Simultaneous bilateral lung transplantation. An experimental study.

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SIMULTANEOUS BILATERAL LUNG TRANSPLANTATION

an experimental study

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Stellingen

1. De overlevingsduur van longallotransplantaten kan worden verlengd door deze transplantaties uit te voeren tussen verwante honden met een gelijk serologisch bepaald deel van het hoofd histocompatibiliteits-complex.

2. Het transplanteren van beide longen en-bloc, waarbij de arteria pulmonalis, het linker atrium en de trachea van de donor worden verbonden met dezelfde structuren van de recipient, is chirurgisch-technisch goed mogelijk in het dierexperiment en vereist geen tijdelijke extracorporele zuurstofvoorziening van het bloed.

3. De recirculatie van bilateraal getransplanteerde longen in de recipient gaat gepaard met een circulatoire shock, die het gevolg is van het verlies van circulerend bloedvolume in de tevoren bloedeloze longen en van een verminderde functie van het hart.

4. Door stoornissen in de natuurlijke reinigingsmechanismen van getransplanteerde longen is infectie een belangrijke doodsoorzaak na longtransplantaties.

5. Door een toren van tien kilometer hoogte te bouwen kan gebruik worden gemaakt van het aanzienlijke temperatuursverschil tussen met zonnewarmte verhit water en de koude lucht op zeer grote hoogte van de atmosfeer en kan een wereldomvattende en onuitputtelijke energie-voorziening worden geschapen.

6. De peroperatieve calibrering van de pylorus tijdens de hoog-selectieve vagotomie kan achterwege blijven, indien bij pre-operatief uitgevoerde oesophago-gastro-duodenoscopie de endoscoop met een buitendiameter van 13 millimeter zonder moeite in het duodenum kan worden gebracht.

7. Het vaststellen van de immunologische reactiviteit als prognostisch parameter bij chirurgische patienten verdient een plaats naast de bepaling van het haemoglobinegehalte.

8. Het verdient aanbeveling om in kleine orthodontische prothesen een röntgenabsorberend deel te verwerken om lokalisatie na accidentele aspiratie of na inslikken te vereenvoudigen.

9. Het laten fungeren van de patient als bode van hem onbekende en hem betreffende medische gegevens door het meegeven van collegiale correspondentie in gesloten couvert is een verwerpelijk gebruik.

10. De werkgever heeft meer mogelijkheden dan de werknemer om de vertrouwensrelatie, noodzakelijk voor het goed functioneren van een bedrijfsarts, in zijn belang te gebruiken.

11. Het verdient aanbeveling om akoestische signalen van apparatuur voor de bewaking van vitale functies van de patient na circa vijf minuten automatisch te laten uitschakelen, daar er na het verstrijken van deze periode zonder medisch ingrijpen niet meer van bewaking kan worden gesproken.

12. Het toenemend tempo van veranderingen in de samenleving vindt zijn weerslag in het groeiend aantal losbladige standaardwerken.

13. De uitspraak „voorkomen is beter dan genezen" vindt gestalte in de vergelijking van het door de Wereld Gezondheidsorganisatie in 1979 bereikte resultaat bij de uitroeiing van pokken met de resultaten van het onderzoek naar de mogelijkheden tot transplantatie van de long.

Stellingen behorend bij het proefschrift van Zacharias Jan de Langen "Simultaneous Bilateral Lung Transplantation, an experimental study", 1980.

rijksuniversiteit te groningen

SIMULTANEOUS BILATERAL LUNG TRANSPLANTATION

an experimental study

proefschrift

ter verkrijging van het doctoraat in de geneeskunde
aan de rijksuniversiteit te groningen
op gezag van de rector magnificus dr. j. borgman

in het openbaar te verdedigen
op woensdag achtentwintig mei negentienhonderdentachtig
des namiddags te vier uur
door

zacharias jan de langen

geboren te delft

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drukkerij van denderen b.v.
groningen

promotores:
prof. dr. ch. r. h. wildevuur
prof. dr. h. heemstra

coreferent:
prof. dr. j. r. benfield

"one man's artifact is another man's assumption."
jere mead (1962)

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CHAPTER 1
INTRODUCTION

Lung transplantation has been regarded the ultimate therapy for patients with progressive pulmonary disease leading to a deterioration of lung function in spite of receiving the best available treatment. It has been the subject of an increasing amount of investigative effort since the earliest experimental transplantations of pulmonary tissue by Carrel (1907) and Demikhov in 1947 (Demikhov, 1962). More than 500 titles regarding this subject have been published since. Reviews of the experimental effort have been published by Trummer (1965), Blumenstock (1967), Trummer and Berg (1968), Veith (1968), Trummer et al. (1971) and Veith and Blumenstock (1971). The initial efforts regarding experimental lung transplantation were concerned primarily with solving technical problems. In 1950 Metras reported a major technical advance. He was able to decrease the incidence of thrombosis of the pulmonary venous anastomoses by transecting a cuff of left atrium including the orifices of the pulmonary veins and thereby eliminating the need for individual vein anastomoses. This technique has been generally adopted since. The importance of achieving perfection in the venous anastomoses of canine lung transplants was originally emphasized by Benfield and Coon (1967) who showed that elevated vascular resistance and poor function resulted from even minor imperfections at the left atrial cuff anastomosis. An adequate lumen at the site of the pulmonary artery anastomosis has been shown to be essential for the maintenance of normal function and vascular resistance in unilaterally transplanted lungs (Veith and Richards, 1969). Wildevuur (1967) showed in dogs after unilateral lung reimplantation that, with structural defects at the site of the anastomoses of vessels or bronchi, changes in lung function could always be explained by the mechanical interference with blood flow or ventilation. Technical problems have also arisen with the bronchial anastomosis because of the precarious vascularity of the transplant bronchus that can derive its blood supply only from collaterals between the pulmonary and bronchial arteries. As a result all workers have noted instances of bronchial leakage and stenosis due to varying degrees of ischemic necrosis of the transplant bronchus. Various methods have been designed to minimize these bronchial anastomotic problems.

Many investigations of lung transplantation were concerned with the consequences of division of pulmonary hilar structures. The tolerance of the lung to withstand temporary ischemia from two to three hours has been

demonstrated by Veith et al. (1971c) in their experiments of unilateral lung reimplantation with immediate ligation of the contralateral pulmonary artery in dogs. In experiments of Bogardus (1958) and Stone et al. (1966) it was shown that simple division of the bronchial arteries by stripping the pulmonary hilus was tolerated without necrosis of the main stem bronchus. The effects of denervation on lung function have been studied by stripping all structures around the bronchi and vessels in the pulmonary hilus (Bogardus, 1958; Faber and Beattie, 1958; Eraslan et al., 1966). In dogs bilateral pulmonary denervation was at first thought to be associated with death due to the inability of the animal to ventilate adequately with loss of lung innervation (Howard and Webb, 1958; Alican and Hardy, 1963; Benfield et al., 1963; Ballinger et al., 1964; Nakae et al., 1967). However, it is now realized that acute bilateral denervation can be compatible with survival in the dog (Lower et al., 1961; Faber et al., 1965; Wildevuur et al., 1969; Alican et al., 1971; Veith et al., 1971a). Hirsch et al. (1968a, 1968b) and Nigro et al. (1968) have presented detailed studies of nerve end-organs in the pulmonary parenchyma and demonstrated that nerves fail to regenerate in dogs observed for several years after autotransplantation of the lung. Several authors reported Hering-Breuer reflexes to be absent up to four-and-a-half years after reimplantation of a single lung in some dogs (Nigro et al., 1968; Peset et al., 1969) while others reported some evidence that the Hering-Breuer reflexes return from as early as the seventh month after lung autotransplantation (Marshall and Gunning, 1966; Trummer and Berg, 1968, pp. 6-10). Division of lymphatics has not been found to be associated with changes in ventilation or oxygen consumption. The lymphatic circulation from the transplanted lung is restored within three weeks and should not impose any long-term impairment of lung function (Eraslan et al., 1964). Blank et al. (1966) and Siegelman et al. (1977) showed that spontaneous re-establishment of the bronchial artery supply occurs between one week and one year after unilateral lung reimplantation.

In view of these considerations with individual hilar structures one would not expect severely altered function when all the hilar structures are sacrified as a consequence of the procedure of reimplantation of the lung. Numerous studies of the function of reimplanted lungs have been performed. Most of these studies have demonstrated a transient and in some instances a persistent decrease in the proportion of ventilation and oxygen uptake

contributed by the reimplanted lung (Hughes et al., 1954; Hardy et al., 1963a; Nigro et al., 1963; Reemtsma et al., 1963; Bücherl et al., 1964; Trummer and Christiansen, 1965; Sharma et al., 1966; Birch et al., 1968; Veith et al., 1969b).

Allograft rejection of the transplanted lungs has been the subject of many investigations. The results of most of these studies are in accordance with the features and time sequence of lung allograft rejection in dogs as has been originally described by Barnes et al. (1963). The allografted lung in dogs will without immunosuppressive treatment be rejected within five to ten days. Various methods of immunosuppression have been employed to enhance lung allograft survival. However, no consistent prolonged lung allograft survival and function has been achieved yet.

The limited success of the clinical application of lung transplantation thus far is in sharp contrast to the enormous experimental effort. After the first human lung allotransplantation by Hardy and coworkers (1963b) 35 more allogenic lung transplantations in men have been reported and were summarized by Wildevuur and Benfield (1970) and Veith and Koerner (1974). One patient survived ten months (Derom et al., 1971), another six months (Veith et al., 1973), and several others survived one to two months after the transplantation. Although the patients who survived for the longer periods were substantially palliated, no unmitigated long-term success has been achieved yet. A progressive respiratory insufficiency was the major problem encountered in the postoperative period of these patients. The malfunction of the transplant was in approximately half of these cases primarily introduced by the remaining diseased own lung of the patient. Patients with chronic obstructive pulmonary disease had higher expiratory airway resistance in the remaining lung than in the transplanted lung. The consequences of this and associated differences in compliance were demonstrated by a gradual progressive expansion of the patient's own remaining lung. This led to compression of the transplant. High pulmonary vascular resistance in the recipient's own lung resulted in the deviation of an intolerable large share of the cardiac output to the transplanted lobe in one case. Discrepancies of ventilation and perfusion between the recipient's own lung and the transplant were demonstrated in two cases. Massive

pneumonia was a major cause of death in the cases of human lung transplantation. Cross-infection from the diseased remaining own lung of the patient has been held responsible for many of these infections.

Since most candidates for lung transplantation suffer from bilaterally diseased lungs, the experimental efforts to overcome the problems introduced by the remaining lung progressed in two directions. The majority of investigators tried to obtain a single pulmonary transplant with sufficient function to permit the elimination of the contralateral lung. Veith et al., (1969a, 1971b) were able to obtain survival after unilateral auto- and allotransplantation of the lung in dogs with immediate ligation of the contralateral pulmonary artery. Others have tried to overcome this problem by resection and transplantation of both lungs simultaneously (Vuillard et al., 1969; Wildevuur et al., 1969; Veith et al., 1971a; Alican et al., 1971; Vanderhoeft et al., 1972; Kondo et al., 1972; Alican et al., 1973).

Simultaneous bilateral lung allotransplantation might overcome the problems encountered from the presence of the remaining lung after unilateral lung transplantation, provided that the simultaneous transplantation of both lungs is technically feasible and immediate adequate function can be achieved. The possibility to transplant both lungs simultaneously has not yet been studied intensively. Therefore experiments were performed in dogs to study the possibility of simultaneous bilateral lung transplantation with preservation of lung function. The results of this experimental work are presented in this thesis.

outline of this thesis

The first purpose was to define the influence of donor selection for major histocompatibility antigens on lung allograft survival in related inbred dogs and to achieve an experimental model in which functional aspects after simultaneous bilateral lung allotransplantation can be studied not immediately influenced by rejection phenomena (chapter 2).

The methods for the measurements of cardiopulmonary function before and after simultaneous bilateral allotransplantation of the lungs are described separately (chapter 3).

The second purpose was to develop an operative technique for the simultaneous transplantation of both lungs en-bloc with a minimal number of three anastomoses and without the need for temporary cardiopulmonary bypass (chapter 4).

The third purpose was to study the immediate functional and morphological changes of the simultaneously transplanted lungs and to determine the factors responsible for immediate death after this procedure (chapter 5).

The fourth purpose was to define the factors which interfere with prolonged survival and function after simultaneous bilateral lung allotransplantations (chapter 6).

CHAPTER 2
THE INFLUENCE OF THE MAJOR HISTOCOMPATIBILITY COMPLEX ON LUNG ALLOGRAFT SURVIVAL

2.1 introduction

The influence of donor selection for organ transplantation has been well established nowadays. The ultimate success or failure ("rejection") of a tissue graft is determined by differences in genetically controlled structures in donor and recipient. The genes controlling these structures have been called histocompatibility genes. A striking similarity between the human major histocompatibility system (HL-A) and the canine major histocompatibility system (DL-A) has been observed (Vriesendorp et al., 1973a). The major histocompatibility complex in dogs consists of two different series of multiple alleles which control antigens that can be recognized serologically (SD = serological defined) and a third and closely linked system (LD = lymphocyte defined) which controls structures that determine reactivity in the mixed lymphocyte culture (Vriesendorp et al., 1973b). The influence of prospective DL-A typing on allograft survival in littermate donor-recipient pairs has been defined for skin (Vriesendorp et al., 1971), heteropic small bowel (Westbroek et al., 1971), pancreas (de Gruyl et al., 1973), kidney (Westbroek et al., 1972) and orthotopic heart (Bos et al., 1972) but not for the lung. Lung allograft survival has been studied mostly in non-typed donor-recipient combinations. Two reports are available on immunologically matched lung allografts between inbred littermate and non-littermate dogs. Blumenstock et al. (1971) indicated that prospective typing of donor and recipient beagles and mongrel dogs for 11 lymphocyte antigens influences lung allograft survival. Benfield et al. (1972) experienced longer lung allograft survivals in related dogs without lymphocyte antigen incompatibilities compared to a similar group of unmatched mongrel dogs.

The purpose of this study was to define the influence of prospective DL-A typing on the lung allograft survival in related inbred donor-recipient combinations of dogs, to define the histological phenomena of rejection, and to achieve an experimental model in which bilateral allotransplantations of the lungs could be performed with a minimal immunological reaction to study functional aspects not immediately influenced by rejection phenomena.

2.2 materials and methods

The experimental animals consisted of nine littermate pairs of inbred labrador-retrievers selected on the basis of serological criteria from a colony of the Central Institute for the Breeding of Laboratory Animals TNO, Austerlitz, The Netherlands. The serological typing was done with a battery of approximately 80 antisera recognizing DL groups 1, 2, 3, 7, 8, 9, 10 of the first series and DL groups 4, 5, 6, and 13 of the second series of multiple alleles. The unassigned group DL 11 and 12 were also determined. A previously described one-stage microcytotoxicity test was used (Smid Mercx et al., 1975) by H. M. Vriesendorp and co-workers (Laboratory for Experimental Surgery, Erasmus University, Rotterdam, The Netherlands) and later by J. A. Kaars Sijpesteijn and co-workers (Laboratory for Bloodgroup Serology, University Hospital, State University, Groningen, The Netherlands). Selection of pairs of dogs for transplantation was done on serological criteria resulting in three groups of littermate pairs with two identical haplotypes (group 1), one haplotype difference (group 2) and two haplotype difference (group 3).

The selected pairs of dogs in the three different groups were randomized. Between three pairs of dogs in each group unilateral lung transplantations were performed by interchanging the left lungs. The standardized technique for unilateral autotransplantation was used (Wildevuur, 1967, pp. 18-19). After harvesting, the donor lung was immediately flushed through the pulmonary artery with 500 ml glucose 5% of 4°C containing 25 mg heparine. The transplantation was done one after the other resulting in a longer ischemia time for the second implanted graft. This graft was preserved by submerging with ligated bronchus in glucose 5% solution of 4°C. Penicillin (400,000 U i.m.) and streptomycin (.5 g i.m.) was administered daily starting before transplantation and continued for seven days in surviving dogs. No immunosuppressive drugs were given.

The assessment of graft survival was done by sequential open lung biopsies

before transplantation, 30 minutes after recirculation of the graft, and in the surviving dogs 4 or 5, and 7 days, 2, 3, 5, 10, and 15 weeks after transplantation. X-ray pictures of the chest in anterior-posterior direction were taken regular during the first week and prior to each open lung biopsy. The dogs were sacrificed when the X-ray picture showed complete homogeneous density of the left hemithorax and when the macroscopic aspect of the lung at the time of lung biopsy showed a complete consolidation of the graft. At autopsy the anastomoses were judged for patency and excision biopsies of central and peripheral parts of all lobes of transplanted and own lung were taken. All specimens were preserved in formalin and processed by routine procedures for light microscopy by one investigator (Prof. Dr. Ch. R. Jerusalem, head Laboratory for Cytology and Histology, Catholic University, School of Medicine, Nijmegen, The Netherlands). Graft survival time was defined as the time between transplantation and the occurrence of homogeneous density of the left hemithorax on X-ray picture.

2.3 results

There were no operative deaths and none of the dogs required blood transfusions. The individual and mean ischemia times and survival times of the

table (2.1) individual and mean results in the three different groups

group	number	graft ischemia time (min)		graft survival time (days)
		first	second	
GROUP 1	1813	116		105
	1809		102	19
	1888	57		20
	1889		155	1
	1877	45		21
	1890		112	49
	mean	73	123	35.8
	s	± 38	± 28	± 37.2
GROUP 2	1806	67		6
	1807		100	6
	1892	46		6
	1891		128	6
	1808	51		15
	1810		114	12
	mean	55	114	8.5
	s	± 11	± 14	± 4.0
GROUP 3	1876	78		5
	1887		125	4
	1878	66		1
	1879		105	2
	1886	50		3
	1896		113	3
	mean	65	114	3.0
	s	± 15	± 10	± 1.4

Individual and mean graft ischemia times and graft survival times with standard deviation of the mean (s) in the three experimental groups: identical (group 1), one-haplotype different (group 2) and two-haplotype different (group 3). The first and second implanted graft of the paired interchanging procedure are indicated as such.

lung grafts in the three different groups are given in table (2.1) and figure (2.1). The first and second transplanted lungs of the paired interchanging procedure were subjected to an ischemia time of approximately one and two hours respectively. No significant difference in ischemia time between the three different groups was observed. Also ischemia time did not correlate with graft survival time. A significant difference in mean lung graft survival time was observed between the two-haplotype different (3 ± 1.4 days) and both the one-haplotype (8.5 ± 4 days) and identical (35.8 ± 37.2) combinations ($p<.05$, Student t-test). No significant difference in graft

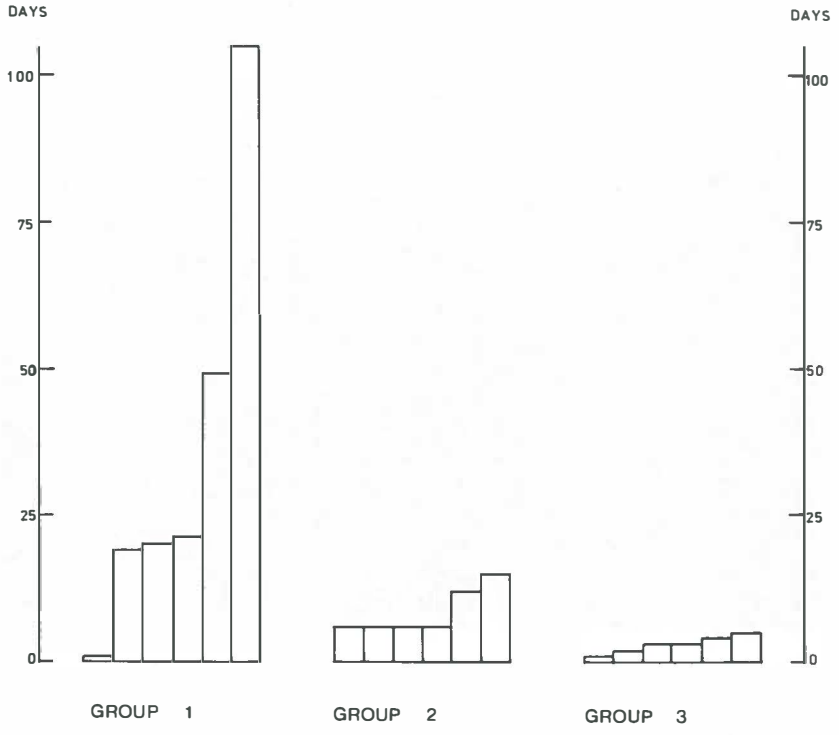


figure (2.1)
graphical representation of the individual lung allograft survival times in the three different groups: 1, identical; 2, one haplotype difference; 3, two haplotype difference.

survival time was found between the identical and the one-haplotype different dogs. However, one exceptional case of short graft survival, i.e. one day, occurred in group 1.

group 1 - identical -

In five of the six dogs the histopathological changes in lung biopsies taken 30 minutes after recirculation of the grafts consisted of dilated and congested alveolar capillaries, occasionally with local endothelial damage in small vessels, and a frayed appearance of alveolar epithelium with an increased number of free alveolar macrophages (figure 2.2).

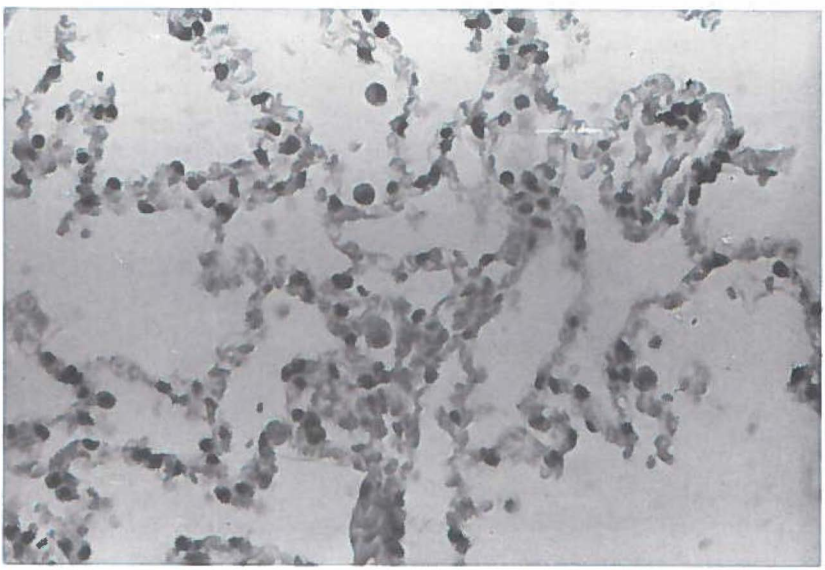


figure (2.2)
frayed appearance of alveolar epithelium, congested capillaries, some polymorphonuclear cells and free alveolar macrophages in a lung biopsy taken 30 minutes after recirculation of the lung allograft in H 1877.

In one graft (H 1889) however, more pronounced endothelial damage with

attachment of polymorphonuclear cells (pmn cells) in small vessels was apparent. In this dog the X-ray picture showed a complete homogeneous density of the left lung on the first postoperative day. At autopsy five days after transplantation the graft was completely consolidated and areas with hemorrhage were observed. Microscopy exhibited a complete hemorrhagic necrosis with a diffuse infiltration of the graft by pmn cells. Abscess-like accumulations and infiltrations of pmn cells in bronchioli and bronchi and thrombotic material in several veins were apparent. Mononuclear cells (mn cells) were notably absent. In the contralateral lung of this dog the number of pmn cells was increased and small areas of hemorrhagic necrosis were present. This graft had been subjected to the longest ischemia time of approximately two-and-a-half hours.

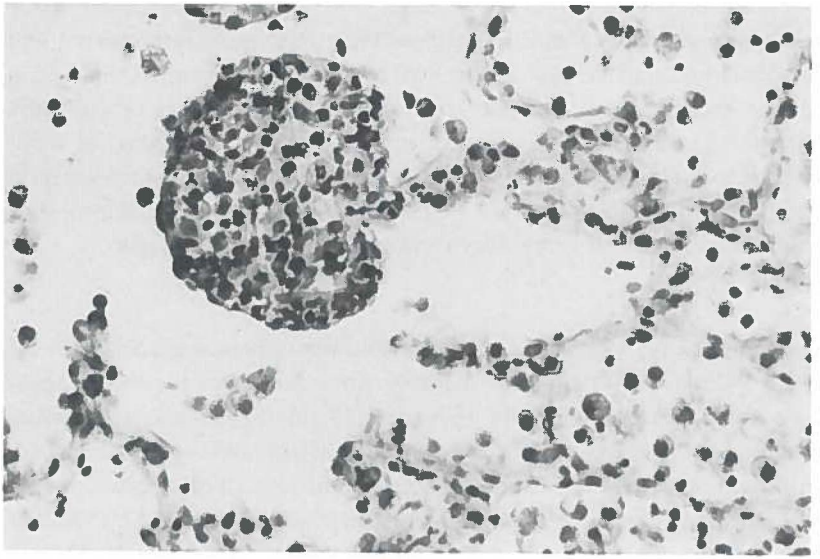


figure (2.3)
balloon-like enlargement of an alveolar septum by proliferation of interstitial cells, interspersed with several mononuclear cells and a few polymorphonuclear cells in a lung biopsy 12 days after left lung allotransplantation in H 1877.

In three of the remaining five dogs (H 1809, H 1888 and H 1877) a complete density of the transplant on X-ray picture was seen at the end of the third

postoperative week. The macroscopic aspect of the transplanted lungs of these dogs at the time of biopsy five and seven days after operation was normal. Microscopy of the specimens showed aspecific changes such as interstitial edema, focal peribronchial hemorrhage, and some exudate with free alveolar macrophages. In the biopsies 12 and 13 days after transplantation a distinct increase of mn cells around blood vessels, interstitial cell proliferations, thickened alveolar septa, and an increased number of free alveolar macrophages were observed in two dogs (H 1877 and H 1809) (figure 2.3). In one dog (H 1888) only proliferative changes were seen. At the end of the third postoperative week these three grafts were completely consolidated. Microscopy exhibited essentially the same changes with varying intensity. Mononuclear cells predominantly small lymphocytes were accumulated around bloodvessels and in the peribronchial tissue frequently showing a cuffing pattern. Pmn cells were interspersed and exfoliation of alveolar epithelium with free alveolar macrophages and mn cells in exudates were apparent (figure 2.4). The destructive changes of a hemorrhagic necrosis were only seen in the transplant of one dog (H 1888) in which autopsy was postponed to the 34th day after transplantation notwithstanding the opacity on X-ray picture two weeks before. The contralateral lungs of these dogs showed only aspecific changes.

