EXPERIMENTAL PULMONARY TRANSPLANTATION

by

GEORGE HANNAH
M.B.,Ch.B., F.R.C.S.(Glas.) F.R.C.S.(Edin.).

A thesis submitted for the Degree of Master of Surgery to Glasgow University.

June 1970.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

EXPERIMENTAL PULMONARY TRANSPLANTATION

Preface

Chapter I — Introduction

Chapter II — Surgical Technique of Pulmonary Transplantation.

Chapter III — Immunosuppression in Pulmonary Transplantation.

Chapter IV — The Function of Pulmonary Transplants.

Chapter V — Discussion

Bibliography

PREFACE

The challenge of pulmonary transplantation embodies the twin disciplines of surgical expertise and scientific enquiry. My interest in this subject has been stimulated by my colleagues in both surgery and scientific research. Pre-eminent amongst these has been my former surgical tutor, Mr. J. Hutchison, Consultant Thoracic Surgeon, Glasgow Royal Infirmary. I acknowledge my indebtedness to him, not only for my early surgical training but also his constant help and unfailing guidance in this research. I also wish to thank Mr. J.M. Anderson, Consultant Surgeon, Glasgow Royal Infirmary, for his enthusiastic support and expert help in both the use of local infusion therapy and in the principles of controlled biological experiments. It is axiomatic that this type of research calls for surgical teamwork of the highest order. In this respect I am indebted to Mr. M. Neely, for his considerable surgical skill and assistance, and to a succession of surgical registrars from Glasgow Royal Infirmary. Professor A. Forrester and Dr. G. Robinson of the Department of Anaesthetics, Glasgow Royal Infirmary, have co-operated enthusiastically as members of the surgical and anaesthetic team and I record my

appreciation of their efforts. Dr. W. Spilg, Department of Pathology, Victoria Infirmary has kindly assisted with the preparation and interpretation of the histological specimens, and his assistance has been invaluable.

Finally, I acknowledge the willing help of Miss M. Burnside for typing the manuscript, Mr. W. Towler for his aid with photography and Miss J. MacDonald for her artistic skill in the preparation of the diagrams.

CHAPTER I

INTRODUCTION

The successful establishment of transplantation of pulmonary tissue would fulfil a need in clinical practice. Out of a total of 33,311 deaths from all causes compiled by the Registrar General for Scotland in 1968, 3,195 deaths (approximately 9.8%) were due to carcinoma of the lung, and 2,675 deaths (8%) could be attributed to chronic respiratory disease (including emphysema and asthma). The annual death rate from these causes has been increasing progressively with atmospheric pollution and consumption of cigarettes. Our present methods of treatment of both chronic respiratory failure and carcinoma of the lung are extremely unsatisfactory.

The operability rate of patients with carcinoma of the lung is approximately one-third. Of the remainder age, poor general condition and extensive disease preclude surgery. There is, however, a significant group in whom pre-operative studies show that pulmonary function after resection would be inadequate to sustain life in comfort.

Similarly, survival in patients with chronic respiratory disease can be estimated by the appropriate study of lung function. Burrows

and Earle (1969) in a detailed study of over 200 patients with chronic airways obstruction, determined, by multiple regression analysis with survival as the dependent variable, that the 5 year life expectancy was only 40% if the forced expiratory volume was below 0.75 litres.

It would appear, therefore, that once the immunological problems of transplantation have been solved, there will be many possible indications for the use of lung transplants.

The earliest attempt at lung transplantation was performed by Carrel and Guthrie in 1905, after the pioneering work of Ullmann, Dec Astello and Floresco in their successful experimental kidney transplants in 1902-1905. Carrel established the method of the vascular anastomosis for transplants of kidney, ovary, limbs and thyroid, and also transplanted the heart and lungs of a kitten into the neck of a cat. The lungs became engorged within two days and the experiment was concluded. Carrel's magnificent achievements in this field were accompanied by truly remarkable foresight when he stated that "the present surgery of organs is in its infancy ..... When the extirpation of an organ is necessary, the ideal treatment would be the immediate transplantation of a sound organ to take its

place." His statement that "the future of transplantation of organs for therapeutic purposes depends on the feasibility of hetero-transplantation", is prophetic.

Demikhov, on 15th May 1947, reported his technique of transplanting the right lower lobe in animals to the First All-Union Conference on Thoracic Surgery in Moscow, and the result became available in a publication by Fegiz to the Western World in 1959, and in a translation into English by Basil Haigh in 1962 of Demikhov's book "Experimental Transplantation of Vital Organs". Two animals survived for 7 days both dying of pneumothorax due to disruption of the bronchus. In three similar experiments, technical failure was attributed to both arterial and venous thrombosis.

An upsurge in experimental work occurred in 1950 when Staudacher, Bellinazzo and Pulin performed both lobar reimplantation and homograft procedures using vitallium tubes for the vascular anastomoses. The reimplanted lobes functioned for an average of 12 days, whereas the homografts were rejected within 7 days. In the same year, Lanari, Mollins and Croxatto carried out lobar transplant experiments with a similar result. In 1950, Metras made a further advance in the surgical technique when he replaced anastomosis of the individual

pulmonary veins by an anastomosis through the wall of the left atrium. A similar anastomosis was described by Neptune, Weller and Bailey, one year later and has now become the standard procedure, overcoming one of the main technical difficulties, namely venous thrombosis.

Although Blades (1952) recorded the first successful pulmonary reimplantation procedure in a dog, carried out by Beattie in 1950, most of the literature ascribes to Juvenelle the first successful long surviving autotransplantation of the canine lung in 1951. Good function of this transplant at 35 months after operation was demonstrated by bronchospirometric methods. Since that time, many experimental lobar and whole lung transplants have been successfully applied in dogs as well as other species. Evaluation of the technical and physiological problems of transplantation in sheep (Borrie and Montgomery, 1958, and Davies et al., 1965) baboons (Haglin et al., 1963, and Haglin and Orn, 1964) and calves (Borrie and Lichter, 1964) have shown them to be similar to that of the canine preparation. Long term survival with functioning autograft lungs has been demonstrated by many workers, although initial attempts at simultaneous bilaterial reimplantation and unilateral reimplantation followed by contralateral pneumonectomy were accompanied by death of the animals from

respiratory failure. It was assumed that this was due to denervation and the loss of the Hering-Breuer reflex. It has been shown, however, that survival with relatively normal pulmonary function can occur after bilateral pulmonary autotransplantation (Slim et al., 1964, Lempert and Blumenstock, 1964, Haglin and Orn, 1964, and Faber et al., 1965) and chronic survival on only a reimplanted lung has been reported by several workers (Nigro et al., 1963, Duvoisin et al., 1964, Yeh et al., 1966, and Shaw and Hill, 1968). These studies have demonstrated conclusively that the gross functional capability of the denervated organ can support life.

With the advance of immunosuppressive therapy in the late 1950's and its relative success in human renal transplantation, an attempt at pulmonary allotransplantation became a practical possibility. Hardy and his associates, with their unparalleled experience of over 400 experimental lung transplants in dogs, and their considerable expertise in the modification of graft rejection by azathioprine, performed the first human lung allotransplant in 1963. Hardy's team transplanted a cadaver left lung into a 58 year-old man with a carcinoma of the left main stem bronchus and a markedly diminished pulmonary reserve. Tissue typing was not carried out. The immunosuppressive

therapy used was azathioprine, prednisolone and cobalt therapy, directed to the mediastinum. The patient died on the 18th post-operative day from renal failure, which had been present before operation. The patient's arterial oxygen saturation was 87.3% pre-operatively (due to a functional arterio-venous shunt through his atelectatic left lung) and rose to 98.59% after transplantation. At autopsy compliance of the graft was grossly equivalent to that of the right lung. Alveolar architecture of the transplant was well preserved and no cellular infiltration characteristic of impending rejection was present in the graft.

Up to the present time 21 human lung allotransplants have been reported in the medical or scientific publications and in the national press (Table 1). In addition, Meshalkin and Felofilov (1964) have resected and reimplanted the lung in the treatment of severe bronchial asthma in man. Two patients out of 7 in this series died from profuse pulmonary haemorrhage in the post-operative period. The other five patients survived and the asthma was said to be improved.

Of the human lung allotransplant procedures 4 were left lower lobe transplants and the rest were whole lung transplantations. Only 7 patients out of the 21 allotransplants lived longer than 10 days with

TABLE I

HUMAN LUNG ALLOTRANSPLANTATION

DATE	CENTRE	TYPE OF PROCEDURE	SURVIVAL TIME
11.6.63.	J.D. Hardy, University of Mississippi, U.S.A.	Left Whole Lung	18 Days
6.6.63.	G.J. Magovern, University of Pittsburgh, U.S.A.	Left Whole Lung	8 Days
25.6.65.	K. Shinoi, Tokyo Medical College, Japan.	Left Lower Lobe	Transplant Removed 18 Days Post-operatively
13.9.65.	W.E. Neville, Veterans Administrative Hospital, Ill. U.S.A.	Right Whole Lung	5 Hours
29.9.65.	J.J. White, Royal Victoria Hospital, Montreal, Canada.	Left Whole Lung	7 Days
15.3.66.	Y. Tsuji, University of Nagasaki Medical School, Japan.	Left Lower Lobe	Transplant Removed Post-operatively
5.1.67.	E.S. Bucherl, Municipal Hospital, Berlin, Germany.	Left Whole Lung	1 Hour
4.67.	Y. Hayata, Tokyo Medical College, Tokyo, Japan.	Left Lower Lobe	Transplant Removed 8 Days Post-operatively
15.6.67.	O.B. Gago, University of Michigan Hospital, U.S.A.	Left Lower Lobe	$\frac{1}{2}$ Hour
67.	E.S. Bucherl, Municipal Hospital, Berlin, Germany.	Right Whole Lung	2 Days

/-

TABLE I

DATE	CENTRE	TYPE OF PROCEDURE	SURVIVAL TIME
15.5.68.	A. Logan, Royal Infirmary, Edinburgh.	Left Whole Lung	13 Days
14.5.68.	J.J. Haglin, Hennepin Country General Hospital, U.S.A.	Left Whole Lung	8 Hours
31.8.68.	A.C. Beall, Jr., Baylor University College of Medicine, Texas, U.S.A.	Left Whole Lung	26 Days
8.11.68.	G.L. Hallman, St. Lukes Episcopal Hospital, Texas, U.S.A.	Left Whole Lung	6 Days
14.11.68.	F. Derom, Academisch Ziekenhuis Rijksuniversiteit, Belgium.	Right Whole Lung	10 Months
18.1.69.	J.D. Hardy, University of Mississippi, U.S.A.	Right Whole Lung	29 Days
6.3.69.	F.J. Veith, Montefiore Hospital, N.Y., U.S.A.	Right Whole Lung	14 Days
26.3.69	D.L. Ross, Guy's Hospital, London.	Left Whole Lung	10 Days
9.4.69.	A.C. Beall, Jr., Baylor University College of Medicine, Texas, U.S.A.	Left Whole Lung	10 Days
1.6.69.	P.J. Vanderhoeft, Hopital Saint-Pierre, Bruxelles, Belgium.	Left Whole Lung	11 Days
69.	D.R. Kahn, Ann Arbor, Michigan, U.S.A.	Left Whole Lung	4 Days

the transplant intact.

The second human lung transplant was performed in 1964 by McGovern and Yates. They transplanted a whole left lung in a 44 year-old man suffering from severe obstructive emphysema. The immunosuppressive therapy used on this occasion was methotrexate. A marked lymphopenia occurred and the patient died on the 8th day after operation from bronchopneumonia. Some of the other cases have been similarly instructive. Shinoi and his team from Japan reported in 1966 the first left lobar transplant in a man of 44 years who required resection of the lingula and lower lobe of the left lung for recurrent haemoptysis due to bilateral bronchiectasis. On the 17th post-operative day, however, the patient developed pulmonary oedema and re-exploration was necessary to remove the transplanted lobe, following which his symptoms subsided. The lobe showed thickening of the alveolar walls, the alveoli being filled with exudate and there was peri-vascular cuffing with mononuclear cells. In 1966, White and his colleagues in Canada reported their attempt to transplant a whole left lung into a 31 year-old man with respiratory failure from advanced silicosis. The immunosuppressive regime of azathioprine, steroids and actinomycin was similar to that which they used for renal transplantation.

Some improvement in pulmonary function occurred but the patient died suddenly on the 7th post-operative day from extensive pneumonitis and infarction of the main stem bronchus.

In this country, the best documented case is that of Matthew and Logan in 1968, who transplanted a whole lung into a 15 year-old boy who developed severe respiratory distress from proliferative alveolitis and bronchiolitis after ingesting the dangerous weedkiller paraquat. He died 13 days after the operation from respiratory failure due to the continuing action of the weedkiller. The histological features of rejection in the transplanted organ were reputed to be absent.

Most of the other lung transplants have suffered a similar fate with the exception of one of the Belgian patients (Derom, University Surgical Clinic, Belgium). This solitary case provides some hope for the future of human pulmonary allotransplantation. The patient was a 23 year-old man suffering from severe silicosis with progressive respiratory failure and pulmonary infection with staphylococci and candida albicans. He had a cadaveric whole right lung transplant performed on 14th November 1968, by Derom's team. All of the facets in this procedure were favourable. Tissue typing showed that most groups were identical. In addition to azathioprine, prednisolone and

actinomycin C, anti-lymphocytic serum was utilised to good effect for the first time in human lung transplantation. There were no technical complications and the transplanted lung began to function immediately after operation. The arterial oxygen saturation on spontaneous respiration 20 hours after transplantation was normal. From a virtually bedridden state pre-operatively, the patient was able to run and climb stairs within a few weeks after operation. It was reported, however, that he died of infection 300 days after the procedure.

The very poor results in human lung transplantation demands caution in future attempts before the two main problems of infection and rejection have been solved on an experimental basis.

The Present Problems in Experimental Pulmonary Transplantation

The experience gained by experimental lung transplantation over the last 15 years has defined the major problems in the following categories:-

1. The effects of surgical techniques in pulmonary transplant procedures. The technical problems arise from devascularisation of the bronchus and from the difficulties in preventing venous occlusion in the low pressure pulmonary vascular system.

2. The assessment of the transplant procedure on all aspects of pulmonary function such as blood flow, ventilation, alveolar volume, gas exchange, compliance, surfactant activity, bronchial clearance and ciliary activity.

3. Graft preservation and the effects on function of the methods utilised to prevent graft deterioration.

4. The prevention of rejection in an organ which is open to atmospheric contamination with the consequent liability to infection.

5. The monitoring of transplant function.

   (a) to detect the early changes attributable to rejection crises.

   (b) to assess continually the long term function of the graft by reliable parameters.

An attempt has been made here to demonstrate a satisfactory surgical technique, to devise and analyse the effect of a method of local immunosuppression, whose systemic effects are minimised, and finally to assess the function of a pulmonary graft by a method

which is reliable, repeatable and readily applicable to the human being without resort to the need for general anaesthesia with its consequent deleterious effects.

CHAPTER II

THE SURGICAL TECHNIQUE OF PULMONARY TRANSPLANTATION

Introduction and Principles of Surgical Technique

Division of all the structures in the pulmonary hilum is required for lung transplantation. The structures divided are the pulmonary artery, the pulmonary veins, the bronchi, the bronchial arterial supply and the lymphatic and nerve supply. The transplantation procedure therefore poses several problems specific to the lung, both for its viability and for successful function.

Lymphatic and Nerve Supply

It is not possible to reunite the lymphatic and nerve supply with present day surgical techniques.

Dissection of lymphatics with consequent lymphoedema of the graft may occasion the temporary ventilatory disfunction seen 2-5 days after transplantation. It has been shown (Hardy et al., 1963) that regeneration of lymphatics occurs towards the end of the first week in the canine preparation and it would seem therefore unlikely that division of the lymphatic supply has any prolonged effect on the

functional capability of a graft.

The denervation which occurs following pulmonary transplantation has been thought however to be of more importance. The loss of the afferent arc of the Hering-Breuer reflex with the resultant deprivation of the normal respiratory pattern has been previously postulated by the early workers to be an insuperable barrier to bilateral transplantation or total pulmonary denervation, especially since Portin et al. (1960) could find no evidence of nerve regeneration in a transplant at 35 months. The early work of Alican and Hardy (1963) and Yeh and his co-workers (1962) suggested that an intact Hering-Breuer reflex was necessary for spontaneous respiration. Both groups of workers were unable to obtain survivors following total pulmonary denervation either by contralateral pneumonectomy following re-implantation or bilateral pulmonary re-implantations.

With improvement in surgical techniques and aftercare, it has more recently been shown that survival with relatively normal pulmonary function can occur after bilateral autotransplantation in both dogs (Lempert and Blumenstock, 1964, Slim et al., 1964, and Faber et al. 1965) and in baboons (Haglin and Orn, 1964). Similarly prolonged survival on only a re-implanted lung has been demonstrated by several workers

(Nigro et al., 1963, Duvoisin et al., 1964, Yeh et al., 1966, and Shaw and Hill, 1968). It would seem therefore, that survival depends more on alveolar-capillary exchange than on the presence of functional stretch receptor activity. This conclusion is re-enforced by the absence of alteration in tidal volume, respiratory rate or pattern of air flow, after bilateral block of the vagus nerves in the neck (Guz et al., 1964) or after complete stripping of the pulmonary hilum (Faber et al., 1965).

Although the Hering-Breuer reflex is apparently not necessary for spontaneous respiration, the other effects of denervation (namely the loss of the cough reflex, deprivation of ciliary movement, and the effects on the secretory activity of the bronchial mucous membrane) have been less widely studied. The recent observations of Hirsch and his co-workers (1968) suggests that there may be more deleterious effects especially with regard to retention of secretions and consequent infection with foci of fibroplasia following transplantation.

Pulmonary Vascular Anastomoses

Two major problems have been encountered in pulmonary haemodynamics of the transplanted lung - namely pulmonary arterial hypertension and the frequent occurrence in the early experiments of venous occlusion.

The precise factors causing a rise in pulmonary vascular resistance following transplantation are still disputed. The studies of Yeh (1962), Nigro (1963) and Christiansen et al. (1965) showed that in the canine preparation after autotransplantation, the pulmonary arterial pressures are elevated and become markedly so (by approximately 50%) when the contralateral pulmonary artery is clamped. More recently Susuki et al. (1968) have found by lung scanning techniques that there is an increase in the pulmonary vascular resistance of a graft after clamping the contralateral pulmonary artery. Kahn et al. (1965) studied the response of the reimplanted lung to hypoxia and suggested that this was the aetiological factor in the production of the increased pulmonary vascular resistance. Allgood, Ebert and Sabiston, (1967) and Kottmeier, Cheng and Fitzgerald, (1969) have claimed that the alteration in pulmonary haemodynamics is due to denervation. It seems more probable, however, that pulmonary hypertension may be the end result of several factors, the most common being stenosis of the arterial suture line and venous occlusion. Sharma et al. (1966) and Daly and Waldhausen (1966) claim that the rise in vascular resistance after transplantation is due to either a decrease in pulmonary arterial flow or an increased resistance to pulmonary outflow rather than denervation. This issupported by Faber's

report of normal vascular resistance after a complete stripping of the pulmonary hilum.

