AUTOTRANSPLANTATION OF THE LUNG: EXPERIMENTAL STUDIES ON THE CAPE BABOON*

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SUMMARY AND CONCLUSIONS

Autotransplantation of the left lung was performed on 15 adult baboons with an 8-day survival rate of 80% and a long-term survival rate of 60%. On 5 of the survivors a subsequent contralateral pneumonectomy was performed without mortality. Most deaths occurred early in the series due to technical problems. A surgical technique has been standardized and with further experience in this field the mortality associated with autotransplantation of the lung in baboons should be less than 10%.

Xenon-133 ventilation-perfusion studies of the transplanted lung demonstrated a significant reduction in perfusion, and to a lesser extent of ventilation. Ventilation rapidly recovered towards normal but perfusion only approached normal values several months after transplantation. These physiological alterations may be due to the denervation attendant upon complete removal of the lung with reimplantation. The $\dot{V}/\dot{Q}$ imbalance does not appear to be of major consequence when a contralateral pneumonectomy is performed.

The autotransplanted lung appears to be a valuable model for studying the effects of lung transplantation alone, without the problems of rejection. These studies seem to be particularly valuable in the primate who closely resembles the human anatomically and physiologically. Further studies are currently in progress.

The greatest obstacle to the successful transplantation of organs remains the immunologic reaction of the host to an allograft, but the physiological sequelae of the procedure itself may be important as well. Unmodified lung allografts usually reject within 8 days; the autografted lung, on the other hand, is a valuable model for study of the pathophysiological effects of transplantation without the rejection reaction. The present experiments were undertaken to investigate the technique of lung transplantation in subhuman primates, and to determine lung function differentially and quantitatively with xenon-133 ventilation-perfusion studies in reimplanted lungs.

Early studies of lung transplantation were primarily concerned with development of techniques of auto- and allotransplantation. The earliest recorded case is that of Carrell and Guthrie in 1905.[1] They transplanted the heart and lungs of a kitten to the neck vessels of an adult cat. The lungs soon became oedematous and distension of the right ventricle occurred. The experiment was terminated after 2 days because of infection.

Demikhov[5] described techniques of whole-lung and lung-lobe transplantation at the first All-Union Conference on Thoracic Surgery in Moscow in 1947. Staudacher[23] used autotransplantation of the lung in the dog as a control to study the effects of homotransplantation of pulmonary lobes. The autotransplanted right lower lobes functioned for an average of 12 days whereas the homotransplanted lobes were rejected within 7 days.

Juvenelle *et al.*[15] described the first successful autotransplantation of the right lung in a dog in 1950. The animal survived for 35 months and was sacrificed. Bronchospirometry revealed only moderate loss of function at this time. Autotransplantation of the lung has been used clinically in the treatment of severe bronchial asthma in man,[17,18] with some improvement reported.

MATERIAL AND METHODS

Fifteen adult male baboons (*Papio ursinus*), weighing between 20 and 28 kg were used. Differential lung perfusion and ventilation studies were done pre-operatively and at varying intervals postoperatively, using ^{133}Xe.[3,5,20,24] The animals were tranquillized with phencyclidine hydrochloride† and intubated with a cuffed endotracheal tube. A catheter was inserted in a forearm vein for injection of the ^{133}Xe. The airway was connected to a manually operated pump fitted with a two-way valve and a pressure gauge (Fig. 1). The animal was placed in a sitting position in a specially built chair. Succinylcholine‡ was injected intravenously and respiration assisted manually with the pump.