In the remaining two dogs confluent infiltrates appeared gradually on the X-ray pictures and a complete homogeneous density of the transplanted lung had developed seven (H 1890) and 15 (H 1813) weeks after transplantation. The grafts were consolidated at that time and the dogs sacrificed. Proliferation of interstitial cells and numerous free alveolar macrophages were observed three weeks after transplantation in one dog (H 1890) but had disappeared five weeks after transplantation. At autopsy of this dog seven weeks after transplantation various changes were observed. Areas of ischemic necrosis were alternated with areas of granulation tissue. An increased number of pmn cells, macrophages, lymphocytes, and fibrin deposits were seen. The bronchial anastomosis showed dense infiltration with pmn cells. The contralateral lung in this dog exhibited interstitial edema and proliferation of interstitial cells. In the other dog (H 1813) peribronchiolar and occasionally perivascular accumulation of macrophages and mononuclear cells with interstitial edema and an increased number of free alveolar macrophages were the first serious histopathological changes

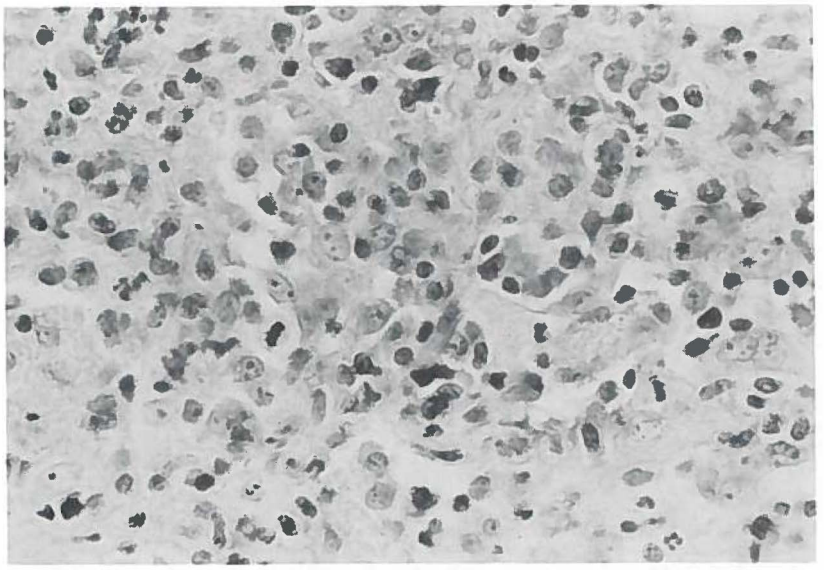


figure (2.4)
carnacious aspect of the left lung three weeks after allotransplantation in H 1877 due to proliferation of alveolar epithelial cells; interstitial cells, macrophages, a few polymorphonuclear cells and mononuclear cells are interspersed.

observed three weeks after transplantation. In the biopsies taken five and ten weeks after transplantation these infiltrates became more sharply defined and in the autopsy specimens 105 days postoperatively distinct small perivascular and peribronchiolar granulomas mainly consisting of small mn cells were apparent (figure 2.5). Fibrotic changes were seen in the very periphery of this transplant. Apart from slight interstitial edema and alveolar exudate no distinct parenchymal changes were observed in the contralateral lung.

group 2 - one haptotype difference -

The histopathological changes observed in the lung biopsies taken 30

minutes after recirculation of the graft in this group resembled the picture in the dogs of group 1.

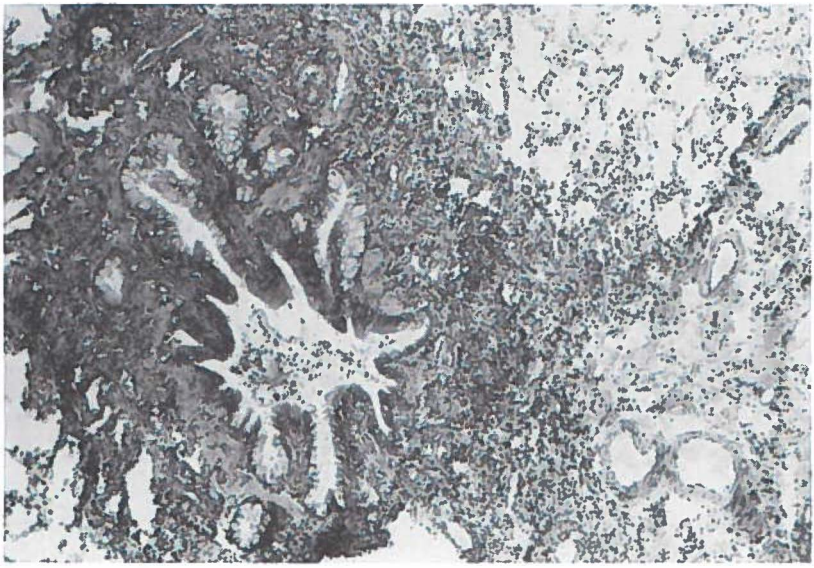


figure (2.5)
massive peribronchial accumulation of mononuclear cells ten weeks after left lung allotransplantation in H 1813.

Confluent infiltrates in the left lung on X-ray pictures made on the fourth and fifth day after transplantation were apparent in these dogs although to a varying extent. Microscopy of the lung biopsies at that time showed accumulations of mn cells in small vessels and cuffs of mn cell infiltrates around bloodvessels and bronchioli (figure 2.6). Interstitial infiltrates contained mn cells, macrophages, erythrocytes and occasionally pmn cells. These cells were also observed within the alveoli with various degrees of exudates. Proliferative changes of interstitial cells and alveolar epithelium were present in some cases.

In four dogs (H 1806, H 1807, H 1892 and H 1891) a complete opacity of the left lung on X-ray pictures was seen on the sixth postoperative day. The lung

grafts were consolidated at biopsy and the dogs therefore sacrificed. Autopsy showed occluding thrombi on the left atrial and pulmonary artery

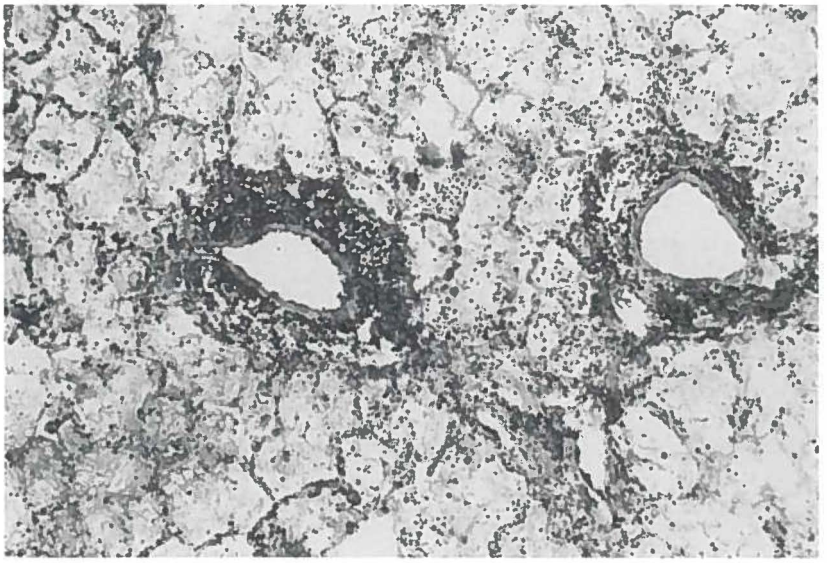


figure (2.6)
accumulations of mononuclear cells around pulmonary vessels five days after left lung allotransplantations in H 1891.

anastomoses in two dogs (H 1892 and H 1891). In the other two dogs (H 1808 and H 1810) the lungs remained aerated untill the 12th and 15th day. Complete consolidation of the transplanted lungs was observed on the 12th (H 1810) and 19th (H 1808) day. Hemorrhagic necrosis was prominent in the autopsy specimens of all lung grafts except one (H 1808) in which a generalized accumulation of mn cells without severe destructive changes was seen. In and adjacent to the bronchi of four dogs (H 1892, H 1891, H 1808 and H 1810) pmn cell infiltrations with mucoid material were apparent. These changes were also present in the contralateral lung of three of these dogs (H 1891, H 1808 and H 1810). In one dog the pmn cells showed no bronchial predilection in the contralateral lung but hemorrhagic infiltrations and intense proliferation of macrophages were more prominent.

group 3 - two haplotype difference

An often pronounced damage of endothelial cells with attachment of polymorphonuclear cells was the first histopathological change observed in the lung biopsies taken 30 minutes after recirculation of the grafts in all dogs except one (figure 2.7). In this dog (H 1876) only aspecific changes observed in all transplants at this moment were seen.

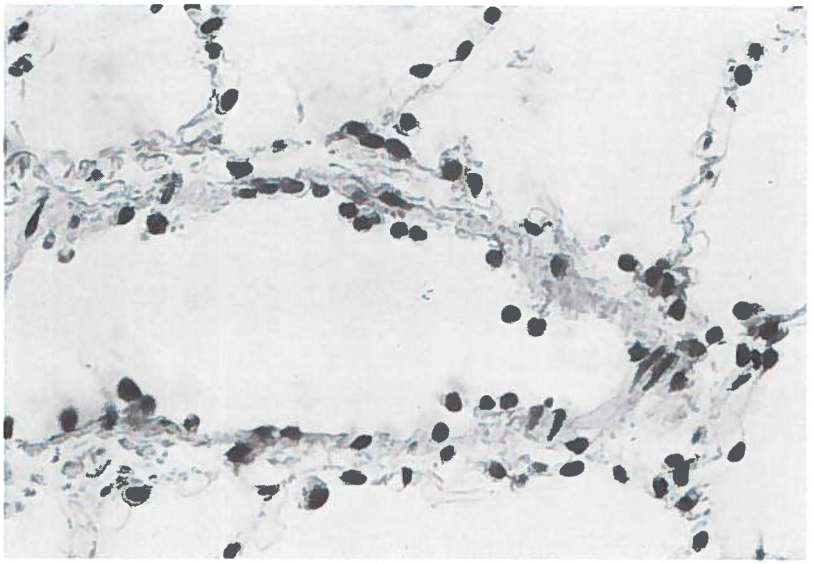


figure (2.7)
almost complete absence of vascular endothelial cells; several polymorphonuclear cells are attached to the damaged surfaces.

Opacity of the left lung was seen in all the first taken X-ray pictures between the first and fifth postoperative day. Because of the hostile behaviour of these inbred labrador-retrievers X-ray pictures could not be made on every day of the first postoperative week in all cases. All grafts were consolidated at the time of the first biopsy on the fourth and fifth day postoperatively.

Autopsy showed occluding thrombi at the anastomotic site of both left atrium and pulmonary artery in two dogs (H 1878 and H 1879) whereas it was confined to the left atrium in one dog (H 1876) and to the pulmonary artery in another dog (H 1896).

Histopathological changes of the autopsy specimens of the grafts ranged in all dogs from moderate alveolar and perivascular hemorrhage to complete hemorrhagic necrosis. Pmn cell accumulations were seen in all with preferential localisation in and around bronchi and bronchioli in the three dogs (H 1876, H 1887 and H 1878) which contralateral lungs also showed these abnormalities. Congestion of alveolar capillaries, edema, and occasionally hemorrhage were observed in the contralateral lungs of the remaining dogs.

2.4 discussion and conclusions

This study indicates that probably three different patterns of lung allograft rejection in immunologically well-defined littermate dogs can occur and at least significant differences in survival time were observed. To determine the actual time of lung allograft rejection the sequentially taken X-ray pictures serve as a reasonable indicator considering that the observed radiological abnormalities are verified by histological examination of either a biopsy of the lung graft from the living dog or of autopsy specimens. The criterium for lung allograft rejection in this study, i.e. complete homogeneous density of the transplanted lung on X-ray pictures, correlated with the occurrence of hemorrhagic necrosis of the lungs transplanted between one or two-haplotype different dogs except in one in which the mentioned condition has been violated. In the identical dogs however, this criterium was not associated with hemorrhagic necrosis although the morphological features in these lungs were severe and considered as an end-stage of chronic rejection. This discordance partially explains the small difference in graft survival times between identical and one-haplotype different dogs. The presence of a very short graft survival time of one day in one dog which could not be definitely related to rejection excludes a significant difference in mean survival times where otherwise the two groups would have differed. Since the radiological abnormalities are not specific for rejection Shimada et al. (1973) used bronchoscopy for differentiation of rejection from infection. The absence of endobronchial signs of pneumonia correlated with the occurrence of rejection without infection. The determination of antibodies by immunofluorescent techniques, complement measurements and cytotoxic assays by Benfield et al. (1972) did not give a predictive value for lung graft rejection. ^{131}I-fibrinogen activity was found to be non-specific for pulmonary rejection (Bardfield et al., 1976). So far no better indicator of pulmonary allograft rejection is available than the sequential X-ray pictures combined with histological examination.

The classical morphological picture and time sequence of lung allograft

rejection was observed in all dogs with one haplotype difference in the serologically defined part of the major histocompatibility complex. According to Barnes et al. (1963) the morphological features of pulmonary allograft rejection generally proceed in the following sequence: starting at the fourth or fifth day after transplantation prominent perivascular mononuclear cell (mn cell) infiltration involving some small vessels and focal areas of alveolar edema appear. Scattered areas of acute purulent bronchopneumonia may be present. These changes become more generalized during the next few days. The widespread edema is accompanied by a pleiomorphic intra-alveolar infiltrate of predominantly round cells as well as polymorphonuclear cells (pmn cells). At eight to ten days there is more widespread edema and hemorrhagic necrosis of the alveolar tissue. In the ultimate stage hemorrhagic necrosis is apparent. The remains of perivascular infiltration is overwhelmed by the general tissue destruction in this stage. The histopathological changes observed at different stages after transplantation of the left lungs in our dogs in the one-haplotype different group closely resemble this cellular component of rejection of allografted lungs. The observed pmn cell accumulation with mucoid material in the bronchi of four transplants and in the contralateral lungs of three of these indicates the occurrence of bronchopneumonic changes probably secondary to the general destruction of the pulmonary parenchyma. Whether the partially necrotic transplant functioned as focus of infection for the own lung remains uncertain but is likely. The observed thrombotic events are considered to be secondary to the destruction of the transplant and not the result of technical imperfections of the anastomoses since the majority of these events were observed in the early rejected lungs and only once in the longer survivals. Thrombosis was probably the result of severe vasculitis preceding the hemorrhagic necrosis and of the stasis and slow bloodflow through the destructed transplant.

The histopathological changes observed in five of the lung grafts of the identical combinations at different stages indicates that the accumulation of mn cells is probably retarded for one to three weeks. This rejection phenomenon seemed also to be less destructive since reparative changes and proliferative changes were seen concomittantly. Complete destruction of the graft by hemorrhagic necrosis was only seen in one dog approximately five weeks after transplantation. The course of events in two dogs in this

group does not allow a solid conclusion whether infection or rejection was the primary cause of the observed changes. The perivascular and peribronchiolar granulomas mainly consisting of small mn cells observed in the autopsy specimens of the transplant in one dog (H 1813) resembled those seen in the biopsy of its donor (H 1809) prior to transplantation. This might point to a chronic inflammatory reaction to an unknown infectious agent already present before transplantation. The transformation of a pathological microscopic picture of the graft in the other dog (H 1890) three weeks after transplantation to normal five weeks after transplantation at least indicates that regeneration of initial events remains a possibility.

Obviously chronic rejection is not a distinct morphological classification but only a continuation of the events comprising the rejection reaction. As has been shown in kidney allotransplants (Foker and Najarian, 1972, pp. 137-138) the pathological changes of the kidney cells are the most striking aspect of chronic rejection although host immunological effectors are represented by mn cells, pmn cells, platelets, and fibrin deposits. Proliferation of glomerular endothelial and mesangial cells frequently occur in these chronically rejected kidneys. Proliferation is accompanied by an increased number of macrophages. The chronic rejection phenomena observed by Kondo et al. (1974) in immunosuppressed dogs 396, 177, 84, and 72 days after one-stage bilateral lung allotransplantation resemble the changes observed in our experiments in the identical combinations. The observation of Veith and Hagstrom (1972) of a distinct alveolar pattern of rejection consisting of fibrinous alveolar exudate and desquamation of alveolar epithelium without any associated perivascular or peribronchiolar mn cell infiltration could not be substantiated by our morphological findings.

The presence of the histopathological changes in lung specimens taken 30 minutes after blood flow to the transplanted lung had been restored, and the subsequent hemorrhagic necrosis within five days in nearly all two-haplotype different combinations might indicate an hyperacute and accelerated rejection. The hemorrhagic necrosis might actually have occurred even earlier than we observed since X-ray pictures could not be taken on every day of the first postoperative week in all these dogs because of their hostile behaviour. The relative absence of these early vascular changes

in the identical and one-haplotype different combinations suggests a positive correlation between the serologically defined difference in histocompatibility antigens and this hyperacute rejection. The same observations have been made by Wildevuur et al. (1973) in some of the bilaterally transplanted lungs in mongrel dogs not prospectively typed for histocompatibility antigens. The morphological observations by Wildevuur and also in our experiments resemble some of the histopathological features in hyperacute and accelerated renal allograft (Starzl, 1964) and xenograft rejection (Perper and Najarian, 1966). The mechanism of hyperacute rejection of kidney allografts has been described by Hume (1971) as a consequence of pre-existing antigenic exposure of the recipients resulting in antibody formation. If tissue is transplanted from a donor whose antigens are similar to those producing the immunization antibodies become attached to the endothelium of small bloodvessels as soon as circulation of the transplanted tissue is restored. Polymorphonuclear cells adhere to the formed antibody-complement layer on the endothelium leading to its destruction. Jap et al. (1973, pp. 159-179) demonstrated by electron microscopic examination of lung biopsies taken 30 minutes after recirculation of simultaneous bilateral transplanted lungs in mongrel dogs that various kinds and degrees of endothelial lesions ranging from swelling and vesiculation, local damage to total loss of epithelium occurred. Neutrophilic granulocytes attached to endothelial cells by finger-like cytoplasmic protrusions, disintegrated cells and pmn cells within endothelium were frequently observed in his preparations. Wildevuur et al. (1973) postulated the presence of natural occurring and heterospecific antibodies as the immunological cause of these early graft failures. The presence of these antibodies and the way in which they effect the ultimate cellular efferent chain of this hyperacute rejection has not yet been demonstrated in lung allografts. Although the observed early changes in our cases resemble those seen in cases of acute hemodynamic shock (Wilson et al., 1970) and in animals subjected to endotoxic or septic shock (Coalson et al., 1970; McKay et al., 1966, 1967) it is unlikely that hemodynamic disturbances are responsible for our observations after unilateral lung transplantation. A mild shock syndrome which can not be excluded in our experiments would have occurred more or less equally in the dogs of all the three groups and the possible pathological changes not mainly confined to the two-haplotype different combinations. The mild endothelial lesions of small vessels and congestion of alveolar capillaries with slight interstitial

edema observed in the earliest biopsies of most lung allografts from identical and one-haplotype different dogs probably represent the non-specific changes due to the immediate preservation procedure and processing of the lung graft during the period of transplantation since similar changes were observed by Jap et al. (1973) in isolated lungs. The hyperacute graft destruction with the vascular changes already observed 30 minutes after recirculation in one of the dogs of the identical group (H 1889) resembles the observations of hyperacute rejection in the two-haplotype different combinations. The coincidence of this hyperacute graft destruction in this matched combination with the longest ischemia time observed (155 min.) might as well be an explanation since the time limit of donor lung preservation of two hours (Veith et al., 1966; Homatos et al., 1968) was exceeded. However, the occurrence of hyperacute rejection of lung allografts in donor-recipient combinations with an identical major histocompatibility complex cannot be excluded.

Although the observed histopathological changes in these small numbers of lung allografts in the three different groups could not be solely attributed to rejection in all cases, the conclusion that prospective typing for the serologically defined part of the major histocompatibility complex influences the rejection of lung allografts in dogs seems to be tentative. It is tempting to explain the differences in histological signs of rejection by qualitative or quantitative differences in transplantation antigens in the donor tissues. However, the immune response of the recipient toward the allograft is influenced by several other factors such as the affinity of the effectors (humoral and cellular) of the recipient immune system for the donor transplantation antigens and the accessibility of the different structures in the allograft to either antibodies or cells.

In prospect of our simultaneous bilateral lung allotransplantations in dogs, the use of littermates matched for their major histocompatibility complex gives the opportunity to study the physiological effects of the transplantation procedure without interference by rejection of the graft in the first three weeks in contrast to the early rejection response in non-typed mongrels. Since azothioprine has been proven to attenuate the lung allograft response in non-typed mongrel dogs (Barnes and Flax, 1964) the rejection of

lung grafts from matched littermates might as well be additionally retarded or even avoided by this immunosuppressive treatment.

CHAPTER 3
METHODS FOR MEASUREMENT OF CARDIOPULMONARY FUNCTION

symbols

the used symbols are letters, groups of letters, figures or signs, that represent a magnitude, element or calculation. Indices are added as subscript or superscript giving answer to the question "where?" as superscript and "what?" as subscript. When only one index is needed it is used as subscript. A dash above a symbol gives the mean of the variable.

symbols

A	surface area
BE	base excess
C	compliance
c	concentration, specific heat
D	diffusion coefficient
F	volume fraction, fractional concentration
f	frequency
m	mass
p	partial pressure, tension, pressure
pH	negative logarithm of hydrogen concentration
q	mass flow rate, volume flow rate
R	resistance
RQ	respiratory quotient, respiratory exchange ratio
S	oxygen saturation
T	period, periodic time
T	absolute temperature
t	time
t	temperature
U	galvanometer deflection
V	volume
γ	specific weight

indices

A	alveolar
a	arterial
B	barometric
b	blood
b	body
br	breath
cal	calibration
cel	cellular
D	dead space
dyn	dynamic
E	expiratory
FRC	functional residual capacity
h	heart
i	indicator
I	inspiratory
la	left atrial
lvs	left ventricular stroke
mv	mixed-venous
p	pulmonary
pev	pulmonary extravascular
sh	shunt
sp	specific
T	tidal
w	water

References: Dresner (1971); Mook et al. (1976).

3.1 introduction

Pulmonary function studies after bilateral lung transplantation should provide an evaluation of the function of the transplant as a gas exchange organ. In unilateral lung transplantation the life of the subject does not depend solely on the transplant and the evaluation of its exchange function is complicated by the presence of the contralateral normal lung. In bilateral lung transplantation the subject's gas exchange depends completely on the function of the transplant. At the same time the assessment of function is no longer complicated by the contribution of a parallel normal lung. Function studies after bilateral lung transplantation will give a better insight into the functional status of the transplant in the various stages after operation.

The transport of gases from inspiratory air to blood and vice versa is mediated by pulmonary ventilation and blood flow, and by diffusion through the separating membrane. The distribution of these quantities through the lung as a whole is of importance. In man several measurements of pulmonary function depend on the subject's conscious co-operation. This type of co-operation is not available in experimental animals.

Ventilation is the rhythmic mass movement of gas in and out of the lungs. It can be quantified in terms of frequency of breathing (f_{br}), tidal volume (V_T), and minute volume of ventilation (q_E). The measurement of functional residual capacity (V_{FRC}) is possible in an anesthetized animal.

Circulation through the lungs is closely related to the systemic blood flow. Apart from very small physiological left-to-right shunts, the cardiac output (q_s) of right and left side of the heart is the same in a steady-state. Pulmonary vascular resistance is an important indicator of the status of the pulmonary vessels. In essence the blood flow through the lungs is a pulsatile phenomenon and phase relation between pressure and flow determine the impedance of the system and provide information about its elastic and

resistive properties. In this study only the relation between mean bloodflow and mean pressure gradient across the system, i.e. mean pulmonary vascular resistance (R^{p}_{b}), has been used as an indicator of the vascular status of the lung.

Diffusion of oxygen and carbon dioxide from alveolar air to the blood and vice versa can be described in physical terms. By measuring the diffusion capacity for carbon monoxide (D^{p}_{CO}) instead of oxygen most of the difficulties in calculating the mean capillary and effective alveolar tension of oxygen are obviated (Forster, 1964).