The discrepancies observed by most workers in relation to pulmonary arterial hypertension may well have been attributable to stenosis at the arterial or venous suture lines. The recent work of Veith and Richards (1969) has clearly shown that the indistensible pulmonary arterial anastomosis produced by continuous arterial suture can give rise to hypertension when the contralateral pulmonary artery is occluded, and emphasizes the importance of passive factors rather than denervation in the complex regulation of the pulmonary vasculature. Similarly, Benfield and Coon (1967) have implicated obstruction to the pulmonary venous drainage at the left atrial anastomosis as the cause of abnormal resistance characteristics in the pulmonary arterial circulation. It would appear, therefore, that the cross sectional area of both anastomoses is crucial and it is likely that if this is adequate, pulmonary hypertension will be minimal.

Apart from its effect in increasing vascular resistance, the most serious complication of a technical kind occurring after transplantation is that of venous thrombosis. Alican and Hardy (1963) in their early experiments on lung reimplantation encountered this problem as a major

cause of death in the 52% of dogs who died within the first four weeks after operation. Sharma and his colleagues observed similar effects in their studies of transplants of the left lower lobe when end-to-end anastomosis of the left inferior pulmonary vein was carried out. The technique described by Metras in 1950 and Neptune in 1952 is most effective in reducing the incidence of pulmonary venous occlusion. This entails taking a large cuff of the left atrial wall along with the individual pulmonary veins for a whole lung graft or with a single pulmonary vein in a diaphragmatic lobar graft. Fonkalsrud et al. (1969) have shown that implantation into the atrial appendage of the recipient confers no advantage over an anastomosis in the normal anatomical site in the postero-lateral wall of the left atrium. There is little doubt, that the complication of venous thrombosis and occlusion can be virtually eliminated by the use of this surgical technique.

The Bronchial Anastomosis

The Bronchial Circulation

The classical studies of Miller (1925) have shown that the bronchial arteries supply the main bronchi and their subdivisions down to the distal end of the alveolar duct. It is technically possible by taking a cuff of the aortic wall along with the bronchial arteries to re-establish the

bronchial arterial circulation in the performance of a lung transplant. The technical difficulties of this procedure, however, with its consequent mortality have fortunately been found to be unnecessary. Division of the bronchial arteries does not affect lung function, either immediately or several months after operation (Karsner and Ash, 1912, and Borrie, Campbell and Fulton, 1958). The maintenance of nutrition in the bronchus and parenchyma of a transplanted lobe is of more importance. Ellis, Grindlay and Edwards discovered in 1951 by injecting the plastic vinyl acetate into the right posterior bronchial artery to occlude it, that the pulmonary arterial supply was sufficient to prevent necrosis extending further than the hilar bronchi. It was subsequently found by Huggins (1959 that bronchial disruption could be minimised in the performance of a lobar transplant by dividing the donor bronchus to within less that 10 mm. of the lung parenchyma. Similar studies in whole lung transplants have not been performed. It seems probable that secondary bronchial necrosis can be avoided provided the pulmonary arterial anastomosis is adequate. Nevertheless reduction in nutrition of the bronchus may be responsible for the late development of bronchial stenosis which is encountered in most series.

Ischaemic Time

The short period of ischaemia (approximately 45 minutes) in all of the experiments described here is well within the estimated ischaemic time of several hours at normothermic temperatures before cellular damage results (Blades et al., 1952, Bogardus, 1958, Connaughton et al., 1962, and Borrie and Lichter, 1964). Until more data is available on preservation and storage of the lung it would seem better not to perfuse or ventilate the isolated organ prior to reunion. Milhaud and his group (1969) have shown that ventilation confers no advantage and indeed may lead to destructive emphysema although others (Garzon et al., 1966) claim that hyperbaric oxygen confers viability for approximately 24 hours, and a combination of hypothermia and hyperbaric oxygen for 48 hours (Largiader et al., 1965, and Hino et al., 1968). Perfusion with low molecular weight Dextran and heparin solutions is known (Pegg et al., 1964, Hendry et al., 1968) to cause extensive intimal and endothelial damage in the kidney. Veith (1967) has demonstrated similar findings in the lung when perfused with bloodless media. This has more recently been confirmed by Fonkalsrud et al. (1969) who demonstrated that graft perfusion had a deleterious effect on the mean survival time of dogs subjected to left lung transplants.

The surgical technique used in our experiments has been devised in the full knowledge of the principles outlined above and has been modified in the light of experience to accommodate a method of regional infusion of immunosuppressants.

PREPARATION FOR OPERATION AND ANAESTHESIA

PREPARATION OF ANIMALS

Ninety-five adult mongrel dogs of a known standard weight (20 - 25 Kg.) were used in these experiments. The animals were vaccinated with "Canilep" (Glaxo Laboratories) against distemper, leptospiral fever and infectious hepatitis. They were deprived of food and water from 9 p.m. on the evening prior to operation. After induction of anaesthesia, the left flank and rib cage was clipped and shaved. In these experiments only left lung or lobar transplantations were performed, in accordance with most other series. For anatomical reasons the left lobe or lung transplantation is easier to perform and the left lower "diaphragmatic" lobe is large accounting for approximately 26% of the total lung volume (Rahn et al., 1956, and Zajtchuk et al., 1967) Each dog was given 600 mgm. (600,000 units) of procaine penicillin G. (Glaxo Laboratories) by deep intramuscular injection immediately prior

to operation and this was continued as a daily dose for the first five post-operative days.

ANAESTHESIA

Too little attention has been paid in the past to anaesthesia in major animal experiments but the help of a skilled anaesthetist is accompanied by a significant fall in the operative mortality.

Anaesthesia was induced and maintained by a total dose of approximately 500 mgm. of sodium pentothal intravenously according to the known body weight of the animal. A cuffed bronchus blocking endotracheal tube was inserted into the trachea and manipulated into the appropriate position at the carina. The tube used was a Gordon Greene tube modified by Professor A. Forrester and Dr. G. Robinson (Department of Anaesthetics, Glasgow Royal Infirmary). It was designed specifically for the purpose of canine lung transplantation, and has been found to be invaluable for the prevention of the severe hypoxia resulting from the considerable air leak occurring from the cut end of the host bronchus during the operation. In addition, it eliminates the use of bronchial occlusion clamps which are traumatic and devitalise the divided bronchus. The tracheal cuff on the endotracheal tube was inflated and complete control of respiration obtained by connecting it to

a Barnet ventilator set to a stroke volume of approximately 300 cc. at a rate of 15-20 cycles/min. Anaesthesia was maintained at a light plane by means of a mixture of oxygen, nitrous oxide and halothane. Care was taken that hypoxia did not occur at any time during the procedure. The animals were placed on the operating table lying on their right side. During the operation, 1 litre of 5% Dextrose saline was infused intravenously on the assumption that the dogs would be dehydrated for at least 24 hours, prior to, during, and after the operation. Hypotension from blood loss was rare and blood volume replacement found to be unnecessary.

TECHNIQUE OF LOBAR AUTOTRANSPLANTATION

11 lobar autotranspiants were performed by the following technique.

Surgical Approach. After cleaning the skin with 5% chlorhexidine in alcohol, the operation site was draped and an incision approximately 20 cms. in length was made over the 5th rib on the left side. The thoracic musculature overlying the rib cage was reflected, haemostasis secured and the most convenient rib, either the 4th, 5th or 6th resected subperiostially. The parietal pleura was opened, allowing the lung to retract and a Price-Thomas thoracic retractor inserted. In order to

perform a left diaphragmatic lobe transplant it is necessary to remove the apical and cardiac lobes which are separate in the canine preparation.

Left Apico-Cardiac Lobectomy. The hilar structures of the left lung were completely dissected free by dividing first the inferior pulmonary ligament. The pulmonary veins draining the lobes of the left lung are usually separate and enter the left atrium independently although this was not found to be an invariable feature. The venous drainage of the apical and cardiac lobes were dissected free and doubly ligated with 00 silk and divided, as in Figure 1. The pulmonary artery, found dorsally, was dissected from the surrounding adventitia and the branches supplying the upper two lobes isolated, doubly ligated and divided. A single bronchus supplies these two lobes and takes origin from the main stem bronchus on its ventral aspect lying between the main pulmonary arterial trunk and the venous drainage. The bronchus supplying the apical and cardiac lobes was dissected free and the small bronchial arteries either tied or coagulated with diathermy. After inflating the lowermost cuff on the endotracheal tube and confirming that the tube was occluding the left main bronchus, ventilation of the right lung was observed while the left lung remained collapsed despite manually increasing the ventilatory pressure. The bronchus supplying the apical

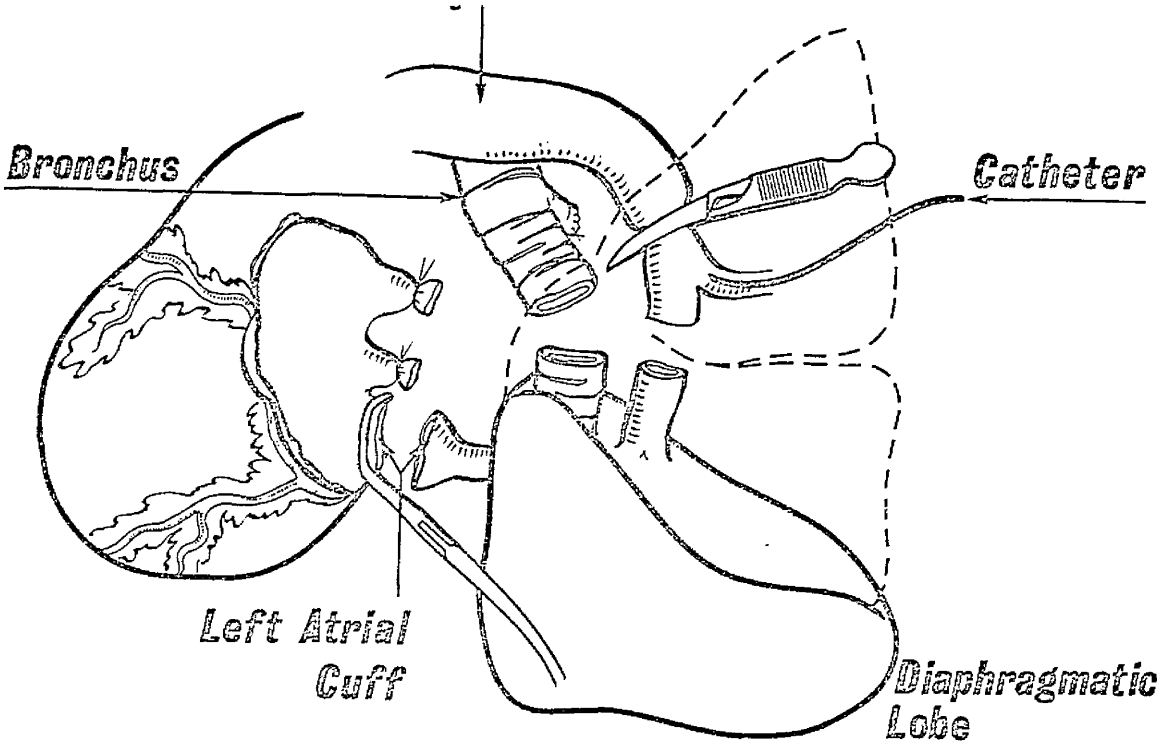

FIGURE 1. THE TECHNIQUE OF LEFT DIAPHRAGMATIC LOBAR TRANSPLANTATION

The interrupted lines show the position of the apical and cardiac lobes which were removed prior to transplantation of the diaphragmatic lobe.

and cardiac lobes was divided flush with the main stem bronchus and closed with interrupted 000 silk sutures thereby completing the apico-cardiac lobectomy. These lobes were thereafter discarded.

Preparation of Left Diaphragmatic Lobe for Transplantation. The pericardium was opened anterior to the pulmonary vein draining the diaphragmatic lobe as in Figure 1. The atrial wall around this vein was dissected free, especially posteriorly, to expose the termination of the venous drainage of the diaphragmatic lobe on the contralateral side, which is in close proximity. Care was necessary at this juncture to prevent opening the mediastinal pleura covering the right lung. Atraumatic bulldog clamps were applied to the pulmonary artery distal to the cardiac and apical lobar arteries and the vessel was transected with the knife. An atraugrip Satinsky clamp was applied to the left atrium at least 2 cms. from the entrance of the left lower pulmonary vein, care being taken not to include the termination of the right pulmonary vein supplying the right diaphragmatic lobe posteriorly. A curved bulldog clamp was applied to the left lower pulmonary vein. The left atrial "cuff" was transected as close to the Satinsky clamp on the atrium as possible, leaving sufficient (5 mm.) distal to this clamp for the anastomosis. Minimal dissection of the bronchus supplying the

diaphragmatic lobe was carried out. The bronchus was cut by the knife as close to the lung parenchyma as possible and certainly no greater than 10 mm. distance from it. The small bronchial arteries found dorsally were tied with 0000 silk or cotton. The lobe was now completely free and ready for the transplant procedure. No attempt was made to perfuse, ventilate or cool the isolated lobe prior to reunion in accordance with the principles outlined above.

Technique of the Transplantation Procedure. The transplantation procedure was begun with the venous anastomosis. Two Blalock mattress everting sutures were inserted as stay sutures through both host and recipient atrial cuffs. The venous anastomosis was accomplished by a continuous everting suture of 00000 Mersilene interrupted twice. The full thickness of the atrial cuff on either side was taken to ensure correct intima to intima apposition.

The anastomosis of the bronchus was performed next. A posterior layer of interrupted 000 silk sutures was inserted through the full thickness of the bronchial wall and tied with the knots external to the bronchial lumen. An anterior layer of sutures was inserted in a similar fashion and the bronchus tested for leakage by releasing the cuff of the endotracheal tube. The arterial anastomosis was carried out in accordance

with the principles enunciated above. Two lateral stay sutures were inserted through both host and donor pulmonary artery. The posterior layer of mattress everting interrupted 5-0 Mersilene sutures were inserted by rotating the pulmonary artery through 180° by means of the lateral stay sutures, which had previously been inserted. The anterior layer of interrupted sutures was completed after flushing the arterial lumen with heparinised saline (1,000 international units of heparin to 500 cc. normal saline).

The atrial clamps were released first and any leakage controlled by insertion of a few interrupted sutures. The arterial clamps were thereafter released and the suture lines inspected for leakage and narrowing. Haemostasis was rarely a problem and blood flow within the graft was easily determined by inspection and palpation. Diminution in blood flow to the graft was usually found to be due to twisting of the arterial anastomosis and required undoing of the sutures and re-anastomosis with proper orientation of the vessels.

The lung was inflated after releasing the cuff on the endotracheal tube and full visual confirmation of graft function determined. It was found necessary to ventilate the graft and compress it manually for a few minutes in order to obtain complete aeration of the lobe. Prior to

closure of the chest the lung was fully inflated and any intrapleural air or blood was aspirated. The chest wall was closed in three layers by continuous chromic catgut sutures with the aid of a Tudor-Edwards rib approximator. No intrapleural drain was found to be necessary, provided that prior to complete closure of the chest wall the lung was fully inflated. The skin was closed with interrupted silk or nylon sutures. The wound was sprayed with Nobecutane, dressed with gauze and an encircling elastoplast bandage applied round the thoracic cage to prevent the animal damaging the wound. Anaesthesia was discontinued. The trachea and bronchial tree were sucked out by means of an endobronchial catheter passed through the endotracheal tube which was thereafter removed.

TECHNIQUE OF WHOLE LUNG TRANSPLANTATION

Three whole lung transplants were performed in these experiments.

Surgical Approach. The skin was cleansed with 5% chlorhexidine in alcohol and an incision approximately 20 cms. in length made over the length of the 5th rib on the left side. The thoracic muscles were reflected and the 5th or 6th rib resected subperiostially in a similar fashion to the lobar transplant procedure. The pleura was opened and a Price-Thomas thoracic retractor inserted.

Preparation of the Lung for Transplantation. The lower cuff on the endotracheal tube was inflated and confirmation was obtained that ventilation was proceeding in the right lung while the left lung remained collapsed. The pericardium anterior to the termination of the pulmonary veins in the left atrium was opened widely as in Figure 2. Dissection of the termination of the three pulmonary veins was necessary in order to obtain a large cuff of the left atrium. The left main pulmonary artery required dissection to the main pulmonary trunk and atraumatic bulldog clamps were applied proximal to the apical lobar artery. The pulmonary artery was thereafter divided between the clamps with the knife. The main stem bronchus to the left lung divides just proximal to the parenchyma into the bronchus supplying the apical cardiac lobes and the large left diaphragmatic lobe. The main stem bronchus required some dissection to expose its branches in order that it be transected as close to the pulmonary parenchyma as possible without transecting the individual lobar bronchi. The small bronchial arteries were found dorsally and were ligated with 3-0 silk. After transecting the bronchus and pulmonary artery, attention was turned to ensuring complete dissection of the termination of the three pulmonary veins and visualising the termination of the veins from the contralateral lung. Two 00000 stay sutures were

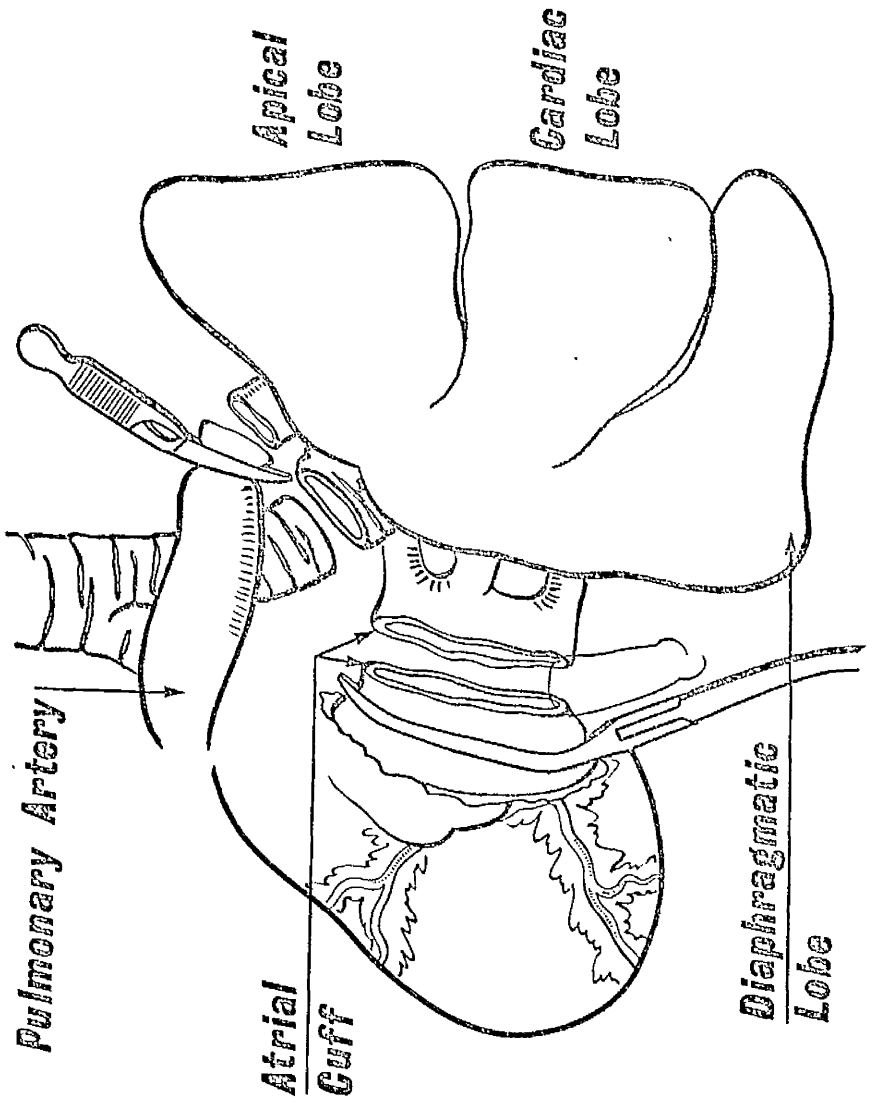

FIGURE 2. Technique of Whole Lung Transplantation.

applied to the left atrium cranial and caudal to the superior and inferior pulmonary veins respectively in order to ensure correct application of the atraugrip Satinsky clamp. This clamp was applied in a cranio-caudal direction at least 1 cm. distal to the insertion of the pulmonary veins. Small bulldog clamps were positioned over the individual pulmonary veins to occlude them. The atriotomy was completed by means of angled Pott's scissors. The whole lung was ready for the transplantation procedure.

