1. Introduction

1.1 Historical Highlights

During the First International Symposium on Plasmapheresis held in Cleveland, Ohio (USA) in 1982, the International Society for Artificial Organs (ISAO) opened a museum to serve as an International Center for Artificial Organs and Transplantation. The museum located now in Houston, Texas, (USA), shows the efforts made during the last centuries and decades to help humanity by healing diseases in a very impressive way.
One can find a collection of instruments, pictures, and documents that focus on „the ancient medical belief that removal of a patient´s blood can also lead to the removal of his disease“ (2545). This simplistic concept of „bloodletting,“ or phlebotomy, although widely accepted in the prescientific era, could not, of course, be upheld in the light of modern medicine. Still, it appears to have returned to our clinical practice within the framework of new theories. Specialists use it to treat hemochromatosis, polycythemia, and acute heart insufficiency. Now, however, phlebotomy is more likely to bring to mind the phlebotomists who draw blood for testing in every hospital.
While the practice of plasmapheresis may also call to mind the ancient practice of bloodletting (1468), since blood content is removed from the patients' body for a period of time, the two therapy methods are, of course, based on entirely different theoretical bases and are used to treat conditions understood within the framework of modern medicine.

Figure 1: A motif of bloodletting on an old Greek vase (1893)

1.1.1 Bloodletting

Throughout human history, people have held an intuitive belief that impurities or poisons in the body cause disease. The priests of ancient Egypt, the healers of classical Greece and imperial Rome, the learned doctors of feudal Europe, all took for granted the necessity of cleansing the bodies of sick patients to get rid of foul and noxious matter. Thus for 3,000 years of Western civilization, physicians and healers required the sick to sweat; to drink copious amounts of potions to rinse their bodies; and to take emetics, purgatives, and enemas to cleanse their bowels. Finally, they were bled. The practice of bloodletting was based on a simple logical analogy: „You must first draw out the old and stagnant water from a drinking well to allow the fresh spring water to flow in“ (2545).

Since its origin the practice of bloodletting, also known as phlebotomy, has been marked by controversy, but proponents were quick to emphasize that little else could be offered as a remedy for disease. As the theories surrounding the procedure grew more complex, so did the devices used to perform it (1468).

More than six thousand years ago, bloodletting or phlebotomy methods for different diseases are mentioned in the Chinese medicine and three thousand years ago, priests in ancient Egypt began the use of bloodletting to treat many diseases (1467). The practice of bloodletting spread to Greece, where the art of medicine in the ancient world reached its prime between 500 B.C. and 500 A.D. (Figure 1). This creative period is symbolized by Hippocrates, known as the „Father of Medicine" (460–377 B.C.). He is known as the author of the Hippocratic Oath, whose principles still guide physicians today: "First, do no harm." Hippocrates brought the use of bloodletting as a way to prevent illness into Western medicine.

The Greek-Roman physician Galen (130–211 A.D.), was a pillar of medical practice. It was Galen who transmitted Hippocrates' teachings to the European medical tradition: both teachers and practioners accepted his medical teachings as dogma for fifteen hundred years. Galen supported bloodletting and cupping in light of the humoral theory, which held that illnesses of various types were caused by an imbalance of one or another of the body´s four humors: blood, yellow bile, black bile, and phlegm. According to this school, bloodletting was a means of correcting such an imbalance (459). It was thought that bloodletting would relieve an excess of blood in a patient and thereby improve his health.

An early 14th century Syrian painting depicts two Arab scribes sitting on top of a bloodletting device counting each drop of blood as it falls to the basin below (Figure 2). The inscription on the right says that they must alternately count three gram units of blood, called dirhans, until approxi-

Figure 2: Bloodletting device from the early 14th century (Syrian painting of 1315 A. D.) (1893)

Figure 3: Porringer, barber´s basin, and bleeding bowl – the trademark of the barbers (14th century) (1468)

mately 20 ounces of blood has been drained (1468). Pulleys are attached to the scribes' arms and move their pens as the basin fills with blood.

In medieval times, it was predominantly the barber surgeons who carried out therapeutic bloodletting (1468). They used special knives for the procedure, and the blood was collected in the famous „bloodletting bowl" which remains the trademark of hairdressers - the former barbers - in Europe (Figure 3) (1468). Special bowls to catch the blood as it flowed from a vein appeared for the first time in the 14th century. The earlier basins were made of clay or brass; later they were of pewter bowls. Some of the pewter bowls had gradations to measure the amount of blood removed. A variety of prewter bowls were used between the 17th and 19th centuries.

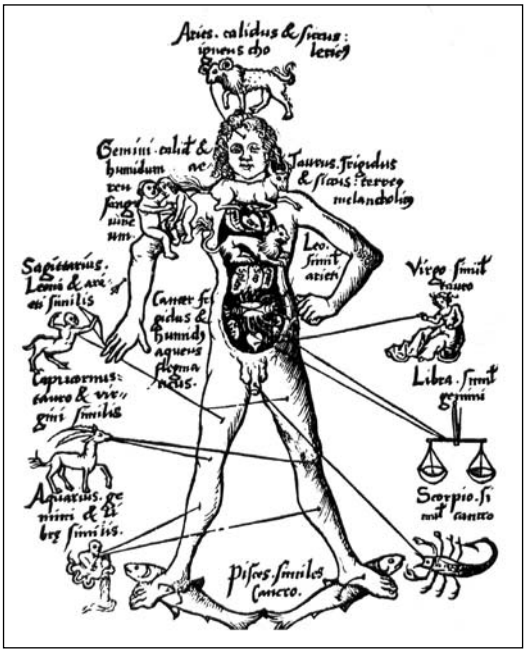


Figure 4: Zodiac man, an aide-memoire used from the 12th to the 18th century (1468)

Well into the late 18th century, the „bloodletting-man“ was widely used as an aide-memoire to recall when and for what illness to go to the barber for bloodletting (Figure 4). Around the figure one can see the signs of the Zodiak. They determine the optimal time for the cure of the various ailments in different parts of the body. Behind this image stands the ancient belief in a connection between different parts of the body and the stars and planets in the sky (459). During the Middle Ages, astrological influences played a part in deciding the best time to bleed a person suffering from a particular illness.
In the early 18th century, bloodletting and related procedures reached their height of popularity. With the increased knowledge of anatomy and hematology in the middle of the 19th century, bloodletting began to decline in the Western World. Figure 5 shows the technique and instruments of bloodletting used in the 18th century.