Distribution

The over-all exchange function of the lung can be measured as a change in concentration between inspired an expired gas or inflowing and outflowing blood. The oxygen consumption (qo_2) and carbon dioxide production (qco_2) can be calculated from the minute volume of ventilation and the respective concentrations in the inspired and expired air. The ratio of carbon dioxide to oxygen exchange thus obtained is the gas-respiratory quotient (RQ_{gas}). In a similar way the blood flow and blood gas concentrations provide a measure of oxygen and carbon dioxide exchange from the circulatory side. The respiratory quotient in this case is the blood-respiratory quotient (RQ_{blood}). Under steady-state conditions the exchange of nitrogen is zero and the gas and blood respiratory quotient will be equal. With "ideal" diffusion the partial pressures for oxygen and carbon dioxide reach equilibrium at the end of a pulmonary capillary. If in such a case the ventilation and circulation would be uniform throughout the lung the corresponding partial pressures in the alveolar gas and end-capillary blood would represent "ideal" alveolar composition (Riley and Cournand, 1951). At a given composition of inspiratory air and mixed-venous blood it can be proven that a fixed relation exists between alveolar gas composition and respiratory quotient in an "ideal" lung and in a steady state (Rahn and Fenn, 1955). This relationship is defined in the "alveolar air equation". The quotient between alveolar ventilation and alveolar blood flow, the so-called alveolar ventilation-perfusion ratio, must under these conditions also have a well-defined value. In reality the lung is not an "ideal" lung with uniform ventilation and blood flow and uniform alveolar gas tensions. This non-uniformity affects the

apparent equilibrium between gas tension in alveolar air and blood as judged from over-all measurements of these quantities. Especially for oxygen an alveolar-arterial gradient in tension may arise from the non-uniform distribution of ventilation and blood flow even in the presence of ideal diffusion. The **effective** alveolar pressures are the pressures that, if homogeneously present in a model lung, would cause the same exchange of oxygen and carbon dioxide with the capillary blood as the existing gas exchange in the actual lung, provided the composition of the inspiratory air and mixed venous blood are identical in both cases (Riley and Cournand, 1951). **Ideal** pressures are those which the alveolar gas and alveolar capillary blood would have in a lung with homogeneous alveolar composition and with perfect equilibrium between blood and gas phases whereby the quantitative aspects of gas exchange would be identical to the ones existing in the actual lung (Riley and Cournand, 1949). The effective alveolar gas and blood pressures become identical to the ideal values when the oxygen diffusion gradient is negligible (Riley and Cournand, 1951). A lung with inhomogeneous ventilation-perfusion relationships and therefore inhomogeneous alveolar gas composition is less "effective" then a lung with uniform distribution of ventilation-perfusion ratios. This means that the homogeneous lung can under comparable circumstances perform the same over-all gas-exchange with less alveolar ventilation and pulmonary capillary blood flow then the sum of the separate alveolar ventilations and blood flows of the parts composing the inhomogeneously functioning lung. The difference in the ventilation is the so-called alveolar dead space. The difference in capillary blood flow is an apparent venous-admixture. For carbon dioxide the effect of the distribution factor is very small and also the alveolar-arterial diffusion gradient is negligible. Therefore it has become customary to consider the actual mixed arterial carbon dioxide pressure as identical to the effective alveolar carbon dioxide pressure and with known values of over-all carbon dioxide exchange and respiratory quotient to derive effective alveolar oxygen pressure and effective alveolar ventilation using the alveolar air equation and alveolar ventilation equation (Rahn and Fenn, 1955). The difference between actual ventilation and effective alveolar ventilation is physological dead-space ventilation. Physiological dead-space can be considered as the sum of anatomical and alveolar dead-space. To the venous admixture effect which is caused by areas with relatively low ventilation-perfusion ratios may be added a true shunt or true venous admixture caused by blood flowing through non-ventilated areas. A true

shunt retains its value with high oxygen breathing whereas the apparent venous admixture becomes negligible when breathing pure oxygen. For this reason the alveolar-arterial gradient in oxygen tension during oxygen breathing can be considered as an index of true shunt, i.e. blood flow through non ventilated areas. In general the determination of the alveolar-arterial oxygen gradient during air breathing gives an over-all appraisal of the efficiency of the oxygen exchange but does not allow one to distinguish which of the three factors, the "venous admixture", the "diffusion limitation", and the "distribution" is responsible for a given difference. The functional significance of the physiological dead-space and venous admixture is best expressed by the ratio of physiological dead-space volume to tidal volume ($V_D \cdot V_T^{-1}$) and shunt flow to total pulmonary blood flow ($q_{sh} \cdot q_s^{-1}$) respectively. The physiological dead space to tidal volume ratio can then be calculated from known values of mixed arterial and mixed expired carbon dioxide pressures while the percentage shunt flow to total pulmonary blood flow can be calculated by applying the Fick principle.

3.2 measurement techniques

3.2.1 general conditions

All measurements were done under pentobarbital anesthesia (6% solution, .5 ml per kilogram body weight intravenously for induction and repeated doses of one milliliter every hour) with the dogs in the restrained supine position. Arterial blood samples for bloodgas measurements in the postoperative period were taken without anesthesia. After muscle relaxation with a single intravenous dose of succinylcholine (5 mg) the dog was intubated with a cuffed endotracheal tube (Rüsch, int.diam. 9 or 9.5 mm). A continuous intravenous drip of saline was set at rate of approximately 100 milliliters per hour. Generally the animal was breathing room air except for the measurement of pulmonary shunt percentage. The environment was kept at a constant temperature of 20° C. For measurement of systemic and pulmonary arterial blood pressures, catheters (Danula subclavia, 22 gauge and 252 mm long; Bard-I-cath., 24 gauge, 70 cm long) were percutaneously introduced into a femoral artery and the right jugular vein respectively and connected via three-way stopcocks to pressure transducers (Statham P 23Db and P 23BB, respectively). The pulmonary artery catheter was manipulated in correct position under control of the pressure signal. An esophageal balloon catheter with a latex balloon of 10 cm and filled with .2 ml air was introduced in the lower third of the esophagus and connected to a differential pressure transducer (Statham, PM 6). The ECG-signal was continuously monitored from standard three leads (Hewlett-Packard 8811A). Arterial and mixed-venous blood samples could be withdrawn from the three-way stopcocks of the femoral artery and pulmonary artery catheters. All recordings of continuous signals were made on a 8-channel paper recorder with appropriate amplifiers (Hewlett-Packard 7785A).

3.2.2 blood

The **hemoglobin concentration** (c_{Hb}) was measured by photometry of the optical density of hemiglobincyanide of an arterial blood sample (Vitatron universal photometer) and expressed in terms of millimols per liter blood (van Kampen and Zijlstra, 1965, pp. 141-187).
The **hematocrit** (F_{cell}) was measured in arterial blood by the microhematocrit technique and expressed in terms of percentage. By measuring hematocrit in arterial blood the whole-body hematocrit is estimated with a negligible error (Albert, 1965).

3.2.3 blood gases and pH

The apparatus used for measurements of pH, p_{O_2} and p_{CO_2} (Gascheck-AVL, type 936, Radiometer) combines a capillary glass pH electrode, a capillary p_{O_2} electrode and a capillary p_{CO_2} electrode. The apparatus meets the criteria for accurate measurements as discussed by Severinghaus (Severinghaus, 1965). Blood samples for measurements were taken under anaerobic conditions with heparin-filled plastic syringes of 5 or 10 ml and glass syringes of 10 ml after pure oxygen breathing. The measurements were done immediately after blood collection at body temperature. Daily calibration of the apparatus was done according to the specifications of the manufacturer. When high p_{O_2} values were measured a separate calibration was needed. Fractional saturation of hemoglobin with oxygen (S_{O_2}) was calculated using a nomogram designed by Rossing and Cain (1966).

Base excess (BE) was calculated from the values for arterial carbon dioxide tension, pH and hemoglobin concentration. According to SiggaardAndersen (1966) the buffer capacity (β) of blood in vitro depends on hemoglobin concentration. In our units this is:

$$\beta = 9.5 + 2.62\, c_{Hb} \qquad (1)$$

In vivo the buffer capacity is smaller because the carbon dioxide in the blood exchanges freely with the interstitial fluid compartment which is not nearly as well buffered because of its low protein concentration. Since the blood can be assumed to carry almost all the short-term buffer capacity and since its volume is about 37% of total extracellular fluid volume we followed the suggestion of Burnett et al. (1974) taking:

$$\beta = 0.37\,(9.5 + 2.62\,c_{Hb}) \qquad (2)$$

Plasma bicarbonate concentration was determined using the Henderson-Hasselbalch equation. As a normal standard bicarbonate we took a mean preoperative value for 13 dogs in such a way that mean base excess for these dogs was zero. This standard value amounted to 19.53 meq·l^{-1}. The plasma bicarbonate concentration in an acute uncompensated respiratory acidosis or alkalosis is then:

$$c^{R}_{HCO_3^-} = 19.53 + \beta\,(7.40 - pH) \qquad (3)$$

The difference between actual bicarbonate concentration and this value is the base excess (Burnett et al., 1974):

$$BE = (c_{HCO_3^-} - c^{R}_{HCO_3^-})\ \ meq \cdot l^{-1} \qquad (4)$$

3.2.4 lung function

Ventilation

As has been noted in the introduction (3.1) the active co-operation needed for the measurement of lungvolumes and capacities is not available in laboratory animals. Therefore we only measured **tidal volume** (V_T), **frequency of breathing** (f_{br}), **minute volume of ventilation** (q_E) and **functional residual capacity** (V_{FRC}).

A pneumotachograph head (Fleish type I) with a dead space of 15 ml, directly connected to the endotracheal tube of the dog, was used for measurement of ventilation parameters (Fleish, 1925). The flow-to-pressure relation of the used pneumotachograph is linear from 0 to 1500 ml per second flow. The ventilatory volumes were obtained by electronic integration of the flow signal from the differential pressure transducer (Jaeger, Pneumotachoscript und Lungenfunctions Computer with Hewlett-Packard bioelectric amplifier 8811A). The accumulated expired volume was recorded every 30 seconds. Calibration of the pneumotachograph was done by flushing with a dry gas mixture of ten liters nitrogen and two liters oxygen per minute from rotameters. The viscosity of this gas mixture closely resembles that of air. Calibration of the accumulated volume was done by emptying a one liter gas syringe through the pneumotachograph six times.

Minute volume of ventilation (q_E) was measured as the mean accumulated expired volume in five minutes and expressed in terms of milliliters per minute. A five minute period of recording was chosen to correct for small variations in the expired volumes.

Frequency of breathing (f_{br}) was calculated as the mean of the same five minute period and expressed in terms of breaths per minute.

Tidal volume (V_T) was calculated by dividing minute (expired) volume of ventilation by frequency of breathing and expressed in terms of milliliters. A systemic error of approximately 5% overestimation in the pneumotachographic respiratory flow measurements can be introduced by differences in temperature, pressure, humidity and composition of the respiratory gas and calibration gas (Grenvik et al., 1966). Small errors are also introduced by the inexactitude of the gas syringe and the rotameters. However such systemic errrs do not invalidate comparison between various conditions.

pulmonary mechanics

Static pulmonary compliance (C^{P}_{st}) was measured as the mean ratio of inflated and deflated volumes to transpulmonary pressure differences under static conditions. After muscle relaxation with a single intravenous injection of succinylcholine (5 mg) the lungs were inflated with increments of 200 ml of air to a maximum of one liter and successively deflated with the same volumes. The transpulmonary pressures were measured with a differential pressure transducer (Statham, PM 6) between tracheal tube and esophageal pressure representing intrapleural pressure (Millic-Emili et al., 1964). When transpulmonary pressure had stabilized, the new volume was given. The static pulmonary compliance was expressed in terms of liters per kilopascal.

Dynamic pulmonary compliance (C^{P}_{dyn}) was measured as the ratio of volume change to transpulmonary pressure change during quiet breathing. The volume change and pressure change were measured between the points of no flow from simultaneous recordings of air flow, tidal volume and transpulmonary pressure according to the techniques described before. Dynamic pulmonary compliance was expressed in terms of liters per kilopascal.

Specific pulmonary compliance (C^{P}_{sp}) was calculated by dividing dynamic pulmonary compliance by the previously measured functional residual capacity and expressed in liters per kilopascal per liter V_{FRC}.

Pulmonary resistance (R^{P}_{dyn}) was calculated as the ratio of the difference between inspiratory and expiratory transpulmonary pressure to the difference between inspiratory and expiratory air flow at mid-tidal volume. The measured value was expressed in kilopascal per liter per second.

Functional residual capacity was measured concomittantly with diffusing capacity and therefore the method of measurement will be described there.

The **alveolar ventilation** (q_A) and **alveolar oxygen tension** (p^Ao_2) were calculated from the alveolar ventilation and gas equations (Rahn and Fenn, 1955). This includes the assumption of a steady-state and a negligible concentration of carbon dioxide in the inspired air.

$$q_A = \frac{q_{CO_2}}{p^a\ co_2}\ 0.115\ \ ml \cdot min^{-1}\ (BTPS) \qquad (5)$$

in which 0.115 is the combined correction factor for conversion of partial pressure to fractional concentration and STPD to BTPS.

and

$$p^A\ o_2 = p^I\ o_2 - p^a\ co_2 \cdot \left(F^I\ o_2 + \frac{1 - F^I\ o_2}{RQ}\right) \qquad kPa \qquad (6)$$

in which $p^I\ o_2 = .2095\ (p_B - p_{H_2O})$
$F^I\ o_2 = .2095$

Alveolar-arterial oxygen pressure **gradient** ($p^Ao_2 - p^ao_2$) was calculated from the measured alveolar oxygen tension and arterial oxygen tension and arterial oxygen tension.

Pulmonary diffusion capacity for carbon monoxide (D^Pco) was measured by a breath-holding technique according to the principle developed by Marie Krogh (1915), modified by Forster et al. (1955) and adapted for use in the anesthetized dog by Jouasset-Strieder et al. (1965). The tracheal tube was connected to two three-way stopcocks to permit spontaneous breathing, lung inflation and alveolar gas sampling. After three previous inflations with one liter air the lungs were inflated with one liter of a helium-carbon monoxide mixture in air. This mixture was contained in a perspex syringe which had been repeatedly flushed with the test gas. The inflation was maintained for about 10 seconds. During the inflation no expiratory efforts were observed. The esophageal pressure rose to an average value of 2 kPa.

After the breath-holding period (T), determined from the continous registration of esophageal pressure, about 200 ml gas was sucked back into the syringe and after turning a three-way stopcock an alveolar gas sample was forced into a rubber collecting bag by compressing the animals chest manually. The alveolar sample was immediately analyzed for the fractional concentration of helium (F^A_{He}) and carbon monoxide (F^A_{CO}) with a katharometer (Meinhardt UG 22) and an infrared carbon monoxide analyzer (Meinhardt UG 52). The alveolar sample was led through silicagel for drying. The analyzers were calibrated with known test gas dilutions. The test gas contained 10% helium and .1% carbon monoxide in air (Hoek-Loos). Diffusing capacity for carbon monoxide was calculated using equation:

$$D^D_{CO} = \left(\frac{V_A}{(p_B - 6.3)\cdot T}\right)\cdot \ln\left(\frac{F^A_{He}\cdot F^I_{CO}}{F^I_{He}\cdot F^A_{CO}}\right) \text{ml}\cdot\text{min}^{-1}\cdot\text{kPa}^{-1}\ (\text{STPD}) \quad (7)$$

in which p_B is the ambient barometric pressure in kPa,
T is the actual time of breath-holding in minutes,
F^A_{He}, F^A_{CO}, F^I_{He} and F^I_{CO} are measured,
V_A is the "alveolar volume" calculated from the dilution of helium. Since inflation started at end-expiration the "alveolar volume" consists of functional residual capacity (V_{FRC}) plus the inspired volume (V_I).

Functional residual capacity (V_{FRC}) was measured from the dilution of the inspired helium in the alveolar air according to equation:

$$V_{FRC} = \frac{F^I_{He} - F^A_{He}}{F^A_{He}} \cdot V_I \quad \text{ml (STPD)} \quad (8)$$

in which V_I is the inspired volume, being 1000 ml,
F^I_{He} and F^A_{He} were measured.
Functional residual capacity was expressed in terms of milliliters (BTPS).

A systemic error is introduced by not including the anatomical dead-space

volume in the equation. With an assumed anatomical dead-space volume of 37 to 77 ml (Stahl, 1967), the measured functional residual capacity is overestimated by 10 to 13% when equation (8) is used in stead of that used by Sikand and Piiper (1966) and by its use in the calculation of diffusion capacity for carbon monoxide this value is equally overestimated. The validity of the method for measuring functional residual capacity is based on the assumption of even distribution of the inspired gas after the breath-holding period. Because of the existence of uneven distribution of inspired gas even in healthy lungs (Fowler, 1952) a breath-holding period of 10 seconds may not be enough to establish a homogeneous distribution of this inspired gas mixture. The effect of uneven distribution in healthy lungs and even more in diseased, cq. transplanted lungs, may give rise to errors in the estimation of functional residual capacity and diffusion capacity for carbon monoxide, expecially in the sense of underestimating the actual values (Bouhuys, 1964; Sikand and Piiper, 1966).

Gasexchange

Oxygen consumption (q_{O_2}) and **carbon dioxide production** (q_{CO_2}) were measured in a steady-state with the dogs breathing room air through a one-way breathing valve. Expired air was collected for five minutes in a plastic bag connected to the expiratory side of the breathing valve. The bag was tested for gas-permeability for oxygen and carbon dioxide during five minutes and was found to be sufficiently gas-tight. The fractional concentrations of oxygen ($F^E_{O_2}$) and carbon dioxide ($F^E_{CO_2}$) were measured immediately with a paramagnetic oxygen analyzer (Taylor O_2 Servomex OA 250) and an infrared carbon dioxide analyzer (Meinhardt UG 51) after drying the gas with silicagel. The concomittant minute volume of ventilation (q_E) was measured with the pneumotachograph between endotracheal tube and breathing valve. The oxygen consumption and carbon dioxide production were calculated using the following equations derived from Otis (1964):

$$q_{O_2} = \frac{F^I_{O_2}\,(1 - F^E_{CO_2}) - F^E_{O_2}}{1 - F^I_{O_2}} \cdot q_E \quad ml \cdot min^{-1} \qquad (9)$$

where $F^{I}o_2$ is .2095 (Diem, 1968)
$F^{E}\ o_2$, $F^{E}\ co_2$ and q_E were measured.

and

$$q_{CO_2} = F^{E}\ co_2 \cdot q_E \quad ml \cdot min^{-1} \tag{10}$$

The values of oxygen consumption and carbon dioxide production were expressed in terms of milliliters per minute and corrected for standard temperature, pressure, dry (STPD).

The **respiratory quotient** was calculated by dividing the measured carbon dioxide production by oxygen consumption (gas RQ).

The ratio of physiological dead-space to tidal volume ($V_D \cdot V_T^{-1}$) was measured using the Enghoff modification (Enghoff, 1938) of the Bohr equation (Bohr, 1891):

$$V_D \cdot V_T^{-1} = \frac{p^{a}\ co_2 - p^{E}\ co_2}{p^{a}\ co_2} \tag{11}$$

in which $p^{E}\ co_2$ was calculated from the fractional concentration of carbon dioxide in collected expired air,
$p^{a}\ co_2$ was measured in arterial blood sampled midway the expired air collection.

intrapulmonary shunt

The percentage true intrapulmonary shunt of cardiac output, the **shunt percentage,** was measured during oxygen breathing. After ten minutes the lungs were manually inflated five times just prior to the simultaneous arterial and mixed venous blood sampling. Partial pressures of oxygen and carbon dioxide in both samples and hemoglobin concentration in arterial blood were measured immediately. The shunt percentage was calculated according to the equation of Finley et al. (1960):

$$q_{sh} \cdot q_s^{-1} = \frac{.23\ (p^B - p^A_{H_2O} - p^A_{CO_2} - p^a_{O_2})}{.23\ (p^B - p^A_{H_2O} - p^A_{CO_2} - p^{mv}_{O_2}) + 21.6\ c_{Hb}\ (S^a_{O_2} - S^{mv}_{O_2})} \quad (12)$$

in which $\bar{p}^B$ is the ambient barometric pressure,
$p^A_{H_2O}$ is 6.28 kPa at 37° C (Altman and Dittmer, 1970; table 10),
$p^A_{CO_2}$ is $p^a_{CO_2}$,
$S^a_{O_2}$ is 1,
$p^a_{O_2}$, $p^{mv}_{O_2}$ and c_{Hb} were measured,
S_{O_2} was derived from a nomogram relating p_{O_2}, pH, temperature and hemoglobin saturation (Rossing and Cain, 1966),
.23 is the solubility coefficient of oxygen in whole blood expressed in $ml \cdot l^{-1} \cdot kPa^{-1}$,
21.6 is the oxygen capacity of hemoglobin expressed in $ml \cdot mmol^{-1}$.

The validity of this measurement is based on the assumptions of complete saturation of mixed arterial blood hemoglobin, a tension equilibrium for oxygen and carbon dioxide in end-capillary blood and alveolar air during oxygen breathing, and a complete wash-out of nitrogen from the lungs.

3.2.5 cardiodynamics

Cardiac output (q_s) was determined by the indicator-dilution technique (Hamilton et al., 1928). A specially designed injection system for repeated instantaneous injections in the pulmonary artery of a fixed volume of a stock solution indocyanide-green (Cardiogreen, Hynson; 5 $mg \cdot ml^{-1}$) was used. This system has previously been described by ten Hoor (1969, p. 173). Blood was withdrawn from the femoral artery catheter through a cuvette densitometer (Waters D 401) at a constant rate of 45.9 ml per minute with an infusion/withdrawal pump (Harvard 900). The downslope part of the dye-concentration curve was extended to the baseline by semilogarithmic extrapolation to correct for recirculation of the dye (Kinsman et al., 1929).

A static calibration of the densitometer was done before and after a series of cardiac output measurements. For this purpose .01 ml of the prepared indicator solution was withdrawn with an Hamilton syringe (Hamilton Comp.) and mixed in 10 ml of the dogs blood in a glass syringe. One drop of heparin was added to prevent coagulation. Under continuous stirring blood was withdrawn from the syringe through the cuvette at a constant rate of 45.9 ml per minute.
Cardiac output was calculated using the equations:

$$q_s = \frac{m_i}{y \cdot A_i} \quad ml \cdot min^{-1} \tag{13}$$

and

$$y = \frac{c_{cal}}{U_{cal}} \tag{14}$$

in which m_i is the amount of injected indicator,
A_i is the surface area of the extrapolated curve determined by planimetry (A.Ott, Kempten, West-Germany, type 30),
y is the calibration factor relating indicator concentration to galvanometer deflection,
c_{cal} is the concentration of indicator used for the calibration,
U_{cal} is the galvanometer deflection of the calibration curve at its plateau.

Possible errors in the concentration of indicator in the stock solution cancel out in equation (13) and (14). The possible total error in the indicator dilution method is approximately 10%. Calibration errors can amount to 5.5% (ten Hoor, 1969, pp. 261-262).

Heart-rate (f_h) was measured from the QRS-complexes of the continuously recorded ECG signal and expressed in terms of beats per minute.

Left ventricular stroke volume (V_{lvs}) was defined as cardiac output divided by heart-rate and expressed in milliliters.

Bloodpressures, i.e. femoral artery, pulmonary artery, and left atrial pressures, were measured with pressure transducers (Statham P 23Db and P 23BB) connected to saline filled intravasal catheters in the appropriate positions. The catheters were flushed repeatedly to prevent clotting. The pressure transducers were calibrated with mercury manometers and zero-pressure was determined with the transducers at heart-base level, i.e. two-third of the anterior-posterior thoracic diameter in supine position. The pressure signals were amplified (Hewlett-Packard 8805A) and recorded. Mean pressures were obtained by electronic integration and expressed in kilopascal. For measurements of left atrial pressure a catheter (Bard-I-cath; 24 gauge, 70 cm long) was inserted through a needle in the left atrium after thoracotomy. The other end of the catheter was brought to the exterior through a separate stabhole incision. It was closed after filling the catheter with diluted heparin and buried after termination of the measurements in a subcutaneous pocket for later use.

Pulmonary vascular resistance (R^{p}_{b}) was calculated as the ratio of the difference between mean pulmonary artery and left atrial pressures ($\bar{p}_{pa} - \bar{p}_{la}$) to cardiac output ($q_s$). When left atrial pressure could not be measured the ratio was determined between mean pulmonary artery pressure and cardiac output. The pulmonary vascular resistance was expressed in terms of kilopascal per liter per second.

3.2.6 blood volumes and pulmonary edema

Total blood volume (V^{b}_{b}) of the dogs was determined by dilution of 2 ml of a stock solution of I^{131} serum albumin injected directly in an accessible vein. Two heparinized arterial blood samples of 10 ml were collected in plastic syringes, one before and one 10 minutes after injection of the tracer.

The activity of the stock solution and the blood samples were counted in a scintillation well-counter. The total blood volume was calculated using the equation:

$$V^{b}{}_{b} = \frac{m_{b}}{c_{i}} \text{ ml} \qquad (15)$$

in which m_b represents the amount of I^{131} albumin
= (count rate of tracer — background) × dilution of stock solution × volume administered,
c_i represents the concentration of tracer in the postinjection sample = (count rate of postinjection sample — background of dog in pre-injection sample).

Since the range of measured hematocrit values in our experiments was .36 to .54, the systemic error introduced by not correcting the measured value for the difference between measured hematocrit and whole body hematocrit accounted for an overestimation of 2.3 to 4.5% (Albert, 1971, pp. 593-602). Inaccuracy of the administered volume gives a small accidental error.

Pulmonary blood volume ($V^{p}{}_{b}$) was measured by sequential injection of indocyanide-green in the pulmonary artery and left atrium and measurement of the sequential indicator dilution curves from the femoral artery as has been described by Spangler et al. (1971). After semilogarithmic extrapolation of the downslope part of both curves the cardiac output was calculated and mean transit times of both curves determined. Pulmonary blood volume was calculated according to equation:

$$V^{p}{}_{b} = (T_{pa} - T_{la}) \cdot \bar{q}_{s} \text{ ml} \qquad (16)$$

in which T_{pa} and T_{la} are the mean transit times of indicator after injection in pulmonary artery and left atrium,
$\bar{q}_s$ is the mean cardiac output calculated from both curves.

The use of the same sampling system for both determinations ensures that any distortion of the curves due to the sampling will affect both curves equally. A discussion of errors due to variations in timing and rate of injection, position of catheters, and sampling system is given by Yu (1969, pp. 67-72).

Pulmonary extravascular water volume (V^{pev}_w) was estimated to serve as an indicator of interstitial edema. The measurement was performed by a double indicator dilution technique according to the principle of Chinard and Enns (1954). Heat served as the diffusable indicator as suggested by Noble and Severinghaus (1972), while indocyanide-green was used as the intravascular indicator. A precise volume of 3 ml saline at room temperature was injected with .25 ml indocyanide green solution (approximately 5 mg ml ^{1}) sequentially in the pulmonary artery and in the left atrium. The indocyanide dilution curves were recorded from one femoral artery as described while the thermodilution curves were recorded with a commercially available thermistor probe (Hewlett-Packard 1412A, 1200 ohm ± 5% at 40°C) introduced into the descending aorta through the other femoral artery and connected to a low-level amplifier (Hewlett-Packard 350-1500A and 350-15). The cardiac output and mean transit times of the two dye-concentration and two thermodilution curves were calculated after semilogarithmic extrapolation of the downslope parts of the curves. Thermodilution cardiac output was calculated according to equation:

$$Q_T = \left\{\frac{\gamma_i \cdot c_i}{\gamma_b \cdot c_b}\right\} \cdot \left\{\frac{V_i \cdot (T_b - T_i)}{\int \Delta T_b\,(t)dt}\right\} \cdot (1 - a) \qquad (17)$$

in which γ_i is specific weight of injectate, being 1.005 $g \cdot cm^{-3}$,
γ_b is specific weight of dog blood (van der Werf, 1965, p. 154),
c_i is specific heat of injectate, being 4.17 $J \cdot g^{-1} \cdot °C^{-1}$,
c_b is specific heat of dog blood (van der Werf, 1965, p. 154),
T_b is temperature of dog blood measured with the thermistor,
T_i is the temperature of the injectate determined with a needle thermometer in the bottle of injection fluid (Ellab, Copenhagen, type TE 3 and K 3),
V_i is the injected volume, being 3 ml,

$\int \Delta T_b(t)dt$ is determined by the surface area of the curve calibrated for paper speed and thermistor,
(1 — a) is a correction factor for heat losses through catheter wall calculated according to Vliers (1970, pp. 37 and 53).