Technique of the Whole Lung Transplantation Procedure. The transplantation procedure was begun with the critical atrial anastomosis. It was found to be essential to orientate the graft prior to anastomosis in order to eliminate any harmful twisting of the anastomosis at the completion of the procedure. Two mattress Blalock sutures were inserted through both donor and host atrial cuffs at the cranial and caudal ends of the vessels. In addition, two lateral stay sutures were inserted through the host atrium alone, in order to separate the margins of this atrial cuff. The anastomosis was performed with everting continuous 5-0 Mersilene sutures interrupted twice as in Figure 2. The full thickness of the atrium was taken by means of these sutures in order to obtain intima to intima apposition. Before completing the

anastomosis the vessel was filled with heparinised saline. The bronchial anastomosis was carried out with interrupted through and through sutures of 000 Mersilene, the posterior layer being carried out first. The bronchus was sucked out before completion of the anastomosis and temporary inflation of the graft carried out to determine whether any air leaks were present. Extra sutures were inserted if leakage occurred through the bronchus. The final anastomosis of the main left pulmonary artery was performed in a similar fashion to the lobar transplant. Two lateral mattress everting sutures were inserted, and the vessel was rotated through 180^{O} to insert the interrupted mattress everting sutures. The anterior layer was completed in a similar fashion after irrigating the lumen with heparinised saline. The bulldog clamps on the pulmonary veins were removed and the Satinsky clamp on the host atrium released slowly in order that any bleeding could be controlled with interrupted sutures. The pulmonary arterial clamps were removed. The anastomosis was inspected both for blood flow and any narrowing. Finally, the cuff on the endotracheal tube was released and the graft manually inflated until full aeriation of any atelectatic segments of the graft was obtained. Confirmation of blood flow, ventilation and graft function was obtained both by inspection and by palpation of the various anastomosis. The

chest cavity was aspirated of blood and air, the thoracic cage was closed in three layers by means of continuous chromic catgut sutures. No intrapleural drains were found to be necessary but full expansion of the lung and aspiration of the pleural cavity of blood and air were carried out prior to complete closure of the chest wall. The wound was dressed with gauze and an encircling elastoplast bandage.

Anaesthesia was discontinued after aspiration of the trachea and bronchus of blood and secretions through the endotracheal tube by means of an endobronchial catheter.

TECHNIQUE OF LOBAR ALLOTRANSPLANTATION

Seventy-seven lobar allografts were carried out in these experiments.

In order to minimise the period of ischaemia and to economise in the use of donor animals, allograft experiments were conducted in pairs, exchanging the diaphragmatic lobes between two dogs. The animals were paired according to the degree of disparity in the physical traits of their major breed components and dogs of a similar breed were never assigned to a donor-recipient pair.

The allograft transplantation procedure was similar in all respects to the lobar autograft technique described above, and the ischaemic time

was no longer than 45 minutes for each procedure.

TECHNIQUE OF LOCAL INFUSION

1. Regional Infusion of Immunosuppressants into the Pulmonary Arteries Supplying the Graft. This method of regional infusion was carried out only in left diaphragmatic lobar allografts and was performed in 45 transplants.

The technique is shown in Figure 3. The procedure was similar to that described above, except that, prior to apico-cardiac lobectomy the lobar artery to the apical lobe was dissected into the parenchyma of the lung for approximately 2 cms. and a small bulldog clamp applied prior to transection of the main vessel distal to this. The pulmonary veins to the apical and cardiac lobes were doubly ligated and divided as was the pulmonary artery branch supplying the cardiac lobe. Following transection of the bronchus supplying the apical and cardiac lobes, the pulmonary artery to the apical lobe was divided as far distally as possible to the bulldog clamp. After performing the left diaphragmatic lobar allotransplant by the standardised technique outlined above, a fine non-irritant Teflon catheter (external diameter 1 mm., internal diameter 0.5 mm.) was inserted through a small stab incision in the chest wall posterior

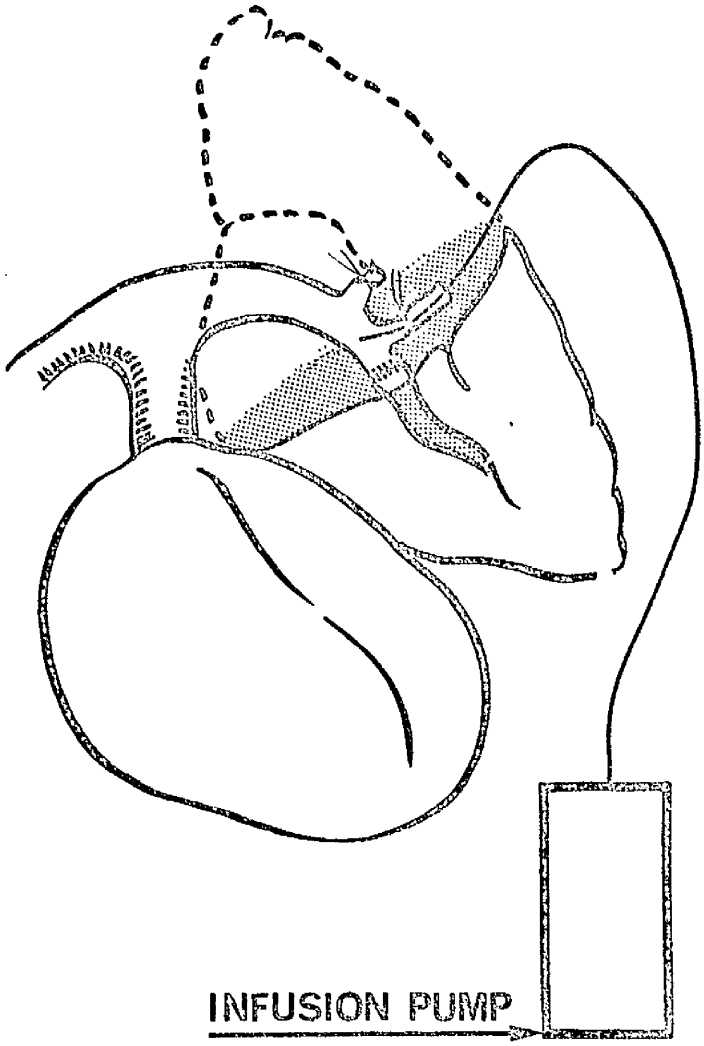

FIGURE 3. Diagmatic illustration of method of local graft infusion in lobar transplants.

to the scapula just behind the neck. A ligature was placed round the apical lobar artery but not tied until the Teflon catheter was inserted into the artery so that its tip lay just within the main pulmonary trunk. The ligature was tightened and the catheter securely sutured into place by means of a stitch through the pericardium. A 3-way tap was connected to the end of a catheter which was thereafter flushed with heparin saline and aspirated to ensure that flow was adequate.

2. Technique of Intra-Aortic Infusion. A similar technique of catheterisation was performed in 12 animals by inserting the Teflon catheter again through the chest wall into the thoracic cavity and into the thoracic aorta through the most convenient intercostal artery. This usually lay at the 6th interspace and, after dividing the parietal pleura the vessel was isolated. Two ligatures were passed around the vessel and the intercostal artery opened. The Teflon catheter was passed in a retrograde fashion into the dorsal aorta, securely tied into the intercostal artery and secured to the parietes by means of a stitch through the parietal pleura.

The Teflon catheter used has been shown (Anderson and Hutchison, 1968

to be effective and non-reactive in the pulmonary vascular system of patients who have received constant intravascular chemotherapy with consistent technical success. These catheters can dwell in large arteries and veins for many months and indeed in one patient for 3 years without untoward effect. Benfield, Coon and Cree (1967) have used a similar indwelling catheter for periods of up to 3 years in normal untransplanted lungs.

Infusion of the immunosuppressants was carried out immediately after the completion of the transplant procedure on release of the arterial and venous clamps in both intrapulmonary arterial and intra-aortic methods. The immunosuppressants (which will be described later) were delivered by connecting the Teflon catheter to either the Watkin's chronofusor microinfusion pump (U.S. Catheter and Instruments Corp., U.S.A.) or to the Sage microflow pump (Horwell Ltd., London). The Watkin's U.S.C.I. chronofusor is a small portable clockwork motor which drives a rotary pump and is illustrated in Figure 4. It is an extremely reliable method of delivering a constant infusion and requires little attention apart from winding the clockwork mechanism twice per day. The silicone rubber bag which contains the infusate empties at a regular

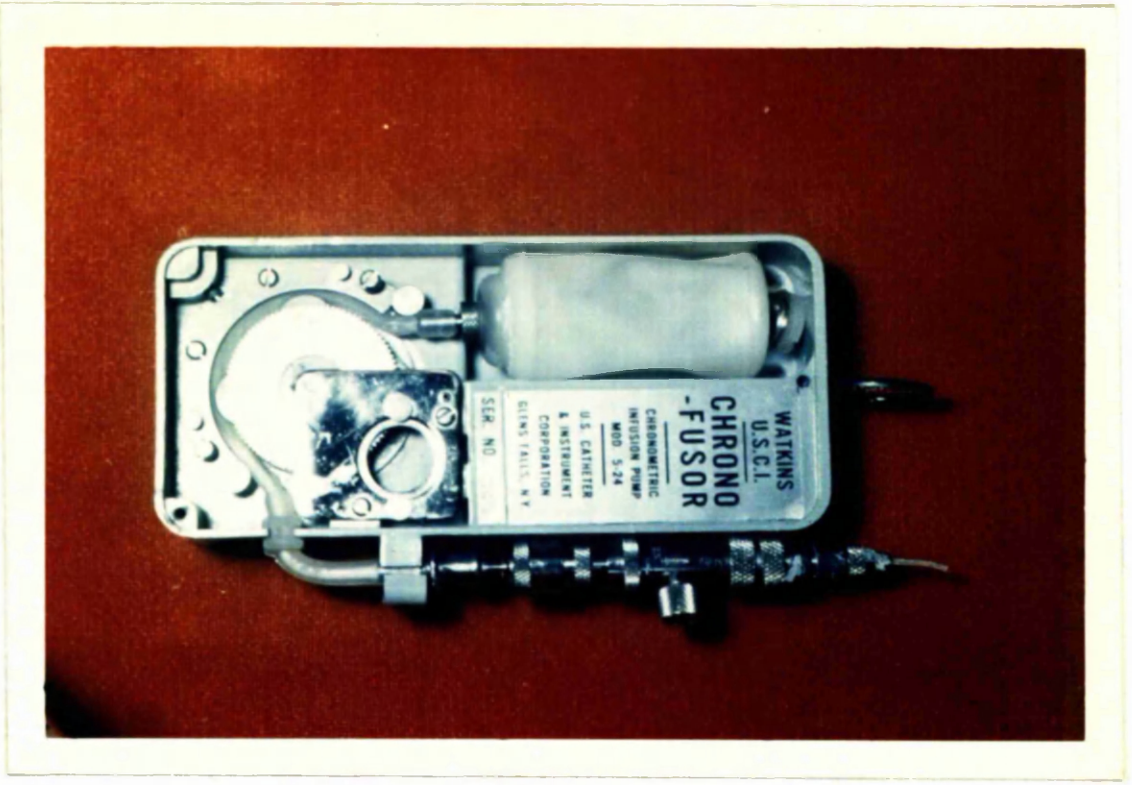

FIGURE 4. Watkin's U.S.C.I. Chronofusor.

FIGURE 5. Sage Microflow Pump (Model 216).

speed within 3 days. The Sage microinfusor illustrated in Figure 5 has a delivery speed which can be varied from 1 ml. in quarter of an hour to 1 ml. in twenty-four hours. It is battery operated and the piston in the syringe is depressed by means of the evolution of gas from a special electrolytic solution.

The chronofusors or microfusors were strapped to the back of the dog after recovery from the anaesthetic for the period of infusion (normally 5 days). During this period the dogs were not restricted in any way and were able to run, eat, drink and sleep without discomfort. Figure 6 demonstrates the catheter and chronofusor in situ in an animal following a lobar transplant.

Post-Operative Management

All of the animals were observed during the immediate post-operative period until the return of full conscious levels. The intravenous infusion was discontinued at the end of the procedure and the animals allowed full freedom to drink and move within a few hours after operation. Waterseal chest drains were not used routinely but pleural aspiration was employed if any effusion developed. Endobronchial suction was necessary in some cases where atelectasis was suspected because of

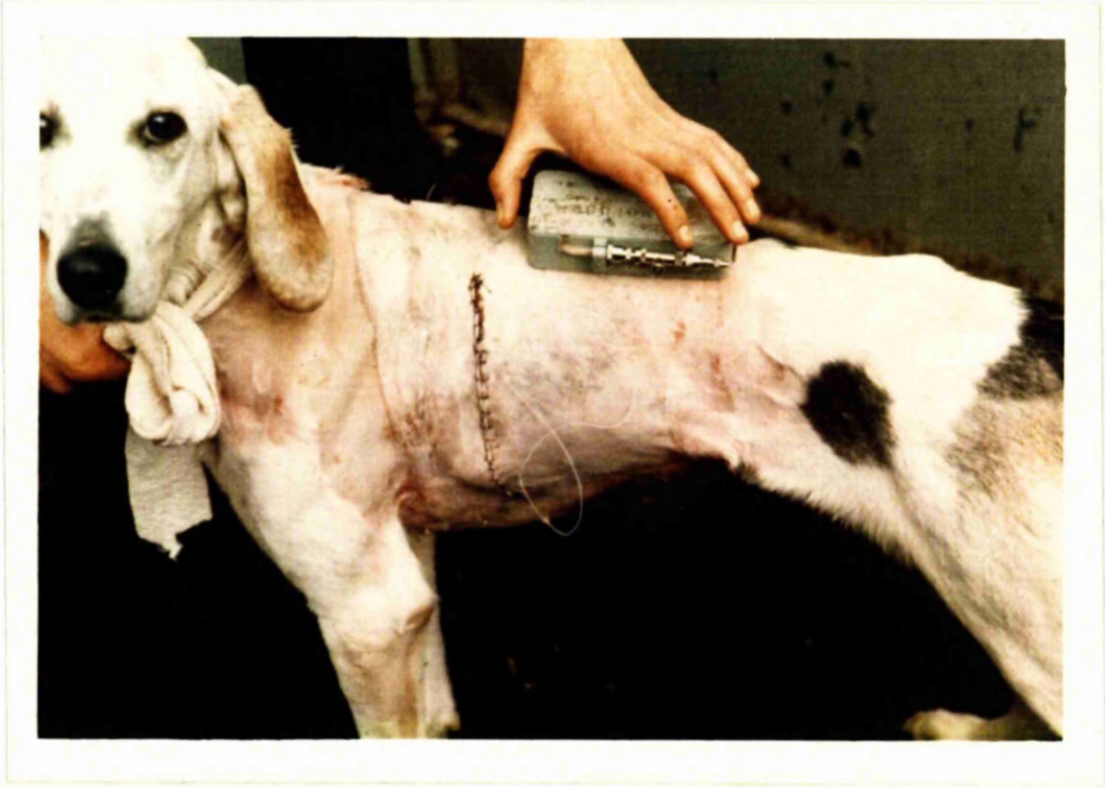

FIGURE 6. The Watkin's U.S.C.I. Chronofusor Pump in situ connected to a Teflon catheter inserted into a pulmonary allograft.

post-operative dyspnoea, cyanosis or auscultatory findings in the chest.

All animals were given 600,000 units of procaine penicillin G. post-operatively. None required either blood replacement or post-operative intravenous feeding. They were returned to a heated cage for 24 hours after operation and temperature and respiratory rates were recorded 3 times daily.

RESULTS AND CAUSES OF DEATHS

The survival time of each preparation was recorded as the number of days to death of the dog from technical reasons, from rejection in the allograft preparation or from wilful or accidental killing for histological purposes.

Post-mortem studies were performed in all animals dying from any cause and histological studies were carried out on appropriate specimens after inflation of the lungs with 10% formal saline delivered through the trachea.

The total number of experiments carried out from December 1968 to January 1970, was 95. Of these, 5 animals were subjected to left

apico-cardiac lobectomy without grafting (3 of whom had left pulmonary artery ligation) for control purposes in the study of pulmonary function.

Results in Autotransplantation

Thirteen autograft experiments were performed. Two in this group had whole lung grafts. The remaining 11 had left diaphragmatic lobar transplants. Four animals died from venous occlusion leaving 9 successful grafts available for further study including assessment of respiratory function. One dog with a successful whole lung graft succumbed to pulmonary oedema 6 weeks after transplantation during an attempt to perform contralateral pneumonectomy. One animal with a lobar graft, proven to be satisfactory on both functional assessment and by post mortem studies, died from an anaesthetic mishap while undergoing Xenon^{133} studies, 2 weeks after operation. At present there are 7 animals with pulmonary autografts alive and well at periods ranging from 6 months to over 2 years. One animal successfully delivered four live puppies 3 months after operation and was undistressed during labour and in the post-partum period.

