in the setting of cardiac transplantation. Preliminary studies in dogs after cardiac transplantation indicated that reperfusion with leucocyte depleted blood increased stroke work and cardiac output, and were soon followed by clinical studies that showed minimal histological changes and lower myocardial creatinine phosphokinase levels after reperfusion with leucocyte depleted blood. However, clinical effects in terms of cardiac function were less clear.

As a result of the effects of leucocyte depleted reperfusion during cardiac transplantation, blood cardioplegia filters were developed. Only the target organ is depleted, while total body and side effects are minimal. This stimulated research in the setting of ischaemia and reperfusion and clinical studies were extended to emergency cardiac operations in patients who developed an acute myocardial infarction. Sawa et al. applied leucocyte depleted blood cardioplegia in elective and emergency patients. The results of leucocyte depletion were better in emergency patients than in elective patients. Lower peak myocardial creatinine phosphokinase levels were measured in the emergency patients and less dopamine was required at weaning off cardiopulmonary bypass. This study was extended to patients with left ventricular hypertrophy, defined as a left ventricle mass >300 g, to investigate if
reperfusion injury was attenuated by the application of a leucocyte depletion filter for blood cardioplegia that was administered for the first 10 minutes after aortic cross clamp release. Left ventricular biopsies had significantly lower scores for myocyte damage and for endothelial cell damage of capillaries in the leucocyte depleted group. The leucocyte depleted group also had lower myocardial creatinine phosphokinase levels, and needed less dopamine for weaning off cardiopulmonary bypass. No side effects of leucocyte depletion were noted. In a subsequent study the patients with left ventricular hypertrophy were divided in a group with a long and in a group with a short aortic cross clamp time. The effects of leucocyte depletion were more pronounced in the group with the longer cross clamp times. Roth et al. also studied patients with depressed left ventricular function, using serial leucocyte depletion filters in the blood cardioplegia line. Less dopamin was needed in their filter group, that also showed an increased left ventricular ejection fraction. Another interesting thing in this study was the use of two blood cardioplegia filters in line. The authors wanted to achieve a high degree of leucocyte depletion and felt that the documented efficacy of a standard blood cardioplegia filter was too low. We also found that the efficacy of a leucocyte depletion filter was lower if cardiopulmonary bypass perfusate was used.

These findings indicate that leucocyte depletion is more beneficial in patients that are clinically in less favourable circumstances. It also indicates that the systemic effects of leucocyte filtration are minimal.

A word of caution should be made with regard to the proper interpretation of the clinical results in the earlier studies. At that time only transfusion filters and cell separator technology were used for leucocyte depletion. The efficacy of these technologies is less than the efficacy of the current generation leucocyte depletion filters. Thus, the clinical effects may have been underestimated in the past.

Another important question that had to be resolved was how long leucocyte depletion should be performed in order to prevent reperfusion injury. This question was addressed by Breda et al. They studied lung preservation in a rabbit model, and found that reperfusion with leucocyte depleted blood preserved lung function. Then they added again leucocytes to the perfusate and found that the addition of leucocytes after one hour of reperfusion did not cause significant injury to the lung.

In contrast with the findings in isolated organ perfusion, Bando et al. were the first to report a favourable systemic effect of leucocyte filtration. They found in dogs, subjected to cardiopulmonary bypass, a reduction of free radicals and a preservation of pulmonary function by the application of a leucocyte depletion filter in the cardiopulmonary bypass circuit. These findings were repeated by Johnson et al. who also studied dogs during cardiopulmonary bypass, using a bubble oxygenator. They found an improvement in pulmonary shunt in the filter group. In addition, histological investigation of the lungs after cardiopulmonary bypass revealed lower oedema scores in the dogs that had leucocyte depletion, which resulted in an improved gas exchange. These two landmark studies served as a starting point regarding the effects of systemic leucocyte depletion.