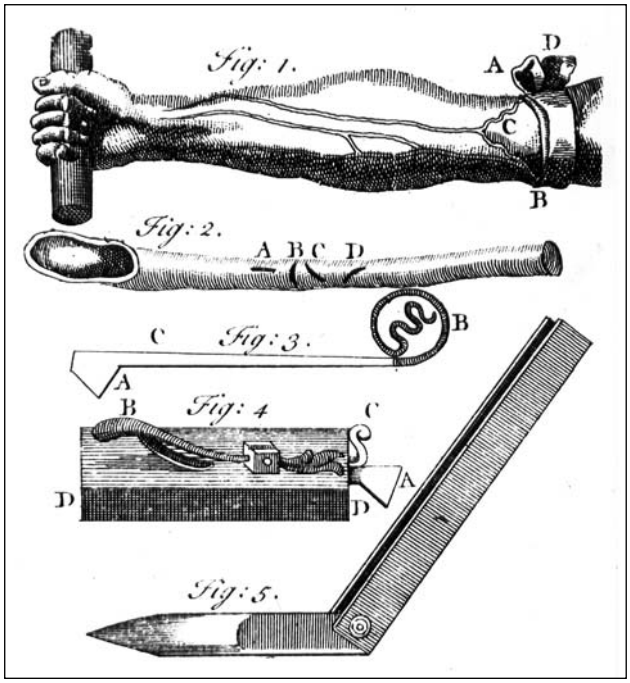


Figure 5: Techniques and instruments for bloodletting of the 18th century.
Fig. 1 illustrates the human veins. Fig. 2 shows the various types of incisions, Fig. 3 depicts a fleam, Fig. 4 shows a spring lancet, and Fig. 5 is an example of a French lancet (1468)

This possibility of treating illness by removing or exchanging the patient´s blood continued to engage medical doctors for centuries. This interest is best documented in a discussion between Samuel Pepys and Dr. William Croone during a meeting of the Royal Society, London, in November, 1666 (1744):

> *...a pretty experiment, of the blood of one Dog let out ... into the body of an other ... This did give occassion to many pretty wishes, as of the blood of a Quaker to be led into an Archbishop, and such like. But, as Dr. Croone says, may if it*

takes be of mighty use to man´s health, for amending of bad blood by borrowing from a better body.

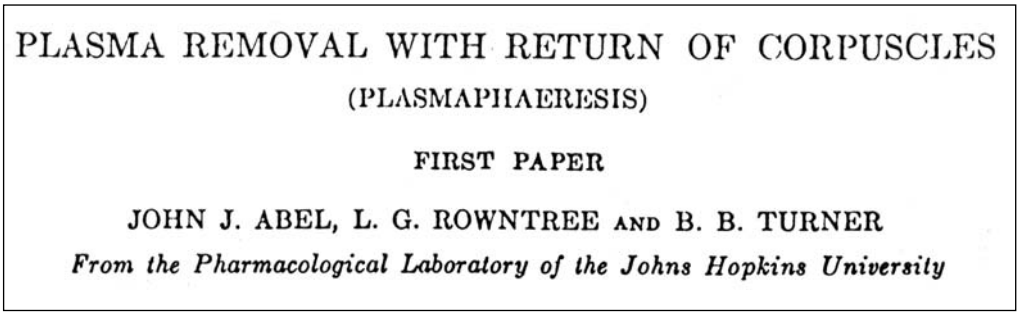
While bloodletting expressed the long-perpetuated theory of the humors over many centuries, there is another history to tell, that of the gradual series of advances – separated by centuries at first - the understanding of how the blood flows in the body and later, of its composition. British physician William Harvey (1578–1657) is known as the one who first grasped the nature of blood circulation. This breakthrough may not, have actually been his. During his travels as a young man, he rediscovered a rare manuscript describing the circulation, *Christianismi Restitutio*, which was written by Spanish physician Michael Servetus, almost a century previously (2750). Some also trace the earliest accurate description of blood circulation to the writings of a 13th century Muslim physician, Ibn Nafis. Harvey penetrated to further key insights through his own investigations and careful quantitative analysis. He performed many experiments on animals and realized that because of the quantity of blood required by the body, the liver could not make it quickly enough, and so had to recycle blood. He further distinguished between two loops made by the blood, that is, pulmonary and systemic circulation. Harvey also realized that blood moved in one direction and from this, grasped the function of the heart in pumping it throughout the body (459). His work represents a paradigm shift in the how the body could be understood and opened the door to modern medicine.
The late 19th century saw a series advances in the understanding and treatment of disease. Basing his work on Jenner´s cowpox vaccination from 1798, Pasteur developed killed and attenuated vaccines in 1881 (1467). The conceptual breakthrough of the germ theory of disease he introduced opened to door to subsequent insights. In 1885, Roux and Yersin presented work on bacterial toxins. Five years later, von Behring and Kitasato described antitoxins. Not long after, in 1983, Buchner introduced the concept of complement. The next year, Pfeiffer and Bordet extended this concept by identifying the action of complement in fighting bacteria (459).
At the turn of the century that Metchnikoff and Ehrlich introduced two perspectives on immune function at almost the same time. The understanding that white blood cells play an important role in immunity dates back to this moment. Ehrlich´s humoral theory focused the role of antibodies, chemical products of cells, in the body´s response to disease (796, 2055). His side-chain theory posited the existence of receptors on the cell surface that reacted to invasive entitites. Metchnikoff´s theory the cellular immunity emphasized the function of white cells in this response. He demonstrated the role of phagocytes in the body´s defense against disease. These two theories, both correct and complementary in their perspectives, laid the groundwork for modern immunology and its several lines of investigation. In 1887, Fodar established the idea of humoral immunity (1467).
The growing insight in the morphology of the blood finally led to the concept of removing only certain components of the blood, specifically the plasma, instead of the whole blood to treat diseases. In 1878, Laval developed in Sweden the first open continuous centrifuge to separate the blood cells from the plasma (1467), which was important for the developments in the 20th century.

1.1.2 Plasmapheresis

Hedon seems to have been the first to discuss the subject of plasmapheresis in 1902. He autotransfused body-own erythrocytes into rabbits (1223). The first therapeutic approaches in humans were taken by Fleig in 1909 (Table 1). He reported investigations of autotransfusions with washed blood cells in toxemia and hetero transfusions in anemia (920).
Five years later in 1914, Abel, Rowntree, and Turner studied the effect of plasma removal in dogs (5). They created the term Plasmapheresis (Figure 6). Two years earlier, these authors had proven the feasibility of dialysis in dogs. In 1926 Gilbert, Tzanck, and Negroni described plasmapheresis

Table 1: Plasmapheresis methods - Overview (I Plasmapheresis, II Selective Separation Methods)