Results in Allotransplantation

It is difficult to distinguish the immunologically mediated histological

changes in the graft from those due to mechanical interruption or infection. This has also been noted in relation to canine liver allotransplants by Fonkalsrud, Ono, Shafey and Longmire (1967). The technical failure rate in this series is therefore an approximation.

Of the 77 allograft procedures, 21 animals were also given skin grafts to determine the site of action of local immunosuppression. Thirteen animals died from causes other than rejection, leaving 64 for study of the effect of local immunosuppression. Of these 13 animals, 7 died of pulmonary venous occlusion and 1 animal died of haemorrhage from the apical lobar artery after it had pulled the Teflon catheter loose. Two animals died of cardiac arrest (one of these probably as a result of anoxia due to atelectasis). Only 2 animals suffered a tension pneumothorax secondary to bronchial disruption and 1 animal died of pulmonary oedema of indeterminate aetiology.

Comment

The overall mortality rate in the 90 transplants(from technical complications) of 18.8% is in accordance with most other series. Fonkalsrud et al. (1969) in a study of 40 lung transplants, using systemic heparin, had a 25% mortality from technical causes within the

first week. Yeh and Ellison (1962) had 14 deaths (26%) from technical causes, mainly venous thrombosis, in a series of 53 left lung transplants. Duvoisin, Fowler, Pain and Ellis recorded an overall mortality rate of 35% in lung reimplantation in 1964. Borrie and Lichter (1964) and Davies, Rosser and West (1965) had the high early mortality in sheep of 60% and 40% respectively. Rangel et al. (1969) using cadaveric lungs in canine transplants had 11 deaths (50%) in 22 dogs from technical complications. Mouritzen et al. (1967) in a series of 32 lung re-implants had a mortality of 19% during the operation and a 53% during the first week.

The bronchial disruption rate in this present series is low (2.2%) compared with that of Neptune, Weller and Bailey (20%) in 1953. Haemorrhage and pulmonary oedema accounts for 2.2%, and cardiac arrest under anaesthesia for 2.2% of the overall figures. The main cause of death from technical failure is vascular thrombosis and the rate in this series is 12.2%. This accords well with Alican and Hardy's initial rate of 52% where the individual pulmonary veins were anastomosed and more recently Fonkalsrud's figures of 8 out of 40 dogs (20%) despite the insertion of the anastomosis into the atrial appendage.

CHAPTER III

IMMUNOSUPPRESSION IN LUNG TRANSPLANTATION

Introduction

Immunological rejection is likely to remain for some considerable time as the central problem in transplantation surgery. Generalised immunosuppressive techniques are accompanied by a high incidence of deaths from infection due to depression of those responses in the hosts which are beneficial and necessary for survival. The early morbidity and mortality in recipients of renal allografts has been attributed to the large continuous systemic doses of immunosuppressive drugs given (Shackman et al., 1963, Hill et al., 1967, and Kramer et al., 1968). The most commonly used method of immunosuppression in experimental pulmonary allografts has been the chemical suppression of the generalised immune centres. Because these modes of interference are non-specific, depression of the resistance to infection in the recipient animal occurs and suppression of host response by these means is not ideal.

Nevertheless, the introduction of chemical immunosuppressants such as methotrexate, azathioprine and actinomycin has successfully led to the

longer survival of pulmonary allografts. Sporadic prolonged survival of animals with functioning grafts on immunosuppressive agents has been reported in several series. Barnes and Flax (1964) claimed to have had survival of a dog with a left lung homograft up to 238 days after grafting with azathioprine. Gago et al. (1965) using methotrexate reported survival with function 10 months after operation. In the series reported by Nettlebad et al., 1964, one dog survived on azathioprine therapy for 296 days, although the mean survival time for the whole series was 31.6 days on azathioprine compared with a mean survival time of 5.3 days for untreated canine allografts. The best results in Blumenstock's series (1964) were obtained with the aggressive suppression of the immunological mechanism by whole body irradiation, methotrexate, and prior exposure of the recipient animals to donor antigen. Although 5 animals in this group were alive 5 months after grafting, it is possible that these results were improved by chance histocompatibility.

Although the reactions within the graft are important, the effects on the regional and generalised immune centres have concentrated attention upon generalised immunosuppression. Attempts to maintain a balance between abrogation of the immune response to achieve adequate protection for graft function and the need to diminish iatrogenic toxcicity

has led to the study of local immunosuppression. In 1967, Kountz and Cohn reported significant abrogation of graft rejection with a 'cocktail' of six methyl-prednisolone, actinomycin D and heparin, infused locally into renal allografts. The prospect of relatively non-toxic immunosuppression led Kountz to use this method in human renal transplant patients. Kountz and Cohn (1969) reported that 58 of 60 human renal transplant patients were well at periods exceeding one year following transplantation.

Aim of Study

In view of the indifferent results of pulmonary transplantation in animals and in humans and the known disadvantages of generalised immunosuppression, a series of experiments have been carried out using regional infusion. Attempts have been made to test the efficiency of the method in terms of survival and function of canine pulmonary transplants, and to determine whether the effect is local or due to recirculation of the infusate.

Two series of experiments were carried out. The first compared the effect of regional graft infusion of the cocktail of drugs used by Kountz and Cohn with that of the same drugs infused systemically into the dorsal aorta. The second series of experiments were undertaken to determine

by means of skin graft from the lung donors, which part of the immune cycle is affected by local graft infusion.

DESIGN OF THE EXPERIMENTS

Seventy-seven mongrel dogs of 20 to 25 Kg. in weight were paired according to the degree of disparity in their physical traits of their major breed components. Dogs of similar breed or general appearance were never assigned to a donor recipient pair. All of the animals were submitted to left apico-cardiac lobectomy and the left diaphragmatic lobes were simultaneously exchanged between pairs of dogs by the standardised technique outlined in Chapter II.

Ventilation and perfusion studies using Xenon^{133} were performed in all groups at regular intervals after pulmonary transplantation, and an estimate of the lung survival time obtained when no function was present in a previously viable graft. The survival time of the preparation was recorded as the number of days to the death of the animal from rejection of the graft. Post-mortem studies were carried out in all animals and rejection assessed by the absence of technical failure and on the histological appearances of the pulmonary tissue. Of 77 dogs, 13

were thus excluded from this study for technical reasons either because there was no blood flow in the transplant as assessed by Xenon^{133} studies or the preparation was found at post-mortem to have venous or arterial occlusion. White blood cell counts were carried out in all animals weekly

Similar rigid criteria were applied in determining survival of skin grafts. All skin grafts were recorded both visually and photographically as being successful takes before being occluded in the series. The skin grafts were inspected daily and appearances recorded. The end point (skin graft survival time) was taken as that day when less than 10% of the epithelium was still intact, the day of grafting being taken as day 0 of the experiment.

SERIES I

A comparison of the effects of local and systemic immunosuppression on pulmonary transplants was undertaken in this series of experiments. As controls, survival was evaluated in recipients of pulmonary allografts unmodified by any treatment and in a group given only the same low dose of oral immunosuppressant given to the recipients of both local and systemic chemotherapy. Twenty-eight of the 56 allograft preparations were used as controls, leaving 28 animals in the test group given intra-pulmonary arterial infusion therapy.

MATERIALS

Group 1

Seven animals received no immunosuppression therapy and were given only the routine antibiotic (600,000 u. penicillin G.) administered to all animals daily.

Group 2

This group consisting of 9 animals received oral immunosuppression only in the form of low doses of azathioprine (Imuran, Burroughs, Wellcome Tuckahoe, New York) 2 mg. per Kg. per day, and prednisolone (Decortisyl, Roussel Corporation, New York) 1 mg. per Kg. per day for the duration of their survival. The first oral dose was administered 18 hours before operation.

Group 3

This group consisted of 12 animals who received in addition to the same oral treatment administered to Group 2, the infusion of immunosuppressants systemically. The infusate was the same as that given to Group 4 in all respects except that it was delivered through a Teflon catheter inserted into the thoracic aorta through an intercostal artery.

Group 4 (Test Group)

This group received the same oral azathioprine and prednisolone as Groups 2 and 3. In addition they were given the following infusate in

daily doses through an indwelling catheter in the pulmonary artery:-

300 mgm. 6 methyl-prednisolone.
(Solu-medrol, Upjohn Co. Kalamazoo, Michigan).

0.1 mgm. actinomycin D.
(Lyovac Cosmegen, Merck, Sharp and Dohme, Rahway, New Jersey).

20 international units heparin.
(Pularin Evans Medical, Liverpool, England).

The Teflon catheter used for infusion into the pulmonary artery or dorsal aorta was connected to a Sage pump or a Watkin's chronofusor immediately after release of the vascular clamps and the infusion was continued in both groups for 5 days after operation.

RESULTS

The results are seen in tabulated form in Table II. The order of the results in each group is determined by increasing duration of survival time, and is not a historical sequence, thus eliminating selection of results with increasing surgical competence.

Group 1

One animal died at 4 days from technical causes leaving 6 for assessment of survival without immunosuppressive therapy. These animals died between 4 and 7 days following transplantation, the mean survival time being 5.8 days. They were well with functioning grafts on the second post-operative day but the onset of the rejection crisis was heralded with

TABLE II

SERIES I

TYPE OF IMMUNOSUPPRESSION	NO. OF DOGS	SURVIVAL TIMES IN DAYS		RANGE OF WHITE CELL COUNTS (c.mm.)
		INDIVIDUAL	MEAN	
GROUP 1 (None)	6	4,5,6,6,7,7.	5.8 Days	
GROUP 2 Oral Only	8	4,4,4,6,6,10,14,30.	9.75 Days	8,600-20,000
GROUP 3 Oral Plus Intra-Aortic	9	6,6,6,8,8,10,15,20,23.	11.3 Days	8,400-68,000
GROUP 4 Oral Plus Intra-Pulmonary (Test Group)	21	4,4,4,4,5,6,7,9,9,12, 13,*18,*19,20,22,29, 29,65,°79,82,570+.	48.1 Days	

* Killed during laboratory epidemic of distemper.

o Killed for histopathological studies.

\+ Alive at present.

lethargy, coughing, dyspnoea, pulmonary oedema, and haemoptysis. In general the host died within 24 hours. Confirmation of rejection was obtained at post-mortem by the typical microscopic and macroscopic appearances and on the absence of technical mishaps.

Morphological Appearances

It is extremely difficult to evaluate the more subtle cellular changes associated with the homograft rejection response in pulmonary tissue, not only because such changes are not widspread but also because of the problem of differentiating those changes due to infection, perfusion and ischaemic damage. Homograft rejection in the transplanted lung of the unmodified host has been studied by various workers (Lanari et al., 1951, Barnes et al., 1963, and Sharma et al., 1966) and in general has three main components.

1. Peri-vascular mononuclear infiltrates.
2. Intra-alveolar oedema and inflammation.
3. Terminal massive pulmonary necrosis and haemorrhage at the end of one week which rapidly leads to death of the animal.

Figure 7 shows the typical macroscopic appearances of rejection in the transplanted lobe. At the time of death a small haemorrhagic pleural effusion was frequently observed. The transplanted lungs were dark red in colour and cut with the consistency of liver tissue. Patchy areas of necrosis were evident and the lung was consolidated throughout. The bronchial mucosa was congested and haemorrhagic and sero-purulent

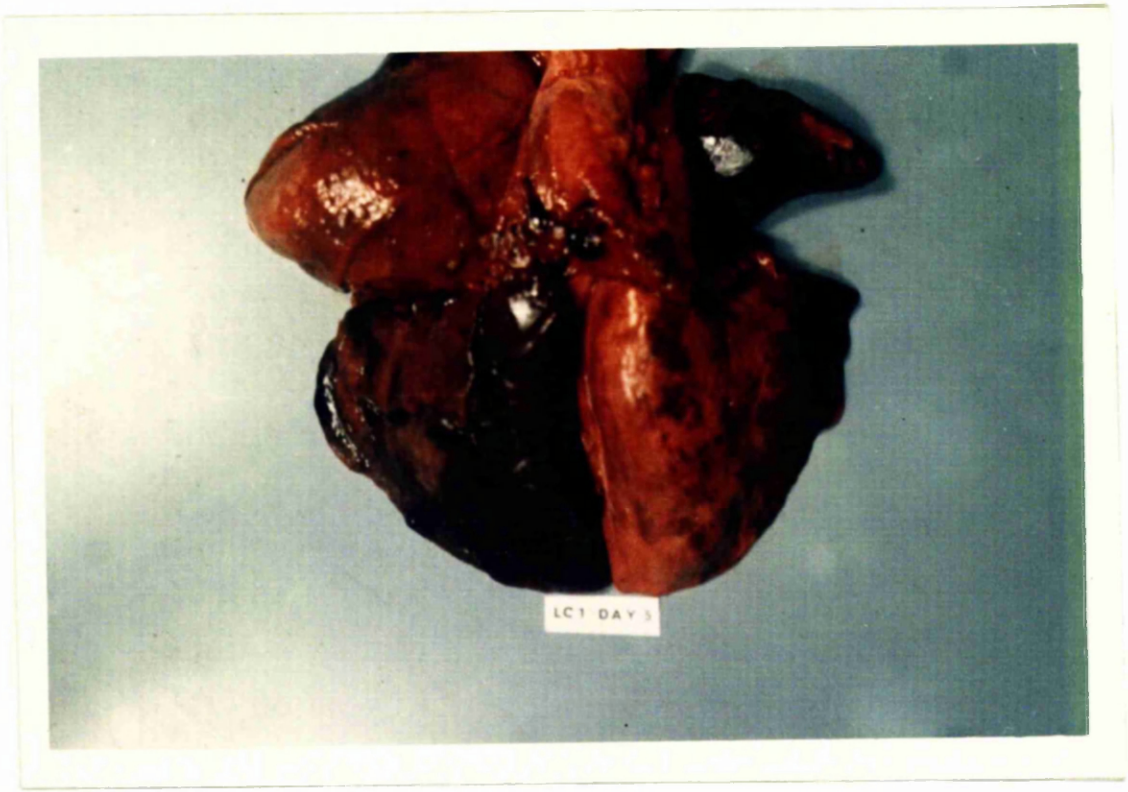

FIGURE 7. Photograph of rejected lobe (on left) 5 days after transplantation.

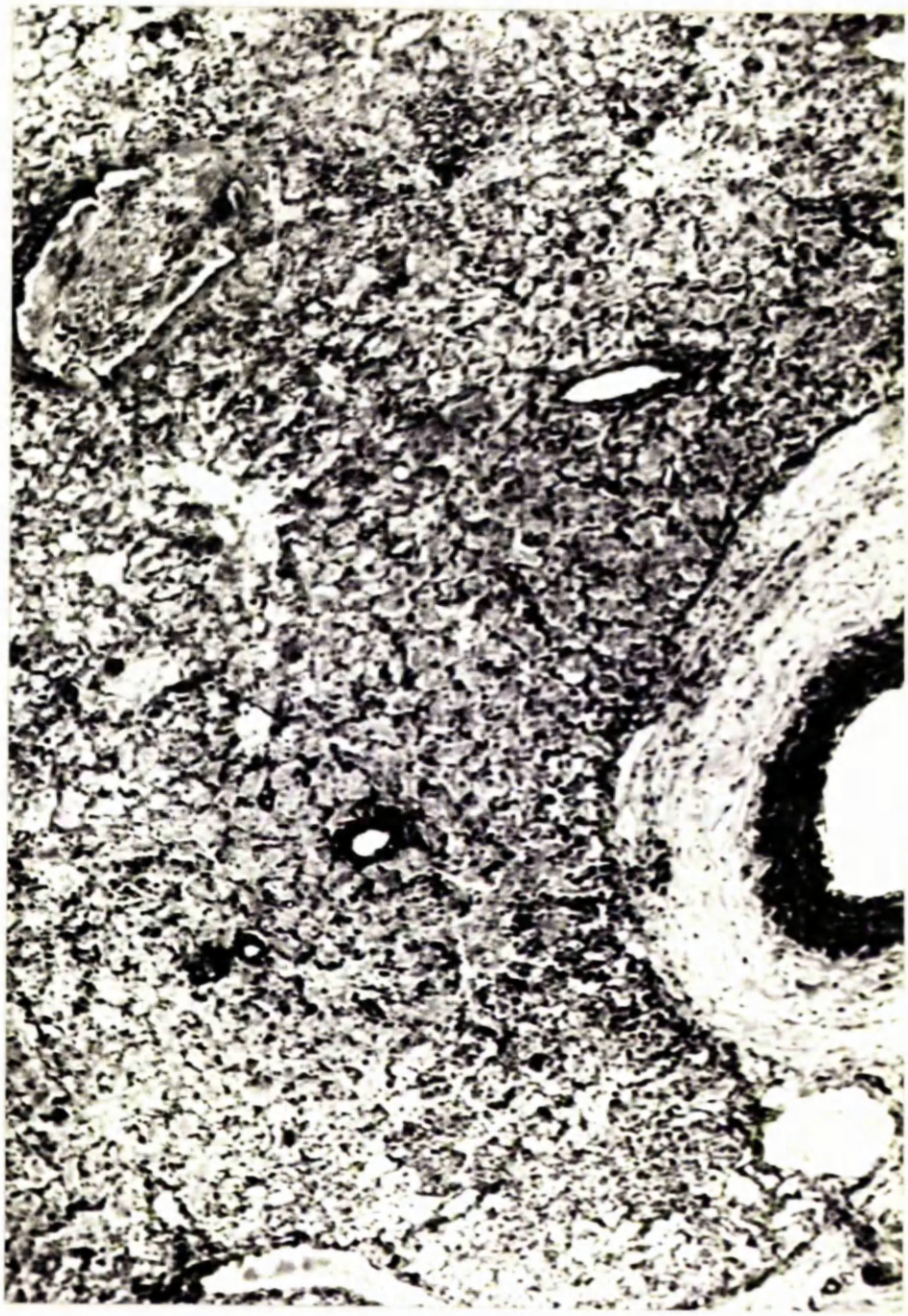

FIGURE 8. Histological appearances of lobar allograft unmodified by immunosuppression. Widespread necrosis, alveolar disruption, intra-alveolar oedema and cellular infiltrates. (H & E x 4)