Most studies on systemic leucocyte depletion during cardiac surgery agree on the fact that filtration reduces postoperative leucocyte counts. However, there is disagreement about the clinical effects on pulmonary or cardiac function. Some studies reported a short term improvement in postoperative oxygenation. Palanzo et al. found an improved arterial blood oxygenation. With 100% oxygen, patients in the
leucocyte depleted group had an arterial oxygen tension of 54.9 kPa vs. 46.4 kPa in the controls. The postoperative time on the ventilator was with 9.2 vs. 13.3 hours also shorter in the leucocyte depleted group than in the control group.\textsuperscript{70} Johnson et al. reported a transient improved oxygenation in the leucocyte depleted group with a transient improved intrapulmonary shunt (19\% filter vs. 24\% controls), which was in agreement with their previous study on dogs.\textsuperscript{71} Lust et al. also found a slight improvement in arterial blood oxygenation.\textsuperscript{72} Others however, did not find an improved arterial blood oxygenation at all.\textsuperscript{73} It should be noted that in all these studies arterial line filtration during the whole period of cardiopulmonary bypass was used.

Several factors may explain the reported differences in filter efficacy. Amongst them are the timing and duration of the filtration procedure during the operation and the type of filter used. For example, leucocyte depletion is commonly achieved with a filter in the arterial line throughout the cardiopulmonary bypass period.\textsuperscript{37,70-74} However, using this procedure, high elastase levels have been demonstrated after the filter in the arterial line.\textsuperscript{73} Elastase is a marker enzyme for leucocyte activation. Mair et al. found elevated systemic levels of elastase by the end of the filtration period,\textsuperscript{74} and also Palanzo et al. could not demonstrate a reduction in systemic plasma elastase levels when applying leucocyte depletion in the arterial line of the cardiopulmonary bypass circuit.\textsuperscript{70} These findings suggest that the leucocytes which were trapped in the arterial line filter become extensively activated. However, it may also be possible that activated leucocytes are preferentially trapped inside the filter. This was first suggested by Thurlow et al. when they investigated the expression of antigens on neutrophils using leucocyte-associated monoclonal antibodies.\textsuperscript{75} In a subsequent small scale study in humans they concluded that the application of an arterial line filter did not result in a significant depletion of the leucocyte load during cardiopulmonary bypass, but that according to their indirect measurements of superoxide the activated forms of the leucocytes appeared to be depleted.\textsuperscript{76}

The good results of leucocyte depletion during reperfusion, the moderate results of leucocyte depletion with an arterial line filter during the whole cardiopulmonary bypass period and the limited capacity of the filters, prompted Hachida et al. to remove the leucocytes from the circulation by a systemic filtration procedure in a restricted but well aimed time span.\textsuperscript{77} They applied leucocyte depletion only in the reperfusion phase after aortic cross clamp release and reported an improved pulmonary index after 3 and 6 hours. Matheis et al. also applied leucocyte filtration in a short time period after aortic cross clamp release and found in the leucocyte depleted group a reduction in inotropic support and a reduction in troponin-T concentrations, indicating less myocardial damage.\textsuperscript{78} However, Baksaas et al., using a similar procedure, found a reduction in circulating leucocyte counts, but could not demonstrate clinical differences with their control group.\textsuperscript{79} They suggested that filtration during release of the aortic cross clamp was too late, because a large population of leucocytes was already activated at that time. Thus, timely and well defined periods of leucocyte depletion may offer an advantage over generalized procedures during the whole cardiopulmonary bypass period.

Does leucocyte depletion have effects on other organs? Tang et al. found a better renal function in the leucocyte depleted group in cardiac surgical patients.\textsuperscript{80} Davies et al. used an interesting approach.\textsuperscript{81} They removed platelets and leucocytes from patients by plasmapheresis preoperatively. This approach resulted in a reduction of postoperative blood loss, an improved pulmonary function and a
reduction in allogenic blood transfusion. However, their approach has not gained wide acceptance because of the costs and complicated logistics involved. Moreover, it is difficult to accept that a reduction of blood loss could be achieved in the absence of platelets.

From the studies mentioned in this review it may be concluded that leucocyte depletion has a beneficial effect on several clinical parameters. However, leucocyte depletion filters are still relatively little used in routine practice. One explanation may be that there are no large scale studies, which demonstrate the clinical effects of leucocyte depletion in terms of reduced organ injury and length of intensive care unit or hospital stay. There is only one study that comprises 100 patients and in this study it was found that leucocyte filtration applied during all stages of cardiac surgery reduced the inflammatory response after cardiopulmonary bypass, was cost-effective and resulted in a shorter hospital stay. However, this study was designed to compare several anti-inflammatory strategies and was not intended to make an in depth assessment of the effects of leucocyte filtration on perioperative organ injury.