	Author	Year	Ref.	Method	System
I	Hedon	1902	(1223)	Reinfusion of erythrocytes in rabbits	-
	Fleig	1909	(920)	Autotransfusion in humans	-
	Abel et al.	1914	(5)	Plasma removal in dogs	-
	Gilbert et al.	1926	(1026)	Plasmapheresis in humans	-
	Tui et al.	1944	(3098)	Plasma donation in humans	Centrifuge
	Grifols - Lucas	1950	(1106)	Plasma donation in humans	Centrifuge
	Greenwalt	1967	(1100)	Lymphocyte concentration, Antilymphocyte globulin	Double bag centrifugation
	Speiser	1967	(2867)	Plasmapheresis indications	-
	Judson et al.	1968	(1447)	Centrifuge plasmapheresis	Closed system
	Yamazaki et al.	1976	(3337)	Membrane plasmapheresis	Balancing system
	Nose`et al.	1976	(2219)	Membrane plasmapheresis	Balancing system
	Castino et al.	1976	(554)	Membrane plasmapheresis	Balancing system
	Glöckner et al.	1978	(1034)	Membrane plasmapheresis	Balancing system
	Bambauer et al.	1981	(143)	Membrane plasmapheresis	Single-needle technique with double head pump
	Cobe 1981	1981	(607)	Plate membrane separation	Cobe TPE system
	Fresenius	1983	(947)	Membrane plasmapheresis	2008 PF, acc. to Bambauer, Fresenius, Germany
	Bambauer et al.	1985	(167)	Membrane plasmapheresis for newborns and premature infants	Single needle with miniaturized double head pump
II	Terman et al.	1979	(3024)	Immunoadsorption	Centrifuge
	Agishi et al.	1980	(25)	Cascade filtration	Balancing system
	Malchesky et al.	1980	(1927)	Cryofiltration	Cryomax
	Nilsson et al.	1981	(2202)	Immunoadsorption (Protein A - Sepharose)	Closed system (later Citem 10)
	Borberg et al.	1983	(392)	Immunoadsorption	Anti - LDL sepharose columns
	Wieland et al.	1983	(3276)	Heparin induced LDL precipitation	HELP system, B. Braun, Germany
	Heininger et al.	1985	(1233)	Semiselective adsorption	Asahi, Japan
	Nose`et al.	1985	(2226)	Thermofiltration, elimination of cholesterol by plasma heating	Thermoregulating system
	Antwiller et al.	1986	(69)	Dextran sulfate induced LDL-precipitation	TPE system, Cobe, USA
	Mabuchi et al.	1987	(1903)	LDL adsorption by dextran sulfate	Kaneka system, Kaneka, Japan
	Knober	1987	(1605)	Photopheresis	Therakos, Johnson and Johnson, USA
	Lentz	1989	(1781)	Ultrapheresis cytokine inhibitors	Ultrapheresis system
	Pokrovsky et al.	1991	(2370)	Lp(a)-aphreresis	Anti-Lp(a) columns
	Bosch et al.	1993	(409)	LDL hemoperfusion	DALI system, Fresenius Germany
	Wallukat et al.	1996	(3218)	Immunoadsorption	Anti-human-immunoglobulin-ab columns
	Klingel et al.	2002	(1585)	Lipidfiltration	Asaki, Japan
	Otto et al.	2003	(2285)	LDL hemoperfusion	Liposorber D system, Kaneka, Japan



PLASMA REMOVAL WITH RETURN OF CORPUSCLES

(PLASMAPHAERESIS)

FIRST PAPER

JOHN J. ABEL, L. G. ROWNTREE AND B. B. TURNER

From the Pharmacological Laboratory of the Johns Hopkins University

Figure 6: Introduction to the "First Paper" on plasmapheresis by Abel et al. 1914 (5)

applied in humans (1026). Only 18 years later, this new therapeutic method of plasma removal would find further applications in human medicine.

In 1944 Tui, Bartter, Wright, and Holt carried out plasma separation using centrifuges for the first time. Their research showed that the donor could donate plasma up to four times per week or one time weekly over 2 hours with no significant effect on the composition of the blood. Since that time, it has become possible to produce fractions of plasma for transfusions (3098). In 1950 Grifols-Lucas established plasma collection on a routine basis in Lisbon (1106). In 1952 Adams and his colleagues for the first time successfully treated a patient with multiple myeloma by plasmapheresis (16). In 1967 Greenwalt isolated lymphocytes and anti-lymphocyte globulins by centrifuging blood in double bags (1100).

In that same year, Speiser reported on plasmapheresis in both healthy and sick patients, and established a list of indications for plasmapheresis (2867). Judson developed in 1968 the closed centrifuge which allowed the separation of plasma from cellular components in discontinous and later in continous blood-flow (1094, 1447).

During the mid-1970s, an additional process for plasmapheresis became available which used membrane modules instead of centrifuges. The first successful application of the so-called membrane plasma separation in humans was reported by Yamazaki et al., Castino et al., and Nose´ et al. in 1976 (554, 555, 2219, 3337). In Europe, Glöckner and Sieberth reported in 1978 for the first time the use of plasmapheresis with hollow fiber modules (1034).

The advantages of this method are a complete separation of the corpuscular components from the plasma and due to increased blood flow rate higher effacay (Table 2). Furthermore, cell damage - especially to thrombocytes - occurs less using membranes than centrifuges for cell separation (1143). The plasmapheresis equipment currently available are, however, not yet perfect, because the filtered plasma fractions have to be discarded. Substitution solutions, electrolyte solutions supplemented with human albumin, human serum protein solutions, or fresh frozen plasma are used to replace the discarded fractions.

Table 2: Advantages of membrane plasma separation

1) Simple method
2) Effective elimination of macromolecular substances
3) Complete separation of corpuscular components from plasma
4) Less cell damage, especially of thrombocytes

The development of new, more sophisticated membranes has allowed to new techniques, such as double and triple filtration (e.g., cryo-, thermo- and cascade filtration). These procedures allow for a more selective plasma component removal. Agishi et al. and Malchesky et al reported the introduction of the cascade filtration in patients in 1980 (25, 1927). Malchesky et al. noted possibilities with cryofiltration (1926). Nose´et al. developed in 1985 a method they called thermofiltration, which eliminated cholesterol by heating the plasma (1950, 2226). Table 1 gives a chronological overview of the various plasmapheresis methods.
The adsorption techologies allow the most selective separation of plasma components. In 1979 Terman et al. treated for the first time a 29 year-old female patient with lupus nephritis with extracorporeal immunoad-sorption (3024). Nilsson et al. described in 1981 a procedure for removing antibodies by extracorporeal protein-A-sepharose adsorption in hemophilia (2202). In 1983 Borberg et al. reported using immunoadsorption with anti-LDL-sepharose columns to eliminate LDL (392). In the same year, Wieland et al. described a method of heparin induced precipitation of LDL (3276). Heininger et al. published in 1985 their results with columns which contain material of polyvinylalcohol gel with covanlently bound tryptophan for the adsorption of anti-acetylcholine-receptor antibodies in the treatment of patient with myasthenia gravis (1233). Mabuchi et al. reported in 1987 the introduction of LDL-adsorption by dextran sulfate in hypercholesterolemia (1903).
In 1987 Knober reported the first extracorporeal photopheresis therapy for skin manifestation of cutaneous T-cell lymphoma (1605). Patients who underwent photopheresis were administered methoxsalen orally. The drug enters the nucleus of white blood cells and binds to the DNA. After centrifugation, the separated plasma and the lymphocytes are combined with saline and enter into the photopheresis machine, where a thin film of the lymphocyte enriched blood fraction passes through a channel between twin banks of high-intensity and long wavelength ultraviolet lamps. The drug is photoactivated and locked across the DNA helix and blocks the cells replication. After irradiation the blood cells and plasma are returned to the patient (781, 2596).
In 1989 Lentz reported the clinical effects of removing a middle molecular weight plasma protein fraction (less than 150,000) from the blood of patients with a variety of advanced metastatic cancers.This technique is designed to effectively remove proteins that are distributed not only in plasma but in the entire extracellular water compartment. This protein fraction when removed from the blood of pregnant animals caused the onset of the first stage of labor (1782). In cancer patients the removal of this protein fraction was shown to cause tumor specific inflammation and tumor necrosis. This protein fraction was also proven to contain soluble receptor/inhibitors to tumor necrosis factor and other pro-infammatory cytokines (991, 1781).
Efforts continue to develop new selective separation methods to eliminate specific solutes or groups of solutes from plasma or blood. One of these methods is LDL-hemoperfusion using a modified polyacrylate adsorber. The clinical use of this device was first reported by Bosch and colleagues (409, 410, 411). Another LDL-hemoperfusion system which was used by Otto et al., the Liposorber system contains columns with negative charged dextran sulfate covalenty bound to cellulose (2285). In 1996 reported Wallukat et al. the immunoadsorption in idiopathic dilated cardiomyopathy with anti-human-immunoglobulin-ab columns (3218). The development of a new lipid filter for membrane differntial filtration in the treatment of hypercholesterolemia was reported by Klingel et al. in 2002 (1585).