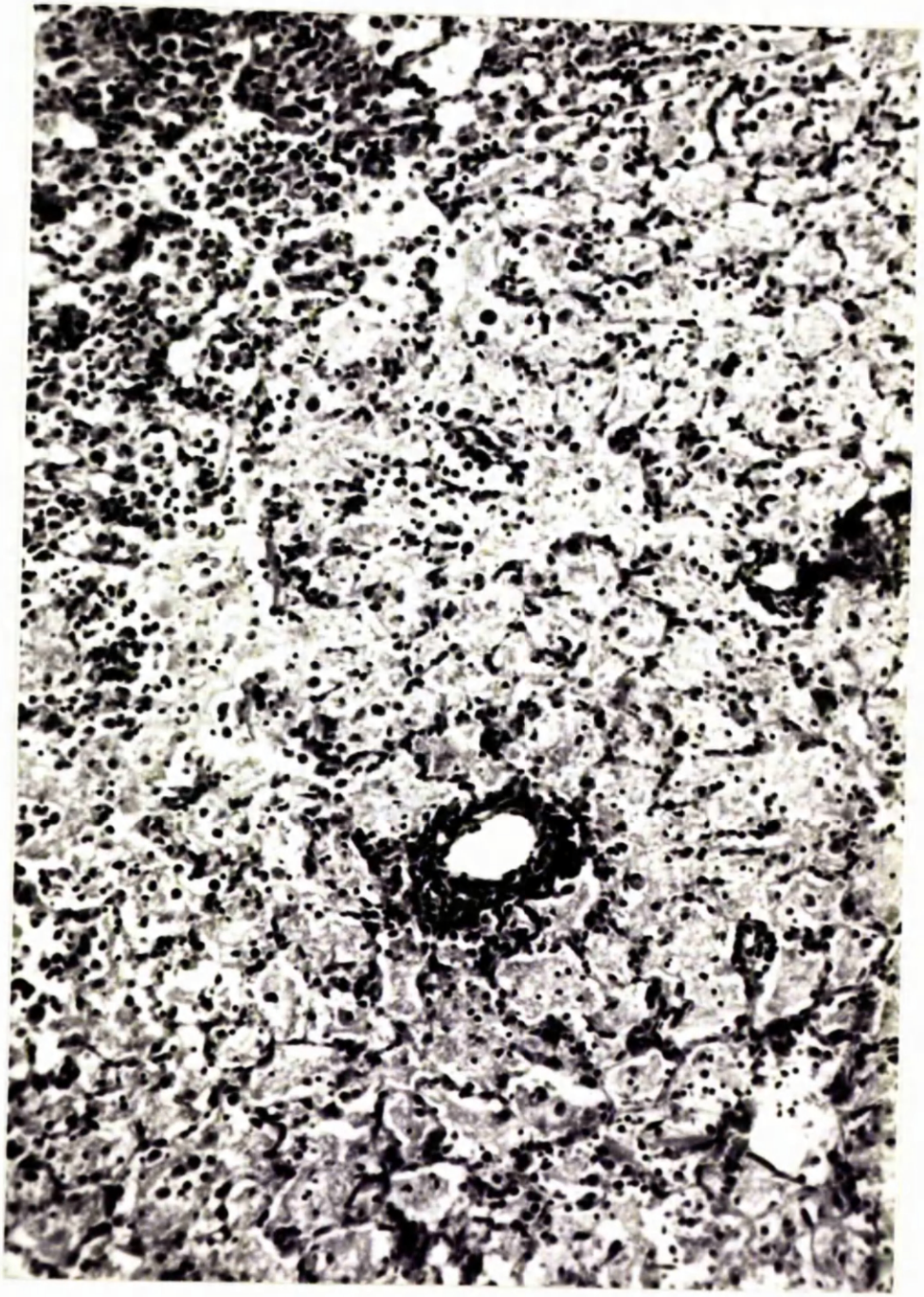

FIGURE 9. Rejected lobar allograft. Dense perivascular cuffing with mononuclear cells. (H & E x 10)

fluid exuded from the alveoli and small bronchi. Figures 8 and 9 demonstrate the typical histological appearances found in this group. There was patchy necrosis of pulmonary tissue and widespread disruption of alveolar pattern. There was widespread oedema within the alveoli accompanied by a pleomorphic intra-alveolar infiltrate consisting predominantly of round cells as well as polymorphonuclear cells. The small arterioles showed marked haemorrhage within their walls with focal necrosis and peri-vascular cuffing with mononuclear cells. This latter feature has been documented by Sharma et al. 1966, as being the characteristic feature of graft rejection in pulmonary tissue.

Group 2

In this group the animals received only oral immunosuppressive therapy. Of the 9 animals, 1 died from technical causes and was eliminated from the study. The mean survival time in the remaining 8 animals was 9.75 days. The fulminating clinical course of the rejection response was similar to that in Group 1 and was not modified by the low dose oral immunosuppression. The morphological appearances of the graft were similar in all respects to those in Group 1.

Group 3

In this group the animals received oral and systemic infusion of the

'cocktail' in the same doses as the test animals and for the same duration, namely 5 days. Twelve animals were used in this group but 3 died in the immediate post-operative period from technical failure, leaving 9 for assessment of systemic infusion of immunosuppressants. There was slightly prolonged survival time, the mean being 11.3 days. However, only 1 animal survived beyond 20 days and the clinical course was similar to that of Groups 1 and 2.

Group 4

The test group consisted to 28 animals which were treated by both oral and localised immunosuppression to the graft. Seven animals were excluded from the study. Four of these died within 3 days of operation from vascular occlusion, and 3 were excluded because there was no evidence of blood flow in the graft immediately after operation. The mean survival time of the remaining 21 animals was 48.1 days and there was evidence of prolonged survival with graft function, 7 animals surviving beyond 20 days. The mean survival time in this group has, in fact, been reduced because two animals with functioning grafts were killed during a laboratory distemper outbreak at 13 and 18 days, respectively after operation, and one was purposely killed at 65 days for histological study of its functioning graft. One animal is still alive

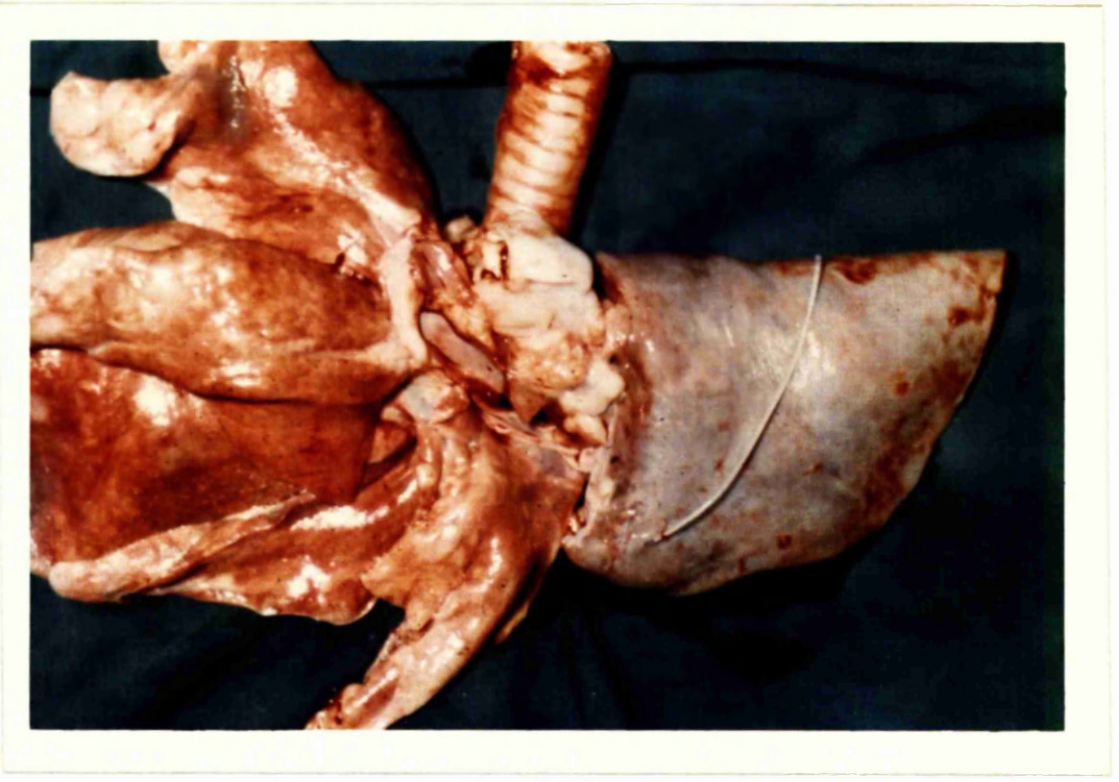

FIGURE 10. Photograph of post mortem specimen. Viable lobar allograft (on the right) at 65 days, treated by local immunosuppression.

at 570 days with a well functioning graft assessed by both Xenon^{133} studies and confirmed by lung biopsy.

There was no clinical evidence of massive sepsis on long term therapy following local immunosuppression, although 1 long term surviving animal developed pustular sores on its trunk and limbs, which was attributed to mange. No animal died of drug toxicity as evidenced by a reduction in the white blood cell counts.

Morphological Features

Figure 10 shows the macroscopic appearances of a functioning graft at 65 days following treatment with local immunosuppression. The pleural surface of the transplant on the right was slightly thickened and dull but the lung ventilated well and was of the same consistency as the contralateral lung. Both anastomoses were patent; the bronchus showed no evidence of an inflammatory response. Histological sections demonstrate little evidence of necrosis and only a minimal inflammatory response. The anticipated classic cellular pattern of the homograft reaction was absent in the long term surviving allografts. There is minimum intra-alveolar exudate. The alveoli are well formed and there is little cellular reaction. Figure 11 shows the histological appearances of the attenuated cellular response. One of the features of this response was the oedema

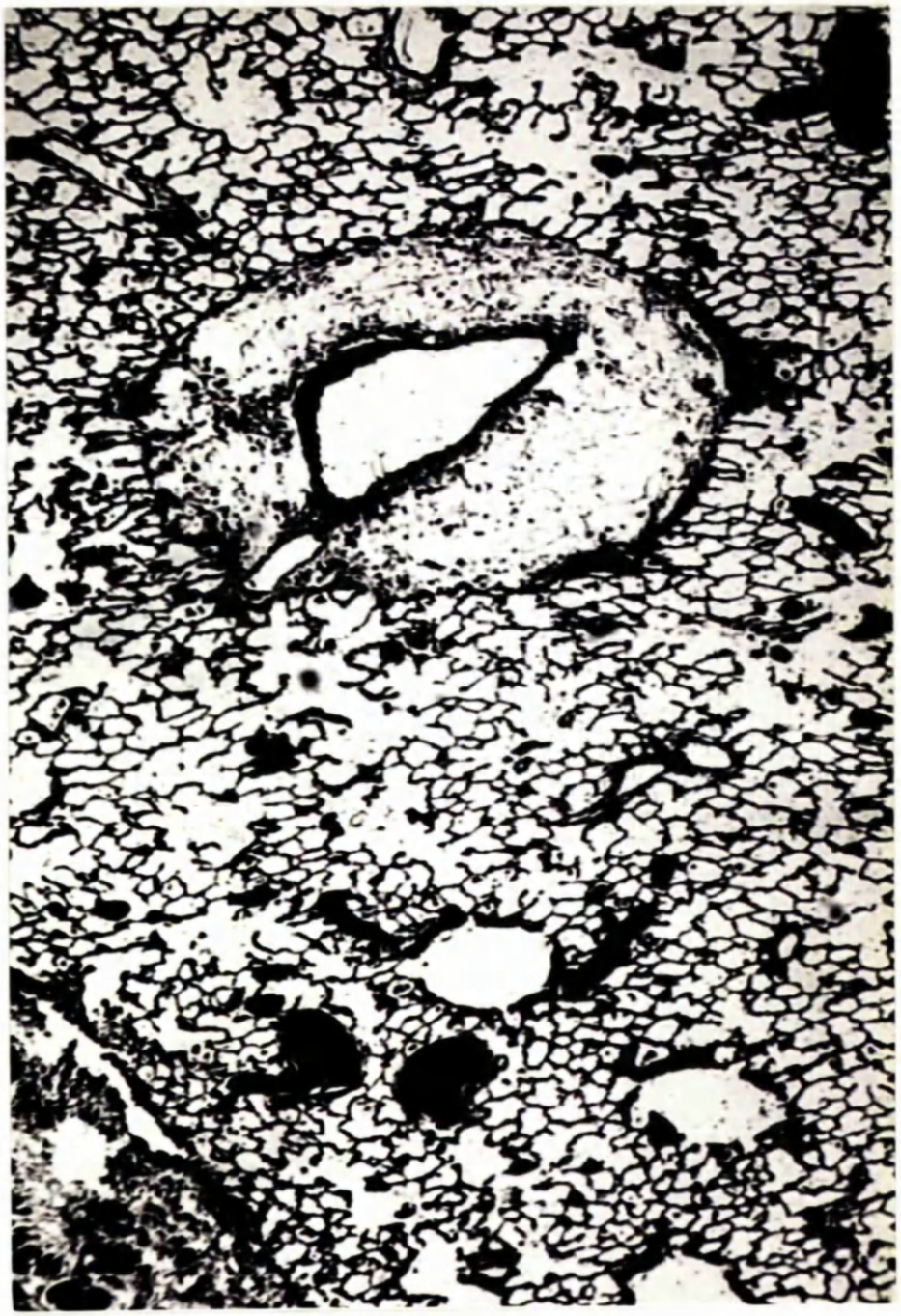

FIGURE 11 (a). Histological features in rejection modified by immunosuppression. Normal alveolar architecture with no cellular or fluid infiltrates. Oedema of small arterial wall. (H & E x 4)

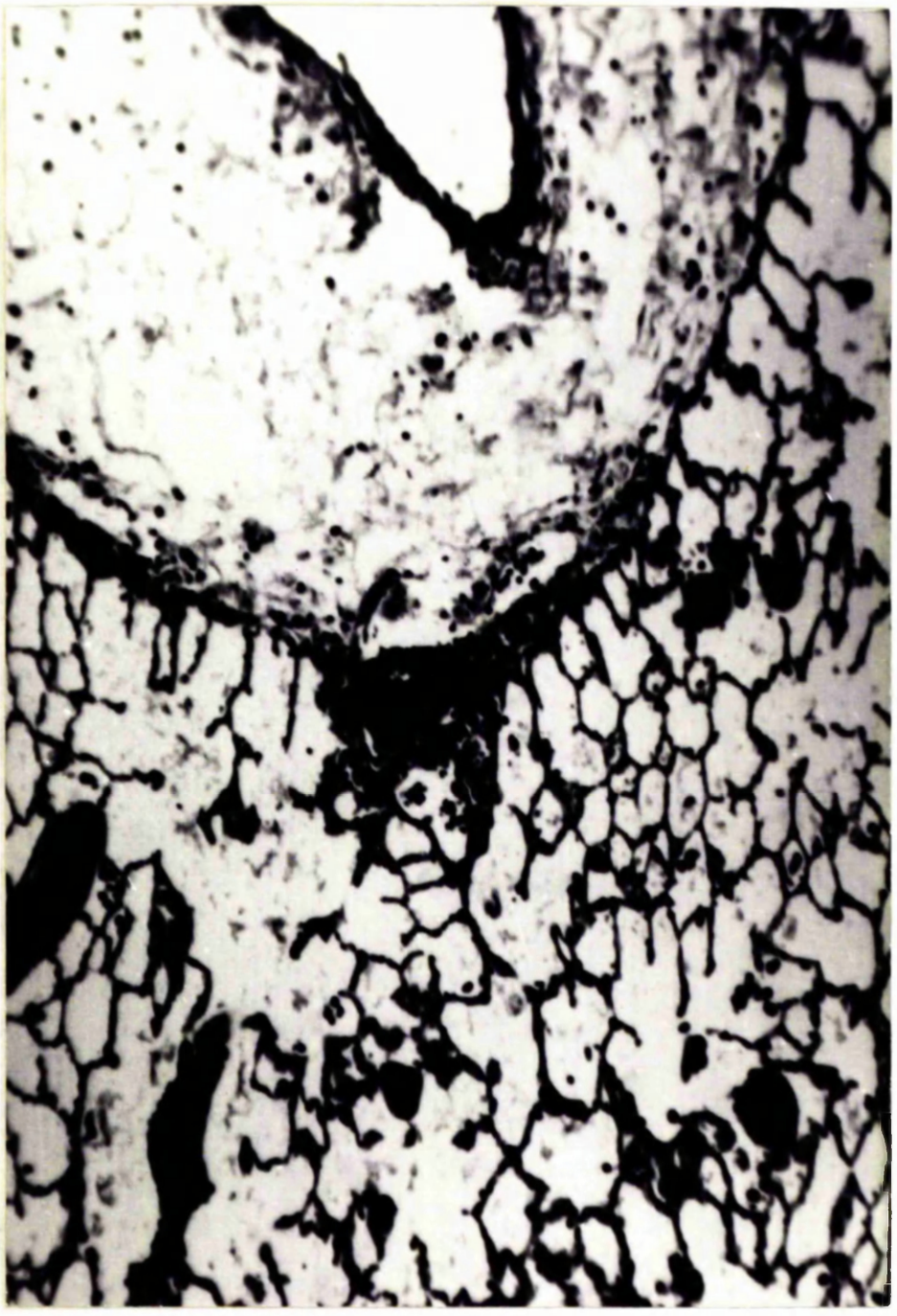

FIGURE 11 (b). High power view of changes in small vessels in modified rejection. Marked swelling due to oedema ; little mononuclear "cuffing".

present within the wall of the small arteries and arterioles often with little or no cellular infiltrate. This characteristic feature has been described by Lanari et al., in pulmonary homografts and has been noted by Porter et al. 1965, to occur in human renal transplants. Porter has demonstrated that human renal grafts rejected after some months show damage to arteries and arterioles often with little or no cellular infiltrate and he suggests that circulating antibodies may be important in chronic rejection.

SERIES II

In this separate series of studies, 21 dogs were given skin grafts from the lung donors,

1. immediately after the conclusion of the pulmonary transplant procedure to determine whether reconstitution of the afferent arc of the immune response in this way would invalidate the beneficial results of local pulmonary graft infusion, and
2. two weeks prior to pulmonary transplantation in order to assess the effect of local immunosuppression on the second set response.

The Skin Graft Technique

The skin graft technique was standardised in all experiments. Under general anaesthesia the right flank was shaved and cleaned with 5% chlorhexidine in alcohol. The recipient area for the skin graft on the right flank was prepared by excision of a rectangular area (4 cms. x 5 cms.) of the epidermis down to, but not including, the panniculus. A standard skin graft (4 cms. x 5 cms. in area with 1 mm. thickness) was obtained from the right flank of the lung donor by means of a Pagett dermatome. The graft was applied by interrupted 000 silk sutures tied over a roll of gauze.

Group 1

Of the 21 animals who were given skin grafts, 14 had contemporaneou skin grafts. Skin grafting in this group was carried out immediately after conclusion of the pulmonary transplant procedure. All of the animals were treated by the same oral and local immunosuppressive regime given to animals in Series I. One animal died of technical failure leaving 20 for study.