The acceptance of fat processing may have a comparable course. Several studies suggest that the major source of the cerebral microemboli that occur after cardiopulmonary bypass is lipid droplets of the patient’s fat that drip into the blood in the surgical field. This lipid-laden blood is aspirated and then returned to the patient via the cardiopulmonary bypass circuit. This was the reason for Arrants et al. in 1973 to use a filter for the removal of fat. An ordinary blood filter with a pore size of 35-40 μm was inserted on either the arterial line of the bypass circuit or the cardiotomy suction line or both. Despite a uniform postoperative rise in blood lipids in all studied patient groups, the use of the filter was disappointing as pulmonary and neurological complications did not decrease in the filter group.

Hill et al. used a dacron wool filter in the cardiotomy suction line. They found a reduction in postoperative cerebral dysfunction which was paralleled by a reduction in cerebral microemboli on autopsy studies. Clark et al. studied the fat filtration characteristics of a packed polyester wool filter when used alone in the cardiotomy suction and in the arterial line during clinical extracorporeal circulation. The total lipid extracted from the cardiotomy filters averaged 376 ± 72 mg and from the arterial line filters 512 ± 95mg. They concluded that significant quantities of solids, two thirds of which were fat, were removed by the filters during cardiopulmonary bypass. Most of the fat was derived from the cardiotomy suction system. Recently, Kaza et al. placed an additional filter after the cardiotomy reservoir of the heart-lung machine and looked for circulating fat. There were no large emboli detected after the filter. However, neurocognitive outcome was not measured. In a study in orthopaedic patients after spine fusion, shed wound blood was retransfused either through a 40 μm pore transfusion filter or through a leucocyte removal filter. Leucocytes and fat were measured after the filter. It appeared that a leucocyte removal filter was very effective for the reduction of fat, but that use of a transfusion filter was ineffective.

Realizing that recycling shed blood with cardiotomy suction is an important source of cerebral fat microemboli, Jewell et al. performed a pilot study in patients undergoing cardiac surgery using a cell saver or unprocessed retransfusion of cardiotomy suction blood. They measured the circulating fat. Although they did not find a difference in the postoperative use of blood or blood products, haemoglobin, or bleeding between the two groups, they concluded that use of a cell saver resulted in less fat being recycled during cardiopulmonary bypass. The findings of this study are
in line with a previous study in dogs subjected to cardiopulmonary bypass. An arterial line filter, aimed to reduce leucocytes and fat, was compared with a cell saver, used to process the cardiotomy suction blood. Less cerebral microemboli were observed in the cell saver group. Interestingly, two different types of cell saver were used in this study, which resulted in measurable differences in cerebral microemboli. However, this was a small animal study and the results should be interpreted with caution. In another small clinical study, cardiotomy suction blood and the residual heart lung machine blood were processed by a cell saver. The processed cell saver blood was retransfused using a fat removal filter in conjunction with a leucocyte removal filter. This approach resulted in an improvement of pulmonary function in the filter group vs. unfiltered controls.

These studies suggest that techniques to reduce circulating fat are promising, but further clinical studies are needed to demonstrate effects on outcome.

5. Outline of the thesis

The next four chapters of this thesis consider leucocyte filtration, chapters 6-8 consider the concept and the effects of fat filtration.

Conflicting results have been reported regarding the clinical effects of leucocyte depletion using a leucocyte filter incorporated in the arterial line of the cardiopulmonary bypass circuit. In chapter 2 a new approach is described. We used a leucocyte depletion filter for the residual heart-lung machine blood that was retransfused in the patient after cardiopulmonary bypass. The hypothesis was that blood that is retransfused in a vein first passes the lungs. The lungs act as an endogenous filter and remove activated leucocytes and debris from this blood. If these activated leucocytes and debris were trapped in a filter this would result in less pulmonary injury and improved postoperative lung function.

In chapter 3 we used the same approach in children. The hypothesis was that the effects of leucocyte depletion would be larger than in adults as especially children with a cyanosis would have less possibilities to cope with oxygen radicals that cause postoperative tissue injury.

Chapter 4 addresses the important question whether a leucocyte depletion filter removes all leucocytes, or more specific the activated leucocytes. Depletion of only the activated leucocytes would be beneficial, because these cells play a major role in the reperfusion injury that occurs in the patient after cardiopulmonary bypass as explained in the introduction. In contrast, removal of all leucocytes may be harmful and raise concern about infectious complications.