1.2 Definitions

The term *apheresis* is derived from a Greek root meaning to remove, grasp, seize, or take away. In modern medicine, apheresis means the use of a procedure to separate components of the blood, followed by removal of one or more of these components. Because of the different usage of the terms

associated with apheresis, the International Society for Artificial Organs (ISAO) established a committee in 1983 to define and standardize the terminology (1140). At the International Workshop on Plasma Separation and Plasma Fractionation held in Rottach-Egern, Germany on March 1983, the Committee on Nomenclature was convened with the two objectives of:

1. Clarifying and defining the nomenclature in areas where its meaning has become confused.
2. Attempting to standardize nomenclature in instances where numerous alternative expressions have developed to describe the same process (3163).

The Committee listed the definitions and preferred terminologies as follows:

1) Apheresis

The Committee agreed that for the reasons outlined above the form apheresis is preferred to pheresis.

2) Plasmapheresis / Plasma Exchange

The term plasmapheresis was widely used to describe the removal of plasma from normal donors for use in transfusions or for the preparation of components. Some authors have preferred to use plasma exchange for this process on the logical grounds that plasma is removed and exchanged for a normal substitute fluid. Therapeutic plasmapheresis or plasma exchange could be used interchangeably to describe this process (871, 1093, 1094, 2000, 2837). It may be helpful to differentiate centrifugal plasmapheresis from membrane plasmapheresis.

3) The Use of Compound Words with Apheresis

Operations in which formed components of the blood are removed may be carried out on either blood cells or plasma and its constituents. The following compound words conveniently described these processes:

– *Erythrocytapheresis:* the selective removal of circulating erythrocytes
– *Thrombocytapheresis:* the separation and removal of platelets as commonly carried out both as a preparative procedure in donors and therapeutically in patients with thrombocythemia.
– *Leuk(c)ocytapheresis:* a general term indicating the removal of white blood cells of all types.
– *Granulocytapheresis:* the selective removal of circulating granulocytes.
– *Lymphocytapheresis:* since the procedure involves the removal of lymphocytes rather than the drainage of lymph, lymphocytapheresis is the preferred term, the selective removal of lymphocytes.
– *Lymphocytoplasmapheresis:* the appropriate compound word to describe the simultaneous removal of lymphocytes and plasma.
– *Neocytapheresis:* describes the removal of young erythrocytes from the circulation by centrifugation.

4) Filtration Procedures

Plasma filtration has two meanings: first the removal of plasma from blood cells by a filtration process, and second, the modification by filtration of separated plasma. The second process is the one to which the term plasma filtration should logically be applied. The Committee suggested that the first process should more logically be referred to as *plasma separation by filtration*. The terms *hemodialysis, hemofiltration* and *hemodiafiltration* must be differentiated.

5) Plasma Cryofiltration

Cryofiltration refers to the process by which separated plasma is cooled to produce microaggregates and then passed through a second stage filter in which the microaggregates are removed.

6) Plasma Cascade Filtration
The term describes the use of two or more filters for the staged or differential fractionation of plasma.

7) Hemoperfusion
Is a procedure for the perfusion of whole blood through a column for the purpose of adsorption of a targeted component. This refers to the removal of components of plasma by circulating whole blood through columns or absorbent materials.

8) Plasma Perfusion
This term refers to the passage of plasma across materials which adsorb specific solutes. Examples include:
– *Immunoadsorption:* used to describe two processes which are essentially different. In one case, plasma containing antibodies may be perfused through a column bearing antigen in an attempt to reduce the level of antibodies in the effluent. In the other case, columns loaded with antibodies directed against plasma constituents have been used to lower the level of antigen in the effluent. The term immunoadsorption is used for both types of procedures. By analogy, the term affinity chromatography is used to describe the selective removal of either antigen or antibody.
– *Selective plasma adsorption:* a number of techniques are now available in which plasma components are removed by binding to ligands other than antigens or antibodies.

9) Selective Adsorption of Cellular Components
For a number of years, various techniques have been available and have been used in animal experiments. In recent years, techniques have come into use for removing subsets of circulating blood cells in patients. Thus, B lymphocytes can be isolated by binding them to immobilized antibodies or immunoglobulins. Such techniques will be used increasingly in patients. It has been suggested that they could be referred to as *selective B lymphocytes adsorption, selective adsorption of T suppressor cells*, and so forth.

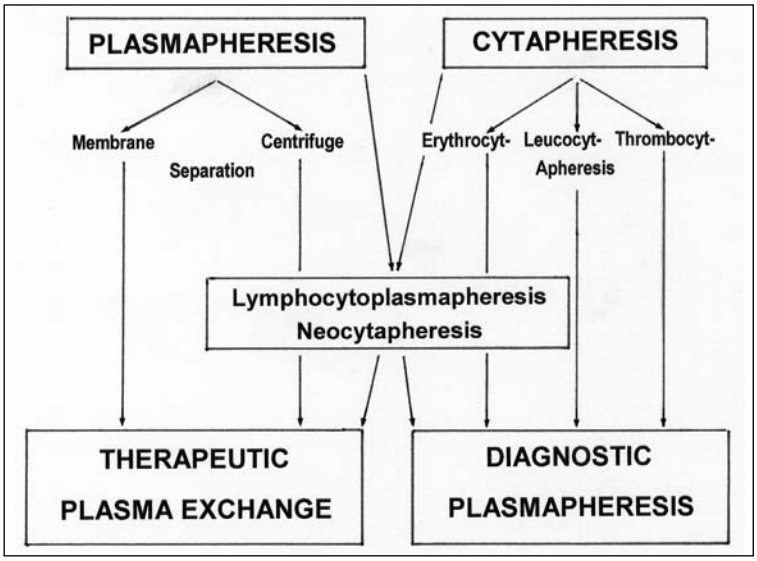


Figure 7: Schemes of Apheresis (238)

These procedures can be used either therapeutically to remove pathologic cells or solutes, or for collection to obtain plasma or blood cells from normal donors (1680) (Figure 7). The erythrocyte, leucocyte, and thrombocyte fractions are very important for therapeutics. In recent years, more and more erythrocyte-, leucocyte-, and thrombocytapheresis procedures are used and combined as therapeutic methods. Examples include the removal of pathologic cells in polyarthritis or reduction of B lymphocytes in transplant patients, as well as patients with progressive multiple sclerosis, and myasthenia gravis (48, 2531, 3128, 3211). Erythrocytapheresis, which reduces the levels of erythrocytes, is employed more and more in the treatment of sickle cell anemia, severe autoimmune hemolytic anemia, and severe parasitemia such as malaria and babesiasis (523, 556, 1534, 1573, 1710, 2740).Thrombocytapheresis is used in thrombocythemia (1530, 2408, 2981, 2982).
Therapeutic plasmapheresis can be carried out with membrane devices or with centrifugal cell separators. The separated plasma can be further modulated in subsequent filtration or adsorptions steps. Examples of secondary separation processes are cryofiltration, thermofiltration, cascade filtration, hydrogelation, adsorption, and immunoadsorption. Figure 8 displays these processes and their uses. Cascade filtration is a general term used to describe the use of two or more membranes staged for the differential fractionation of plasma. The membranes have different molecular weight cut-offs for separating the plasma into different fractions (25, 26).
Cryofiltration refers to the process by which separated plasma is cooled to a selected temperature not lower than its freezing point and filtered in the cold state. The separated plasma is warmed again and returned to the patient (1927, 2840). In thermofiltration, the plasma is kept or warmed above physiological temperature, then filtered. Thermofiltration is useful in removing total cholesterol, LDL, and VLDL from HDL in patients with hypercholesterolemia (1950, 2226). Hydrogelation is a method used with high flux dialyzers pre- and post the membrane separator to filter out water and low molecular substances (2243).
Other methods for plasma modulation are plasma adsorption techniques that adsorb various solutes. In these techniques, physicians typically combine the selective adsorption system with the primary plasma separation system (centrifuge or membrane) (393, 1953, 2231). Over the past several decades, physicians have developed and used sorbents in hemoperfusion as online plasma perfusion to treat drug overdose, uremia, liver insufficiency, autoimmune disorders, and familial and non-fa-