Calibration was done after each pair of thermodilution curves by replacing the thermistor in the Wheatstone bridge by a variable resistance using the relation of temperature change to resistance change given in the calibration chart of the thermistor. The maximal difference between the four measured cardiac output values was approximately 10%.
Pulmonary extravascular water volume was calculated as the difference in mean transit time from pulmonary artery to left atrium between dye and heat, multiplied by the mean of the four cardiac output values.
The errors caused by catheter delay and differences in detection position of thermodilution and dye dilution curves cancel out.

Wet-weight to dry-weight ratio (W/D ratio) of the pulmonary tissue was determined of a small biopsy taken from the periphery of a suitable lobe. After weighing the specimen immediately after sampling (Mettlar balance H 33) it was dried at 36°C during 48 hours in a stove and weighed again. No corrections were made for the water content of the residual blood because the water fraction of normal blood is approximately .83 close enough to that of the lung (Staub, 1974).

CHAPTER 4
OPERATIVE TECHNIQUE FOR THE SIMULTANEOUS BILATERAL ALLOTRANSPLANTATION OF THE LUNGS

4 content

4.1 introduction

In the past simultaneous bilateral lung transplantation has been unsuccessful in the experimental animal and has been considered impossible because of the complete denervation of the lungs (Juvenelle et al., 1951). In 1964 Slim and co-workers (Slim et al., 1964) reported the first successful two-stage bilateral lung reimplantation in the dog. This was followed by a report of Lempert and Blumenstock (1964) and later in 1971 by a detailed study of five dogs that were alive five months following bilateral lung reimplantation (Lempert et al., 1971). Both investigators staged the reimplantations from two to 12 months apart. Faber et al. (1965) reported four long-term survivals in dogs after staged bilateral lung reimplantations with an interval of ten days between the left and right reimplantations. They concluded that the total severance of lymphatic vessels, bronchial arteries and nerves of the lung did not have the deleterious effect on survival and function as was expected before. Alican et al. (1971) were the first to report upon successful one-stage bilateral lung reimplantations in dogs. A double unilateral technique was used through bilateral posterolateral thoracotomies in 26 dogs. A total of six anastomoses was needed and the whole procedure took approximately three hours. Ten dogs survived more than one month with normal bloodgas values, lungscans, pulmonary angiographies and pulmonary artery pressures. Sixteen dogs died within one week after operation mainly because of pulmonary edema. Thrombosis of the left atrial anastomosis was the main technical problem experienced. No bronchial anastomotic failures were found. It was followed by a report of 33 one-stage bilateral allotransplantations using the identical technique (Alican et al., 1973). Without immunosuppression all dogs died during the first five days. With immunosuppression (azothioprine or methotrexate combined with prednisolone) three dogs survived more than one week. Severe pulmonary edema was the main cause of death during the first few postoperative days. Thrombosis at the left atrial suture line of either lung comprising its pulmonary venous outflow occurred in three dogs. In the dogs dying between the second and seventh day epithelial desquamation and necrosis of bronchial mucosa were frequently encountered. In our view this double implantation technique with a total of six anastomoses is time consuming

and has a cumulative risk of complications especially leakage and thrombosis. Vuillard et al. (1969) were the first to describe the implantation of a lung-bloc instead of two separate lungs thereby diminishing the number of anastomoses. The operation was performed through a left-sided thoracotomy. After a left pneumonectomy the lung-bloc was implanted by anastomosing its main pulmonary artery to the recipient's left pulmonary artery, its left atrium to the side of the opened entrances of the left pulmonary veins and its trachea to the left bronchus. However, the animal still had to be turned over and the right-sided thoracotomy performed in order to do the right pneumonectomy and to position the right lung in the right hemithorax. This technique has important advantages. It minimizes the anastomoses to three and obviates the need for extracorporeal circulation. Vuillard reported of three dogs which survived more than 36 hours and mentioned technical failures such as pneumothorax and thrombosis of the left atrial anastomosis but no further details were given. The technical concept was ingenious but we felt it to be impractible if a second thoracotomy could not be excluded. Access to the left lung through the posterior mediastinum from the right side had been used by us for bilateral hilus stripping and seemed also to permit a pneumonectomy. For this reason Vuillard's technique was modified. A right-sided thoracotomy was used. The left pneumonectomy and the positioning of the implanted left lung in the left hemithorax was done through the posterior mediastinum. In 1972 Vanderhoeft reported the first experience with this technique, however, with some important differences (Vanderhoeft et al., 1972). After a right pneumonectomy an extracorporeal circulation was installed. This made it possible to do the left pneumonectomy before implantation. The positioning of the lungs, especially the left lung, through the posterior mediastinum could be done in a non-functioning state. Suturing of the pulmonary artery anastomosis, left atrial anastomosis and both right and left bronchi was done during total cardiopulmonary bypass. The results, however, were disappointing because of technical problems. Of the 11 allotransplantations only six survived more than one hour after operation with the longest one approximately four-and-a-half hours. Hemorrhage was the main complication encountered: four dogs with bleeding from the anastomoses and three due to post-perfusion coagulation defects. We felt that the benefits of using an extracorporeal circulation thereby facilitating the anastomosing technique did not outweigh the disadvantages of introducing the additional risks of the extracorporeal circulation.

4.2. materials and methods

4.2.1 materials

The operation technique was first tested in a series of 10 dogs. After establishing the technique 54 allotransplantations were performed. In the first 28, random pairs of mongrel dogs with an average body weight of 26.9 kg (± 5.9) were used. In the remaining 26 allotransplantations, littermate pairs of labrador-retrievers were used prospectively typed for their major histocompatibility complex (chapter 2). The mean body weight of the second group was 24.4 kg (± 4.0). Donor and recipient in each combination were of an approximately equal weight.

4.2.2 anesthesia

Premedication consisting of atropine (.25 to .50 mg i.m.) and pethidine-HCL (50 mg i.m.) was given. Anesthesia was induced by intravenous thiopenthal sodium (30 mg/kg body weight). Intubation with a cuffed endotracheal tube (Rüsch, i.d. 9 or 9.5 mm) was facilitated by a single intravenous dosage of succinylcholinechloride (5 mg). Artificial ventilation was used either by hand (Water's open-bag system) or with an artificial ventilator (Monaghan 300/DM in experiments 1 to 40 and Engström Respirator ER 300 in experiments 41-54). The minute volume of ventilation was approximately 6 to 10 liters per minute. Anesthesia was maintained with an oxygen and nitrous-oxide mixture (1 : 2 volues) and in experiments 38 to 54 combined with repeated doses of phenobarbital 6% (1 to 2 ml every 3 hours). Analgesia was maintained by repeated doses of pethidine-HCL (25 mg i.v. every 2-3 hours) and muscle relaxation by repeated doses of succinylcholinechloride (5 mg/dose).
A glucose-saline solution (glucose 2.5 mg%, saline .45 mg%) was infused

continuously at a rate of approximately 100 ml per hour. A urine bladder catheter was introduced before operation and removed after finishing the postoperative measurements. The body temperature of the dogs was kept at approximately 37° C by feed-back of the continuously measured rectal temperature (Ellab TE 3 and RK 2) on the temperature setting of a surgical water mattress (Churchill).

4.2.3 operative technique

Donor: the lungs are harvested through a bilateral thoracotomy from a living anesthetized dog in the supine position. The inferior pulmonary ligament is divided and the pericardium resected. The pulmonary artery and its branches are dissected free from the aorta and a nylon tape is placed around the main pulmonary artery. After heparinization (3 mg/kg body weight, i.v.) a wide-bore cannula is inserted through the right atrium into the inferior caval vein. After filling the cannula with blood the distal end is closed. By ventilation with slight positive pressures macroscopic atelectases are removed. A stiff nylon catheter with an egg-shaped tip and an internal diameter of eight mm connected to a standard infusion system filled with the appropriate perfusion solution is inserted through a stabhole incision in the right ventricle and secured by a purse-string suture to prevent bleeding. The tip of the cannula is placed in the main pulmonary artery and the pulmonary artery occluded with the nylon tape around it. The perfusion of the lung is started with one of the solutions summarized in table (4.1) with a perfusion pressure of approximately 4 kPa (= 40 cm H_2O) at 4° C. At the same time the dog is bled from the cannula placed in the inferior caval vein. This blood is collected in citrated bottles for transfusion purposes (2.3 mg sodium citrate and 3 mg glucose per 500 ml). Shortly thereafter the superior caval vein is clamped and the left auricle opened to allow drainage of blood and perfusion fluid from the pulmonary veins. The perfusion is continued untill the effluent becomes clear and the color of the lung tissue has changed to white. Approximately 500 to 1500 ml perfusion solution is needed. In the meantime the ascending aorta is transsected and the pulmonary artery divided just above the pulmonary valve. The heart is removed by resecting

the left ventricle along the atrioventricular groove and by removing the right atrium from the atrial septum. This leaves the lung bloc with the main pulmonary artery and the whole left atrium intact. The lung bloc is finally removed by dissecting it free from the esophagus and transsecting the trachea. The trachea is intubated and air tight fixation accomplished by the use of a ligature. The lungs are kept continuously inflated.

table (4.1) solutions used for donor lung perfusion

solution	number
none	8
glucose	3
krebs	2
modified krebs[1])	15
collins III[2])	2
sacks[3])	24

[1]) Proctor, 1972
[2]) Collins et al., 1969
[3]) Sacks et al., 1973

Recipient: A right lateral thoracotomy is performed through the fifth intercostal space with the dog in the left lateral position. The pulmonary ligament is divided and the whole right lung including the paracardial lobe is retracted through the thoracotomy. The individual hilar structures and the azygos vein are doubly ligated and divided. The right main bronchus is proximally ligated and transsected. The posterior pericardium covering the pulmonary artery is incised and the right pulmonary artery is dissected free from the surrounding loose connective tissues. The right pulmonary artery is then doubly ligated and cut. The right pneumonectomy is completed by ligating and transsecting the pulmonary veins. The stump of the right pulmonary artery is now dissected free towards the main pulmonary artery permitting the placement of an appropriate Satinsky clamp in such a way

that the main pulmonary artery is partially occluded without interrupting the blood flow through the left pulmonary artery. The right pulmonary artery is opened cephalically with extension into the main pulmonary artery in order to permit a wide anastomosis. The pulmonary artery anastomosis is now performed. The donor's pulmonary artery is clamped with a hooked vascular clamp and held adjacent to the right pulmonary artery of the recipient. The donor lungs are retracted anteriorly. The cephalic side of the recipient's pulmonary artery is connected with an everting mattress suture (4-0 silk; atraumatic) to the donor's main pulmonary artery on the side of the right pulmonary artery branch. The caudal side of the recipient's pulmonary artery is similarly connected to the donor's main pulmonary artery on the side of the left pulmonary artery branch. The posterior aspect of the anastomosis is then closed with a continuous everting suture. After completion the lungs are moved posteriorly and the anterior side of the pulmonary artery anastomosis is closed in the same fashion. The pulmonary artery clamp on the recipient's side is opened and the anastomosis is checked for bleeding. Additional sutures if necessary have to be placed at this stage because the anastomosis cannot easily be reached later. An appropriate large Satinsky clamp is placed on the recipient's left atrium including all the entrances of the right pulmonary veins. The goal is to obtain a large atrial cuff without occluding the pulmonary veins on the left side. During these manipulations the cardiac function has to be watched carefully. When the clamp is placed satisfactorily the right pulmonary veins are opened and the atrial wall between the veins is incised. The auricular side of the donor's left atrium is sutured to the middle of the posterior wall of the recipient's left atrium. A continuous suture (4-0 silk; atraumatic) is used and the posterior wall of the anastomosis is closed from the inside. The anterior wall of the anastomosis is similarly sutured from the outside. After the completion of this anastomosis the distal pulmonary artery clamp is opened and residual air vented through an opening in the left auricle which is ligated at termination of the procedure. Thereafter the clamp on the left atrium is opened. The circulation through the donor lungs is restored in this stage to minimize the ischemia time. The recipient's left main bronchus is now intubated through the thoracotomy by way of the reopened proximal right bronchial stump. A ligature is tied around the left main bronchus to prevent air leakage. The recipient's trachea is divided above the bifurcation and the donor's trachea is trimmed down to the bifurcation. Interrupted sutures (3-0 mersilene; atraumatic) telescoping the donor's trachea into the

recipient's trachea are preferred. After completion of the tracheal anastomosis ventilation of the donor lungs is restored. The donor lungs are partially outside the thorax at this time. A left pneumonectomy is performed through the posterior mediastinum from the right side. The left recipient bronchus is detubated and the left lung retracted behind the heart into the right hemithorax. The left pulmonary artery and the pulmonary veins are then doubly ligated and divided. The remaining hilar structures have to be ligated to complete the pneumonectomy. The left vagus nerve can easily be injured during this dissection because it will be retracted to the right side. To position the donor lungs in the appropriate position in the thoracic cage the left lung is pulled into the left hemithorax through the posterior mediastinum by the surgeon's left hand entering the left side from the anterior mediastinum above the heart. The left upper lobe is first pulled through the posterior mediastinum behind the inferior caval vein and pulled upwards with the left hand followed by the lower lobe. Cardiac function has to be watched carefully during this procedure. The paracardial lobe and the right lung are easily positioned thereafter. Only one thoracic drainage cannula is used on the right side. The thorax is closed in layers in the usual way.

In figure (4.1) the different steps of the operative procedure in the recipient have been layed out schematically.

4.2.4 per- and postoperative treatment

All donor dogs received an intramuscular injection of 400,000 I.U. penicillin and .5 g streptomycin with their premedication. All recipient dogs received the same antibiotics for five days starting on the day of operation. All recipient dogs starting from number 38 were digitalized. All dogs, recipient and donor, starting from number 38 received 40 mg methylprednisolone intravenously with their premedication. In the recipient dogs this was repeated after the operation. On the following days prednisolone acetate was

given in a dose of .5 mg per kg body weight. Immunosuppressive treatment of the surviving dogs was started on the first postoperative day. All mongrel dogs received azothioprine intravenously in a daily dose of 3 mg per kg body weight. Azothioprine was also given to the last 14 labrador-retrievers in a daily dose of 1 mg per kg body weight but not to the first 12 labrador-retrievers.

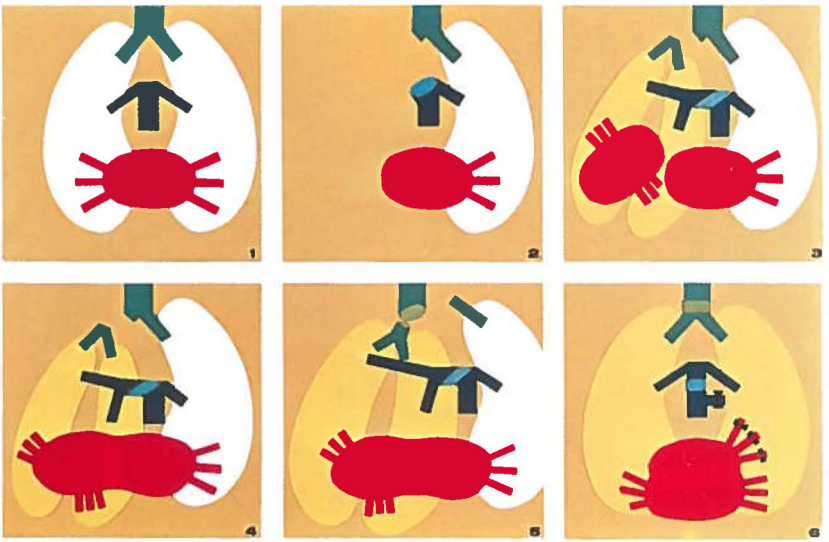


figure (4.1)
schematic drawing of the different stages of simultaneous bilateral lung allotransplantations:

1. the intact lungs in the recipient
2. the right pneumonectomy has been performed
3. the main pulmonary artery of the donor lung bloc has been anastomosed to the right pulmonary artery of the recipient
4. the donor's left atrium has been sutured to the recipient's left atrium and recirculation of the graft starts.
5. the donor trachea is sutured to the recipient's one while the gas exchange is maintained by the remaining left lung
6. the transplantation is completed by the left pneumonectomy and the positioning of the lungs in the thoracic cage.

4.3 results

A compilation of the results is given in table (4.2). In the 54 experiments the mean total ischemia time of the graft, defined as the period between start of in-vivo hypothermic donor lung perfusion and its recirculation with blood in the recipient, was 84 minutes. The mean second warm ischemia time between the end of hypothermic donor lung perfusion and its recirculation amounted to 59 minutes. The mean operation time between opening and closure of the thorax was 210 minutes.

Of the 54 dogs ten died during operation as a result of a lethal complication, seven related to one of the anastomoses and three due to cardiac arrest. In the postoperative period two dogs died because of a complication related to the surgical technique (total operative mortality of 22%). Seven of these complications occurred in mongrel dogs and five in the labrador-retrievers. In 42 dogs the bilateral lung allotransplantation was technically successful (78%). The fate of these 42 animals will be described in chapter (6).

table (4.2) compilation of results

total number of experiments		54
mean total ischemia time	84 ± 20 min.	
mean second warm ischemia time	59 ± 14 min.	
mean operation time	210 ± 42 min.	
total operative mortality		12
successful experiments		42

The surgical complications designated to the different surgical anastomoses and the peroperative cardiac arrests are summarized in table (4.3).

table (4.3) surgical complications

	related to death	not related to death
pulmonary artery anastomosis:		
bleeding	5	
twisting	1	
mural thrombus		2
occluding thrombus		1
left atrial anastomosis:		
bleeding	2	
stenosis		1
mural thrombus		6
tracheal anastomosis:		
leakage	1	
cardiac arrest during operation:	3	
total	12	10

The **pulmonary artery anastomosis** accounted for six complications leading to the immediate death of the animal during the operation. In five of these an irreparable tear of the anastomosis caused a lethal bleeding. In one animal twisting of the pulmonary artery anastomosis due to malpositioning of the anatomic landmarks resulted in complete obstruction to blood flow.

Bleeding from the **left atrial anastomosis** with an accompanying lethal hemorrhagic shock occurred in two dogs, one during operation and the other in the immediate postoperative period. In one dog an intraluminal extending abscess in the left atrial anastomosis covered by normal epithelium impeded the outflow of upper right and left pulmonary veins. This dog died 30 days after transplantation from histologically proven chronic rejection.

Persistent air leakage from the **tracheal anastomosis** was experienced in one dog resulting in a lethal pneumothorax after removal of the thoracic drainage tube three hours after operation.

Mural **thrombus** formations on the suture-line of the pulmonary artery without an obvious narrowing of the anastomosis at autopsy was observed in two dogs. In another dog a thrombus occluding the right pulmonary artery was observed at autopsy ten days after transplantation after an initial normal lung function and a subsequent severe septic period due to a generalized bronchopneumonia. Occluding thrombi on the left atrial anastomosis or in the pulmonary venous entrances were never encountered. In six dogs autopsied between one and seven days after operation mural thrombi were observed on the atrial suture-line. Thrombus formations were nearly always accompanied by a lack of endothelial adaptation or by intraluminal suture threads.

4.4 discussion and conclusions

Regarding the fact that any new experimental method is characterized by a high incidence of failure in the early learning stages, the resulting direct operative mortality rate of only 22 percent is promising. Our results indicate that simultaneous bilateral en-bloc lung transplantation through a right-sided thoracotomy without the use of an extracorporeal circulation can be performed with a high percentage of immediate survival (78%) in the dog. The operation can be performed in a reasonable time of approximately three-and-a-half hours including the harvesting procedure and perfusion. The mean total ischemia time of 84 minutes is well within the range of viability limits for lung grafts of nearly 2 hours (Veith et al., 1966; Homatos et al., 1968).

In judging the feasibility of the operative technique the complications arising from the different sites of anastomoses are of primary importance. Bleeding from a tear of the pulmonary artery anastomosis is the principal cause of peroperative death. The pulmonary artery of dogs is extremely fragile and uncareful handling of this structure during or after anastomosing will easily result in a tear and profuse bleeding. Because of the difference in diameter of the donor's main pulmonary artery and the recipient's right pulmonary artery a widening technique on the right pulmonary artery of the recipient has been used. To achieve this a Satinsky clamp is placed in such a way that the main pulmonary artery of the recipient is partially occluded without interrupting the bloodflow through the left pulmonary artery. Only a small margin of the pulmonary artery on the cephalic side of the recipient is thus available for suturing and might cause problems. The first dangerous moment arises when the posterior wall of the pulmonary artery anastomosis is done. For anastomosing the anterior wall the total lung-bloc has to be turned over. It is very important to keep the two Satinsky clamps on donor's and recipient's pulmonary artery securely adjacent during this movement to prevent traction on the sutures. The second dangerous period arises when the pulmonary artery anastomosis is finished and the proximal clamp is released. Movement of the lung-bloc can easily result in traction on and

tearing of the anastomosis especially on its cephalic side. It is impossible to redo the anastomosis whenever such a tear occurs at this stage of the operation because the margin of the recipient's pulmonary artery is too small to place another Satinsky clamp more proximally without obstructing the flow to the left pulmonary artery of the recipient. Although the suturing of the pulmonary artery anastomosis can be facilitated by the use of a cardiopulmonary bypass, postperfusion coagulopathy will then probably lead to other postoperative complications. Vanderhoeft et al. (1972), using cardiopulmonary bypass for simultaneous allotransplantations of a lung-bloc, reported hemorrhage in seven out of eleven experiments. Four were bleedings from one of the vascular anastomoses and three were the result of postperfusion coagulopathy. Reviewing the literature, bleeding from the pulmonary artery anastomosis cannot be considered as a major technical problem. Regarding the difficulties in bilateral lung transplantations, Vuillard et al. (1969) who were the first to describe the simultaneous en-bloc technique for bilateral lung transplantations in dogs did not report upon the details of complications. Lempert et al. (1971) mentioned only one case of hematothorax after 11 staged bilateral lung transplantations in dogs but did not give the origin of the bleeding. Alican et al. (1971) did not experience any complication of bleeding after one-stage reimplantation of both lungs in 26 dogs. In the numerous reports of unilateral lung reimplantation and allotransplantation bleeding from the pulmonary artery anastomosis is never mentioned as a problem. In the known reviews of human lung transplantations (Wildevuur and Benfield, 1970; Veith and Koerner, 1974) this complication is not mentioned. It can be concluded that the pulmonary artery anastomosis in our technique is a difficult part of the procedure and more difficult than in the unilateral procedure. However, these bleeding complications are considered to be avoidable.

A special problem in our operative technique for the simultaneous bilateral lung transplantation is the secure positioning of the pulmonary artery of donor and recipient for suturing. The described landmarks have to be followed securely otherwise repositioning of the lungs into the thoracic cage will result in twisting of the pulmonary artery. A major malpositioning occurred once and resulted in a complete obstruction to bloodflow. Lesser degrees of twisting can theoretically result in narrowing of the pulmonary artery but this was never observed in our autopsy specimens. Also no

stenosis of the anastomosis has been observed. Veith and Richards (1969) mentioned the importance of an angioplastic widening of the pulmonary artery anastomosis in unilateral lung transplantation with ligation of the contralateral pulmonary artery to prevent the fixed vascular resistance otherwise observed in transplanted lungs (Allgood et al., 1968; Baranski et al., 1963). This problem is specific for this model because the artery of the transplanted lung is forced to accept the entire pulmonary bloodflow. In our experiments the main pulmonary artery was used and a less critical situation existed as compared to the experimental model of Veith.

Where the majority of bleeding problems resulted from the pulmonary artery anastomosis, bleeding from the atrial anastomosis was only experienced twice. Bleeding from the left atrial anastomosis was apparently the result of a not well adapted atrial wall during suturing and should be avoidable. The only specific study on the importance of the left atrial anastomosis has been published by Benfield and Coon (1967). They studied the possibility of obstruction to pulmonary venous return. Isolated division and reanastomosis of a left atrial cuff resulted in an increase in mean pulmonary artery bloodpressure concomittantly with a delayed pulmonary venous return in those dogs in which angiographically a constricted anastomosis was seen. Although we did not control our anastomoses angiographically, we never saw a stenosis of the left atrial anastomoses at autopsy except in one dog. However, this stenosis only partially obstructed the left and right superior pulmonary vein and was the result of an abscess formation in the atrial suture line. Although in our technique the whole left atrium of the donor was transplanted and deprived of its coronary blood supply, stenosis due to ischemic fibrosis was never observed.

Although thrombi on the suture-lines occurred in nine dogs, thromboembolic processes did not play an important role in the final outcome of the experiments. Only in one dog an occluding thrombus was found in the right pulmonary artery at autopsy ten days after transplantation. In the remaining eight dogs small mural thrombi were found on the suture lines of left atrium and pulmonary artery but never to such an extent that it would have compromised the pulmonary blood flow. Histological examination of the anastomoses in these dogs showed in nearly

all cases a lack of endothelial adaptation and intraluminal suture threads at the site of the thrombus foration. Alican et al. (1971, 1973) reported a higher incidence of thrombosis at the left atrial suture line after bilateral lung reimplantation (20%) as compared to bilateral allotransplantation (9%). The small margin of atrial wall available for perfect endothelial adaptation by lung reimplantation explains this difference in incidence of thrombosis. So, in the majority of cases imperfections in the anastomosing technique seemed to be the prime cause of thrombus formation. In fact it is surprising that even without anticoagulation therapy no more thromboembolic complications occurred, because it is well known that the dog is more liable to thrombosis than man.