Group 2 and 3

Six dogs were presensitised with skin grafts from the lung donors two weeks before lung transplantation. As immunosuppressive therapy did not commence until pulmonary transplantation, 6 controls were thus

available for the estimation of survival of homograft skin without immunosuppression. Following pulmonary lobar allotransplantation, 3 animals were treated by the same oral and local immunosuppressive regime given to animals in Series I. The other 3 dogs (Group 3) were given no immunosuppressive therapy following pulmonary lobar allotransplantation and were thus used to assess the survival of the second set response following pulmonary grafting.

RESULTS

Group 1

Table III shows the results obtained in 14 animals with lobar allografts treated by local immunosuppression and having a skin graft from the lung donor immediately on conclusion of the pulmonary transplant procedure. One animal lived for 73 days but has been shown by bronchography and Xenon functional studies to have had a non-functioning graft at 23 days. The survival time of the lung was estimated by Xenon studies as being that day when no blood flow could be demonstrated in a previously viable graft. The mean survival time of the grafted lung in this series was 9 days. The mean survival time for the animals was 11.5 days (excluding that animal which lived 73 days with a non-functioning graft). This survival time is markedly reduced when compared with those

TABLE III

SERIES II

GROUP 1. PULMONARY ALLOGRAFTS TREATED WITH LOCAL GRAFT INFUSION AND CONTEMPORANEOUS SKIN GRAFTS FROM LUNG DONOR		
SURVIVAL TIME IN DAYS	INDIVIDUAL	MEAN
LUNG SURVIVAL TIME	9,9,15,6,6,5,10,2,7,8,6, 12,8,23.	9.0 Days
DOG SURVIVAL TIME	11,9,21,6,6,5,10,7,8,6, 24,8,29.	11.5 Days
SKIN GRAFT SURVIVAL TIME	9,9,12,6,6,5,10,12,7,8, 6,15,8,11.	8.9 Days

animals with lobar allografts treated by local immunosuppression without the application of contemporaneous skin grafts (Series I, Group 4). The skin graft survival time in this group is not prolonged (mean survival time = 8.9 days) when compared to allograft skin applied to dogs without lung transplantation (mean survival time 9.5 days, Table IV).

Group 2

Table IV indicates the results obtained from those animals with lobar allografts treated by local immunosuppression, who were presensitised with skin from the donor of the lung graft two weeks prior to transplantatior All of the animals in both the treated and untreated groups were shown to have died from rejection of the lung by post-mortem studies. The mean survival time of the skin allografts (prior to transplantation of the lung) was 9.5 days and this is in accordance with the survival of canine allo-graft skin in most series. The animals in Group 2, which were treated by local immunosuppression, showed a markedly reduced mean survival time of 4.0 days, indicating an accelerated rejection response despite the use of local immunosuppression.

Group 3

The 3 animals in this group showed a fulminating clinical course with the onset of dyspnoea, haemoptysis and frothing pulmonary oedema

TABLE IV

PULMONARY ALLOGRAFTS PRESENSITISED WITH SKIN GRAFTS	LUNG SURVIVAL TIME		DOG SURVIVAL TIME		SKIN GRAFT SURVIVAL TIME	
	INDIVIDUAL	MEAN	INDIVIDUAL	MEAN	INDIVIDUAL	MEAN
GROUP 2 Treated with Local Graft Infusion	7,4,1.	4.0	7,4,1.	4.0	8,7,11.	9.5
GROUP 3 Untreated	1,6,1	2.7	1,6,1.	2.7	7,13,11.	

within several days of lung transplantation. The mean survival time of both lung and animals in this group was 2.7 days (Table IV) and is characteristic of the accelerated second set response following presensitisation with skin grafts (Hardin, 1956).

CONCLUSIONS

1. Prompt rejection of a canine allograft lung in the host unmodified by immunosuppressive therapy occurs within the first week and is followed by a rapid death of the host. The mean survival time of 5.8 days in this group is similar to that of other series. Some recipient animals may survive despite the necrotic graft but more commonly they die soon after necrosis has become apparent. Zajtchuk et al. (1967) have demonstrated diminished function at 3 days in unmodified allografts following which irreversible graft necrosis and death ensued. Yeh and Ellison found that none of their animals survived beyond two weeks if no immunosuppressive therapy was given. The mean survival times quoted in all other series are remarkably constant. Faber's group had a mean survival time of 6.5 days in 10 animals and Sharma's series (1966) reports 5.3 days in 11 animals. Neptune, Weller and Baillie similarly found the average survival time in their untreated

group as being 6 days, and most of the animals in the series reported by Barnes and Flax (1964) were dead between 7 and 9 days. None of the 7 animals in Susuki's series lived longer than 20 days and the mean survival time for the whole group was 10.3 days. In the series reported by Sharmaet al. (1966) and Nettlebad et al. (1964) the mean survival times were approximately 5 days in unmodified recipients of pulmonary allotransplants. The constancy in all series implies that the survival time in the canine pulmonary allo-transplant preparation relates closely to rejection.

2. The results obtained by local immunosuppression bear favourable comparison with other non-biological methods of generalised immuno-suppression. Hardin (1954) using steroids obtained a mean survival time of 14 days and Neptune and Baillie increased this to 25 days with ACTH. In 1961, Blumenstock reported sporadic survival of animals 6 months to a year when the recipient was treated with methotrexate. De Bono and Brock (1964) also reported prolongation of survival and function with this same drug. Both Hardy et al. (1963) and Parsa et al. (1964) were unable to obtain similar prolongation of survival with methotrexate, the mean survival time in their series being 14 days and 15 days respectively. Similarly, Gago (1964)

was only able to obtain one survivor with a functioning lobe at 6 months out of 30 animals treated with this same drug. Sharma (1966) demonstrated prolongation of survival with myeleran and 6-mercaptopurine, the mean survival time being 31.6 days.

The introduction of azathioprine, actinomycin and cyclophosphamide has led to the extension of survival in pulmonary allotransplants. McPhee and Wright evaluated therapy with cyclophosphamide and actinomycin C and increased survival to a maximum of 49 days, although the average survival time was 28.3 days.

Azathioprine in large doses of approximately 5-10 mgm. per Kgm. body weight (i.e., several times the doses in these experiments) has been used by most workers with some success. Hardy (1964) found an average survival time of 30 days; both Parsa et al. (1964) and Nettlebad et al. (1964) reported similar mean survival times of 27 days and 31.6 days respectively. In Nettlebad's series, 1 survived with a functioning graft for 296 days after transplantation. In Vuillard's series (1969) the longest survival was 35 days with azathioprine. Yeh et al. (1965) demonstrated prolonged survival to a maximum of 80 days and Richards and Asway-Pinto (1964) to 92 days. Susuki et al. (1968), despite the

use of full doses of azathioprine in addition to whole body irradiation found that the mean survival time of 31.6 days which he obtained by this method could be increased to 38.8 days with the supplementary infusion of donor spleen cells.

Although the figures quoted and the average survival time of 48.1 days for local immunosuppression cannot be statistically analysed due to the sporadic nature of survival in such biological investigations (Calne, 1963) it is apparent that the results of local immunosuppression are as good as those obtained by large doses of systemically administered therapy.

The mean survival time in this series approaches the overall figure of 51.5 days reported by Blumenstock et al. (1967) who compared combinations of methotrexate and anti-lymphocytic serum. They had 7 dogs alive and well from 37 to 79 days after transplantation although these results may have been influenced by chance histo-compatibility. More recently Veith, Richards and Lalezari (1969) have experimented with anti-lymphocytic serum and azathioprine and the results (mean survival time 28.5 days) are not outstandingly impressive.

3. Confirmation of the results obtained by Kountz and Cohn (1967)

in renal transplantation is suggested by similar effects in pulmonary allografts. Even such a short course of infusion used in these experiments has prolonged survival in the treated group beyond that of the control groups. Long term therapy by the intra-arterial route is feasible, as is intermittent infusion for rejection crisis and might reasonably be expected to approach the results obtained by Kountz (1969) in human renal transplants.

4. It seems likely that infusion of the immunosuppressive drugs locally into the graft is responsible for the observed effects. There is little difference in survival between the group treated by low dose oral immunosuppression and the untreated group, and the mean survival times of these groups does not compare with that of the group treated by local graft infusion. The less violent clinical course and the absence of the classical histological signs of rejection in the pulmonary tissue of locally infused grafts suggests modification of the immune response by local infusion.

5. The possibility that recirculation of immunosuppressants, with subsequent central depression of the immune response, is responsible for the observations, cannot wholly be excluded from the data presented, but is unlikely for the following reasons:-

(a) When the same infusate of immunosuppressants was administered systemically in otherwise identical circumstances, there was no marked prolongation of survival beyond that of the group treated by oral immunosuppression only, and the survival time did not approach that of the test preparation.

(b) There was no prolongation of skin graft survival time which one might expect when these grafts were applied to recipients of lung allografts treated by local infusion.

(c) White blood cell counts (Table II) did not show the typical leucopenia characteristic of generalised immunosuppression.

6. The effect of local immunosuppression cannot be wholly explained but some deductions may be postulated from the skin graft experiments. It is known (Hardin, 1956) that skin and lung share common transplantation antigens but that the methods whereby graft antigens are processed are not yet clearly defined. It is possible that local immunosuppression prevents antigen release or

recognition and that this effect is overcome by the induction of immunity by the application of contemporaneous skin grafts from the lung donors. Hardin has shown that presensitisation with skin grafts leads to rejection of untreated pulmonary allografts within 3 days of operation. The similar accelerated rejection response induced in the group which received lung allografts after presensitisation with skin from the lung donors, implies that the afferent arc of the immune response was inviolate and that deleterious effects of rejection upon the lung graft continued despite local immunosuppression.

Further evidence as to the mode of action of immunosuppressant drugs will be necessary to establish that phase of the immune response which is most sensitive.

CHAPTER IV

THE FUNCTION OF PULMONARY TRANSPLANTS

Introduction

The assessment of the functional progress of a grafted lung cannot be as easily forecasted as that of other organs. The progress of rejection in renal transplantation may be heralded by the determination of the secretory functions and early treatment instituted (Calne, 1961). Similarly, Starzl et al. (1961) and Goodrich et al. (1956) have assessed the functional capacity of the liver transplants by investigation of the constituents of both blood and biliary secretions. The electrocardiographic changes in cardiac transplants are also well documented and early rejection can be determined (Hallmann et al., 1969, and Stinson et al., 1969).

The gross physiological changes which occur after lung transplantation have been analysed indirectly by contralateral pneumonectomy (Neptune, 1953) bilateral autotransplantation (Lempert and Blumenstock, 1964, Slim et al., 1964, and Faber et al., 1965) and by estimation of arterial oxygen and carbon dioxide measurements and with the simultaneous collection of expired air (Yeh et al., 1962,

and Nigro et al., 1963). Other workers have assessed function by measurement of pulmonary arterial and venous oxygen differences but these methods require the emplacement of catheters under fluoroscopic control, are difficult to perform and are time consuming. Differential bronchospirometry utilising a double lumen endotracheal tube has provided much useful information on the oxygen uptake and exchange in pulmonary grafting (Juvenelle, 1951, Portin, 1960, Linberg and Armstrong, 1961, and Davies and West, 1965). As noted by Faber, however, bronchospirometry is difficult to perform, is frequently unsuccessful, and frequent collection of expired gases is not easily obtainable. The difficulties encountered have led to attempts to collect samples of expired gases by temporary tracheostomy (Borrie and Montgomery, 1958, and Borrie and Lichter, 1964) and to the development of Hedley Jones (1967) of cervical bronchostomy, whereby the implanted left diaphragmatic lobe bronchus was exteriorised in the neck and allowed isolation and control of the expired gases. These methods, albeit useful in the experimental situation, have a high failure rate and cannot be applied to the human situation. Similarly, repeated serial lung biopsy has been used by Sharma (1966) to determine the changes occurring but, as with all of such procedures, early determination

of rejection by these methods is difficult because of the considerable reserve of lung function and the fact that even histological changes do not equate adequately with changes in lung function. Considerable skill is required to obtain valid results by bronchospirometric methods and by pulmonary arteriography, although the latter is invaluable for demonstrating blood flow in a purely qualitative way.

The introduction of methods utilising radio-active gases for measuring ventilation and perfusion in human subjects (Knipping et al., 1955) has given a method which can readily be used in the determination of flow and ventilation in pulmonary transplants. Accurate, direct and quantitative data concerning these parameters in the transplant situation can more readily be obtained in this way than by any method so far described.

The use of gaseous radioactive isotopes for topographic evaluation of lung perfusion and ventilation in man is well documented (Ball et al., 1962, Dollery et al., 1962, West, 1962, and Bryan et al., 1964). Rahn et al. (1956) demonstrated the distribution of ventilation and perfusion in the canine lung and its geographic variability with posture using radio-isotope methods. Both Strieder's group (1967) and Pain and his associates (1967) in this country have used the radio-active gas Xenon^{133} to demonstrate

the early changes occurring in a few canine lung transplant procedures.

The present series of experiments using Xenon^{133} has been undertaken to evaluate sequential changes occurring after transplantation, to determine the validity of the procedure in relation to transplants, to detect changes occurring during rejection, and to study the physiological consequences after long term transplantation by the comparison of both allografts and autografts.

DESIGN OF THE STUDY

1. Materials

Fifty-seven mongrel dogs weighing approximately between 20 and 25 Kgm. were used in these experiments. Eleven had left diaphragmatic lobe reimplantations, 2 had whole left lung re-implantations and 44 had left diaphragmatic lobar allografts. Controls were obtained in 2 normal dogs, and 3 dogs with apico-cardiac lobectomy alone. Three others with pulmonary artery ligation (after apicocardiac lobectomy) and 4 with venous occlusion of the diaphragmatic lobe were utilised to determine the effect of technical failure on the functional studies.

A total of 288 tests of pulmonary function were carried out using Xenon^{133}. Long term surviving allografts were obtained for study

by the method of local immunosuppression outlined above.

2. Method

The animals were anaesthetised using sodium thiopentone, halothane nitrous oxide and oxygen, and were intubated with a well fitting cuffed endotracheal tube. An intravenous cannula was inserted into the most convenient foreleg vein. The animal was thereafter placed in a wooden trough as shown in Figure 12 and paired collimated scintillation counters with 1 inch sodium iodide crystals placed beneath the dog, one on either side to scan the lower lobes. This was achieved by placing the collimators at a standard position $\frac{1}{2}$ inch cranial to the ziphisternum and inclined not more than 30^{O} from the vertical. The collimators were connected to amplifier analysers and ratemeters to a dual channelled servoscribe recorder on which the gamma emissions were recorded graphically.

The endotracheal tube was connected through a three-way tap to a Boyle's anaesthetic machine and to a 1 litre handpump as shown in the diagram.

Perfusion Studies

The animals were ventilated to apnoea, and two millicuries of Xenon^{133} dissolved in saline were injected into the intravenous cannula. Xenon is a chemically insert gas about three times as soluble as oxygen and

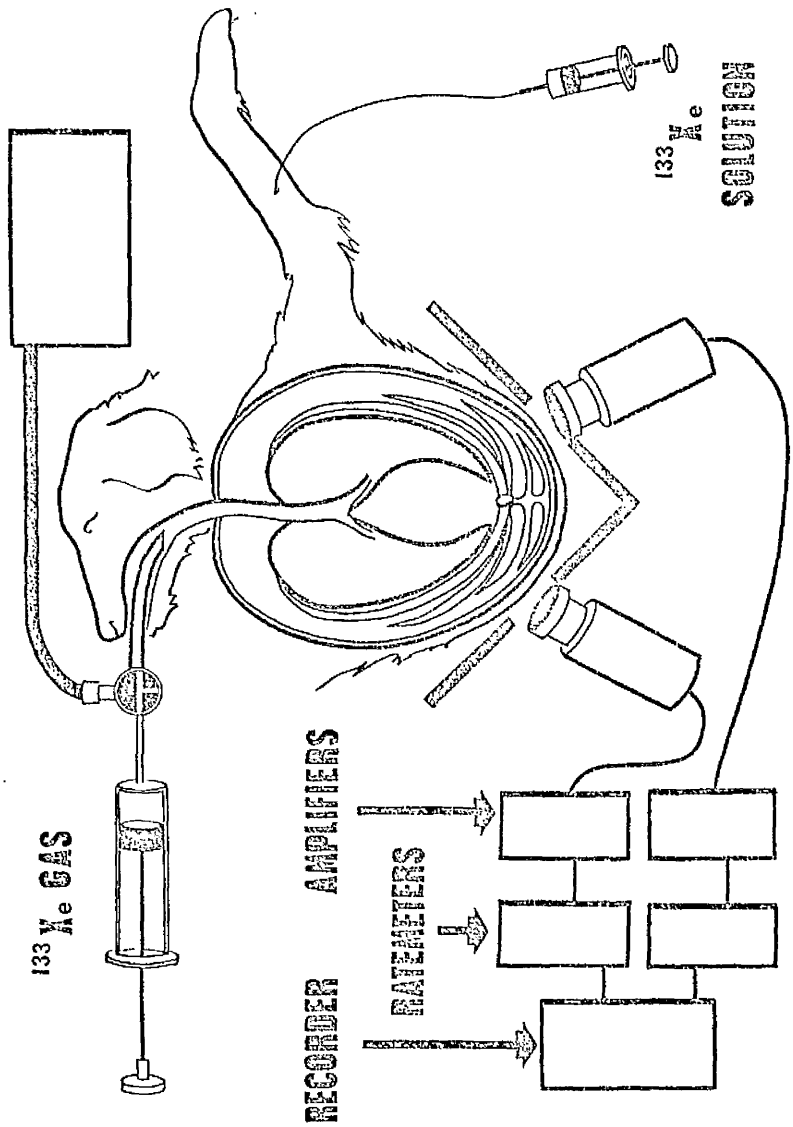

FIGURE 12. Diagram illustrating method of Xenon ^{133} studies.

one-seventh as soluble as carbon dioxide at body tempeatures. The isotope Xenon^{133} decays to stable cesium with a half life of 5.27 days emitting a negative beta particle of maximum energy 0.347 Mev. This is absorbed by less than 1 mm. of tissue and is therefore of no usefulness in external counting. The nucleus formed by beta decay reaches a stable state by emitting a gamma ray of energy 0.081 Mev. which can be recorded by the paired collimated scintillation counters. Each counter responds to radiation from a truncated cone of lung with a sensitivity that diminishes quite rapidly with increasing depth. The gas is evolved during one passage through the lungs into the alveoli after injection. Following the injection of Xenon intravenously, 500 ml. of air were insufflated to the lungs using the handpump and inspiration held to obtain the first plateau of activity for 12 seconds.

Rebreathing was thereafter started using the pump operated by hand at the same tidal volume to obtain mixtures of the gases and even distribution of the Xenon within the lungs. Following this inflation was maintained again to give a second plateau of activity indicative of lung volume. The airway was then connected again to the anaesthetic machine to give a recording of open system washout. This concluded the examination by the intravenous method.