In chapter 5 the effect of several leucocyte depletion strategies is compared in order to find the optimal approach for leucocyte depletion. The usual application of a leucocyte filter in the arterial line of the cardiopulmonary bypass circuit, the application of a filter in the retransfused heart-lung machine blood, and a novel approach via a venous bypass line in the cardiopulmonary bypass circuit are compared. This last approach was chosen because it would be easy to remove a filter after its use from the circuit during the cardiopulmonary bypass procedure. This is necessary because we feel that leucocytes that are trapped in the filter should subsequently be removed from the circulation to prevent the release of activation products.

Chapter 6 serves as an introduction to chapters 7 and 8 and gives an introduction into the concept of fat filtration. There is evidence that leucocytes, fat and particulate
all contribute to postoperative tissue injury. It may well be that the concept of leucocyte filtration has to be extended to include also fat and particulate.

In chapter 7 the clinical application of a recently developed fat depletion filter is evaluated. This filter was positioned after the cardiotomy reservoir of the heart-lung machine, and thus used for all the cardiotomy suction blood. This blood is known to contain a large amount of fat globules.

In chapter 8 the effects of the fat filtration filter on biochemical markers of brain injury during and after the operation are more closely investigated. Neurocognitive dysfunction is common after cardiac surgery and it is known that cerebral fat microemboli play a role in its origin.

In chapter 9 a summary and conclusions are given.

In chapter 10 a summary in Dutch is given.

References
1. Means to achieve a reduction of the inflammatory response in cardiac surgical patients

The generalized inflammatory response that occurs after cardiac surgery, elicits a combined reaction from at least the immune system, the coagulation system and the endothelial cell system. Activated leukocytes play a key role in this process by their interaction with the endothelium, by their interaction with the cardiopulmonary bypass circuit, and by their role in reperfusion injury, where they form platelet-leukocyte complexes. To minimize or even prevent postoperative tissue injury, it is thus attractive to target the leukocytes. This has been tried with various methods for nearly all stages of the inflammatory pathway. Currently, the most important strategies focus either on the prevention of leukocyte activation, or on the modulation of the inflammatory response, or on ultrafiltration techniques.

Leukocyte activation may be prevented with heparin coated bypass circuits, which reduce contact activation and possess an enhanced biocompatibility. However, in a large European trial, clinical benefits could not be demonstrated in low-risk patients. In high-risk patients, the use of a heparin coated bypass circuit reduced the time for postoperative ventilatory support and intensive care unit stay, but this could not be confirmed in another study.

Pharmacological agents may also prevent leukocyte activation. A serine protease inhibitor with a variety of actions, aprotinin, is frequently used. Aprotinin and prednisolone have been found to attenuate the generation of tumor necrosis factor and the upregulation of leukocyte adhesion molecules. A meta-analysis showed that aprotinin, besides a reduction in blood use, reduced perioperative mortality. However, two recently published studies could not demonstrate an anti-inflammatory effect of aprotinin, although postoperative blood loss was reduced. Due to the cost and to the sensibilisation that occurs in 5% of the patients, aprotinin is generally reserved for redo-operations.

Pharmacological agents are also used to modify the inflammatory response. For this purpose corticosteroids, and particular dexamethasone, are often used. Corticosteroids probably change the cytokine balance from proinflammatory to anti-inflammatory. Corticosteroids reduce leukocyte activation and pulmonary leukocyte sequestration. Although dexamethasone has been shown to decrease the concentration of C-reactive protein on the first postoperative day, clear clinical benefits in terms of postoperative oxygenation, time on mechanical ventilation, or intensive care unit stay have not been demonstrated. Use of dexamethasone may even be detrimental by delaying early postoperative tracheal extubation, and initiating postoperative hyperglycemia. In addition, there is concern about the systemic effects, for example the associated immunosuppression.

A relative novel pharmacological agent is the phosphodiesterase inhibitor pentoxifylline, which has recently been used in a porcine model of lung transplantation. In this animal study, the use of pentoxifylline was as effective as leukocyte depletion in preventing reperfusion injury. Several clinical studies are available in humans during cardiac surgery that also demonstrate an effect on pulmonary leukocyte sequestration after cardiopulmonary bypass. However, whether pretreatment with pentoxifylline will improve outcome in patients remains to be elucidated. Pentoxifylline pretreatment in cardiac surgical patients attenuated a postoperative deterioration of endothelial, renal, and liver function, but in a patient