The optimal technique for tracheal or bronchial anastomosis is very much disputed in the literature. The bronchial anastomosis has been considered the Achilles-heel in lung transplantation (Veith and Koerner, 1974). Problems included leakage, stenosis, hemorrhage and mucosal necrosis leading to aspiration pneumonia. Such problems have contributed to the death of many of the human lung transplant recipients (Wildevuur and Benfield, 1970; Veith and Koerner, 1974). On this basis several investigators have advocated immediate revascularization of the bronchial arteries of the transplant by implanting into the recipient's aorta a button of donor's aorta containing the origins of the bronchial arteries. Metras (1950) first reported upon restoration of the bronchial arteries but gave little information of its effects on the healing of the bronchial anastomosis. Eight years later Bogardus (1958) again suggested re-establishment of the bronchial arterial circulation and in 1964 Nettleblad et al. (1964) restored the bronchial circulation in canine left lower lobe allotransplants with success. Mills et al. (1970) saw 82 percent bronchial complications after left lung allotransplantations compared to 20 percent when the bronchial arteries had been reconstituted. However, the origin of the bronchial artery supply is not constant (Notkovich, 1957) and its reconstitution will add to the complexity of the operation. For this reason many investigators attempted to minimize bronchial anastomotic complications by other methods. These included keeping the bronchial stump of the donor lung as short as possible (Ebert and Hudson, 1971; Alican et al., 1971), reinforcing the anastomosis with surrounding vascularized recipient tissue (Trummer and Berg, 1968; Blumenstock et al., 1967) or employing an intussuscepting technique (Veith

and Richards, 1970). We advocated the intussuscepting technique for our tracheal anastomoses and this proved to prevent leakage and fistulation. Only one leakage was seen in the immediate postoperative period. Our results are comparable with those of Castenada et al. (1972) who experienced only one disruption of the tracheal anastomosis after 21 successful cardiopulmonary autotransplantations. Lower et al. (1961) found normal healing of the tracheal anastomosis in two dogs which survived cardiopulmonary allotransplantation for five days.

Apart from the complications arising from the three different anastomoses three dogs died during operation from a cardiac arrest. They developed an unexpected and sudden ventricular fibrillation imediately after recirculation of the graft that could not be converted. In one dog air in the coronary arteries could be detected which points to the danger of entrapment of air in the left atrium. For this reason we systematically vented the transplanted left atrium through the left auricle before the clamp on the recipient's left atrium was released. In the other two dogs no air could be detected in the coronary arteries but it is still suggestive because fibrillation started just after recirculation of the graft.

In conclusion, the described operative technique for the simultaneous bilateral allotransplantation of the lungs proved to be feasible and offers a relative low mortality rate from technical failures whereas the majority of the experienced complications should be avoidable by a more precise technique. Bleeding from the pulmonary artery anastomosis can be avoided by more awareness of the fragile structure of the pulmonary artery. Thrombosis is preventable by perfect adaptation of the vascular endothelium and air leakage by employing an intussuscepting technique for the tracheal anastomosis. The next step in the investigation of the possibility to transplant both lungs simultaneously had to be directed toward the analysis of the function of the transplanted lungs.

CHAPTER 5
IMMEDIATE FUNCTION AFTER SIMULTANEOUS BILATERAL ALLOTRANSPLANTATION OF THE LUNGS

5 **contents**

5.1 introduction

Although simultaneous bilateral allotransplantation of the lungs in dogs has been shown to be technically feasible (chapter 4), the required conditions to overcome the insult of the transplantation procedure and to achieve immediate adequate function is of primary importance for survival of the animal. The factors which determine the initial function of the transplanted lungs and the death of the recipient in the early period after transplantation should therefore be analysed before the factors which interfere with prolonged survival and function are to be studied.

The functional status of the lungs immediately after their bilateral allotransplantation will be affected by the combined effects of ischemic damage to the lungs, the severance of bronchial arteries, lymphatics and nerves, the imperfections of the anastomoses of pulmonary artery, left atrium and trachea, the surgical trauma per se, and the occurrence of early rejection. The effect of allograft rejection can be eliminated by reimplantation in stead of allotransplantation of the lungs. However, the employed surgical technique for simultaneous bilateral transplantation of the lungs cannot be used in a reimplantation model. From our results of the influence of prospective typing for the major histocompatibility complex on lung allograft survival in chapter 2, it is not to be expected that rejection will influence the function of lungs transplanted between serologically identical littermate dogs in the early postoperative period. The immediate changes of function after allotransplantation of the lungs in these dogs can therefore be expected to be similar to a reimplantation response.
The immediate functional sequelae of our transplantation procedure and the possibility to survive the immediate postoperative period of 24 hours were therefore analyzed in a selection of dogs which received bilateral lung transplants from SD-identical littermates and in which cardiopulmonary function studies could be done.

5.2 materials and methods

Simultaneous bilateral allotransplantation of the lungs was technically successfully performed in 42 dogs (chapter 4). Twenty-one of these were labrador-retrievers which received their transplants from SD-identical littermates prospectively typed according to the technique described in chapter 2. Eight of these dogs were excluded from this study, four because of their immediate death when spontaneous breathing of air was allowed, and four because cardiopulmonary function measurements were not performed. Eventually cardiopulmonary measurements could be done in 13 dogs immediately after transplantation and the results are presented here.

The operative technique for the simultaneous transplantation of both lungs has been described in chapter 4. Sacks' solution was used for the flushing of the donor lungs in these experiments (Sack's et al., 1973).

The therapeutic regimen consisted of daily intramuscular injections of penicilline (400,000 U) and streptomycine (.5 g), starting with the premedication of both recipient and donor and continuing for five days in the surviving recipient dogs. During operation mannitol (100 ml 20%) was given intravenously as soon as recirculation of the graft was obtained. Furosemide (40 mg) was administered intravenously as soon as reventilation of the graft started. During or immediately after operation sodium bicarbonate (8.3%) was administered in various amounts (50 - 200 ml). Immediately after operation and preceding the cardiopulmonary measurements artificial ventilation was continued with positive end-expiratory pressure of 5 cm H_2O for a period of 30 to 60 minutes (Engström Respirator, ER 300). In the first seven experiments donor blood was not transfused a priori, but only when the circulation of the dogs deteriorated immediately after operation. In the remaining six experiments all recipient dogs received at least 500 ml donor blood as soon as recirculation of the graft was obtained and a second transfusion was given in most of these dogs after the end of the operation. After every transfusion of

500 ml blood .5 g calcium laevulate was administered intravenously. The last six dogs also received methylprednisolone (40 mg i.v.) with the premedication, repeated approximately eight hours later. The same dose of methylprednisolone was given to the donor animal with its premedication. Digoxin was given preoperatively to the last six recipient dogs in a dose of .5 mg and repeated four and eight hours later in doses of .3 mg each. No maintenance dose of digoxin was given thereafter.

The measurement of cardiopulmonary variables was done according to the techniques described in chapter 3. Preoperative measurements were performed in all donor and recipient dogs approximately one week before the operation. These measurements were repeated after transplantation as soon as the dog could resume spontaneous breathing of air without immediate signs of respiratory distress. Generally this was approximately one to two hours after operation. For the measurement of left atrial pressure and other variables which require catheterisation of the left atrium, the actual transplantation was in some dogs preceded by the implantation of a catheter in the left atrium after the initial right thoracotomy. In these dogs cardiodynamic variables and blood and pulmonary extravascular water volumes were measured anew with open thorax. These values served as preoperative control values for these dogs. Statistical analysis of the mean values of the measured variables, calculated from the available pre- and postoperative data of the 13 experiments, was done by a Wilcoxon matched-pairs signed ranks test (Siegel, 1956, pp. 75-83).

An X-ray picture of the chest was made in the anterior-posterior direction with the dog still in the restrained supine position and after removal of the thoracic drainage tube before the dogs were brought to their cage.

In the first seven experiments an incision biopsy of the periphery of a suitable lobe of the transplanted lungs was taken 30 minutes after recirculation of the graft. Autopsy was done in all dogs which died and their thoracic contents were inspected for macroscopic abnormalities. The three anastomoses were excised and a peripheral and central incision biopsy was taken from all lung lobes. All specimens were fixed in formalin and

processed by routine procedures for light microscopy. The histology of these specimens was judged by one investigator (Prof. Dr. Ch. R. Jerusalem, head Laboratory for Cytology and Histology, Catholic University, School of Medicine, Nijmegen, The Netherlands).

5.3 results

The individual and mean characteristics of the 13 experiments are compiled in table (5.1). Donor lungs were flushed with one to two liters Sacks' solution during 11 to 49 minutes. Mean total ischemia time of the grafts was 79 ± 21 minutes and mean second warm ischemia time amounted to 60 ± 18 minutes. No correlation was found between the ischemia times and survival times of the dogs. The estimated blood losses and amounts of donor blood transfused during or immediately after operation are given in this table. Blood loss never exceded 500 ml. In the first seven experiments blood transfusions were not given to four dogs and in three cases only after the operation was finished. In the last six experiments between 800 and 1500 ml donor blood was transfused of which 500 ml was given to all recipient dogs as soon as recirculation of the transplanted lungs started. Of the 13 dogs five died within the first 24 hours after transplantation while eight dogs survived for a varying period longer than one day.

5.3.1 immediate effect on morphology

The histopathological changes observed in the peripheral lung biopsies, taken 30 minutes after recirculation of the graft in the first seven experiments, are summarized in table (5.2). These seven dogs included only the dogs which neither received blood transfusions immediately after recirculation of the graft nor immunosuppressive drugs or digoxin. Interstitial edema and congestion of alveolar capillaries and small vessels were consistent findings in all the specimens. Localized damage of the endothelium of small vessels with occasionally attachment of polymorphonuclear cells (pmn cells) was observed in two dogs, one dying within the first 24 hours after operation and the other ten days after operation. In four dogs small thrombi were seen in the alveolar capillaries but not in larger blood vessels. Distinct perivascular hemorrhage was apparent in one dog which survived for 30 days. A frayed appearance of alveolar surfaces was seen in one dog.

table (5.1) compilation of results

experiment	perfusion		ischemia times		blood balance		survival
	amount	time	total	second warm	loss	transfused	time
no.	(ml)	(min)	(min)	(min)	(ml)	(ml)	(days)
1	1500	16	113	97	500	700	0
2	1000	49	104	55	200	500	0
3	1000	12	61	49	200	1000	0
4	1000	23	103	80	200	0	2
5	2000	21	100	79	100	0	30
6	1000	15	85	60	150	0	7
7	1000	17	83	66	100	0	10
8	1300	14	44	30	250	1000	0
9	1800	20	80	60	450	800	0
10	1400	14	56	42	400	1150	5
11	1500	11	64	53	100	1500	2
12	1500	13	76	63	200	1000	4
13	1500	17	60	43	100	1230	17
means:	1346	19	79	60	—	—	—
s:	±333	±10	±21	±18			

The amounts and time periods of donor lung perfusion, the total and second warm ischemia times, the estimated blood losses and the transfused amounts of donor blood, and the survival times of the 13 individual experiments are given with the mean and standard deviation of the first four variables.

table (5.2) histopathological changes in lungs 30 minutes after their simultaneous bilateral transplantation in seven dogs.

number of case	1	2	3	4	5	6	7
survival time (days)	0	0	0	2	30	7	10
damaged endothelium with pmn cells	●						●
congestion of capillaries	●	●	●	●	●	●	●
microthrombi in capillaries	●	●		●		●	
interstitial edema	●	●	●	●	●	●	●
perivascular hemorrhage					●		
frayed appearance of alveolar surfaces						●	

5.3.2 immediate effect on function

The results of the pre- and postoperative measurements in the dogs disconnected from the ventilator and breathing spontaneously are described on the following pages.

blood, blood gases, pH and base excess: (table 5.3)

Hemoglobin concentration (c_{Hb}) and hematocrit (F_{cell}) did not show significant changes.
Mean arterial oxygen tension ($p^a o_2$) decreased from 12.9 to 10.1 kPa. This change was significant ($p<.01$). Mean arterial oxygen saturation ($S^a o_2$), arterial carbon dioxide tension ($p^a co_2$), and pH were normal at this stage. In the five dogs which died in the initial 24 hours after operation, the individual values of $p^a o_2$ were below 8.5 kPa in four, of $S^a o_2$ below .90 in two and of pH below 7.20 in one. Mean base excess (BE) increased significantly with a mean change of 2.58 meq·l^{-1} ($p<.05$).

table (5.3) blood, bloodgases, pH and base excess

variable	unit	preoperative		postoperative	
c_{Hb}	mmol·l^{-1}	9.8	(1.4/13)	10.0	(1.4/12)
F_{cel}		.46	(0.4/13)	.46	(.06/12)
pH		7.31	(0.6/13)	7.36	(0.09/13)
$p^a o_2$	kPa	12.9	(2.1/13)	10.1	(1.9/13)**
$p^a co_2$	kPa	5.3	(.8/13)	5.5	(.8/12)
$S^a o_2$		.942	(.025/13)	.907	(.092/13)
BE	meq·l^{-1}	0	(4.4/13)	2.58	(4.9/12)*

mean results of measured preoperative and immediate postoperative variables with standard deviation of the mean and number of measurements (s/n).
* significant difference, $p<.05$
** significant difference, $p<.01$

lung function: (table 5.4)

Minute volume of ventilation ($q_E \cdot m_b^{-1}$) increased significantly ($p<.05$) from 187 to 271 $ml \cdot min^{-1} \cdot kg^{-1}$. A significant increase ($p<.01$) was also observed for tidal volume ($V_T \cdot m_b^{-1}$) from 12 to 20 $ml \cdot kg^{-1}$. Frequency of breathing (f_{br}) and functional residual capacity ($V_{FRC} \cdot m_b^{-1}$) remained at the same level as preoperatively. However, one dog was excluded from the statistical analysis of these variables because of its persistent tachypnoe during the preoperative measurements. Physiological dead-space to tidal volume ratio ($V_D \cdot V_T^{-1}$) did not change. Alveolar ventilation ($q_A \cdot m_b^{-1}$) decreased only in one dog but this change was due to the abnormal high preoperative value in this tachypneic dog. In the remaining five dogs in which alveolar ventilation was measured it increased after operation with a mean value of 71 $ml \cdot min^{-1} \cdot kg^{-1}$. Mean alveolar oxygen tension (p^Ao_2) increased significantly ($p<.05$) from 12.4 to 15.1 kPa. The changes in mean alveolar-arterial oxygen pressure difference ($p^Ao_2 - p^ao_2$) and alveolar ventilation could not be demonstrated to be significant with the used matched-pairs signed ranks test because of a devious change in one of the six dogs. With the Student t-test for paired observations the changes appeared to be just significant ($p<.05$). Intrapulmonary shunt percentage ($q_{sh} \cdot q_s^{-1}$) and diffusion capacity for carbon monoxide (D^p_{CO}) were normal immediately after operation. Carbon dioxide production ($q^E_{CO_2} \cdot m_b^{-1}$) increased significantly with 2.6 $ml \cdot min^{-1} \cdot kg^{-1}$ ($p<.05$) and oxygen consumption ($q^Eo_2 \cdot m_b^{-1}$) did not change. Respiratory quotient (RQ) increased from .80 to 1.04 and this change was significant ($p<.05$). Dynamic pulmonary compliance (C^p_{dyn}), static pulmonary compliance (C^p_{st}), specific pulmonary compliance (C^p_{sp}), and pulmonary resistance (R^p_{dyn}) remained at a normal level immediately after operation. However, a large spread of postoperative values occurred except for C^p_{st}. Comparing the dogs that died within 24 hours after operation with those who survived longer, no differences in lung function variables between both groups were observed at this stage.

table (5.4) lung function

variable	unit	preoperative		postoperative	
$q_E \cdot m_b^{-1}$	$ml \cdot min^{-1} \cdot kg^{-1}$	187	(30/11)	271	(77/11)* [1]
f_{br}	br.p.m.	16	(4/11)	16	(7/11) [1]
$V_T \cdot m_b^{-1}$	$ml \cdot kg^{-1}$	12	(3/12)	20	(12/11)** [1]
$V_{FRC} \cdot m_b^{-1}$	$ml \cdot kg^{-1}$	44	(8/12)	41	(15/6) [1]
$V_D \cdot V_T^{-1}$		.458	(.228/5)	.466	(.114/5)
$q_A \cdot m_b^{-1}$	$ml \cdot min^{-1} kg^{-1}$	83	(29/6)	148	(38/6)* [1]
$p^A o_2$	kPa	12.4	(.7/6)	15.1	(1.1/6)*
$(p^A o_2 - p^a o_2)$	kPa	1.2	(.9/6)	3.9	(1.7/6)* [1]
$q_{sh} \cdot q_s^{-1}$		.087	(.040/11)	.108	(.089/11)
D^p_{CO}	$ml \cdot min^{-1} \cdot kPa^{-1}$	202	(28/12)	202	(51/6)
$q^E_{o_2} \cdot m_b^{-1}$	$ml \cdot min^{-1} \cdot kg^{-1}$	5.3	(1.6/6)	6.4	(2.3/6) [1]
$q^E_{CO} \cdot m_b^{-1}$	$ml \cdot min^{-1} \cdot kg^{-1}$	4.1	(1.0/6)	6.7	(1.7/6)* [1]
RQ		.80	(.06/6)	1.04	(.14/6)* [1]
C^p_{dyn}	$l \cdot kPa^{-1}$	1.06	(.21/12)	1.44	(1.06/12) [1]
C^p_{st}	$l \cdot kPa^{-1}$	1.32	(.20/6)	1.33	(.08/4)
C^p_{sp}	$l \cdot kPa^{-1} \cdot l^{-1}$	1.17	(.34/12)	2.36	(1.37/6)
R^p_{dyn}	$kPa \cdot l^{-1} \cdot sec^{-1}$	.255	(.039/12)	.392	(.206/9)

mean results of measured preoperative and immediate postoperative variables with standard deviation of the mean and number of measurements (s/n).

* significant difference, $p<.05$

** significant difference, $p<.01$

[1] The data of one dog were excluded (see text)

cardiodynamics: (table 5.5)

Cardiac output ($q_s \cdot m_b^{-1}$) decreased significantly ($p<.01$) from 195 to 118 $ml \cdot min^{-1} \cdot kg^{-1}$. A significant decrease was also observed for left ventricular

stroke volume ($V_{lvs} \cdot m_b^{-1}$) from 1.1 to .7 ml·kg $^{-1}$ ($p < .01$). In six of the seven dogs in which blood transfusions were either not given or only after the operation, the individual values of cardiac output fell below 100 ml · kg $^{-1}$ and of left ventricular stroke volume below .7 ml · kg $^{-1}$. Three of these dogs died within 24 hours after operation. However, in the dogs which received blood immediately after recirculation of the graft in the recipient the cardiac output never fell below 150 ml · min $^{-1}$ · kg $^{-1}$ and left ventricular stroke volume never below .8 ml · kg $^{-1}$.

Mean femoral artery pressure ($\bar{p}_{fa}$) remained at a normal level comparing mean pre- and postoperative values. However, in all cases it decreased acutely when the recirculation of the transplanted lungs was restored. It remained low in the three dogs which were not treated with immediate blood transfusions and died in the initial postoperative period. A significant increase was observed for both pulmonary artery pressure ($\bar{p}_{pa}$) and pulmonary vascular resistance (R^p_b) with nearly 1 kPa and 38 kPa · 1 $^{-1}$ · sec $^{-1}$, respectively ($p < .01$). Generally when cardiac output was severely depressed the individual values of $\bar{p}_{pa}$ showed only a moderate increase and the individual values of R^p_b a sharp increase. When cardiac output was only moderately depressed $\bar{p}_{pa}$ was much higher and R^p_b less increased. One dog however showed a high mean pulmonary artery pressure concomittantly with a depressed cardiac output but this high value existed already before operation. Because of the small number of measurements statistical analysis of the observed values of mean left atrial pressure ($\bar{p}_{la}$) and trans-pulmonary pressure difference ($\bar{p}_{pa} - \bar{p}_{la}$) could not be performed. In one dog a more than 100 percent increase of the preoperative value of $\bar{p}_{la}$ was seen. This dog died within 24 hours after operation.

blood volumes and pulmonary edema: (table 5.6)

The total and pulmonary blood volumes and pulmonary extravascular water volume were only measured in four of the dogs which received blood transfusions at the moment of recirculation. Total blood volume ($V^b_b \cdot m_b^{-1}$) increased only in one dog concomittantly with an increase of pulmonary blood volume ($V^p_b \cdot m_b^{-1}$) and pulmonary extravascular water volume

table (5.5) cardiodynamics

variable	unit	preoperative		postoperative	
$q_s \cdot m_b^{-1}$	$ml \cdot min^{-1} kg^{-1}$	195	(57/12)	118	(38/13)**
f_h	b.p.m.	186	(23/12)	172	(34/12)
$V_{lvs} \cdot m_b^{-1}$	$ml \cdot kg^{-1}$	1.1	(.3/12)	.7	(.3/13)**
$\bar{p}_{fa}$	kPa	17.6	(1.6/12)	16.4	(2.8/12)
$\bar{p}_{pa}$	kPa	2.3	(5/13)	3.2	(.7/12)**
$\bar{p}_{la}$	kPa	.4	(.1/ 5)	.7	(.1/ 3)
$(\bar{p}_{pa}-\bar{p}_{la})$	kPa	1.6	(.4/ 5)	3.1	(.7/ 3)
$R^p{}_b$	$kPa \cdot l^{-1} \cdot sec^{-1}$	30.5	(3.9/11)	68.5	(21.9/ 7)**

mean results of measured preoperative and immediate postoperative variables with standard deviation of the mean and number of measurements (s/n). Since preoperative measurements of left atrial pressure was only done with open thorax preceding the transplantation the concomittant measurements in these dogs were taken as preoperative values.

* significant difference, $p<.05$

** significant difference, $p<.01$

table (5.6) bloodvolumes and edema

variable	unit	preoperative		postoperative	
$V^b{}_b \cdot m_b^{-1}$	$ml \cdot kg^{-1}$	102	(11/6)	109	(32/6)
$V^p{}_b \cdot m_b^{-1}$	$ml \cdot kg^{-1}$	7.4	(1.9/6)	10.1	(2.8/4)
$V^p{}_b \cdot V^b{}_b^{-1}$		.070	(.017/6)	.088	(.016/4)
$V^{pev}{}_w \cdot m_b^{-1}$	$ml \cdot kg^{-1}$	4.4	(1.3/5)	8.6	(1.6/4)
W/D ratio		5.03	(.23/12)	6.26	(.55/5)[1])

mean results of measured preoperative and immediate postoperative variables with standard deviation of the mean and number of measurements (s/n). The mean value of W/D ratio measured in 12 normal dogs was taken as preoperative value. All the preoperative measurements of the variables were performed with open thorax preceding the transplantation.

* significant difference, $p<.05$

[1]) significant difference, $p<.05$ (Student t-test for two samples)

($V^{pev}{}_{w} \cdot m_b^{-1}$). In all four dogs the increases of pulmonary blood volume were less than 60 percent of the original values. In three dogs the increase of pulmonary extravascular water volume was more than 100 percent of the preoperative value. Wet-weight to dry-weight ratio (W/D) measured immediately after operation was significantly higher then comparable values measured in 12 normal labrador-retrievers (Student t-test, $p<.05$).

5.3.3 postoperative course of five dogs dying within 24 hours

Five of the 13 dogs died within the first 24 hours after transplantation. Three of these did not receive blood transfusions before the operation was completed and exhibited a severe decrease of cardiac output with approximately 57 percent, of left ventricular stroke volume with 54 percent, and a decrease of mean femoral artery pressure with 23 percent. Transfusion of blood in excess of the estimated loss in these three dogs did not restore cardiac output and left ventricular stroke volume but it aggravated the already existing hypoxemia. The two other dogs received blood transfusions immediately after the recirculation of the graft in the recipient. Here a less pronounced decline of cardiac output with 27 percent and of left ventricular stroke volume with 38 percent was accompanied by a slightly elevated mean femoral artery pressure. However, a severe hypoxemia existed in these two dogs after operation. The X-ray pictures of all five dogs showed a homogeneous density of both lungs with signs of vascular congestion. At autopsy the lungs were swollen with rounding of the normal sharp edges. The surfaces of the cut lungs exuded hemorrhagic fluid. In one dog small non-occluding mural thrombi were observed on the suture-line of both vascular anastomoses. Otherwise no abnormalities of the anastomoses were seen at autopsy of any of these five dogs.

The histopathological changes in the autopsy specimens of all these dogs consisted of a severe congestion of alveolar capillaries and small vessels, interstitial and alveolar edema and peribronchial, perivascular and intra-alveolar hemorrhage. In two dogs hemorrhagic necrosis was already

apparent at this stage after operation. An increased number of free alveolar macrophages was present in two dogs. Microthrombi in alveolar capillaries and small vessels and damage of endothelium was seen in some dogs, however without attachment of polymorphonuclear cells to the damaged surfaces.

5.4 discussion and conclusions

The histopathological changes observed in the lung biopsies taken 30 minutes after recirculation of the bilaterally transplanted lungs resemble the changes seen at the same stage in the left lungs after its unilateral transplantation between comparable SD-identical littermate labrador-retrievers as shown in chapter 2. The relative absence of endothelial damage of small pulmonary vessels with attachment of many polymorphonuclear cells to the damaged surfaces in the lung biopsies taken 30 minutes after recirculation of the bilaterally transplanted lungs indicates that hyperacute and accelerated rejection of the lungs in these matched combinations of dogs did probably not occur. These histological findings were frequently seen in bilaterally transplanted lungs in mongrel dogs at the same stage (Wildevuur et al., 1973) and were characteristic for unilateral lung allografts in the two-haplotype different combinations described in chapter 2. The observed functional and morphological changes immediately after simultaneous bilateral allotransplantation of the lungs in the described 13 labrador-retrievers can therefore safely be regarded as similar to a reimplantation response.

The immediate postoperative data on lung function can be considered in relation to the morphological picture of the freshly transplanted lungs as apparent from lung biopsies taken before closure of the thorax. Congestion and edema would be expected to lead to important disturbances in perfusion, diffusion, ventilation and their mutual relations. Although oxygen uptake immediately after operation was comparable to preoperative values, the exchange function of the lungs as judged by the magnitude of the alveolar-arterial oxygen pressure difference was apparently insufficient at this stage and resulted in hypoxemia after spontaneous breathing of air was resumed. Hypoxemia was more pronounced in the dogs which died within 24 hours after operation but could not be related to specific disturbances of lung function. With the available data on alveolar-arterial oxygen pressure difference, diffusion capacity for carbon monoxide, and shunt percentage only a partial analysis of the exchange function of the lungs could be made.