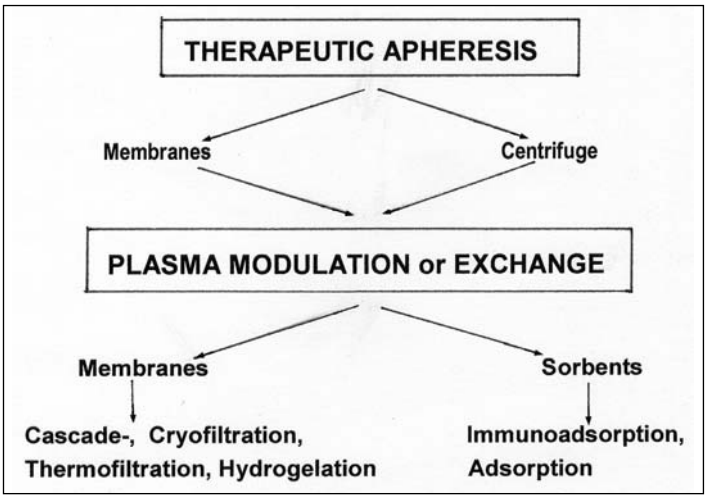


Figure 8: Possibilities for selective plasma separation methods (238)

Table 3: Sorbents for plasma adsorption (2611)

Ligand or Material of Adsorption	Agent sorbed
Polylysin methylated albumin	T 4 phage DNA
Anion - exchange resin Polyanion Dextran sulfate	 Bilrubin LDL
Heparin: heparin agarose	LDL
Tryptophan IM - TR	Anti - acethylcholine receptor ab, IC*, RF*
Phenylalanine IM - PH	Anti - MBP ab, IC, RF
Modified PVA* gel I - 02	RF, IC Anti - DNA ab Anti - RNP ab Anti - SM ab
Oligosaccharide	Anti - blood type ab
Charcoal	Bilirubin, creatinine, urea, potassium, amino acids
DNA	Anti - DNA Ab
Ag, blood - type ag Insulin Factor VIII Factor IX Anti - LDL ab	Anti - blood type ab Anti - Insulin ab Anti - factor VIII ab Anti - factor IX ab LDL
Ab anti - alpha feto ab	Alpha fetoprotein
Anti - HBs ab	HBs
Anti - IgE ab	IgE
C1q	IC
Protein A	IC, IgG, C1
sTNFRs, pro-inflammatory cytokine receptor / inhibitors	Anti-TNFR and pro-inflammatory cytokine receptor ab

* PVA: polyvinyl alcohol, RF: rheumatoid factor, IC: immune complex, TNFR: tumor necrosis factor receptor

milial hypercholesterolemia. Two major classes of sorbents are used: those that are immunologically selective, and those that are non-immunologically selective (1928). Immunologically selective sorbents are characterized by the presence of sites that are immunologically specific and recognize a particular immunoreactive region on circulating plasma factors. Non-immunologically selective sorbents are non-specific from an immunologic point of view and generally adsorb a broader range of plasma solutes. Table 3 outlines various ligands or materials for adsorption and the agents adsorbed. Most of these have been investigated clinically.

Plasma fractionation may also be carried out by other methods such as on-line precipitation of LDL or the globulin fraction of plasma (2843). Convective electrophoresis has also been investigated for

protein separation. Other on-line therapeutic processes include using bioreactors and various cellular devices in which biologically active materials are housed for plasma perfusion (2843).
Selective separation methods are generally not as widely available clinically but are used in the experimental setting. Such techniques hold much future promise.

2. Methods

2.1 Membrane Plasmpheresis

Until the development of hollow fiber membranes, therapeutic plasma exchange (TPE) was almost exclusively carried out by the centrifugal technique. The first centrifuges only worked in discontinous blood flow. Since the beginning of the 1970s, there have also been systems available working continously (387, 1093, 1370). These devices are mainly used today - aside from the conventional TPE and selective separation process - for the production of plasma fractions and blood cell concentrates. While in North America TPE is mostly carried out with centrifuges, since several years membrane plasmapheresis is used more and more. In Europe and Japan, membrane plasmapheresis is mainly used.
The membranes used for plasma filtration separation and fractionation may be distinguished from conventional dialysis membranes and high-flux membranes used in hemofiltration by their very high or select passage of plasma proteins. Membrane techniques are simple and safe to apply and can be competitive to other plasma separation and treatment technologies (1957, 2882). The advantages of membrane plasmapheresis include its simplicity to use with blood pumps and no observed white blood cell or platelet loss, compared with centrifuges.
The devices for membrane plasmapheresis have to be operated with the control of the transmembrane pressure. The hemofiltration systems can be used; however, several changes are still desirable. Since the filtration rate depends upon the transmembrane pressure (TMP), the deposition of blood cells, as well as their damage by shear rate, increases with TMP, one has to work with lower TMP values (mostly below 150 mm Hg) (585, 872, 1135, 1520, 2602) (see Chapter 2.2). Moreover, in TPE (2–6 liters), the fluid quantities which are to be exchanged are considerably lower than in hemofiltration (18–36 liters), where the primary objective is fluid removal alone. The computer-controlled and expensive hemofiltration devices, which many groups use for TPE, balance the continously filtered plasma quantity by means of a weighing mechanism (318, 1138, 1926, 2816, 2877). Now several special devices for TPE, cascade filtration, and adsorption techniques are available from different companies.
In the second half of 1979, Bambauer et al. introduced membrane plasmapheresis for immunological, neurological, and other diseases (143). Their work was based on many years of experience with extracorporeal circulation and with the treatment of acute kidney injury (AKI), as well as endstage renal disease (ESRD). At first, the treatments were carried out with a hemofiltration apparatus already available on the market. This apparatus worked according to the two-needle-technique. The technical expenditure for TPE was too high, however, so the system was simplified from the technical point of view. Figure 9 depicts this process.