Ventilation Studies

A second recording was made by injecting 2 millicuries of Xenon^{133} into the endotracheal tube followed by 500 mls. of air from the handpump. Thereafter, the record was obtained in an exactly similar manner to the intravenous technique and from this ventilation measurements could be assessed.

The above method follows closely the method described by Pain and his associates in their studies. They have determined the standard deviation of a single observation and have shown this to be 7% for blood flow measurements and 8% for ventilation studies, confirming the reliability of repeat estimations.

In these experiments, ventilation and perfusion studies were measured in the recipients of transplanted lungs at 3, 9 and 30 days after operation and usually at monthly intervals thereafter.

EXPRESSION OF RESULTS

1. Perfusion Studies

Figure 13 shows the tracing obtained in a dog by the intravenous method. It will be seen that the records of activity obtained over each lung are virtually identical. In this and all subsequent tracings the record from the left lung is in heavier lines. From such a record

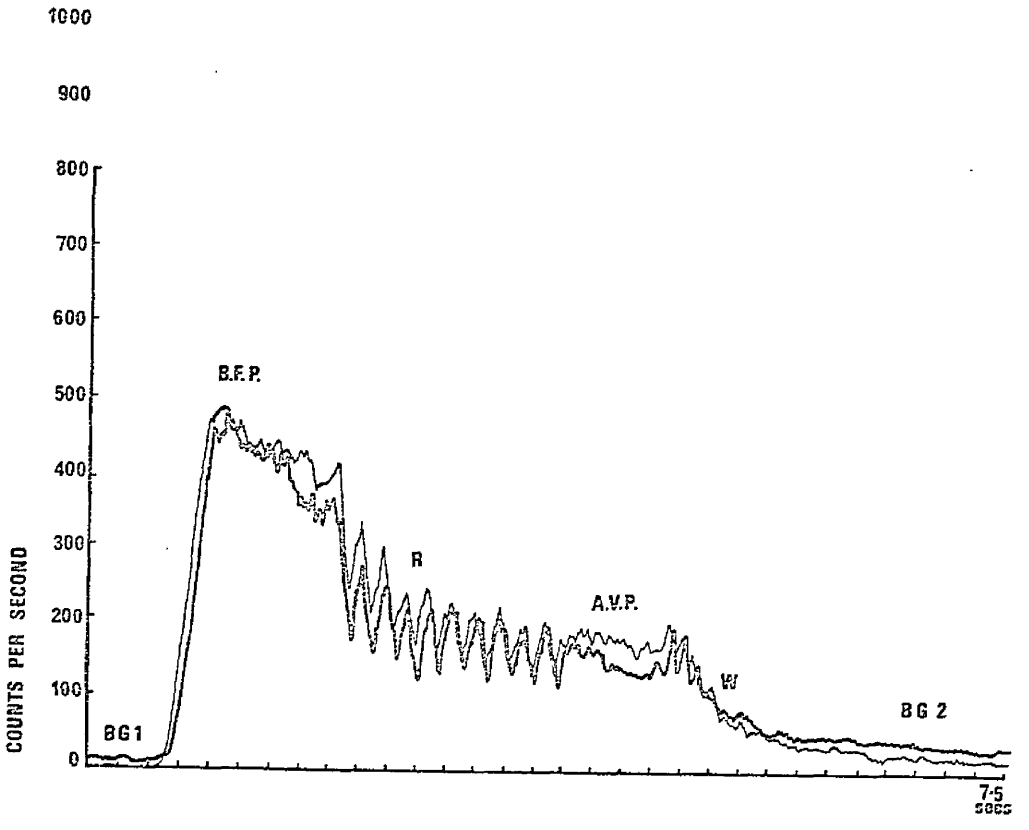

FIGURE 13. Xenon^{133} perfusion tracing in normal dog. B.G.1 = initial background reading. B.F.P. = blood flow plateau. R. = rebreathing oscillations. A.V.P. = alveolar volume plateau. W. = open system washout. B.G.2 = final background reading. (Activity recorded from left lung is in heavier type in all records).

the following may be determined.

(1) The first plateau of activity (B.F.P.) is reached rapidly within 5 seconds from a steady pre-existing background level of activity (B.G.1). This represents the complete evolution of the gas into the alveoli across the alveolar membrane and is an index of both blood flow and diffusion. It should be noted that this plateau is horizontal indicating that there is no evidence of leakage in the closed system during breath holding, and in addition there is no second peak of activity which might occur with recirculation of the gas.

(2) The second phase of the tracing (R) shows good ventilatory oscillation dropping to a second plateau (A.V.P.) when even distribution of the isotope has occurred within the lung. This plateau is lower than the first and expresses ventilated alveolar volume. The final part of the tracing (B.G.2) records the background level of activity after open system washout (W) and is usually higher than the initial background level.

2. Ventilation Studies

Figure 14 shows the tracing of a normal dog after inhalation of the isotope and is similar to that obtained by the intravenous method. Given uniform distribution, the initial plateau (V.P.) represents ventilated lung volume and again is equal on both sides. The second plateau after ventilation within the closed system is an index of effective ventilated alveolar volume (A.V.P.).

CALCULATIONS

From the above tracings the following calculations may be made.

1. Blood Flow L/R

This is obtained by subtracting the initial background level from the blood flow plateau for each lung separately and expressing this as a ratio of the left to right. This should approximate to unity in the normal animal. For this tracing (Figure 13).

$$\text{Blood Flow L/R Ratio} = \frac{\text{BFP - BG (1) (Left Lung)}}{\text{BFP - BG (1) (Right Lung)}}$$

$$= 0.95$$

2. Blood Flow per Unit Lung Volume L/R

This is calculated by dividing the corrected evaluate of the initial injection plateau by the corrected ordinate of the rebreathing

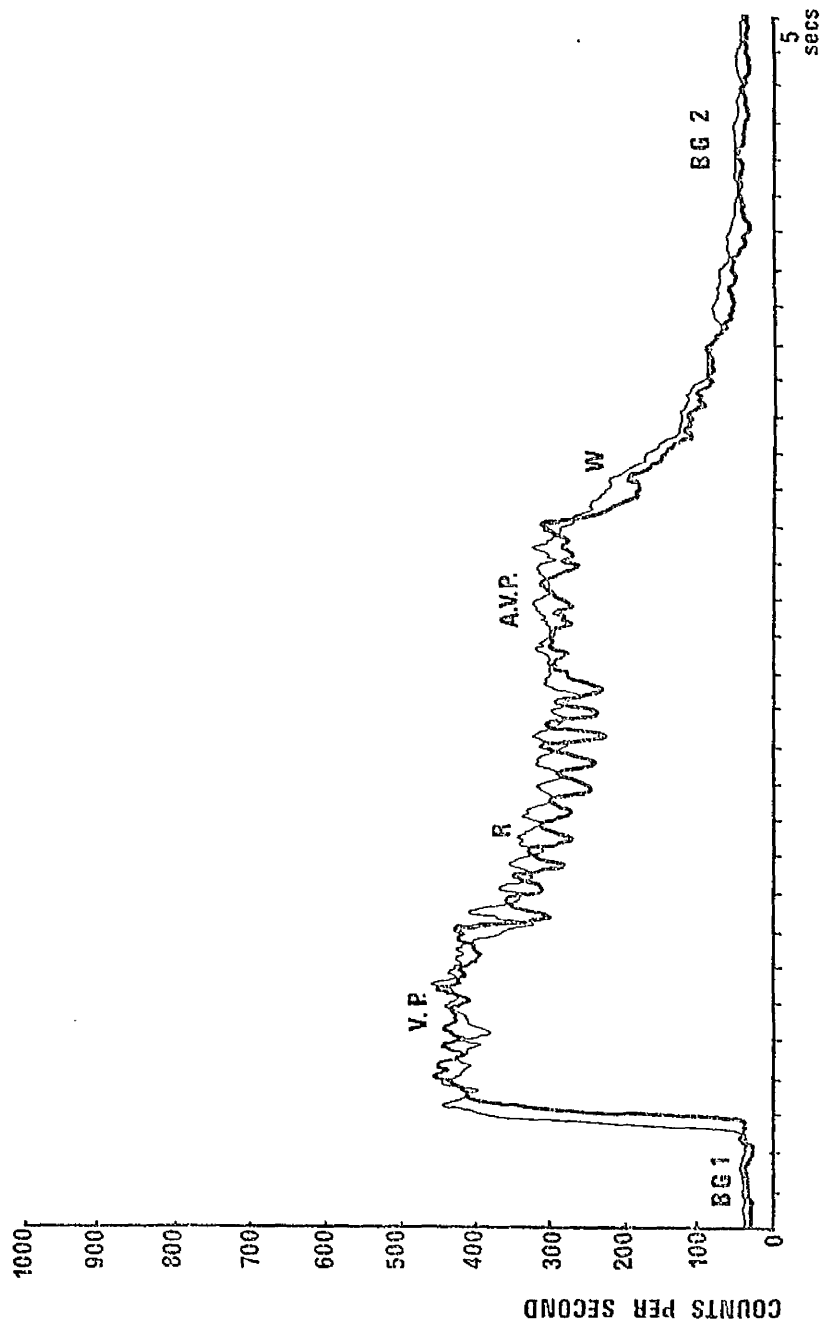

FIGURE 14. Xenon^{133} ventilation tracing in normal dog.
B.G.1 = initial background reading. V.P. = ventilation plateau.
R. = rebreathing oscillations. A.V.P. = alveolar volume plateau.
W. = open system washout. B.G.2 = final background reading.

plateau for each lung separately and expressing these as a ratio of left to right e.g.

Blood Flow per Unit Volume L/R =

$$\frac{BFP - BG\ (1)}{AVP - BG\ (2)}\text{(Left Lung)} - \frac{BFP - BG\ (1)}{AVP - BG\ (2)}\text{(Right Lung)}$$

$$= 1.4$$

3. Alveolar Volume L/R

Alveolar volume is calculated by subtracting the final background level from the alveolar volume for each lung and expressing these as a ratio L/R.

$$\text{Alveolar Volume L/R} = \frac{AVP - BG\ (2)\ \text{(Left Lung)}}{AVP - BG\ (2)\ \text{(Right Lung)}}$$

$$= 0.88$$

4. Ventilation L/R Ratio

This is estimated by subtracting the background level from the initial ventilation plateau and expressed again as a ratio of left to right.

$$L/R = \frac{VP - BG\ (1)\ \text{(Left Lung)}}{VP - BG\ (1)\ \text{(Right Lung)}}$$

$$= 1.00$$

5. Ventilation per Unit Volume L/R

This is determined by dividing the corrected ordinate of the

of the initial plateau by the corrected ordinate of the rebreathing plateau for each lung and expresses this as a ratio of left to right.

Ventilation per Unit Volume L/R =

$$\frac{VP - BG\ (1)}{AVP-BG\ (2)}\ (\text{Left Lung}) \ - \ \frac{VP - BG\ (1)}{AVP- BG\ (2)}\ (\text{Right Lung})$$

$$= \quad 1.00$$

VALIDITY OF XENON^{133} STUDIES IN RELATION TO LOBAR TRANSPLANTS

It has been shown by Ball that the use of Xenon^{133} scanning in the human subject is reliable in the appraisal of the regional variation in the function of the lung. Determination, however, of the validity of the method in relation to lobar transplants is not documented.

The following controls were used to provide validity for the conclusions in the transplant situation, and to determine the effect of technical failure on the functional studies.

1. Function in a Diaphragmatic Lobe

Two animals were subjected to left apico-cardiac lobectomy leaving the left diaphragmatic lobe intact. Figure 15 shows a representative tracing of the perfusion studies carried out. There was only minimal diminution in blood flow of the left compared to the right lung. The mean blood flow L/R calculated for 8 estimations

in 2 animals was o.98. Figure 16 demonstrates a similar example in the ventilation studies and again shows equivalence comparing the left and right lungs. The mean ventilation per unit volume L/R = 0.96, ventilation L/R = 0.61 and alveolar volume L/R = 0.6.

2. Function after Vascular Occlusion

As in the transplantation of other organs, graft failure occurs in the early stages due to the problems of technique. Thrombosis of the pulmonary vascular anastomosis is the most frequent technical mishap.

(a) Pulmonary Arterial Occlusion

Three animals were submitted to left apico-cardiac lobectomy followed by ligature of the artery supply to the left diaphragmatic lobe. Blood flow studies at 30 days post-operatively are exemplified by Figure 17. The first (blood flow) plateau was absent. Following ventilation there was slow rise as mixing of gas from both sides proceeded, to give a second reduced plateau indicative of volume in the devascularised lobe. Table V shows the results. The mean blood flow ratios for 9 readings in three preparations over a period of 30 days was 0.14 (SD $\pm$ 0.06). The mean blood flow per unit volume L/R =

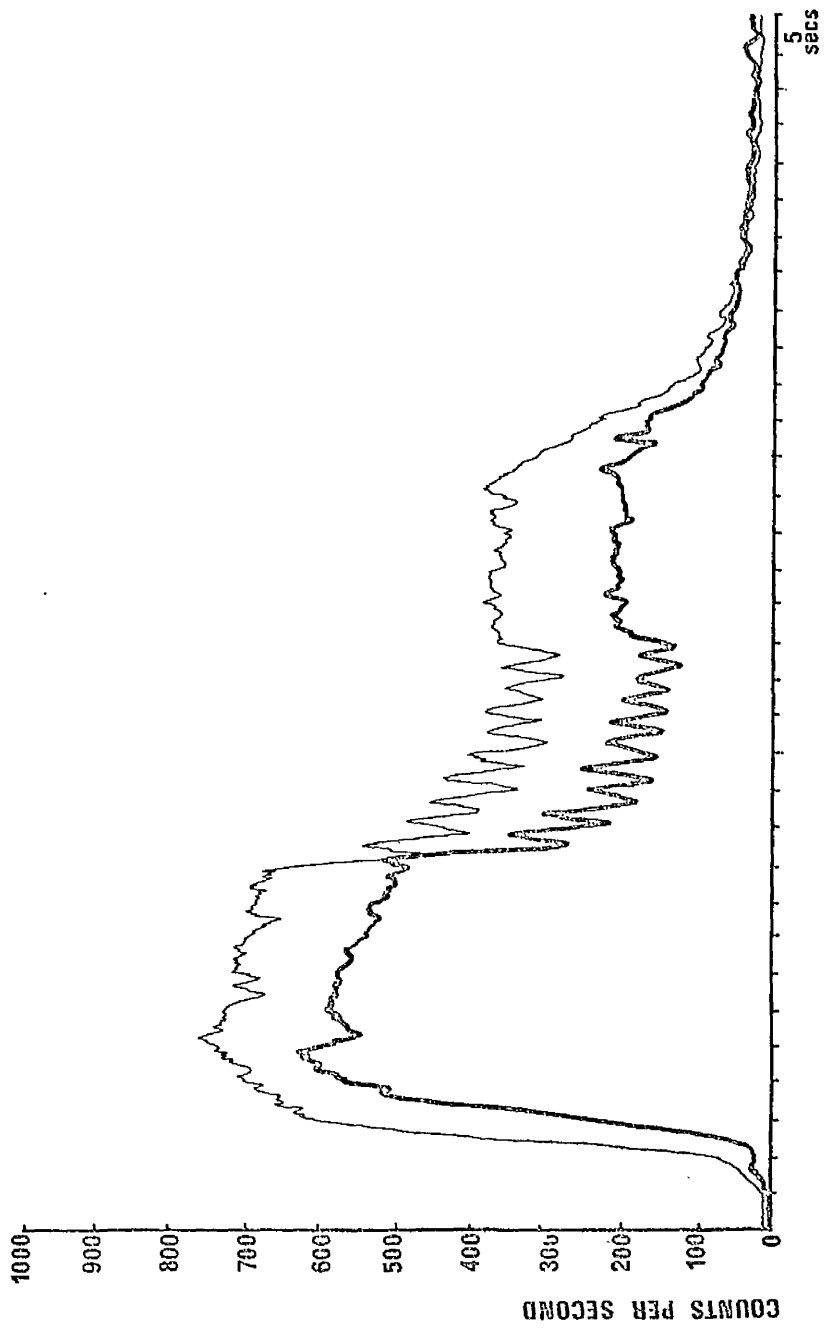

FIGURE 15. Xenon^{133} perfusion study of a left diaphragmatic lobe 2 days after left apico-cardiac lobectomy.

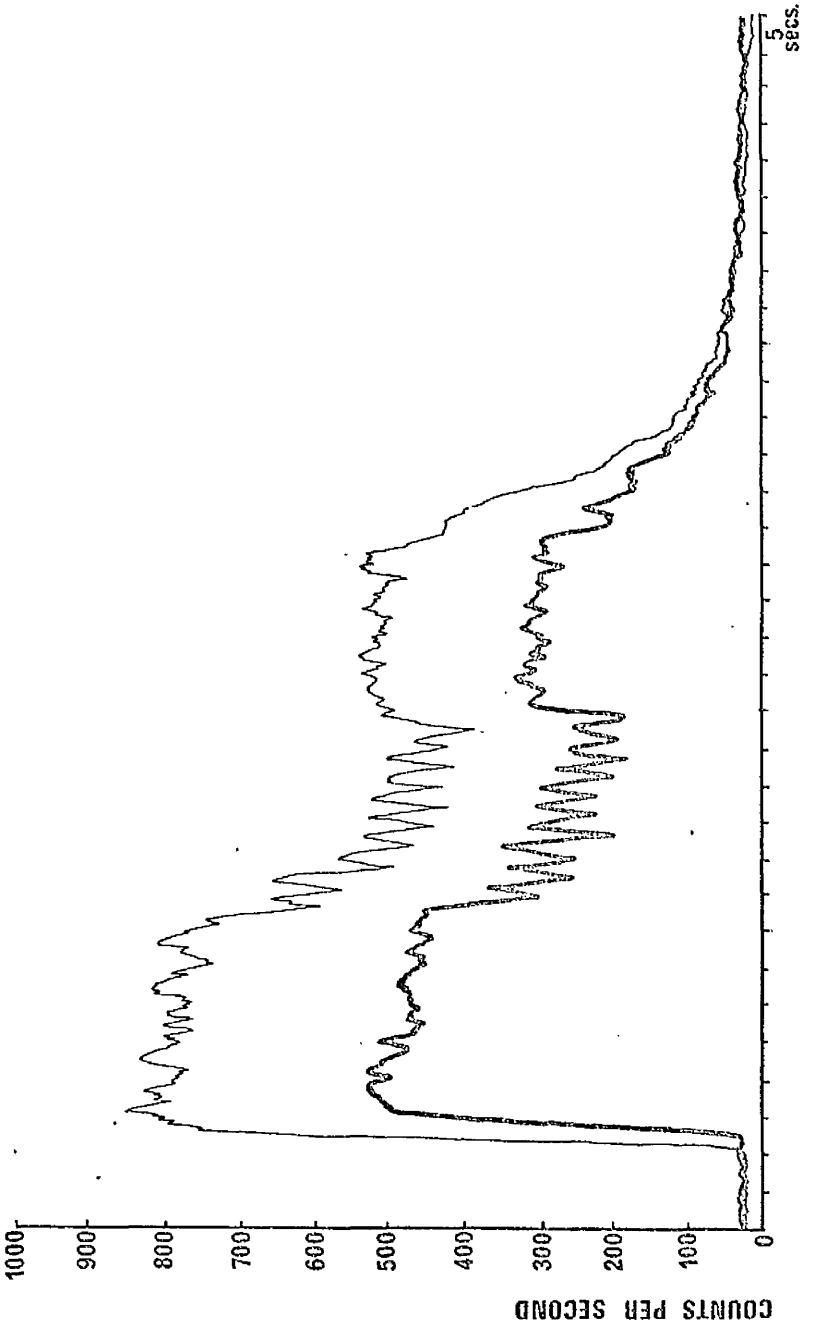

FIGURE 16. ^{133}Xenon ventilation study of a left diaphragmatic lobe 2 days after left apico-cardiac lobectomy.