The diffusion capacity for carbon monoxide was normal at this stage. Our values, however, contain a systemic error by inserting anatomical dead-space in the determination of alveolar volume. However, assuming an unchanged anatomical dead-space after transplantation, it remains well possible to compare pre- and postoperative values. As Sikand and Piiper (1966) have shown, the presence of inequality in ventilation-perfusion relations leads to an underestimation of alveolar volume and of diffusion capacity. We do not know how diffusion capacity is distributed in relation to ventilation and perfusion. When diffusion capacity resides in areas which are badly perfused as is suggested by the histological picture, the remaining capacity in well-perfused parts may be insufficient. The observed significant increase of alveolar-arterial oxygen pressure difference may be due to inequality in ventilation-perfusion relations and to inequality in diffusion-perfusion relations but not to the presence of large true shunts in the pulmonary circulation of our dogs. This explains the observed increase in alveolar ventilation and alveolar oxygen tension in spite of a moderate hypoxemia. The increased carbon dioxide production immediately after transplantation can be explained as a respiratory compensation of an acidosis developed during the operation which was eventually supported by the administration of sodium bicarbonate. Transfusion of blood may also add some bicarbonate to the blood (Stortenbeek, 1971, p. 220). The respiratory quotient rose to one but this was not accompanied by a clear rise in arterial carbon dioxide tension. The carbon dioxide exchange of the freshly transplanted lungs can therefore be considered to be sufficient. Because an acidosis was always corrected by the administration of sodium bicarbonate, the determination of base excess did not serve as a reliable indicator for the development of a metabolic acidosis due to the hypoxemia.

The increased minute volume of ventilation, tidal volume and alveolar ventilation and the unchanged physiological dead-space to tidal volume ratio indicate that the transplanted lungs can be ventilated adequately at this stage. In bilateral lung transplantation the lungs are completely denervated as shown by the absence of the inspiratory-inhibitory reflex of Hering-Breuer after bilateral hilus stripping in dogs (Sugawara et al., 1972). The increase in tidal volume after bilateral lung transplantation is in accordance with the effect of lung denervation (Lim et al., 1958). The expected lower frequency of breathing, however, was not uniformly seen. The relative

normal frequency of breathing can be explained as a relatively increased frequency with respect to the expected low frequency after lung denervation and probably resulted from an increased chemical drive.

Pulmonary edema and congestion can be expected to result in an elevated pulmonary tissue resistance and decreased dynamic and static pulmonary compliance (Noble et al., 1975). In some of our dogs an increase of dynamic pulmonary compliance was seen immediately after operation. This could not be explained by the observed morphological changes. In these dogs the static pulmonary compliance did not increase. The apparent paradox difference between static and dynamic pulmonary compliance in operated dogs may be related to the significance of esophageal pressure as an index of pleural pressure in these dogs either when breathing spontaneously as in the case of dynamic measurements or upon inflation as in the case of static measurements. We did not study surfactant activity after our transplantations but evidence exists that surfactant activity remains unchanged after unilateral lung reimplantations (Portin et al., 1960; Yeh et al., 1963; Waldhausen et al., 1965) and after lung allotransplantations in dogs (Yakeishi et al., 1969). A decrease of surfactant activity after lung transplantation could only be demonstrated by Trimble et al. (1966) in their cases of unilateral lung reimplantation.

The most prominent functional change immediately after transplantation was the severe decrease of mean cardiac output to approximately sixty percent of the preoperative value. The recirculation of the transplanted lungs was accompanied by an acute decrease of mean systemic blood pressure which gradually returned to normal at the end of the operation in most dogs that survived longer than one day. In the dogs surviving longer than one day cardiac output was normal or only slightly depressed. In three of the dogs which died during the first 24 hours after operation it was however severely depressed. A severe decrease of cardiac output could be prevented by transfusion of blood when it was given immediately after the recirculation of the graft in the recipient, but not when it was given after the operation was finished. The immediate transfusion of blood was, however, very critical since it could aggravate the already existing congestion and edema in the freshly transplanted lungs as apparent from the two dogs which died within

24 hours after operation notwithstanding the recovery of cardiac output by this transfusion. The acute changes in the circulation are considered as a shock occurring at the recirculation of the transplanted lungs in the recipient. This recirculation shock appeared to be reversible by the immediate transfusion of donor blood. Untreated it soon passed into the irreversible stage. Several factors might be involved in the appearance of this acute recirculation shock. Hemorrhage during or after operation may be an important cause of shock but it was always less than 500 ml and nearly always replaced by volume. The acute filling of the previously bloodless vascular bed of the transplanted lungs may, however, result in an important immediate loss of circulating blood volume. The pulmonary blood volume measured by the indicatordilution method and amounting to 170 ml after transplantation cannot sufficiently explain the occurrence of an acute shock upon recirculation. This blood volume was about the same as measured preoperatively in our dogs, i.e. 7.4 ml per kg bodyweight and close to the 8.0 ml for normal dogs given by Sanchez et al. (1967). The observed congestion of the pulmonary vascular bed in lung biopsies taken 30 minutes after recirculation and the severe hemorrhage in the pulmonary parenchyma apparent in autopsy specimens of dogs which died within 24 hours after transplantation is in discordance with the measured normal pulmonary blood volume. This discordance can be explained by an incomplete mixing of the injected indicator in the pulmonary vascular bed because of stasis and sequestration of blood in the transplanted lungs (Yu, 1969, pp. 67-72). Probably a larger volume of blood than measured with the indicator-dilution method is withdrawn from the circulating blood volume at the recirculation of the lungs. The acutely occurring pulmonary edema might be another source of loss of circulating volume. In several dogs of this study we have tried to quantify the pulmonary edema by measuring the pulmonary extravascular water volume and by determining the wet-weight to dry-weight ratio of peripheral lung biopsies. Although the measurements were scarce and mostly done in dogs without apparent clinical signs of pulmonary edema, an important rise of pulmonary extravascular water volume was observed in these dogs after transplantation which correlated with the morphological findings. The wet-dry ratio of peripheral lung biopsies may not accurately represent that of whole lungs but probably the direction of the observed changes from normal lungs is correct. Considering the possible causes of the congestion and edema occurring at the recirculation of the transplanted lungs, we believe that the severance of perihilar nerves and

lymphatics and the temporary ischemia including the employed way to protect the donor lungs from its damaging effect are the most important factors responsible for the observed changes. Pulmonary congestion and edema was also observed immediately after bilateral hilus stripping in dogs in experiments performed in our laboratory (Homma et al., to be published). Staub (1974) considered pulmonary edema as a derangement of normal dynamic fluid physiology of the lung in which the lymphatic flow plays a primary role. According to him an increase in microvascular hydrostatic pressure and changes in membrane qualities also contribute to the development of pulmonary edema. An important additional cause of the observed morphological abnormalities and the resulting functional derangements may reside in the temporary ischemia and the employed way to protect the donor lungs from its damaging effect. As has been summarized by Abbot and Weinerth (1971) methods for organ preservation may be divided in two distinct categories considering the physiological sequence of events after death of the donor or the extirpation of the organ. The vital flow to the organ is interrupted depriving it of its supply of nutrients and removal of wastes. This creates an abnormal environment which if not corrected soon leads to injury and death. Methods such as hypothermia, hyperbaria and pharmacological agents all fall into a first preservation category which acts by increasing the resistance to the abnormal environment but as such only delays the ultimate injury and death sequence. Continuous and interrupted perfusion of a stored organ falls into a second preservation category, renewed supply, which is more physiological since it acts to recreate a normal physiological environment. After severalauthors studied the time limits of cadaver lung viability Veith et al. (1971c) showed that dogs can tolerate contralateral pulmonary artery ligation immediately after autotransplantation of a lung subjected to two to three hours normothermic ischemia without perfusion of the lungs provided the lungs were kept inflated during the ischemic period. Hypothermia alone or combined with hyperbaria allowed lungs to be ischemic up to 24 hours without loss of histological integrity (Blumenstock et al., 1962; Largiader et al., 1965) and with the ability to take up oxygen several weeks after autotransplantation of these lungs (Garzon et al., 1968; Hino et al., 1968). Our employed method of flushing the donor lungs with Sacks' solution of 4° C has the advantage of immediately cooling the organ and washing-out the blood that otherwise might coagulate during the subsequent ischemic period. The use of the solution described by Sacks et al. (1973) was based on

the experience with kidney preservation for transplantation. With the idea of reducing changes in ion distributions on both sides of the cell membrane due to inactivation of the sodium pump under hypothermic conditions Collins et al. (1969) created a solution more or less resembling intracellular composition. A later modification of this solution is nowadays generally used for the clinical kidney preservation for transplantation. With the idea of reducing cellular swelling of preserved kidneys Sacks et al. (1973) modified the Collins solution by adding mannitol and found it to be more effective in kidney preservation than the Collins solution at that time. The observed congestion and pulmonary edema immediately after the bilateral lung transplantation might have been an effect of the ischemic damage not enough protected but even more aggravated by the employed technique of flushing the donor lungs with Sacks' solution. Castagna et al. (1972) already indicated that the perfusion of donor lungs prior to their reimplantation offered no apparent advantage over no perfusion at all. The perfusion of donor lungs with plasma or low molecular-weight dextran even increased the water content of the lungs in their experiments. The lungs in their structural and functional aspect totally different from kidneys might demand a composition of preservation solution different from that used succesfully for kidneys. The severe decrease of cardiac output immediately after operation may not only be the result of loss of circulating blood volume. The decline of cardiac output was the result of a low stroke volume since heart-rate did not change. Considering stroke volume as affected by diastolic filling and its complete or incomplete ejection during systole, an important cause of the low stroke volume may lie in a decreased contractility of the myocardium itself. According to Guyton (1973, pp. 240-241) sympathetic inhibition leads to a hypo-effective state of the heart. It is possible that sympathetic cardiac nerves running close to the bronchi have been interrupted during bilateral lung transplantation since these nerves have been shown to intermingle with the peribronchial nerve plexus (Hirsch et al., 1968a, 1968b). The severance of these sympathetic nerves may have resulted in a decreased contractility of the myocardium itself after the transplantation. The same but less prominent picture of a hypo-effective heart was seen in dogs one week and three months after bilateral hilus stripping in experiments in our laboratory (Irie et al., to be published). Fluid loading of these hilus stripped dogs revealed that ventricular function was depressed. Comparable observations of a decreased stroke volume and an inability to increase heart-rate in response to acute blood loss were made by Lower (1969) in his

experience with the reimplantation of the heart in dogs. According to him the observed changes after reimplantation of the heart were partially the result of cardiac denervation. Diastolic filling of the left ventricle may also be inadequate after bilateral lung transplantation since the normal pressure-flow relations in the left atrium may be disturbed by the addition of a non-contracting donor's left atrium. However, the scarcely measured mean left atrial pressures were not significantly different from normal values but mean left atrial pressure may not represent the information necessary for understanding the actually occurring flow pattern in the left atrium during systole and diastole. Whether the decreased cardiac output resulted also from the cardiac depressant or vasodilative action of substances released from or activated by the ischemic lungs themselves remains uncertain. In later stages of irreversible shock several factors may be held responsible for the permanent decreased contractility of the myocardium as has been summarized by Gersmeyer and Yasargil (1979, pp. 28-67).

The observed perivascular tissue changes consisting of edema and congestion may be an important cause of the increased pulmonary vascular resistance after bilateral lung transplantation. Pulmonary edema alone can be an important source of an increased pulmonary vascular resistance (West et al., 1965). Denervation alone has also been regarded as a cause of a changed pulmonary vascular resistance (Nigro et al., 1963; Ebert and Hudson, 1971), but normal pressure-flow regression slopes were observed after unilateral lung denervation by Wagner et al. (1974). He and also Veith et al. (1971a) demonstrated that the vasculature of a single transplanted lung can dilate with increasing blood flow after ligation of the contralateral pulmonary artery. The increase in pulmonary vascular resistance after our bilateral lung transplantations may also be explained by functional stenosis of the vascular anastomoses. We did not find gross narrowing of the left atrial and pulmonary artery anastomoses but the absence of anatomical stenosis does not strictly rule out functional stenosis of the left atrial anastomosis as a cause of an increase in pulmonary venous pressure and of the pulmonary artery affecting the impedance to the pulsatile output of the right heart. Although the resistance of a normal pulmonary vascular bed is inversely related to blood flow, the increased pulmonary vascular resistance after our bilateral lung transplantations was not solely an effect of the diminished flow since in cases where a normal cardiac output was obtained

after transplantation the pulmonary vascular resistance was also high. In our opinion the high resistance found in most dogs was partially the result of a low cardiac output rather than its cause as suggested by Fujimura et al. (1972a; 1972b) in their cases after staged and simultaneous bilateral lung reimplantations. Moreover the rise in mean pulmonary artery pressure after our transplantations can hardly be reconciled with an insufficiency of the right ventricle as a primary cause of a decreased stroke volume.

In conclusion, the immediate effect of simultaneous bilateral lung allotransplantation in our dogs was an acute shock occurring upon the recirculation of the ischemic donor lungs in the recipient. Three of the 13 dogs of this study apparently died within 24 hours after transplantation as a result of this acute shock. Transfusion of blood immediately after recirculation of our transplanted lungs could restore a decreased cardiac output and prevent an irreversible sequence of events of impaired circulation and gas exchange. However, transfusion at this stage seemed to be very critical since congestion and edema were aggravated and pulmonary hemorrhage occurred. As a consequence of these changes and a resulting hypoxemia two other dogs died within 24 hours after transplantation, although cardiac output was restored by the immediate transfusion. The severance of hilar structures as a result of the transplantation procedure and the ischemic damage of the transplanted lungs explain the immediately occurring pulmonary congestion and edema and may have contributed to a hypo-effective state of the heart. It might be expected that the use of pharmacological agents with a stronger inotropic action than the administered digoxin will have a beneficial effect on the circulation immediately after transplantations of the lungs. In our opinion the treatment or prevention of the circulatory collaps upon recirculation of the transplanted lungs will be the key for reaching survival. Proper treatment with carefully guided transfusion and sustained cardiac function will be the first step. Treatment of pulmonary edema by positive end-expiratory pressure might be limited by its suppressing effect on cardiac output. Membrane lung oxygenation for the temporary support of the transplanted lungs might give the possibility to treat pulmonary edema and congestion by relieving the heart and transplanted lungs from their functional burden and may concomittantly support the failing circulation. In experiments by Corso et al. (1973) the beneficial effect of temporary support of the failing

transplanted lungs in baboons by membrane lung oxygenation was shown by the clearance of edema and an improved arterial oxygen tension. Here probably lies a potentially rewarding field for future experiments.

CHAPTER 6
SURVIVAL AFTER SIMULTANEOUS BILATERAL ALLOTRANSPLANTATION OF THE LUNGS

6 contents

6.1 introduction

As has been shown in the previous chapter, the early failures after simultaneous bilateral allotransplantation of the lungs are accompanied by severe initial functional and morphological reactions. The survival of the animal, completely dependent for its gas exchange on the transplanted lungs, and the functional status of the lungs is influenced by the ability to overcome the initial damage and functional deficit and by the prevention of allograft rejection and infection in later stages. The causes of death of dogs and the functional and morphological changes after technically successfully performed simultaneous bilateral allotransplantations of the lungs were therefore studied to determine the most important factors responsible for the survival or death after this procedure.

6.2 materials and methods

In 42 dogs a technically successful allotransplantation of both lungs was performed as described in chapter 4. These 42 dogs can be divided in two groups of 21 dogs each. The first 21 dogs were mongrels receiving their lung transplants from donors not prospectively typed for transplantation antigens, while the other 21 dogs were labrador-retrievers which received their lung transplants from SD-identical littermates typed according to the method described in chapter 2.

All donor lungs were harvested from heart-beating donors. The lungs from the mongrel dogs were either perfused with various solutions or not perfused at all, as shown in table (4.1). The lungs from labrador-retrievers were perfused with Sacks' solution (Sacks et al., 1973).

The anesthesia and the pre- and postoperative treatment has already been mentioned in chapter 4. Essential differences between the treatment of mongrel dogs and labrador-retrievers were as follows: all surviving mongrel dogs received azothioprine in a daily dose of 3 mg per kg bodyweight starting the first postoperative day. Corticosteroids (150 mg tapered to 30 mg) were given immediately after operation to these dogs. In ten of the 21 labrador-retrievers no immunosuppressive drugs were given while in the other 11 labrador-retrievers azothioprine was given intravenously in a daily dose of 1 mg per kg bodyweight starting the day after operation and methylprednisolone (40 mg) with the premedication and repeated eight hours later. On the following days prednisolone acetate was given to these labrador-retrievers in a dose of .5 mg per kg bodyweight. Digoxin was also administered to the last 11 labrador-retrievers on the day of operation. The peroperative treatment of the labrador retrievers has already been mentioned in chapter 5.

Cardiopulmonary measurements could only be done in later stages after operation in some of the labrador-retrievers. In all surviving dogs arterial blood samples were analysed sequentially.

Autopsy was ultimately done in all dogs and the histology of the lungs was judged as described in chapter 5.

Bronchoscopy, which was occasionally done, was performed by E. Th. Edens and J. S. F. Schudel (Department of E.N.T., head: Prof. Dr. P. E. Hoeksema, University Hospital, Groningen, the Netherlands). An open-tip rigid bronchoscope (Storz, 9 mm) was used and pictures were made with a zero-degree telescope (Wolf) with an attached camera (Nikon RTF) with clear finder and flasher (Wolf) on diapositive color film (Kodak Ektachrome H.S.).

survival times

The individual survival times of the 42 dogs are compiled in table (6.1). In figure (6.1) the number of mongrel dogs and labrador-retrievers still alive on subsequent days after technically successfully performed simultaneous bilateral lung allotransplantations are graphically shown. Fifteen mongrel dogs and nine labrador-retrievers died within 24 hours after transplantation. All remaining mongrel dogs and seven of the remaining labrador-retrievers died during the first postoperative week. Five dogs, all labrador-retrievers, survived more than one week and died 10, 17, 30, 75, and 202 days after transplantation.

table (6.1) survival times after simultaneous bilateral allotransplantation of the lungs in 42 dogs

	mongrels	labrador-retrievers	total
technically successful	21	21	42
survival less than one day	15	9	24
survival between 2 and 7 days	6	7	13
survival beyond 7 days	0	5	5

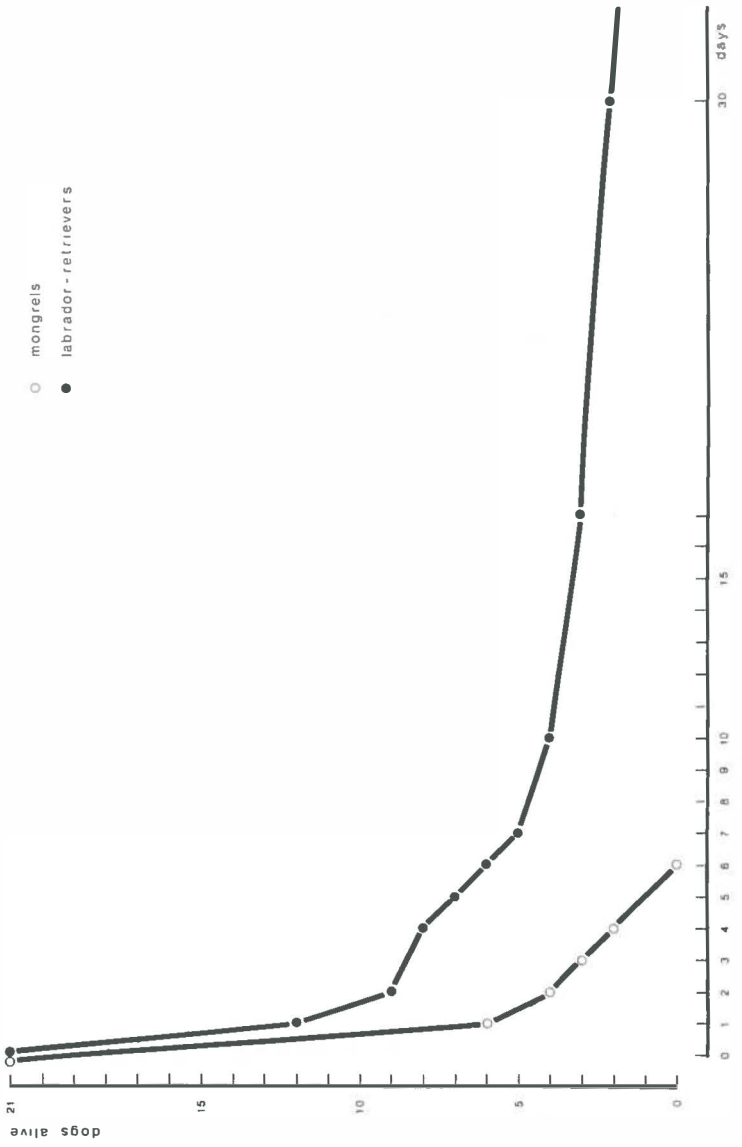


figure (6.1)
graphical representation of the number of mongrel dogs and labrador-retrievers still alive on the subsequent days after technically successfully performed simultaneous bilateral lung allotransplantations.

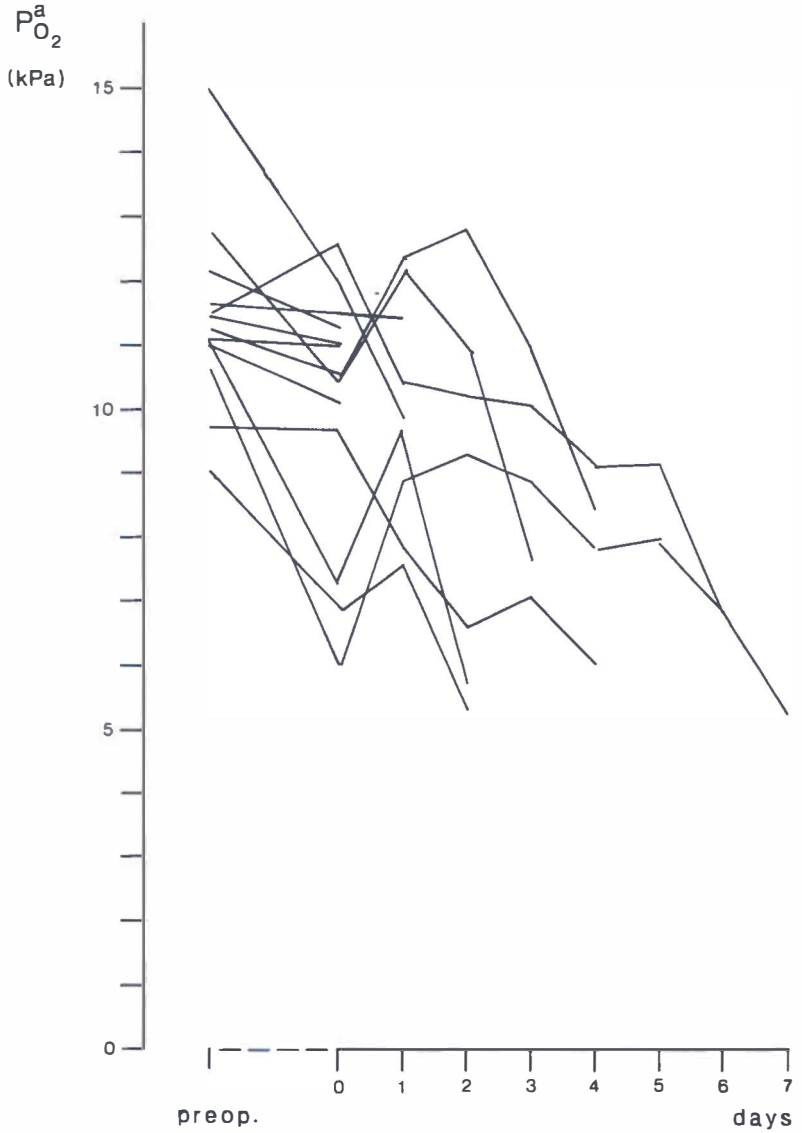


figure (6.2)
graphical representation of the pre- and postoperative arterial oxygen tensions of dogs surviving between one and seven days after simultaneous bilateral lung allotransplantations.

postoperative course and function

Both the 15 mongrel dogs and the nine labrador-retrievers which died in the initial 24 hours after transplantations showed a dyspnoe and signs of alveolar edema in a varying intensity when spontaneous breathing of air was allowed after transplantation. Hypoxemia was present immediately after operation in all these dogs. In four of these 24 dogs blood transfusions were given immediately after recirculation of the graft in the recipient. In all these four labrador-retrievers the immediate transfusion of blood was accompanied by severe alveolar edema and hypoxemia. The X-ray pictures of all the dogs that died in this early stage after transplantation exhibited homogeneous density of both lungs immediately after transplantation. At autopsy the lungs were swollen with rounding of the normal sharp edges. The surfaces of the cut lungs exuded hemorrhagic fluid. In two dogs nonoccluding mural thrombi were observed on the suture line of the vascular anastomoses.

In the dogs which died between one and seven days after transplantation a moderate hypoxemia was present immediately after transplantation. This hypoxemia did not subside on the following days but aggravated toward the end-stage of survival of these dogs (figure 6.2). A continuous fever developed starting on the first postoperative day. The sequentially taken X-ray pictures showed increasing and confluent densities in both lungs that aggravated to a complete opacity on the last days of survival in most dogs. At autopsy the lungs were generally consolidated. Small non-occluding mural thrombi on the vascular anastomoses were seen in three of these dogs.

In dogs surviving more than one week a moderate hypoxemia in the first postoperative week recovered by the end of the first week but death was invariably preceded by a period of aggravating hypoxemia (figure 6.3). A continuous fever was also present in these longer surviving dogs. Anemia developed during the first week, but recovered in most dogs surviving longer than one week.