2.1.1 Single-Needle-Plasmapheresis (SN-TPE)

As early as 1980, physicians adapted the single needle technique to plasmapheresis and simplified the sytem in the process. Investigators used a double pump in combination with a hollow fiber module, a pressure balancing system, and a heater pump (143, 144, 157, 173, 177, 189). Over a period of more than 25 years, this system was used in more than 20.000 treatments. In addition, two level-detectors were added to the system; therefore, the system could work on a semi-automatic principle (149, 157, 173). Based on this principle, Fresenius, Germany, introduced a compact commercial system to the market in 1982 (947). Now different devises for TA methods are on the market available.
First described by Ringoir et al. in 1973, the double pump works according to the principle of pressure-pressure and volume control (2489). It is controlled by a combined air- and level-detector and a volumetric control mechanism (Figure 9). The discontinous mode of action of both pumps pro-

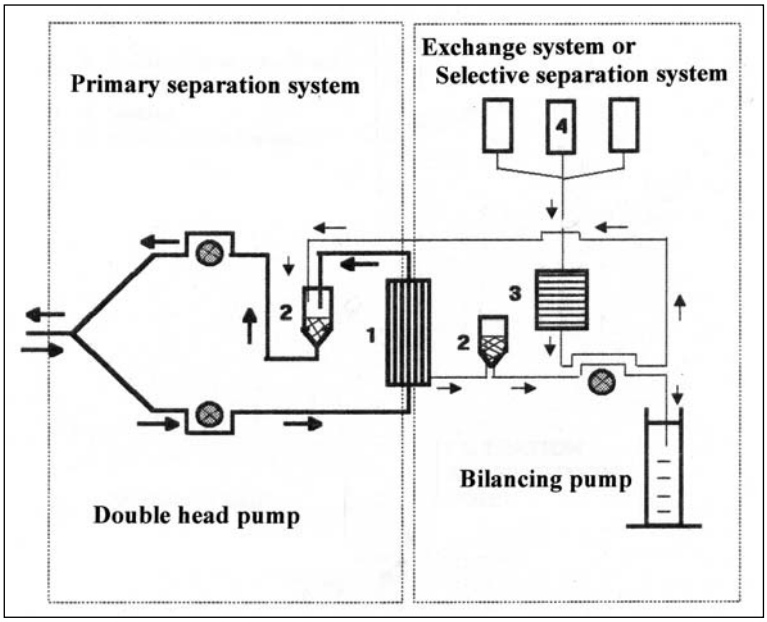


Figure 9: Principles of the single-needle TPE system (149)
(1:hollow fiber module, 2: monitor, 3: heater, 4: substitution solution)

vides for continous variations in flow rate and pressure. Venous backflow is controlled by a second pump. Thus, in contrast to other single needle systems using only one blood pump, in this system high transmembrane pressures are prevented (283, 1641,1644).

The main advantages of the double head pump unit are that:

1. *A relatively large flow rate of plasma through the plasma separator is achieved without the maximum pressure exceeding the safety limit.*
2. *The average pressure in the membrane separator can be adjusted to individual requirements.*
3. *Hemolysis can then occur at various pressures in different membrane separators.*
4. *The discontinous mode of operation of both pumps causes a permanent change in flow and pressure and delays the membrane fouling by blood cells.*

During SN technology no secondary layers are formed because of the continual changing of the transmembrane pressure. This means that mass transport in SN-TPE remains more constant. Therefore the average pressure (P_m) is calculated by Equation 1 (187):

$$P_m = \frac{P_{\min} + P_{\max}}{2} \qquad \text{Equation 1}$$

P_{min}: Minimum pressure
P_{max}: Maximum pressure

In general to prevent hemolysis using common membranes, the pressures noted below should not be exceeded (summarized by the different companies (222)):

Cellulose diacetate membrane	250 mm Hg
Polypropylene membrane	120 mm Hg
Polycarbonate membrane	150 mm Hg
Polyvinylchloride membrane	185 mm Hg

However, operation should be below these pressures to prevent cellular membrane fouling. The average blood flow rate, Q_{Bm}, can be calculated by the following equation:

$$Q_{Bm} = \frac{X + (X - U)}{2(T_1 + T_2)}$$ Equation 2

X: total volume of blood (ml) during the withdrawal phase
X – U: volume of blood (ml) during the return phase
U: volume of plasma (ml) removed
T_1: duration of withdrawal phase (sec.)
T_2: duration of return phase (sec.)

Three examples in Table 4 illustrade how Q_{Bm} changes when time intervals changes (175).These examples demonstrate that the main advantage of this system is that the Q_{Bm} rate is adjustable by regulating the pumps, independent of the blood return flow. Moreover, Q_{Bm} can be kept approximately constant, even in case of minor blood supply difficulties, by reducing the speed of the blood withdrawal pump and increasing that of the blood return pump.
In 1984, a small double head pump was developed which works according to this principle. It is especially useful in newborns and premature infants where blood volume is limited (Figure 10). Hemofiltration, dialysis, and plasmapheresis treatments may be carried out with such a small double head pump (220 x 255 x 95 mm). Plasmapheresis treatment requires a supplementary heater and balancing pump. With a dialysis system, single-needle hemodialysis treatments can be carried out also. The double-needle technique may also be used with this system (167, 176, 184). Special systems of tubing for the blood and filtrate lines, hemofilters, and plasma membrane separators are also available. Table 5 presents the technical data of the hollow fibers used are presented.
The blood flow must be at least 5–15 ml/min. The arterial and venous tubing system and the hollow fiber module may be filled with donor blood or fresh frozen plasma before the treatment is started. The total volume of the extracorporeal circulation is 30–37 ml (185, 187,190).

Table 4: Examples for the average blood flow Q_{Bm} in single-needle technique calculated by Equation 2 (222)

T_1 (sec)	T_2 (sec)	Average blood flow (Q_{Bm}) (ml/sec)	(ml/min)
5	10	2.49	149.4
5	5	3.74	224.4
5	2.5	4.98	298.8
Q_B = 500 ml/min. = 8.3 ml/sec. X = 41.5 ml (Volume during the withdrawal phase) X-U = 32.2 ml (Volume during the venous back flow rate: Withdrawal volume - plasma filtrate volume)			

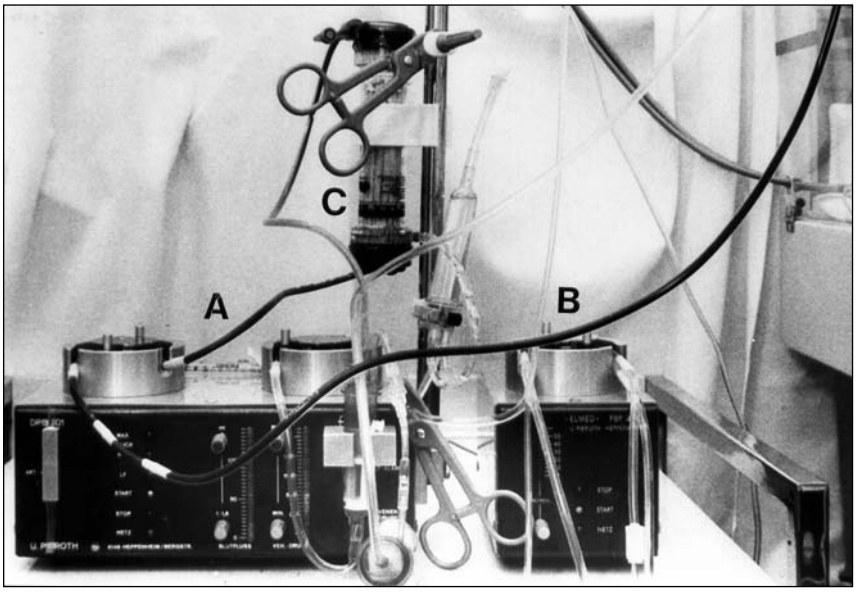


Figure 10: Double head pump unit for treatment of newborns.
(A: double head pump, B: balancing pump, C: membrane separator) (222)