A pair of collimated scintillation counters were placed

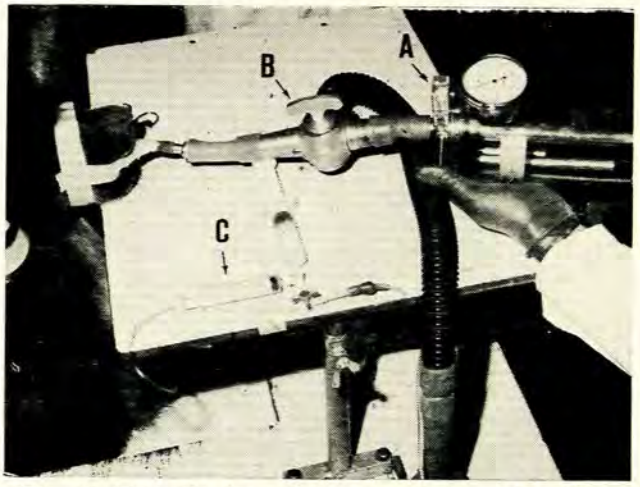

Fig. 1. Determination of lung perfusion with ^{133}Xe. A = calibrated hand pump with pressure gauge; B = two-way valve connected to a gas disposal bag; C = ^{133}Xe dissolved in saline.

in front of the chest and their position marked to ensure accurate geometrical repositioning during future tests.

To estimate pulmonary arterial perfusion, a known quantity of ^{133}Xe (approximately 1 mCi) was rapidly injected into the vein and the catheter flushed with saline. The counts over both lung fields were recorded. Both inflow and wash-out curves were obtained and the amount

*Date received: 9 November 1970.

†Sernylan, Parke Davis.
‡Scoline, Glaxo-Allenbury.

of ^{133}Xe left in the syringe was determined.

For estimation of ventilation, a single inspiration of a known quantity of ^{133}Xe in oxygen was delivered from the pump. Inhalation and wash-out curves were again obtained. Lung volumes were estimated after rebreathing from a closed system, equilibrating the ^{133}Xe throughout both lungs.

A Picker Twin Probe counter with dual channel analyser, dual rate computer, wide-chart two-pen recorder and a high-speed printer were used.

Haematocrit, white cell counts and differential white cell counts were done pre-operatively in all animals. Each received sodium cephalothin* pre-operatively and for 5 days postoperatively. A special chair was built to facilitate pre- and postoperative handling of the animals.

Anaesthesia

Anaesthesia was induced with phencyclidine hydrochloride (1 mg/kg body-weight) intramuscularly, and diazepam† (1 mg/kg body-weight). Following this, all animals were intubated with a cuffed endotracheal tube. A suitable level of anaesthesia was maintained with N_2O, and relaxation was achieved with tubocurarine chloride or succinylcholine as needed. Respiration was manually assisted during operation.

Continuous mean arterial pressure recordings were obtained throughout the operation and during the immediate postoperative period.

The Operation

A standard left posterolateral thoracotomy through the fifth interspace was performed. The left pulmonary artery was dissected to the point where it gives off its branch to the upper lobe. The bronchus was not stripped of adventitia to ensure a good blood supply. The bronchial arteries were divided near the bifurcation of the left main stem bronchus into the upper and lower lobe bronchi. The pericardium was opened circumferentially at the junction of the left pulmonary veins to the left atrium.

The animal was then systemically heparinized. The left pulmonary artery was clamped close to the main pulmonary artery and an additional 10 mg heparin in 5 ml saline was injected rapidly into the pulmonary artery distal to the clamp. The artery was then divided between clamps.

The endotracheal tube was advanced into the right main stem bronchus to occlude the origin of the left main stem bronchus. The bronchus was divided, 3 mm from the bifurcation into the upper and lower lobe bronchi, without using clamps.

A cuff of the left atrium containing the left pulmonary veins was excised with the aid of a Satinsky clamp, care being taken not to occlude the opposite inferior pulmonary vein. The excised lung was immersed in a cold solution of Ringer's lactate for 5 minutes without flooding the bronchial tree. No perfusion of the pulmonary artery was performed.

Reimplantation of the lung was performed using continuous everting sutures to ensure intimal apposition of the vascular anastomoses and mucosal apposition of the bronchus. Ventilation of the lung was resumed after the atrial cuff and bronchial anastomoses were completed. The pulmonary artery anastomosis was performed last. The heparin was not neutralized in any of these experiments and was not continued postoperatively. No blood transfusions were given.

The average total ischaemia time was 75 minutes. The chest tube was removed after 48 - 72 hours. No bronchial leaks occurred in the immediate postoperative period.

*Keflin, Lilly.
†Valium, Roche.