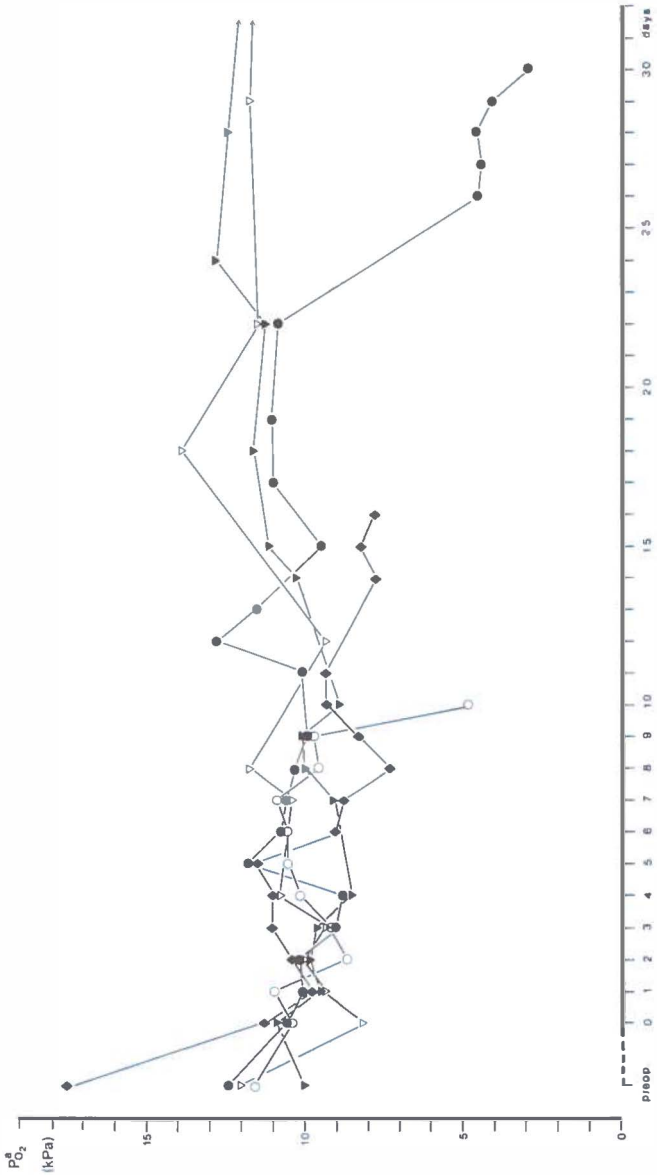


figure (6.3)
graphical representation of pre- and postoperative arterial oxygen tensions of five dogs surviving more than one week after simultaneous bilateral lung allotransplantation.

A typical postoperative course that may be representative for the longer surviving dogs can be illustrated by the case history of a dog surviving for 30 days:

After an uncomplicated operation followed by positive end-expiratory pressure ventilation for 23 minutes, spontaneous breathing was regained and cardiopulmonary function measured. The results are shown in table (6.2). No blood transfusions were given. Sodium bicarbonate (25 ml, 8.3%) was administered during operation. The X-ray picture of the chest after the end of the operation showed well aerated lungs. Cardiopulmonary measurements were repeated 8, 15, 22, 26, and 30 days after transplantation. The results of daily analysis of arterial blood samples and the body temperature are displayed in figure (6.4). In the first postoperative week the general condition improved and X-ray pictures showed only some perihilar densities. Signs of respiratory distress were absent. On the eights day bronchoscopy was done. It showed a pale donor part of the trachea without mucosal defects and no constriction at the site of the anastomosis (figure 6.5). The condition of the dog remained well untill the 22nd day after operation although body temperature was continuously elevated above 38.5°C. Difficulty of breathing and rales became increasingly apparent starting from the 22nd day. Confluent infiltrates in both lungs appeared on the X-ray pictures. Because of a severe respiratory distress on the 26th day after transplantation cardiopulmonary function was assessed and the dog was treated with positive end-expiratory pressure ventilation for three hours. Some clear mucus could be aspirated repeatedly from the trachea. Prednisolone (500 mg i.v.), sodium bicarbonate (100 ml, 8.3%, i.v.) and furosemide (20 mg i.v.) were administered. This did not improve gas exchange. In the following days the condition of the dog deteriorated fast and after a last measurement of cardiopulmonary function the dog died 30 days after transplantation. The last X-ray picture taken showed a homogeneous density of both lungs.
Autopsy revealed pleural adhesions and an atelectatic left upper and right middle lobe. The pulmonary parenchyma was uniformely stiff. A stenosis of the atrial anastomosis comprising the outflow of left and right upper pulmonary veins was seen (figure 6.6). It was caused by an intraluminally extending absces in the cephalid part of the atrial anastomosis. The arterial anastomosis had completely healed and was not constricted. In the donor part of the trachea and larger bronchi the mucosal layer was completely sloughed and lie as a cast in the surrounding tissue (figure 6.7).

comment:
The simultaneous bilateral allotransplantation of the lungs in this dog resulted in an almost normal function of the cardiopulmonary system after operation. The first week after transplantation was characterized by a transient hypoxemia. An initially depressed cardiac output recovered one week after operation and pulmonary artery pressure and pulmonary vascular resistance were normal at that time. The gas exchange function of the transplanted lungs remained sufficient for 22 days although several changes of variables indicated that this function was worsening. Prominent changes were a decrease of functional residual capacity, dynamic and static pulmonary compliance and diffusing capacity for carbon monoxide. Dead-space to tidal volume ratio, true intrapulmonary shunt, mean pulmonary artery pressure and pulmonary vascular resistance gradually increased. In the fourth week after transplantation the function deteriorated at a progressive rate. Diffusion capacity for carbon monoxide was very small and a large true intrapulmonary shunt and physiological dead-space had developed. These changes and the worsened mechanical properties of the lungs at this stage correlated with the observed morphological abnormalities. The dog ultimately died from respiratory insufficiency.

table (6.2) results of measurements of cardiopulmonary function before and sequentially after simultaneous bilateral lung allotransplantation in a dog that survived for 30 days (m_b = 26.5 kg).

variable	pre-oper.	imm.post	8th day	15th day	22nd day	26th day	30th day
σ_{Hb}	9.8	7.0	7.0	7.9	9.2	9.8	8.9
F_{cell}	.47	.31	.32	.38	.46	.45	.43
pH	7.37	7.47	7.36	7.25	7.19	7.50	7.49
p^ao_2	12.4	10.5	10.3	9.6	10.9	4.4	2.9
$p^{a\bullet}o_2$	6.3	4.9	5.3	6.3	6.0	4.0	3.7
S^ao_2	.95	.95	.93	.88	.89	.68	.40
BE	6.3	8.3	2.8	.2	—4.8	5.4	—3.0
$q_E \cdot m_b^{-1}$	181	395	187	212	275		590
f_{b_T}	21	20	12	21	22		42
$V_T \cdot m_b^{-1}$	9	20	21	10	12		14
$V_{FRC} \cdot m_b^{-1}$	39	57	31	31	25	25	20
$q^Eo_2 \cdot m_b^{-1}$	4.7	4.9	3.8	5.3	6.0		11
$q^Eco_2 \cdot m_b^{-1}$	3.7	6.2	4.0	3.8	4.3		4.8
RQ	.80	1.25	1.04	.71	.71		.44
$q_A \cdot m_b^{-1}$	69	145	86	60	83		149
$V_D \cdot V_T^{-1}$	.619	.583	.528	.660	.685		.84
p^Ao_2	12.4	16.8	14.7	11.6	12.3		7.9
$(p^Ao_2\text{-}p^ao_2)$	0	6.3	4.4	2.0	1.4		5.0
$q_{sh} \cdot q_t^{-1}$	.045	.045	.050	.128	.163	.423	.635
D^P_{CO}	195	227	141	135	113	62	45
C^P_{dyn}	1.35	2.23	1.18	.56	.57	.33	.12
C^P_{st}	1.54	1.28		.85	1.36	.28	.18
R^P_{dyn}	.265	.530	.564	.343	.834	.343	.510
C^P_{sp}	1.29	1.48	1.44	.68	.87	.51	.23
$q_t \cdot m_b^{-1}$	158	91	170	113	154	106	146
f_h	180	170	250	210	220	210	160
$V_{tv} \cdot m_b^{-1}$	.87	.53	.68	.53	.72	.49	.91
$\bar{p}_{pa}$	20.7	14.7	15.0	16.3	14.0	10.7	12.0
$\bar{p}_{pa}$	2.3	2.4	2.9	3.1	4.0	4.0	3.6
R^P_b	32.5	60.8	39.0	62.8	58.6	85.6	55.6

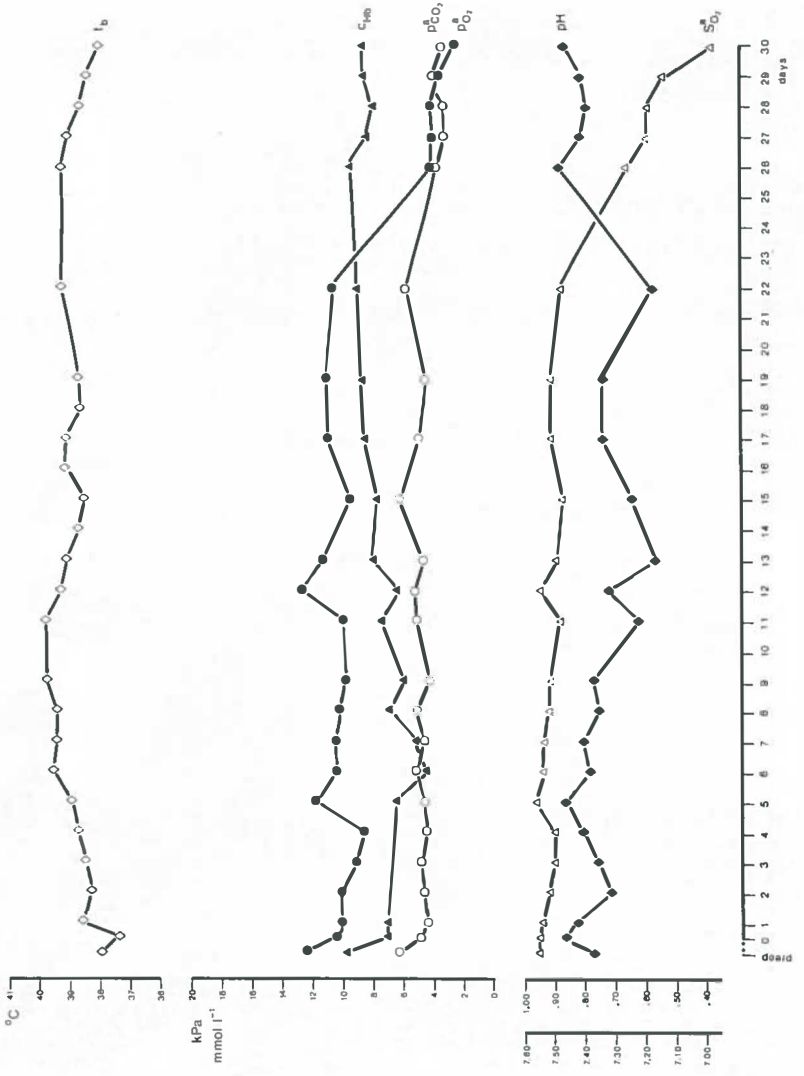
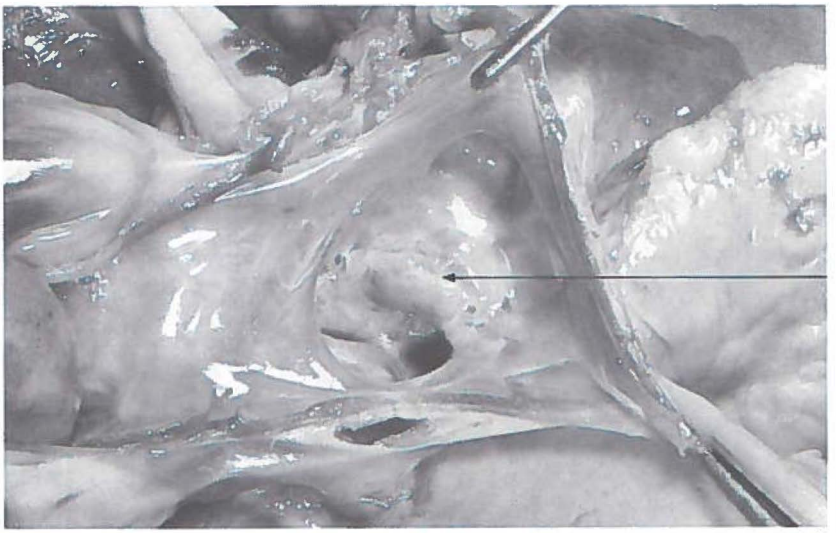
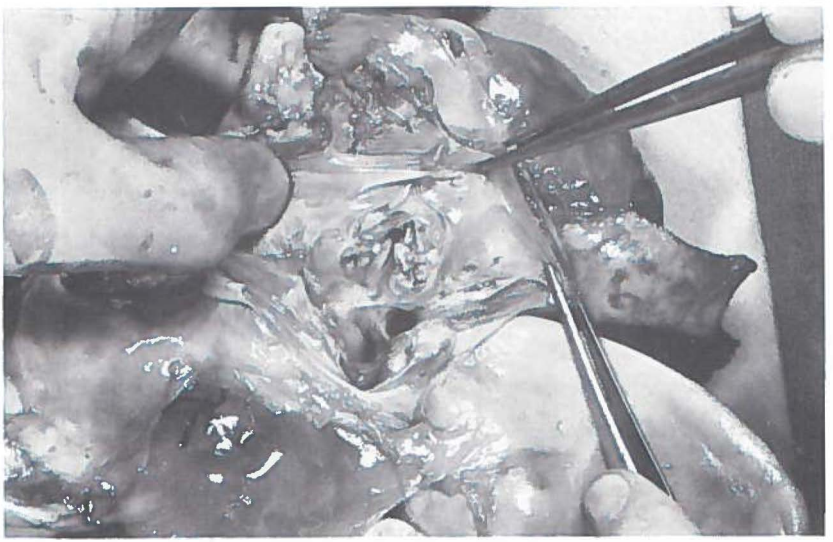


figure (6.4)
graphical representation of the results of sequentially analysed arterial blood samples for oxygen and carbon dioxide tension, oxygen saturation, pH and hemoglobin concentration, combined with the course of body temperature in a dog before and after simultaneous bilateral allotransplantation of the lungs.



a.



b.

figure (6.6)

picture of the left atrial anastomosis 30 days after simultaneous bilateral allotransplantation of the lungs in a dog. The left atrium is opened on its recipient's side.

a. the arrow points to the intraluminal extending abscess

b. the abscess is opened and the entrance of the left upper pulmonary vein is visible beneath it.

figure (6.7)
the tracheal anastomosis 30 days after simultaneous bilateral lung allotransplantation in a dog. Note the sloughed mucosal layer lying as a cast in the surrounding tracheal wall.

The two dogs which survived for 75 and 202 days received blood transfusions immediately after recirculation of the graft in the recipient, digoxin on the day of operation, and azothioprine and prednisolone daily during the period of survival. In these dogs cardiopulmonary measurements were not performed except for sequentially analysis of arterial blood samples and the measurement of cardiac output immediately after transplantation. Cardiac output was nearly normal in these dogs. The general condition of these dogs improved during the first week after operation and a complete recovery was achieved. Sequentially taken X-ray pictures did not show gross abnormalities and arterial blood gases and pH were normal untill deterioration of gas exchange started on the 64th and 98th day respectively.

Body temperature which was continuously slightly elevated rose to approximately 40° C at this stage. The aggravating hypoxemia proceded the death of the animals and the X-ray pictures at that time showed a complete opacity of both lungs. Autopsy revealed non-compliant lungs with pale and dark-red areas. The anastomoses had healed.

histopathological changes in the autopsy specimens (table 6.3)
The histopathological changes observed in the mongrel dogs have been described earlier by Wildevuur et al. (1973) and will only be summarized here. The most striking feature in all lung transplants was interstitial and alveolar edema with peribronchial, perivascular and intra-alveolar hemorrhage. In nine of these dogs endothelial damage with attachment of polymorphonuclear cells frequently showing a pavementing pattern was already apparent in biopsies taken 30 minutes after recirculation of the graft. Hemorrhagic necrosis was present in almost all 15 dogs which died in the first 24 hours after transplantation. In the six dogs surviving longer than one day mononuclear cell accumulation in the pulmonary interstitium was observed in only two cases. These changes were however accompanied by a general infiltration with polymorphonuclear cells. Purulent bronchiolitis and generalized pneumonitis was observed in the remaining four dogs.

In all nine labrador-retrievers which died in the initial 24 hours after operation a severe congestion of alveolar capillaries and small vessels, interstitial and alveolar edema, and peribronchial, perivascular and intra-alveolar hemorrhage were present. In the 12 labrador-retrievers surviving longer than one day this histological picture was less frequently observed or even absent in the three dogs which survived for 30, 75, and 202 days after transplantation. Hemorrhagic necrosis of the lungs was only seen in the dog which died two days after transplantation immediately after an intravenous injection of azothioprine.

Mononuclear cell infiltrations around blood vessels and accumulations of these cells in the vessels were seen in three labrador-retrievers. In the one

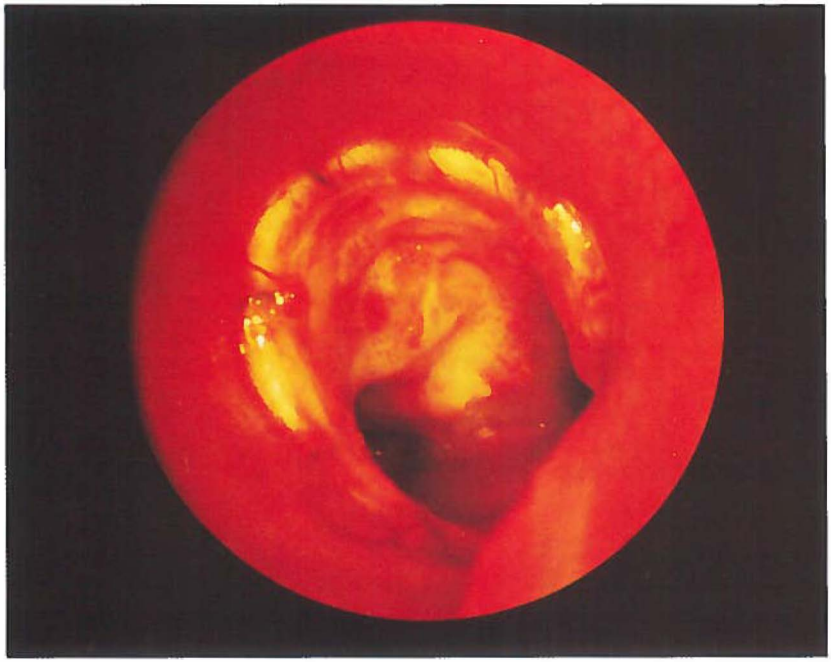


figure (6.5)
bronchoscopic picture of the trachea anastomosis eight days after simultaneous bilateral allotransplantation of the lungs. Note the pale donor part of the trachea.

table (6.3) histopathological changes in the autopsy specimens

death after operation	24 hours		1 to 7 days		more than 7 days	
	mongrels	labradors	mongrels	labradors	mongrels	labradors
congestion, edema, hemorrhage	15	9	6	6		
hemorrhagic necrosis	13	2		1		
endothelial damage with pmn cell attachment	9					
mononuclear cell accumul.			2	2		1
bronchitis, bronchiolitis, pneumonia			6	6		3
helminthiosis						2

dog which survived for 30 days this was the only consistent finding. This dog did not receive azothioprine in contrast to another which died six days after transplantation. Apart from mononuclear cell infiltrations also hemorrhagic changes were present in its autopsy specimens. In still another labrador-retriever mononuclear cells were observed in the autopsy specimens seven days after transplantation but these changes were overwhelmed by the massive infiltration of the pulmonary parenchyma by polymorphonuclear cells.

In the lung specimens of eight labrador-retrievers surviving for 2 to 75 days a general infiltration of the pulmonary parenchyma by polymorphonuclear

cells was the most prominent finding. It was accompanied by interstitial edema and interstitial and alveolar hemorrhage in almost all dogs. In half of these cases the bronchial and brochiolar walls were also infiltrated by polymorphonuclear cells and the mucosal layer desquamated. In one dog, dying from cardiac arrest during bronchoscopy 17 days after operation, the right bronchus was completely obstructed by purulent material and this lung showed the signs of a generalized bronchopneumonia.

In five of the dogs which died between seven and 30 days after transplantation the donor part of the trachea exhibited macroscopically an inner surface denuded of epithelium. This sloughed mucosal layer lie as a cast in the trachea in some dogs (figure 6.5). It was accompanied by infiltration of the walls by polymorphonuclear cells in two dogs. In some, hemorrhagic changes were observed in the tracheal wall but ischemia and mononuclear cell infiltrations were absent. In the two longest surviving dogs the tracheal anastomosis had apparently healed and was macroscopically covered by normal mucosa.

A larvae producing parasite had infiltrated the pulmonary parenchyma of the dog which died 202 days after transplantation. In the lungs of this immunosuppressed dog general or local inflammatory signs were however absent and the normal pulmonary architecture was destructed to a large extent. The same helminthic parasite was observed in the central part of the right middle lobe of the dog which died 75 days after transplantation. Both these dogs shared a common surviving period since they were operated one week after each other. However, the pulmonary parenchyma of this last dog was in large areas destructed by polymorphonuclear cell infiltrations with desquamation of mucosal layers of bronchioli and bronchi. Desquamated alveolar cells and proliferating pneumocytes were a common finding in areas where the pulmonary architecture was still normal.

6.4 discussion and conclusions

Early death after simultaneous bilateral allotransplantation of the lungs was a frequent observation in our experiments. Twenty-four dogs, 15 mongrels and nine labrador-retrievers, died within 24 hours after transplantation. Our study on the immediate effects of simultaneous bilateral transplantation of the lungs in the previous chapter indicated that an acute shock occurred at the recirculation of the transplanted lungs in the recipient. This recirculation shock and the accompanying pulmonary congestion and edema is likely the major cause of early death of 20 dogs, mongrels and labrador-retrievers, which did not receive blood transfusions immediately after recirculation of the grafts. The recirculation shock could be reduced by immediate transfusions, but apparently this treatment resulted in severe alveolar edema and respiratory insufficiency in four labrador-retrievers which died in the initial phase after operation notwithstanding this treatment. The appearance of endothelial damage of pulmonary vessels with attachment of polymorphonuclear cells frequently showing a pavementing pattern with subsequent hemorrhagic necrosis of the lungs within 24 hours after allotransplantation of the lungs in mongrel dogs may indicate the occurrence of hyperacute and accelerated rejection. These histological observations resemble the changes observed in lungs transplanted between littermate labrador-retrievers with two-haplotype difference in the serologically defined part of the major histocompatibility complex as has been described in chapter 2. The occurrence of hyperacute and accelerated rejection of bilaterally transplanted lungs has previously been described by Wildevuur et al. (1973). They already mentioned that the primary occurrence of endothelial lesions and margination of polymorphonuclear cells after recirculation of the transplanted lungs may be caused by shock (Wilson et al., 1970; Coalson et al., 1970; McKay et al., 1966, 1967) or might implicate the presence of naturally occurring and heterospecific antibodies responsible for the hyperacute rejection of the lungs. The severe circulatory failure observed at the acute recirculation of the transplanted lungs described in chapter 5 was not yet appreciated at the time when the experiments in mongrel dogs were performed. This acute recirculation shock might actually have occurred also in the mongrel dogs and might have been the only or

additional cause of the observed histological abnormalities. Discrimination between the occurrence of hyperacute rejection of bilaterally transplanted lungs in mongrel dogs and an acute shock syndrome can not be given.

The higher mortality rate of mongrel dogs in the first 24 hours compared to that of the typed labrador-retrievers may also have been caused by differences in the solutions employed for flushing the donor lungs and by a different pre-, per-, and postoperative treatment. An accumulating experience probably also contributed to the longer survival of labrador-retrievers compared to mongrel dogs since experiments in both groups were not randomized but actually done in subsequent periods. The longest survival after simultaneous bilateral allotransplantation of the lungs, i.e. 75 and 202 days, was reached when the invasive and intensive measurements of cardiopulmonary function were avoided. These measurements are probably strenuous for the dogs and may actually worsen the precarious condition immediately after operation.

From the results of sequentially measured blood gases it is apparent that the initial reactions subsided and that lung function could recover in the longer surviving labrador-retrievers. The temporary fall in arterial oxygen tension in the dogs surviving more than one week has also been observed by Wildevuur et al. (1969) in the first week after bilateral hilus stripping of the lungs in dogs. The accompanying congestion and edema of the lungs of these dogs seem to explain the observed functional deficit. The possibility of bilaterally transplanted lungs to recover from the initial reactions could be confirmed in the dogs which survived 30, 75 and 202 days after transplantation with normal arterial blood gas values untill the end-stage of survival. The presented case history of a dog surviving 30 days after transplantation and ultimately dying from allograft rejection may be illustrative for the possible functional changes after this procedure. The increasing intrapulmonary shunt percentage, decreasing diffusion capacity for carbon monoxide, the decreasing pulmonary compliance and concomittant increase of pulmonary resistance, and the decreasing functional residual capacity in this dog during the period of survival represent aggravating morphological abnormalities of the lungs such as atelectases, infiltrates and changes at the alveolar level.

The classical picture of lung allograft rejection with mononuclear cell accumulations in and around pulmonary vessels was a scarce observation both in mongrel dogs and in typed labrador-retrievers. Because early death occurred in the majority of the mongrel dogs, the complete picture of lung allograft rejection starting at the fourth or fifth day according to Barnes et al. (1963) could not develop in these mongrel dogs. In only two mongrel dogs mononuclear cell accumulations were seen but these changes were overwhelmed by infectious changes of the lungs. Although prolongation of unilateral lung allograft survival in mongrel dogs by the use of azothioprine and corticosteroids has been shown by many authors among others by Barnes and Flax (1964), the influence of this immunosuppressive regimen on graft survival of bilaterally transplanted lungs could not be proven in our experiments in mongrel dogs. This can be explained by the difference between unilaterally and bilaterally transplanted lungs. In the case of unilateral lung transplantation the survival of the animal is not fully dependent on the function of the transplanted lung and derangements of function do not necessarily result in death of the animal as is the case in bilaterally transplanted lungs. In the identical labrador-retriever combinations the pure picture of this mainly cellular-efferent chain of rejection was observed in only two cases of which one did not receive azothioprine and died 30 days after transplantation while the other dog received this immunosuppressive treatment and died six days after transplantation. The relative absence of cellular rejection phenomena in labrador-retrievers surviving more than one week after transplantation indicates that equality of the major histocompatibility complex in littermate dogs favours also the acceptance of bilaterally transplanted lungs. The occurrence of the longest survival periods in dogs which received a small dose of azothioprine and prednisolone may point to an effectivity of this immunosuppressive treatment on prolongation of lung allograft survival in these prospectively typed dogs. The doses which we administered in these dogs were smaller than usual, but we assumed that a smaller dose was justified because the dogs were serologically identical and side effects might be minimized. The occurrence of cellular-mediated rejection in one of the labrador-retrievers which received immunosuppressive treatment indicates that early rejection still remains a possibility in the prospectively typed dogs even when immunosuppressive treatment is given. When infection is superimposed on rejection a clear distinction between either of these processes may become impossible as has been shown in human lung

allotransplants by Cullum et al. (1972) and in canine lung allotransplants by Barnes et al. (1963). Since sequential lung biopsies were not taken in our dogs it remains uncertain whether the observed infectious changes had replaced previous rejection phenomena.