Table 5: Technical data of hollow fiber units for plasmapheresis and hemofiltration in newborns (238)

Material	**Hemofilter⁺**		**Plasmafilter⁺**		
	Ultrafilter* U 2000 Polyamid	**Cardio* BC 16 Polyamid**	**PC - PE 500 * Polycarbonate Polyethylene Copopolymer**	**PP N ** Polypropylene**	**PN N 1000 ** Polypropylene**
Effective surface (m^2)	0.16	0.17	0.14	0.09	0.1
Effective length (mm)	115	120	115	120	105
Number of fibers	2,100	2,100	1,500	700	1,500
Fiber internal diameter (µm)	215	215	315	330	320
Wall thickness (µm)	60	60	70	150	150
Priming volume (ml)	8	8	10	13	15
Max. TMP (mm Hg)	600	600	300	150	150

* Gambro, Germany; ** Dideco, Italy; ⁺ not all hollow fibers are available

In systems for single-needle plasmapheresis, the balancing pump is connected to the blood return air trap of the double head pump through a tubing system. It can pump exact volumes of the substitution solution into this chamber. The filtrate is also pumped off with the substitution fluid through its own tubing. The amount of substitution fluid is equal to the amount of filtrate pumped off with the same balancing pump and over the same pump segments. In contrast, a complex hemofiltration apparatus requires an intricate computer-controlled weighing mechanism to establish the appropriate balance (Figure 9).
A second air trap is included in the filtrate line to prevent misbalance. Filtrate flow rates can be changed by adjusting the speed of the roller pumps and by modifying the transmembrane pressure. This enables the system to be adjusted to the required conditions. On commencing TPE, the filtrate flow rate depends on the blood flow rate and the transmembrane pressure (169).
Ringoir and his group described in 1984 the use of the double head pump membrane plasmapheresis with unipuncture (1727, 3143, 3148, 3158). Kopp et al. reported in 1984 their experience with a single-needle system (1643, 1645). This system is controlled like a pump unit, by micro processors described by Rath et al. (2432). The control value is the pressure of the venous return. If it decreases, the second pump of the double head pump compensates for it by supplying the needed volume. This ensures a relatively steady filtrate flow and avoids periodical *reverse filtration*, which is possible in case of inadequate transmembrane pressures in systems in which the membrane separator is installed between the pumps.
Chmiel et al. and Gupta et al. showed that continuous variations in transmembrane pressure and blood-flow (pulsative flow) lead to considerable retardation in the formation of a secondary membrane. Thus, applying the *pulsative technique* can increase the filtration rate by 25–38 percent compared to the *continuous flow technique* at the same blood flow rate (261, 586, 1133, 1134).
Galletti et al. showed that the properties of the inflow of blood had an influence on the filtrate flow, as did Bauser and Chmiel and Stairmand. These factors have a significant effect on the efficiency of TPE (261, 585 738, 980, 2886). Galletti et al. changed the mode of inflow from continous to pulsatile during the investigation to achieve a rapid increase of filtrate flows (980) (Figure 11). The increase of the filtrate flow is related to an increase in wall shear rate in the pulsatile mode (1792, 1793).

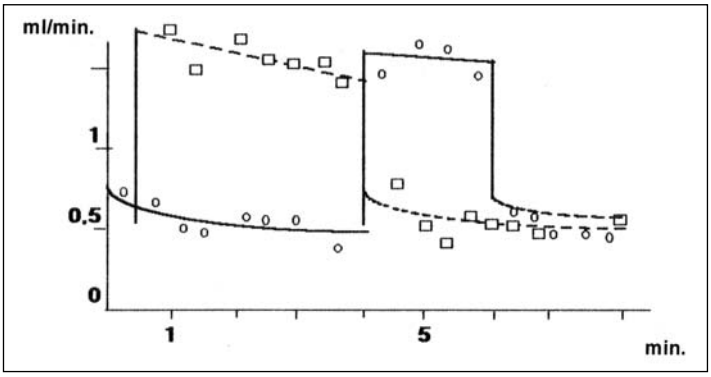


Figure 11: Description of the relationship between the flow-form of the influx, Q_a, (pulsatile-steady) and the filtrate flux, Q_f (222).
In this figure two sucessive experiments are described: Experiment 1: (o) Initially 4 minutes steady flow Q_a, then 2 minutes pulsatile flow (100 cpm), then again steady flow. Experiment 2: (□) Starting from a steady state achieved with a steady flow, then 3.5 min. pulsatile flow, afterwards again steady flow Q_a.The switch of the influx Q_a from steady to pulsatile led to a tripling of the filtrate flux.

Hombrouckx et al. have noted the possibility of hemolysis in single-needle dialysis caused by the roller pumps in the double head pump system, the needles used for access, and the relatively high recirculation rate (1317). Similiar conditions can be observed in TPE. This group developed a bidirectional blood pump. This pump has only one needle and one blood line. The blood is pumped through the needle and the blood line from the blood vessel toward the membrane module to an accumulation bag located above the module. The weight of the bag is monitored, and upon attaining a maximum weight, the pump changes direction and empties the blood from the bag through the module for a second passage back to the patient. The pump is controlled by a minimum and maximum weight of 100 g of blood (1318).
Several groups have studied the single-needle system with its double head pump, but further development and improvements of pump systems are necessary (143, 1297, 1318, 3145). Recently, new pump systems (pulsatile and nonpulsatile) for extracorporeal circulation have found use in clinical practice (434, 1643, 2410, 2412). We must improve on these developments, because by improving of the apheresis systems, physicians can pump the blood more easily, without any disturbance or interruption.

2.1.2 Other Devices for Plasmapheresis

2.1.2.1 Devices for Centrifugal Apheresis Techniques

Several devices are available for therapeutic centrifugal apheresis. These include various models from Haemonetics (30S, 50V50, PEX, V 50 Plus, PSC etc), Cobe (IBM Centrifuge), Fenwal (CS-3000 Plus Cell Separator), Baxter (CS 3000 Cell Separator), Fresenius (AS 104), Dideco (Vivacell BT 789/CE), and others. The devices operate under similiar principles, yet present some differences among them (1202, 1513, 2611).
The most important element of these separators is the disposable plastic bowl in which blood is separated into plasma and cell components. The new models are microprocessor-controlled. Anticoagulated whole blood is introduced into the disposable centrifuge bowl via a rotating seal. As the blood travels down the center channel, the blood fills the bottom portion of the bowl and is centrifuged while traveling through a slit located between the rotating bowl and the shell (Figure 12).