RESULTS

Animal Experience (Table I)

Three animals died within the first 6 hours, due to technical errors. All 3 deaths occurred early in the series. In one case obstruction of the opposite inferior pulmonary vein led to acute pulmonary oedema and haemorrhage into the opposite lung. The operation was not completed. Death in the other 2 animals was due to severe blood loss when the atrial clamp slipped. Not more than 2 - 3 mm of atrial cuff is usually available for anastomosis when the clamp is correctly applied.

One death occurred after 11 days due to severe bronchopneumonia and a thrombus in the left superior pulmonary vein. All suture lines were intact.

Dehiscence of the bronchial suture line with the development of a tension pneumothorax led to the death of one animal 9 days postoperatively. At autopsy necrosis of the left upper lobe was found with signs of aspiration of blood in the right lung.

TABLE I. ANIMAL EXPERIENCE

		No.
A.	Survivors	9
	Survivors with successful contralateral pneumonectomy	5
B.	Deaths:	
	(1) Operative (0 - 6 hours)	
	Clamp disruption	2
	Acute pulmonary oedema	1
	(2) Postoperative (12 days)	
	Bronchial infarction	1
	Bronchopneumonia and thrombosis superior pulmonary vein	1
	(3) Incidental (20 days)	
	Enterocolitis	1

Severe enterocolitis was the cause of death in 1 animal, 20 days after operation. At autopsy all suture lines were intact and there was no macroscopic difference between the two lungs.

No other deaths occurred in the series. Five animals have since had a contralateral pneumonectomy with no mortality.

Lung Function

Ventilation and perfusion data obtained during the first week after operation showed a distinct reduction of perfusion in the reimplanted lung. Ventilation, although affected, was not reduced to the same degree.

Table II and Fig. 2 show the *average* percentage perfusion ($\dot{Q}$) and ventilation ($\dot{V}$) changes in the left lung after left-lung autotransplantation. There was an immediate reduction in perfusion of 18%, with a gradual return to within 7% of the pre-operative value after 14 weeks. Ventilation was less affected immediately and was only 5% less than the pre-operative value after 14 weeks. In some individual cases, ventilation returned to normal

TABLE II. AVERAGE VENTILATION, PERFUSION AND $\dot{V}/\dot{Q}$ RATIOS BEFORE AND AFTER LEFT-LUNG AUTOTRANSPLANTATION AS DETERMINED WITH XENON-133

Perfusion left lung	*Pre-operative*	*1 week*	*2 - 3 weeks*	*5 - 6 weeks*	*14+ weeks*
% of total	50·64	32·48	36·27	38·74	43·77
% of pre-op. value of left lung	100	64·14	71·62	76·50	86·43
Ventilation left lung					
% of total	52·78	43·15	46·11	46·61	47·56
% of pre-op. value of left lung	100	81·75	87·36	88·31	90·10
Index $\dot{V}/\dot{Q}$ left lung	1·04	1·33	1·27	1·22	1·09
Index $\dot{V}/\dot{Q}$ right lung	1·05	0·83	0·87	0·87	0·94

values. Figs. 3 and 4 show these changes graphically in 2 animals.

More pertinent, however, is the reduction of perfusion

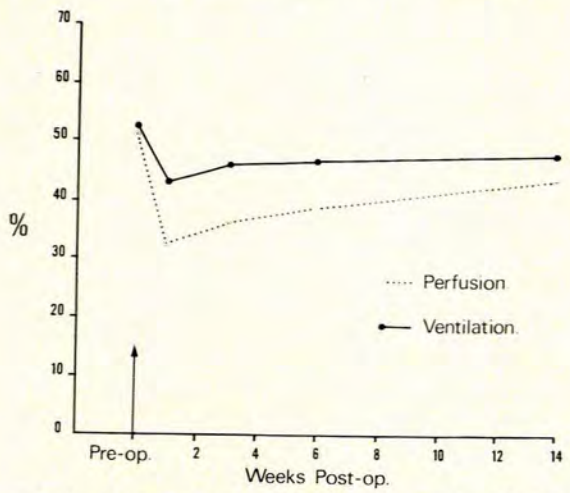

Fig. 2. Average sequential ventilation and perfusion of the left lung in all animals after left lung autotransplantation as determined with ^{133}Xe. Expressed as percentage of *total* perfusion and ventilation of both lungs.