Infection is considered to be a major hazard for allotransplanted lungs, whether performed unilaterally or bilaterally (Trummer and Berg, 1968). It has been an important cause of death after lung transplantation in man (Wildevuur and Benfield, 1970; Veith and Koerner, 1974). As apparent from the histological picture of the autopsy specimens of our bilaterally transplanted lungs, infection presenting as a pneumonitis or bronchopneumonia was the cause of death of 11 of 18 dogs surviving the initial postoperative period. The continuous fever observed in all our surviving dogs may be the expression of a continuously present inflammatory process in these transplants. Several factors contribute to an increased susceptibility of transplanted lungs to infection. As shown by Siegelman et al. (1977) reimplanted lungs are subject to a high incidence of pulmonary infection. The surgical trauma per se, the pulmonary edema and congestion may all present an ideal surrounding for micro-organisms. Denervation of lungs is important in relation to lung defense mechanisms. Denervation of bronchial mucus glands alters mucus secretion (Florey et al., 1932) and delays clearance of tantalum-labeled mucus for 120 to 150 days (Edmunds et al., 1969). The loss of the cough reflex further impairs lung clearance (Edmunds et al., 1969). Susceptibility of transplanted lungs to infection is also increased by the occurrence of rejection. The occurrence of hemorrhagic necrosis and the loss of normal architecture by fulminating rejections makes the transplanted lungs with their continuous contact with the exterior very vulnerable for the introduction of micro-organisms. In most of the mongrel dogs which survived longer than one day after simultaneous bilateral lung allotransplantation severe infectious changes were observed in the first week after operation when rejection can be assumed to occur. Infection might have been superimposed on rejection in these cases but cellular-mediated rejection phenomena could not be observed in the autopsy specimens of these dogs except in two. The increased susceptibility to infection is also enhanced by the immunosuppressive treatment employed in both the mongrel dogs and the labrador-retrievers

The occurrence of an helminthiosis in the two longest surviving dogs which shared a common survival period can be explained as an infection by opportunistic organisms. This resembles the occurrence of infection by opportunistic organisms in the lungs of humans after kidney transplantation when immunosuppressive treatment is given for long periods (Simmons et al., 1972).

The frequent observation of a sloughed mucosal layer of the donor part of the trachea and larger bronchi in all dogs which died between seven and 30 days after transplantation certainly contributed to the inadequate clearing mechanisms of our transplanted lungs. This complication may be the result of a critical retrograde blood supply of the trachea due to the severed bronchial arteries. However, the donor part of the trachea showed capillary filling in all dogs immediately after recirculation of the lungs in the recipient and histological studies early after transplantation failed to show lack of blood supply. Blank et al. (1966) already proved the spontaneous re-establishment of bronchial artery circulation after left lung reimplantations. Recent experiments by Siegelman et al. (1977) showed various degrees of bronchial artery filling beyond the anastomosis more than one week after left lung reimplantation. Although the retrograde blood supply seems to be sufficient immediately after transplantation to cause capillary filling of the mucosal layer, the later observed necrosis of the epithelium indicates a gradual impairment of the mucosal blood supply. This phenomenon was not confined to the lungs that showed cellular-mediated rejection, but also occurring in lungs with an apparent pneumonia. The normal healing of the trachea anastomosis without mucosal necrosis in the two longest surviving dogs which received azothioprine and prednisolone and in which rejection was adequately avoided, suggests that the observed mucosal necrosis may be a specific rejection phenomenon of the tracheal and bronchial walls.

In conclusion, the survival of dogs after technically successfully performed simultaneous bilateral allotransplantation of the lungs depended primarily on the severity of the immediate changes occurring at the recirculation of the lungs in the recipient. These immediate changes consisted of an acute respiratory insufficiency with severe pulmonary edema and congestion. It frequently developed into an irreversible sequence of events leading to early

death after operation. From the results of the analysis of these immediate changes described in the previous chapter it became apparent that an acute recirculation shock was the most prominent change initiating an aggravating sequence of events. Several factors could be held responsible for the occurrence of this recirculation shock. When the acutely occurring deterioration of cardiopulmonary function was only moderate or reversed by transfusions of blood immediately after the recirculation, survival beyond the first 24 hours was reached in several dogs. Hyperacute and accelerated rejection may have contributed to early fatalities when the transplantations were performed between non-related mongrel dogs but could be avoided when littermate pairs of matched donor-recipients were employed. Lung allograft rejection could generally be postponed and even avoided when littermate dogs prospectively typed for the major histocompatibility complex were used and immunosuppression was given with low-dose azothioprine and prednisolone. However, ultimately all surviving dogs died from infection in their transplanted lungs. The increased susceptibility to infection of transplanted lungs is considered to be mainly the result of inadequate clearence mechanisms of the denervated lungs and may also be caused by the general depression of the defense mechanisms of the organisms by the administered immunosuppressive pharmacological agents. A necrosis of the mucosal layer of the donor part of the trachea was frequently observed in dogs surviving more than one week. The exact mechanism of this necrosis is not yet understood but it may be a specific chronic rejection phenomenon.

CHAPTER 7
EPILOGUE

As a consequence of research frequently more questions are raised than can be answered. This is also the case in our experimental studies. Although our results were sometimes far from evidential, an indication of the possibility to transplant both lungs simultaneously in dogs with preservation of the gas exchange function could be given. The operative technique proved to be feasible and the operative mortality could largely be attributed to avoidable technical failures. The required immediate adequate function of the transplanted lungs necessary for the survival of the recipient could only be gained in a small number of dogs. Early fatalities were the result of severe reactions to the transplantation occurring immediately after the recirculation of the transplanted lungs. The nature and the causes of these immediate changes are not yet fully elucidated and require additional studies. The rapidly developing sequence of events leading to the deterioration of cardiopulmonary function requires a more frequent and continuous monitoring of cardiopulmonary functions. This may provide the investigator with the necessary data for an appropriate feed-back on the therapeutic measures and may contribute to more surviving dogs. The absence of a round-the-clock attended intensive treatment of our dogs after transplantation certainly contributed to the high immediate mortality rate. From our results a definite indication of the influence of donor selection on unilateral and bilateral lung allograft survival in littermate dogs could be given. To strengthen this evidence our series have to be extended and additional study on the immunological phenomena of transplanted lungs should be done. The effectiviness of immunosuppressive therapy in avoiding or retarding rejection of allografted lungs should further be evaluated in genetically well-defined animals. Although the dog is a very suitable animal for transplantation experiments because of its size and availability, the use of purely bred dogs as we have employed involves financial problems. Small laboratory animals like the rat with their short generation and gestation time and low cost are preferable for the production of large series of experiments in genetically well-defined populations. Although the transplantation of lungs in small animals like the rat does not seem to be very attractive from a technically point of view, recent experiments in our laboratory indicate that unilateral lung transplantation in rats is technically feasible and can serve as an experimental model to study lung allograft rejection phenomena. The high incidence of postoperative infections of the transplanted lungs in our dogs may be specific for dogs, since the lungs of dogs are known to harbour many infectious agents as commensal in contrast to the lungs of humans.

The inadequate clearing mechanisms of the transplanted lungs may be expected to be treated more effectively in case of human lung transplantations since co-operation, not available in dogs, can be gained in humans. Treatment of infection of the lungs can be improved by chemotherapeutic drugs selected on the basis of culture studies of the mucus.

Many indicative results of our experiments await further proof. The contribution of many different disciplines is required to create the circumstances in which clinical simultaneous bilateral lung transplantation can be performed. As long as this multidisciplinar synthesis has not yet been reached, clinical application of this therapeutic procedure has to be dissuaded.

SUMMARY

In the introduction (chapter 1) the rationale for transplantation of both lungs simultaneously in stead of unilateral lung transplantation is outlined. In approximately one-fourth of the cases of human lung transplantation the experienced malfunction of the unilaterally transplanted lung could be attributed to disturbances introduced by the remaining own lung of the patient. Several studies indicate that a single pulmonary transplant will retain sufficient function to permit the elimination of the contralateral lung in the experimental animal. However, the possibilities to transplant both lungs simultaneously are not yet fully studied. To demonstrate that simultaneous bilateral lung transplantation might be a good alternative for unilateral lung transplantation, experiments were performed in dogs to study the possibilities and limitations of simultaneous bilateral lung transplantation.

Chapter 2 of this thesis deals with the influence of selection of donor and recipient dogs for the serologically defined part (SD) of their major histocompatibility complex on lung allograft survival. Between pairs of littermate dogs with either two identical haplotypes, one haplotype difference, or two haplotype difference, unilateral lung transplantations were performed by interchanging the left lungs. Graft survival was defined as the time between transplantation and the occurrence of homogeneous density of the left lung on sequentially taken X-ray pictures and verified by histology. A significant difference in mean lung graft survival time was observed between the two-haplotype different (3 ± 1.4 days) and both the one-haplotype (8.5 ± 4 days) and identical (35.8 ± 37.2 days) combinations. Three different histological patterns of lung allograft rejection occurred in the three different groups. The classical picture of lung allograft rejection consisting predominantly of mononuclear cell infiltrations was observed in all dogs with one haplotype difference between donor and recipient. The histopathological changes observed in the identical dogs indicated that rejection was retarded for three weeks and that it was also less destructive as in the classical lung allograft rejection observed in the one-haplotype different dogs. In the two-haplotype different dogs the histopathological changes observed in lung biopsies taken 30 minutes after recirculation of the graft in the recipient and the subsequent hemorrhagic necrosis within one to five days indicated the occurrence of a hyperacute and accelerated rejection. It was concluded that, in prospect of our cardiopulmonary function studies

after simultaneous bilateral lung allotransplantations in dogs, the use of littermates matched for their major histocompatibility complex in stead of non-typed mongrel dogs might give the advantage to study the effects of the transplantation procedure without interference by rejection of the lung grafts in the first three weeks.

In the third chapter the methods for the measurements of cardiopulmonary function before and after simultaneous bilateral lung allotransplantation are described.

Chapter 4 deals with the operative technique for the simultaneous transplantation of both lungs in dogs. An earlier described technique for the en-bloc transplantation of both lungs has been modified in such a way that the whole procedure could be performed through only a right thoracotomy. After the right pneumonectomy the transplant's main pulmonary artery was anastomosed to the recipient's right pulmonary artery and the whole donor's left atrium to the recipient's left atrium. By ventilating the remaining left lung of the recipient through the intubated left bronchus the anastomosis between donor's and recipient's trachea could be performed and obviated the need for a temporary extracorporeal oxygenation. After the left pneumonectomy through the posterior mediastinum the transplanted lungs were repositioned in the thoracic cage.
After establishing the technique 54 simultaneous bilateral lung allotransplantations were performed with a direct operative mortality of 22 percent. Bleeding from the pulmonary artery anastomosis was the major technical failure and can be avoided by more awareness of the fragile structure of the pulmonary artery. Thrombosis on the suture lines could invariably be attributed to imperfect adaptation of endothelium. An intussuscepting technique for the tracheal anastomosis prevented air leakage. The chapter ends with the conclusion that the employed technique for simultaneous bilateral allotransplantation of the lungs is technically feasible.

Chapter 5 deals with the analysis of the factors which determine the initial function of the transplanted lungs and the immediate reactions of the

recipient to the transplantation procedure. Since in the case of simultaneous bilateral lung transplantation the survival of the recipient is fully dependent on immediate adequate function, the ability to overcome the insult of the transplantation procedure is mandatory for survival. The immediate functional and morphological sequelae of simultaneous bilateral lung transplantation and the possibility to survive the initial postoperative period of 24 hours were therefore analysed in a selected group of 13 labrador-retrievers which received their transplants from SD-identical littermates. The results of the cardiopulmonary measurements showed that the most prominent reaction to the transplantation procedure was an acute shock, occurring at the recirculation of the ischemic and bloodless donor lungs in the recipient. The ischemic damage of the lungs and the severance of hilar structures by the transplantation procedure are considered to be responsible for the observed congestion and edema of the transplanted lungs upon recirculation. However, even in combination with a moderate hemorrhage during operation this cannot explain sufficiently the observed acute shock after recirculation. The identified decreased stroke volume of the heart, possibly due to the severance of sympathetic cardiac nerves related to the operation, in addition to the loss of circulating blood volume, because of congestion and edema of the lungs, have to be considered as causative factors for the severe decrease of cardiac output. This severe decrease of cardiac output could be prevented by transfusion of blood immediately after recirculation of the graft but the transfusion at this stage seemed to be very critical since it could aggravate the already existing pulmonary congestion and edema. The gas exchange of the transplanted lungs was only moderately disturbed provided that cardiac output was only moderately depressed. The observed congestion and edema of the lungs explained the moderate hypoxemia present in all dogs surviving longer than 24 hours after transplantation. The hypoxemia seemed to be the result of inequalities in the relationships between ventilation, circulation and diffusion of the transplanted lungs. Ventilation and diffusion were however adequate. An increased pulmonary vascular resistance was present and was partially caused by the severe perivascular tissue changes and partially the consequence of a low cardiac output.

This chapter ends with the conclusion that appreciation of the acutely occurring circulatory failure after recirculation of the bilaterally transplanted lungs and adequate treatment at this stage is of primary importance for reaching survival after this procedure.

In chapter 6 the survival of all dogs after a technically successful simultaneous bilateral allotransplantation of the lungs is considered. Of these 42 dogs 21 were mongrel dogs which received their transplants from non-typed donors, and 21 were labrador-retrievers which received their transplants from SD-identical littermates. Of the 42 dogs 24 died within 24 hours after transplantation from an acute respiratory distress due to massive pulmonary edema, congestion and pulmonary hemorrhage. In the prospectively typed labrador-retrievers where rejection phenomena can be expected to be postponed the immediate reactions to the transplantation procedure, as discussed in chapter 5, most likely caused the death of nine dogs in this early stage after transplantation. In the 15 mongrel dogs which died at this early stage a hyperacute and accelerated rejection of the lungs may also have contributed to the early death. The remaining six mongrel dogs which survived the first 24 hours all died within one week mainly from infection of the transplanted lungs. Rejection phenomena were observed in only two of these immunosuppressed mongrel dogs. In the labrador-retrievers rejection phenomena were observed in the autopsy specimens of three dogs which died six, seven and 30 days after transplantation. In one of these three dogs rejection was apparent in spite of the administered azothioprin and prednisolone. Lung allograft rejection could be shown to be postponed and even avoided when dogs were used which received the lungs from SD-identical littermates instead of non-typed mongrel dogs, and when also low-dose azothioprine and prednisolone was given postoperatively. Ultimately all dogs died from infection of their transplanted lungs. The increased susceptibility to infection of transplanted lungs was considered to be mainly the result of the inadequate clearing mechanisms of the denervated lungs. The general suppression of the capability of the organism to combat infection by the administered immunosuppression probably contributed to this increased susceptibility to infection.
The measurements of pulmonary function in later stages after transplantation indicated that the exchange function for oxygen could recover from the initial insult and could remain adequate for several months. Three dogs survived for 30, 75 and 202 days after transplantation and had normal arterial oxygen tensions for almost the whole surviving period.

In chapter 7 the conclusions from the foregoing chapters are reviewed and suggestions for future experiments are given. The possibility to perform

lung transplantations in humans is shortly discussed. The epilogue ends with the conclusion that simultaneous bilateral lung transplantations in men have as yet to be dissuaded untill certain prerequisites are met.

SAMENVATTING

In de inleiding (hoofdstuk 1) wordt de reden aangegeven om beide longen simultaan te transplanteren in plaats van een unilaterale longtransplantatie. In ongeveer één-vierde van alle bij mensen toegepaste longtransplantaties kon de optredende verslechterende functie van de eenzijdig getransplanteerde long worden toegeschreven aan stoornissen, die het gevolg waren van de aanwezigheid van de achtergebleven contralaterale eigen long van de patient. Uit verscheidene onderzoekingen blijkt, dat een unilateraal getransplanteerde long voldoende functie kan behouden om de verwijdering van de contralaterale long toe te laten. De mogelijkheden om beide longen simultaan te transplanteren is echter nog onvoldoende onderzocht. Om aan te kunnen tonen dat simultane bilaterale longtransplantatie een goed alternatief voor unilaterale transplantatie zou kunnen zijn, werden experimenten uitgevoerd bij honden om de mogelijkheden en beperkingen van simultane bilaterale longtransplantatie te bestuderen.

In hoofdstuk 2 van dit proefschrift wordt de invloed van selectie van gever en ontvanger voor het serologisch te bepalen deel (SD) van het zogenaamde hoofd histocompatibiliteitscomplex op de overlevingsduur van longtransplantaten bestudeerd. Unilaterale longtransplantaties werden uitgevoerd door het uitwisselen van de linker long tussen paren van verwante honden met hetzij gelijke haplotypen, één haplotype verschil of twee haplotypen verschil in de serologisch gedefinieerde antigenen van het hoofd histocompatibiliteitscomplex. De overlevingsduur van het longtransplantaat werd gedefinieerd als het tijdsverloop tussen transplantatie en het optreden van een homogene sluiering van de linkerlong op de achtereenvolgens vervaardigde röntgenfoto's en werd getoetst aan het histologisch beeld van de getransplanteerde long. Er werd een significant verschil in gemiddelde overlevingsduur van het transplantaat gevonden tussen de twee haplotypen verschillende (3 ± 1.4 dagen) en zowel de één haplotype (8.5 ± 4 dagen) als identieke (35.8 ± 37.2 dagen) combinaties. In de drie verschillende groepen werden ook drie verschillende beelden van afstotingsreactie van de getransplanteerde long waargenomen. Het klassieke beeld van afstoting van een allotransplantaat van de long, voornamelijk bestaande uit infiltraties met mononucleaire cellen, werd gezien in alle honden met één haplotype verschil tussen gever en ontvanger. De histopathologische veranderingen, die werden waargenomen in de identieke honden, duidden op een vertraging van de afstoting van drie

weken. Deze afstoting ging ook met minder vernielingen gepaard als bij de klassieke vorm van afstoting. De histopathologische veranderingen die werden waargenomen in biopsieën van de longen, dertig minuten na de recirculatie in de ontvanger, bij twee haplotypen verschil tussen gever en ontvanger en de daarop volgende hemorrhagische necrose van de long binnen één tot vijf dagen, duidden op het optreden van een hyperacute en versnelde afstotingsreactie. De conclusie was gerechtvaardigd, dat het gebruik van verwante honden met identieke transplantatie-antigenen in plaats van niet getypeerde bastaard-honden het voordeel zou kunnen hebben, dat de effecten van de simultane bilaterale longtransplantatie gedurende de eerste drie weken kunnen worden bestudeerd zonder de tussenkomst van afstotingsreacties.

In het derde hoofdstuk worden de methoden van metingen van de functie van hart en longen vóór en na simultane bilaterale longtransplantatie beschreven.

In het vierde hoofdstuk wordt de operatietechniek voor de simultane transplantatie van beide longen in honden beschreven. Een reeds eerder gebruikte techniek voor het en-bloc transplanteren van beide longen werd zodanig gewijzigd dat de hele procedure kon worden uitgevoerd geheel vanuit een rechter thoracotomie. Na resectie van de rechter long werd de longslagader van het transplantaat verbonden aan de rechter longslagader van de ontvanger en de gehele linker boezem van de gever aan die van de ontvanger. Door daarna de nog aanwezige linker long van de ontvanger te ventileren na intubatie van de linker hoofdbronchus, kon de verbinding worden gemaakt tussen de trachea van ontvanger en gever. Het gebruik van een tijdelijke extracorporele oxygenatie van bloed kon hiermee worden vermeden. Na resectie van de linker long via het achterste mediastinum konden de getransplanteerde longen op hun plaats worden gelegd.
Nadat de operatietechniek was beproefd, werden 54 simultane bilaterale long-allotransplantaties verricht. De directe operatiemortaliteit was 22 procent. De belangrijkste technische mislukking betrof bloeding uit de anastomose van de longslagader. Dat kan worden vermeden door meer bewust te zijn van de tere struktuur van de longslagader. Stolsels op de vasculaire verbindingen konden onveranderlijk worden toegeschreven aan

een onvolledige adaptatie van het vaatendotheel. Door gebruik te maken van een instulpende techniek voor de verbinding van de trachea kon lekkage van lucht worden vermeden. Concluderend kan gezegd worden dat de voorgestelde operatietechniek voor simultane bilaterale longtransplantatie uitvoerbaar is.

In hoofdstuk 5 worden de faktoren, die de direct postoperatieve functie van de getransplanteerde longen beinvloeden, en de onmiddellijke reacties van de ontvanger op het transplantaat bepaald. De mogelijkheid om van de eerste schade van de transplantatie procedure te herstellen is essentieel voor overleving, aangezien de ontvanger volledig afhankelijk is van de onmiddellijke en voldoende functie van de bilateraal getransplanteerde longen. Om inzicht te verkrijgen in de factoren die het overleven en functioneren direct na een simultane bilaterale longtransplantatie bepalen, werden de gevolgen van deze procedure op de functie en het morfologisch beeld van de longen direct na transplantatie bestudeerd bij een geselecteerde groep van 13 labrador-retrievers, die hun longtransplantaten ontvingen van zg. SD-identieke, verwante honden. De resultaten van metingen van hart- en longfunctie toonden aan dat de belangrijkste reactie op de transplantatie procedure bestond uit een shock, die direct na recirculatie van de ischemische en bloedeloze donorlongen in de ontvanger optrad. De beschadiging van het longweefsel tengevolge van de ischemische periode en de verbreking van de zenuwen, lymfe- en bloedvaten in het hilus gebied van de longen worden als de belangrijkste oorzaken beschouwd van de optredende congestie en het oedeem van de getransplanteerde longen direct na recirculatie. Het herstel van de circulatie in de getransplanteerde longen ging gepaard met een circulatoire shock, die niet uit het opgetreden bloedverlies tijdens de operatie kon worden verklaard. Aangenomen wordt dat het verlies aan circulerend bloedvolume door het uittreden van vocht in het longweefsel en een verminderde hartfunctie door verbreking van sympatische zenuwen naar het hart ten gevolge van de operatie procedure resulteerden in een verminderd hartminuutvolume. Een ernstige daling van het hartminuutvolume kon worden voorkomen door onmiddellijk na recirculatie van de getransplanteerde longen bloed te transfunderen, waarbij echter het gevaar bestond dat de reeds aanwezige overvulling en oedeem van de longen werd

verergerd. Indien de shock niet direct werd behandeld, maar bloedtransfusies pas na de operatie werden gegeven, bleek de behandeling vaak geen effect meer te hebben. Indien het hartminuutvolume slechts matig was gedaald, bleek de gaswisseling van de getransplanteerde longen niet of slechts matig gestoord. De matige hypoxemie, die bij alle honden, die langer dan 24 uur na operatie nog leefden, werd gezien, kon worden verklaard door het optredende longoedeem en de overvulling van de longen. De hypoxemie scheen veroorzaakt te worden door ongelijkheden in de verhouding tussen ventilatie, circulatie en diffusie van de getransplanteerde longen. De verhoogde pulmonale vaatweerstand in de getransplanteerde longen werd deels veroorzaakt door de ernstige weefselveranderingen, maar was ook het gevolg van een verminderde bloeddoorstroming van de longen. Dit hoofdstuk eindigt met de conclusie dat het naar waarde schatten van het falen van de circulatie direct na recirculatie van de bilateraal getransplanteerde longen en een voldoende behandeling van de optredende shock in deze fase van het allergrootste belang zijn om overleving te bereiken.

In hoofdstuk 6 wordt de overleving van alle honden na een technisch geslaagde simultane bilaterale longtransplantatie beschouwd. Van de 42 honden waren 21 niet-verwante bastaardhonden, die hun transplantaten kregen van niet-getypeerde honden en 21 labrador-retrievers, die hun transplantaten kregen van verwante honden met identieke transplantatie antigenen. Van deze 42 honden gingen binnen 24 uur na operatie 24 honden dood ten gevolge van een acute respiratoire insufficientie door longoedeem, overvulling en ook bloeding in het longweefsel. De onmiddellijk na transplantatie optredende reacties, zoals die zijn beschreven in hoofdstuk 5, veroorzaakten waarschijnlijk de dood van negen labrador-retrievers in deze vroege fase na de operatie. Een hyperacute afstotingsreactie heeft waarschijnlijk bijgedragen aan de vroege dood van 15 bastaard-honden. De overlevende bastaard-honden overleden alle binnen één week na transplantatie voornamelijk ten gevolge van infectie van de longen. Afstotingsverschijnselen werden slechts in twee van deze bastaard-honden waargenomen. Bij drie labrador-retrievers werden de longen zes, zeven en dertig dagen na operatie afgestoten. Het was duidelijk dat afstoting van de getransplanteerde longen in deze labrador-retrievers, die hun transplantaten ontvingen van serologisch identieke verwante honden, werd vertraagd en zelfs vermeden wanneer ook nog immunosuppressiva werden

gegeven. Uiteindelijk overleden alle overblijvende labrador-retrievers ten gevolge van infectie van de getransplanteerde longen. Een verhoogde vatbaarheid voor infectie van getransplanteerde longen wordt voornamelijk veroorzaakt door niet goed functionerende reinigingsmechanismen van de gedenerveerde longen. Uit metingen van de longfunctie in latere stadia na transplantatie werd duidelijk dat de uitwisseling van zuurstof door de getransplanteerde longen na een aanvankelijke daling weer normaal werd en zelfs enkele maanden goed bleef. Drie honden bleven 30, 75 en 202 dagen leven. Gedurende vrijwel de gehele periode van overleving bleven de arteriele zuurstofspanningen normaal.

In hoofdstuk 7 worden de belangrijkste conclusies van de voorgaande hoofdstukken nogmaals genoemd en worden enkele aanbevelingen gedaan voor nader onderzoek. De toepassing van dergelijke transplantaties bij mensen moet op grond van de experimentele waarnemingen voorlopig nog worden ontraden, totdat bepaalde voorwaarden zijn vervuld.

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