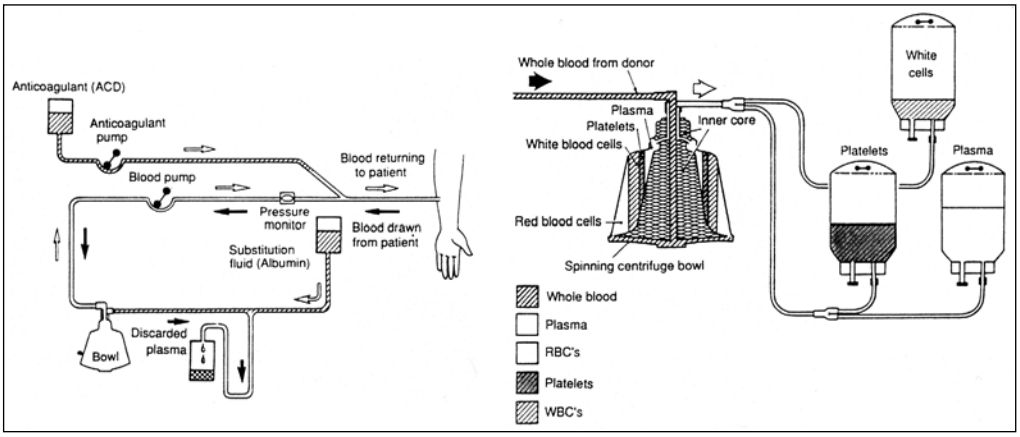


Figure 12: Circuit diagram of a centrifuge apheresis apparatus (left), right: cross-section of a centrifugal bowl (2611)

The centrifugal force separates the blood into plasma, platelets, white blood cell concentrates, and packed red blood cells; it is able to retain any of these components in sterile bags while returning the red blood cells to the patient. The procedure can be performed using one or two venipunctures (388, 389, 1518, 2271, 2515, 2611, 3201).
The various centrifugal devices developed over the past 40 years differ from each other in a number of ways:
– the Haemonetics V50 Plus Cell Separator can use the single-needle technique. When using a repetitive cycle, one can isolate several units of cell components.
– IBM 2997 Continous-Flow Cell Separator (Cobe Laboratories, Lakewood, USA) minimizes the time of the plasma exchange procedure, requiring only a low extracorporeal volume. The separated components can be selectively drawn off by a pump. This machine requires the use of two needles.
– Cobe Spectra is an automated, continous-flow cell separator. A keyboard allows the operator to input information; it will display end-of-procedure results such as predicted yield, volume of cell concentrate, and the time required to complete the procedure.
– Centrifuge Cell Separator (Fenwal) has a centrifuge bowl in which the blood is separated along the periphery. The separated components are then obtainable using pumps. The two-needle system is used in this system.
– CS-3000 Cell Separator (Baxter) has a continous flow coupled with a completely closed system which reduces the potential risk of contamination or possible leaks during plasma or cell collection. A two-needle sytem is required.
– AS 104 (Fresenius) is a computer-controlled apheresis system. The cells are collected outside the gravity field. The machine features safety systems, as well as a particular scanning system to determine the interface position in the separation process.
– Vivacell BT 798/CE (Dideco) incorporates a centrifugal plate heating system designed to prevent the blood from cooling during extracorporeal circulation. Improved separation is achieved, especially when treating disease states such as hyperviscosity syndrome, and cryoglobulinemia, which are temperature-dependent conditions (16, 2072, 3214). In 1986, the Eccentriplate system was introduced. The main difference between the two maschines is in the shape of the separation belt, which is circular in the Vivacell BT 798 and eccentric in the Eccentriplate version (2611).

The plasmapheresis centrifugal technology has recently focused on the collection of peripheral blood stem cells for both autologous and allogenic transplantation in patients with malignancies or hematological diseases and on donor plasmapheresis (1957, 2271).

2.1.2.2 Devices for Membrane Plasmapheresis

In the 1970's, hemofiltration machines were almost exclusively used, since nearly the same functions are required, in membrane plasmapheresis as in hemofiltration. At that time several companies in Europe made such devices (Dialyse Technik, Germany; Sartorius, Germany; Gambro, Sweden). However, because the technical expenditures were high, as early as 1980 technical simplifications were introduced, which were based upon the single-needle technique by means of a double pump and an additional balancing pump (143).
In 1982, a compact device was put on the market from Fresenius, Germany. The balancing also occurs by means of a special balancing pump. The transmembrane pressure is likewise separately adjustable to prevent hemolysis (947).
In 1981, Cobe/USA independently developed a compact flat plate membrane device which works exclusively by using the double-needle method. It is especially suitable for peripheral vascular con-

nection with a blood flow of at least 40 ml/min. The plate membrane module has a surface area of 0.13 m² (Table 9). Thus, depending to the blood flow by which the pressure on the membrane causes stretching, the wall shear-rate is controlled within narrow limits. The filtration performance can thus be kept constant (607). The filtrate flow increases with blood flow.
Other companies have offered devices for membrane plasmapheresis, such as Organon Teknika (Belgium), Dideco (Italy), Gambro (Sweden), Excorim (Sweden), Fasting Bioteck (Norway), Travenol (USA), Kuraray, Kawasumi, Nikiso, Asahi (Japan), etc. These devices also work with the double-needle access technique (1135, 2595). An essential limitation of all these devices is the fact that – aside from their technical complexity - they are only for use in TPE and not in combination with other extracorporeal detoxification methods. These methods include hemodialysis, hemofiltration, cascade filtration, or immunoadsorption. Therefore, several groups have developed their own systems.
Simple and user-friendly systems are necessary for routine clinical use. The preferred systems are the double-pump systems, since they can be combined with nearly all detoxification methods (143). Roller pumps are nearly exclusively used for the extracorporeal circulation. An advantage of this technique is that these pumps are easy to control. The rollers serve as valves to prevent backflow. Because they have demonstrated their advantages during the last decades, roller pumps in the extracorporeal circuit are widely employed.
The roller pump has some disadvantages to be overcome, mainly red blood cell and platelet trauma. Important components need to be improved continuously. They include achieving precise blood flow control to minimize blood cell damage and finding ways to reduce tubing wear, including resolving tubing biocompatibility. Improving blood recirculation in the vascular access is also an issue to be addressed (180, 1388).
Despite the fact that the roller pump, with all its advantages and disadvantages, represents the most used pump in the field of heart, lung surgery, as well as hemodialysis and plasma treatment systems, research groups should continue to develop new pump principles.
In recent years, there has been a great deal of work on developing new blood pumps, as was described at the First International Workshop on Rotary Blood Pumps in Austria in 1988 (3048). Since 1988, there has been ongoing discussion on rotary pump systems at the annual meetings of the International Society for Rotary Blood Pumps (ISRBP) (1668). Of special interest are heart assist pump systems and centrifugal pumps and their influence on blood cells, as well as their antithrombogenicity and hemolytic properties (1662, 2234, 2258, 2988, 2989). In view of the fact that is important to promote high blood volumes (heart surgery, hemodialysis, etc.), as well as very low ones (plasma exchange in adults or hemodialysis and plasmapheresis treatment in children), new pump designs will be needed in the future.
It is therefore desirable that only one machine (hardware) is available for all different extracorporeal methods. This hardware should be equipped with various software programs for running different apheresis methods. The membrane, adsorber and tube sets should be different for different methods. These sets should be only used with special software programs. Only one hardware system for all different apheresis methods could be a great advantage because the machine park in nephrological and intensive medical care units could be reduced. For the physicians and the staff this will be a simplification of their daily work, because they can work with different sets and only one hardware system.
New approaches are made in the last few years. Some companies have developed machines which can be used as well as for intermittend and continous dialysis methods, as hemoperfusion and plasmapheresis but not for immunoadsorption, a successful way.