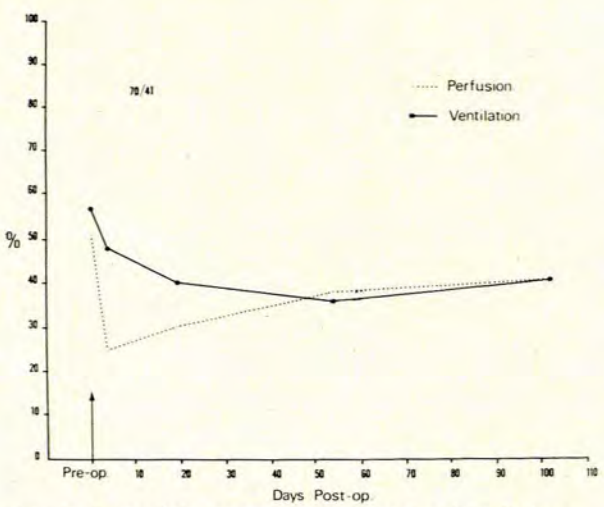

Fig. 3. Sequential changes in ventilation and perfusion of the left lung after left-lung autotransplantation, expressed as percentage of *total* perfusion and ventilation of both lungs (animal No. 70/41).

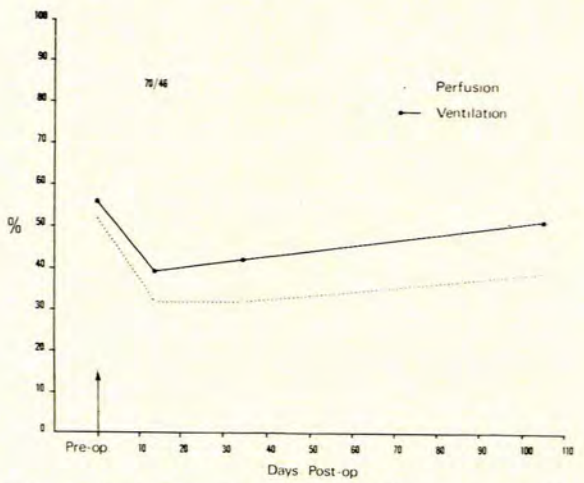

Fig. 4. Sequential changes in ventilation and perfusion of the left lung after left-lung autotransplantation, expressed as percentage of *total* perfusion and ventilation of both lungs (animal No. 70/46).

and ventilation for the operated affected lung. The percentage in perfusion and ventilation is shown in Table II and Fig. 5. From these it can be seen that the immediate reduction in flow to the left lung as compared with the pre-operative flow, is 36%. The perfusion increases over the next few months and after 14 weeks the blood flow to the left lung is 86% of the pre-operative value.

Looking at the changes in ventilation in the left lung only, it can be seen that the immediate reduction of ventilation was 18% with an improvement to 90% of the pre-operative ventilation after 14 weeks.

These changes are also reflected in the ventilation perfusion index [I($\dot{V}/\dot{Q}$)] (Fig. 6 and Table II).

The $\dot{V}/\dot{Q}$ index in the left lung increased immediately after left-lung autotransplantation, indicating that perfusion is more severely affected than ventilation. The $\dot{V}/\dot{Q}$ index declined in the right lung because of increased blood flow. The $\dot{V}/\dot{Q}$ indices of both left and right lungs returned to near normal after 14 weeks.

DISCUSSION

Numerous reports have appeared in the literature since 1960 on autotransplantation of the canine lung.[1,2,6,9,11,16,17,21,22] For technical reasons the left lung was mostly reimplanted. Most dogs, however, died when a subsequent contralateral pneumonectomy was performed.[27]

Baboons have been used for lung transplantation by

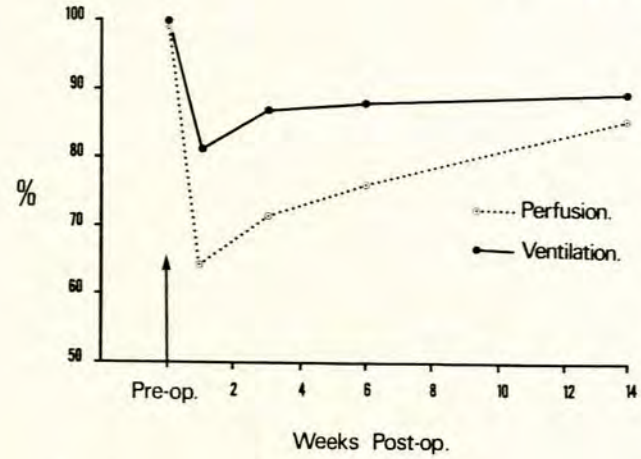

Fig. 5. Average sequential ventilation and perfusion changes in the left lung only. The pre-operative ventilation and perfusion values are taken as 100%.

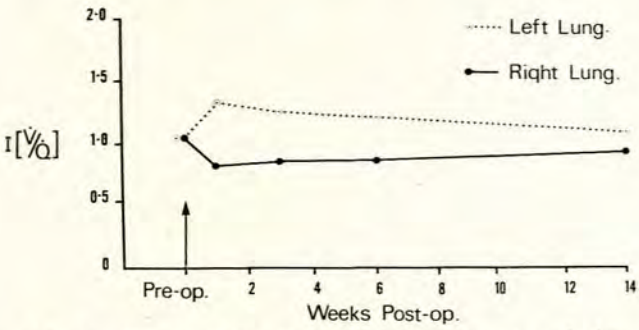

Fig. 6. Average index of ventilation perfusion [I($\dot{V}/\dot{Q}$)] of the left and right lungs in all animals as determined with ^{133}Xe studies.

Haglin and Arnar.[4,11,12] These workers concluded that autotransplantation of the lung could be performed on the baboon with better results than in dogs. Autotransplantation of one lung, both lungs or one lung with contralateral ligation of pulmonary artery or contralateral pneumonectomy have been performed by them with varying degrees of success.

The major cause of death in most series is pulmonary venous thrombosis. This problem was encountered in only one of our primates. Perfect intimal approximation seems to be an important aspect of this problem. The value of heparin is challenged by Arnar and co-workers.[4] There was not a single instance of thrombosis of a vascular anastomosis in their series of 30 consecutive autotransplantations where heparin was omitted. Prolonged systemic heparinization is advocated by some investigators, but others feel that this is not effective in the prevention of vascular thrombosis. We employed systemic heparinization supplemented by temporary local heparinization of the lung, feeling it offered a measure of protection.

The bronchial arterial supply is apparently not essential for adequate lung function. Bronchial necrosis following transplantation has been reported by various investigators in animals[9] and by White *et al.* in their human case.[24] Bronchial anastomosis performed too far from the bifurcation of the left main stem bronchus into upper and lower lobe bronchi, or stripping of the adventitial arteries appears to interfere with the blood supply and may lead to infarction or stenosis at a later stage.[9,24] This may be responsible for the one case of bronchial disruption in this series. Hardy *et al.*[13] have demonstrated that regeneration of the pulmonary lymphatics can be detected 2 - 3 weeks after their division. The problem of bilateral denervation of the lungs is particularly important in dogs, but apparently not to the same degree in baboons.[4,10,19] In a previous experiment by one of us (J.J.W.) it was shown that there was an immediate fall in perfusion of the affected side as measured by ^{133}Xe testing following both denervation and reimplantation, but not in control animals.[25] Ventilation was not affected to the same degree. It is not possible to state at present to what extent nerve regeneration occurs, but it may be responsible for the gradual improvement in perfusion of the denervated or reimplanted lung over the succeeding postoperative period.

The ^{133}Xe ventilation-perfusion studies appear to give accurate quantitative measurements of differential lung function. This method seems to be superior to X-ray and clinical evidence, both of which are unreliable and undiagnostic of lung function following transplantation. It is a logical alternative to bronchospirometry which presents technical problems in baboons and it has the advantage of being a direct evaluation of lung ventilation and perfusion rather than an estimation based on reflected gas exchange.

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