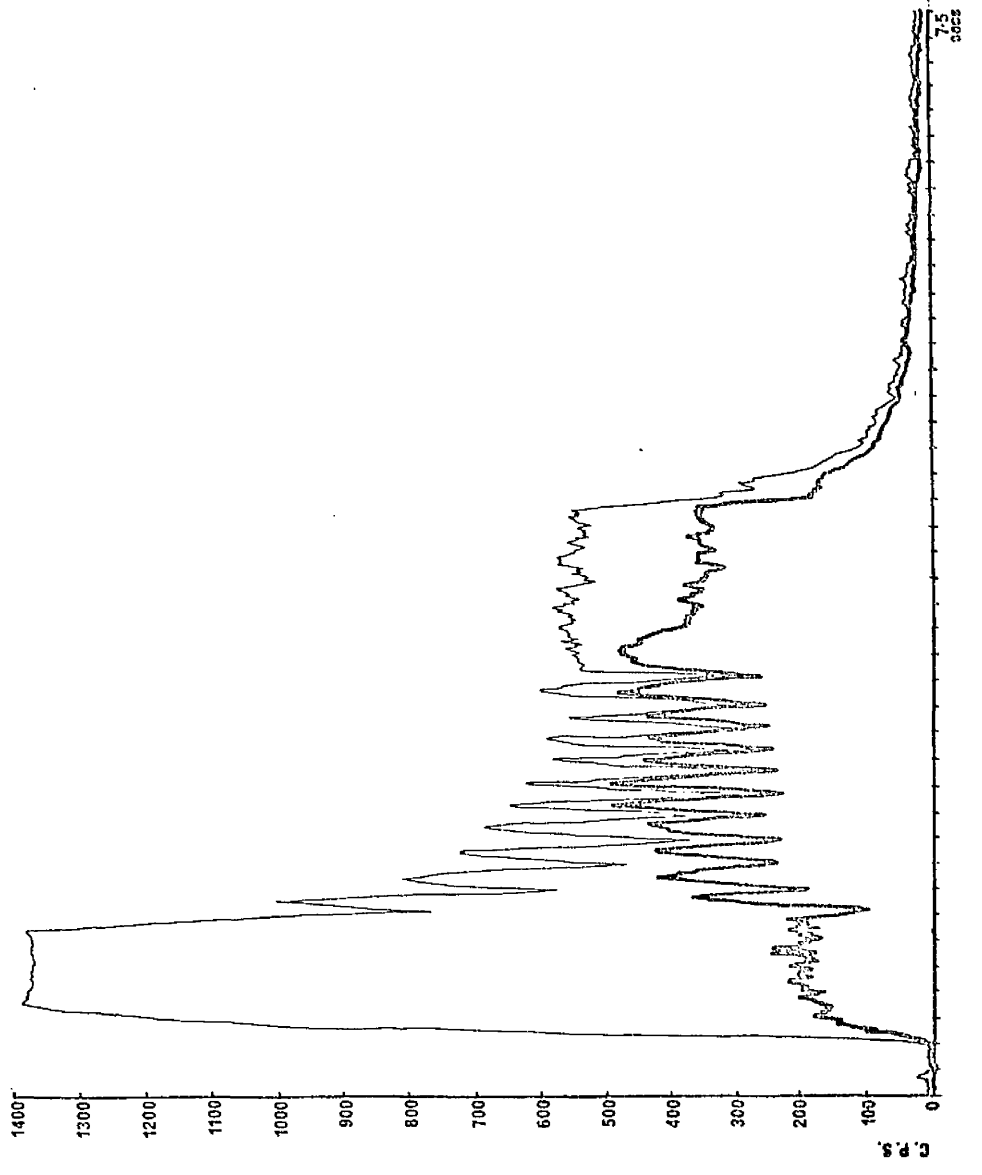

FIGURE 17. $Xenon^{133}$ perfusion tracing 30 days after ligature of pulmonary arterial supply to left diaphragmatic lobe.

TABLE V

VENTILATION/PERFUSION INDICES AFTER ARTERIAL LIGATION OF LEFT DIAPHRAGMATIC LOBE.			
RATIOS L/R	INDIVIDUAL RATIOS	MEAN	STANDARD DEVIATION
Blood Flow per Unit Volume.	0.013, 0.4, 0.2, 0.28, 0.21, 0.73, 0.41, 0.38, 0.38.	0.33	± 0.19
Blood Flow.	0.16, 0.12, 0.058, 0.23, 0.14, 0.17, 0.08, 0.20, 0.13.	0.14	± 0.06
Alveolar Volume.	1.25, 0.3, 0.29, 0.81, 0.66, 0.29, 0.21, 0.53, 0.34.	0.32	± 0.32
Ventilation.	0.27, 0.75, 0.50, 0.35, 0.19, 0.38, 0.34.	0.40	± 0.16
Ventilation per Unit Volume.	0.92, 0.91, 0.95, 0.73, 0.90, 0.93, 0.81.	0.90	± 0.18

0.33 (SD ± 0.19). Figure 18 is a representative example of a ventilatory study at 30 days and indicates that ventilation of the lobe is still present although reduced. The mean ventilation per unit volume L/R over 7 readings = 0.9 (SD ± 0.18).

One can conclude that Xenon^{133} measurements indicate that blood flow in the lobe is virtually absent and that the lung volume is reduced although ventilation per unit volume of lung remain unaltered, i.e., division of the pulmonary artery results in fibrosis of the lung but not necrosis. This work confirms the studies of Blades et al. (1962) who demonstrated experimentally that complete infarction of the lung was not an invariable consequence of pulmonary arterial occlusion.

(b) Venous Occlusion

Figures 19 and 20 are perfusion and the ventilation studies respectively on a dog which had venous thrombosis of its diaphragmatic lobe proven at autopsy. Both blood flow and ventilated alveolar volume are markedly reduced. Table VI shows the mean ventilation/perfusion values in 4 dogs who had venous occlusion at autopsy. The mean blood flow L/R for 7 estimations in 4 dogs was 0.12 (SD ± 0.06). Ventilation L/R was 0.23 (SD ± 0.08) and alveolar volume L/R was 0.19 (SD ± 0.05).

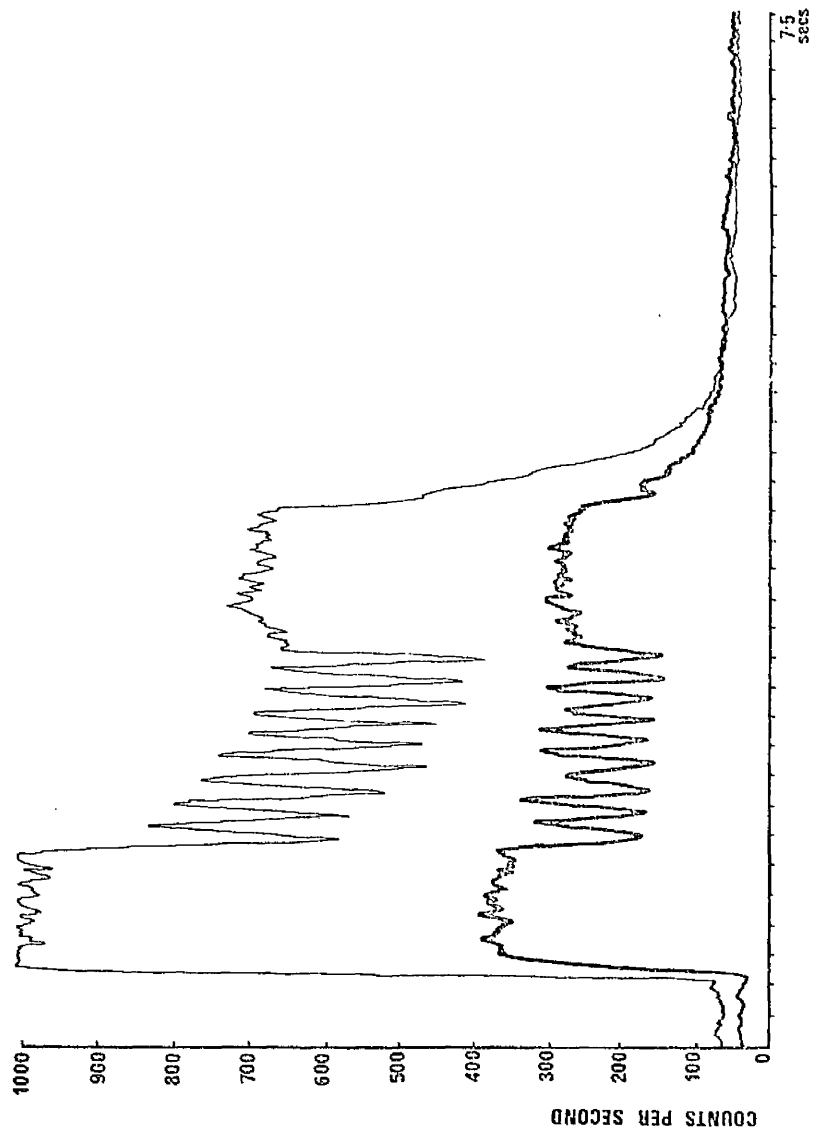

FIGURE 18. $Xenon^{133}$ ventilation tracing 30 days after ligature of pulmonary arterial supply to left diaphragmatic lobe.

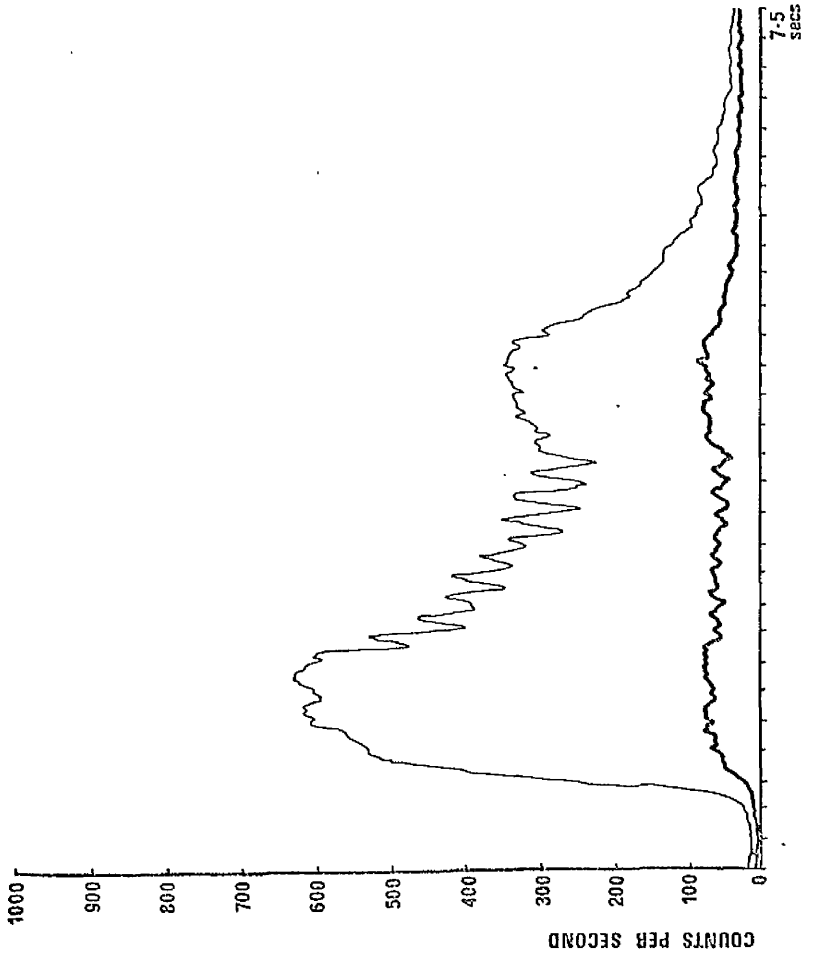

FIGURE 19. Perfusion tracing in venous occlusion of diaphragmatic lobe.

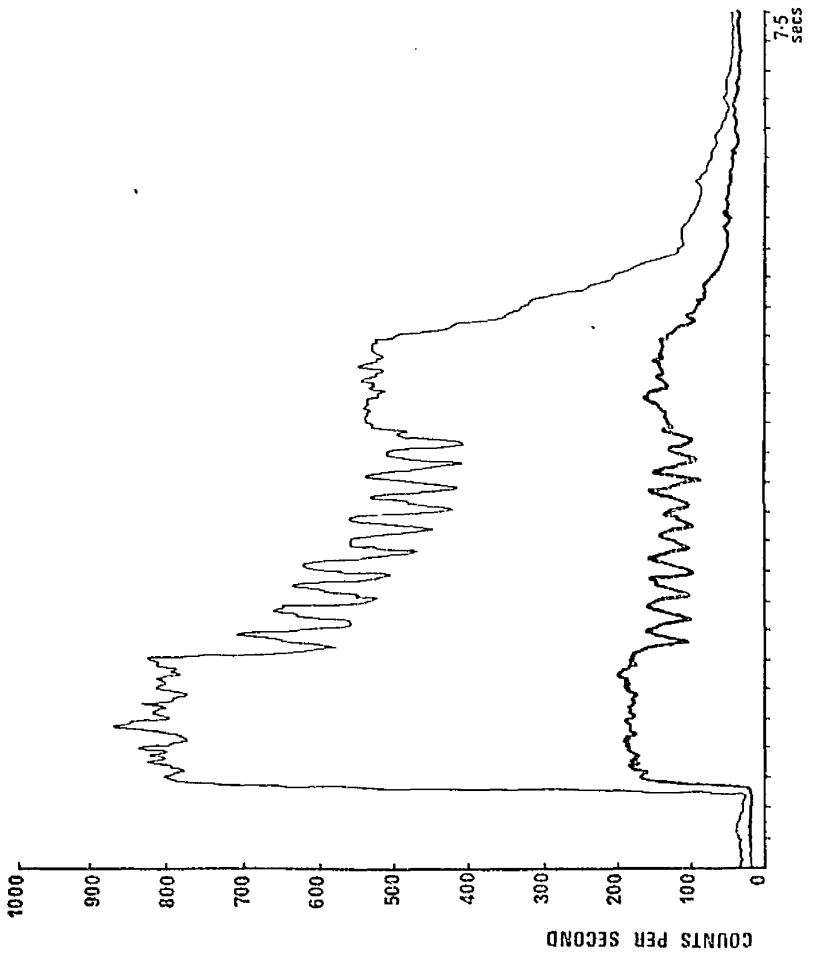

FIGURE 20. Ventilation tracing in venous occlusion of diaphragmatic lobe.

TABLE VI

VENTILATION/PERFUSION INDICES FOLLOWING VENOUS OCCLUSION OF THE LEFT DIAPHRAGMATIC LOBE.			
RATIOS L/R	INDIVIDUAL RATIOS	MEAN	STANDARD DEVIATION
Blood Flow per Unit Volume.	0.68, 0.58, 0.5, 0.58, 0.66 0.46, 0.71.	0.59	± 0.08
Blood Flow.	0.15, 0.09, 0.16, 0.12, 0.11 0.07, 0.12.	0.12	± 0.06
Alveolar Volume.	0.15, 0.19, 0.18, 0.32, 0.16, 0.22, 0.17.	0.19	± 0.05
Ventilation.	0.31, 0.30, 0.25, 0.10, 0.21.	0.23	± 0.08
Ventilation per Unit Volume.	1.10, 1.49, 1.23, 0.81, 0.98.	1.12	± 0.23

In this preparation both blood flow and alveolar volume are minimal, which correlates with the typical macroscopic appearances of the large hepatised lobe found in venous occlusion.

One can conclude from the above studies that measurements of blood by Xenon^{133} is a reliable index of failure of graft function from technical causes.

If the initial blood flow ratio is less than 0.30 in the first few post-operative days, then vascular occlusion has occurred and the graft is likely to be non-viable. Survival cannot, however, be predicted from the ratios of alveolar volume or ventilation. This conclusion was confirmed by study of the blood flow L/R in autograft preparations which did not survive beyond two weeks due to vascular occlusion. Out of 11 lobar autografts, 4 died within the first two weeks. The mean blood flow ratio L/R (Table VII) over the first 5 days in these animals was 0.18 in comparison to the survivors whose ratio was 0.76. Similarly, the mean blood flow per unit volume L/R in the non-survivors was 0.27 and in survivors 0.83. It should be noted that blood flow per unit lung volume L/R may be misinterpreted if there is a simultaneous reduction in lung volume and

TABLE VII

VENTILATION/PERFUSION INDICES IN NON-SURVIVING AUTOGRAFTS			
RATIOS L/R	INDIVIDUAL RATIOS	MEAN	STANDARD DEVIATION
Blood Flow per Unit Volume.	0.20, 0.29, 0.28, 0.33, 0.26.	0.27	± 0.05
Blood Flow.	0.20, 0.23, 0.13, 0.19, 0.16.	0.18	± 0.03
Alveolar Volume.	1.00, 0.78, 0.45, 0.56, 0.60.	0.68	± 0.19

for this reason alveolar volume and blood flow ratios should be examined together.

3. The Effect of Mediastinal Shift on Function

In the canine preparation, the narrow thoracic cavity and the mobile mediastinum may make separation of the activity recorded by each collimator difficult if mediastinal shift occurs subsequent to collapse of a grafted lobe from any cause. The bronchogram in Figure 21 demonstrates the problem. Collapse of the left diaphragmatic lobe occurred and the right diaphragmatic lobe shifted across the left pleural cavity. This could give rise to an error in the interpretation of the Xenon studies. Figures 22 and 23 were perfusion and ventilation studies respectively on this animal and would tend to suggest that perfusion and ventilation were still present at this time (40 days after operation). That this was not correct was predicted from the blood flow L/R ratio in this animal on day 2 after operation, which was less than 0.30.

Bronchography is therefore necessary to determine that "cross-over" has not occurred when a false positive result is suspected from an estimate of previous readings.

In order to get an accurate evaluation of the level of activity

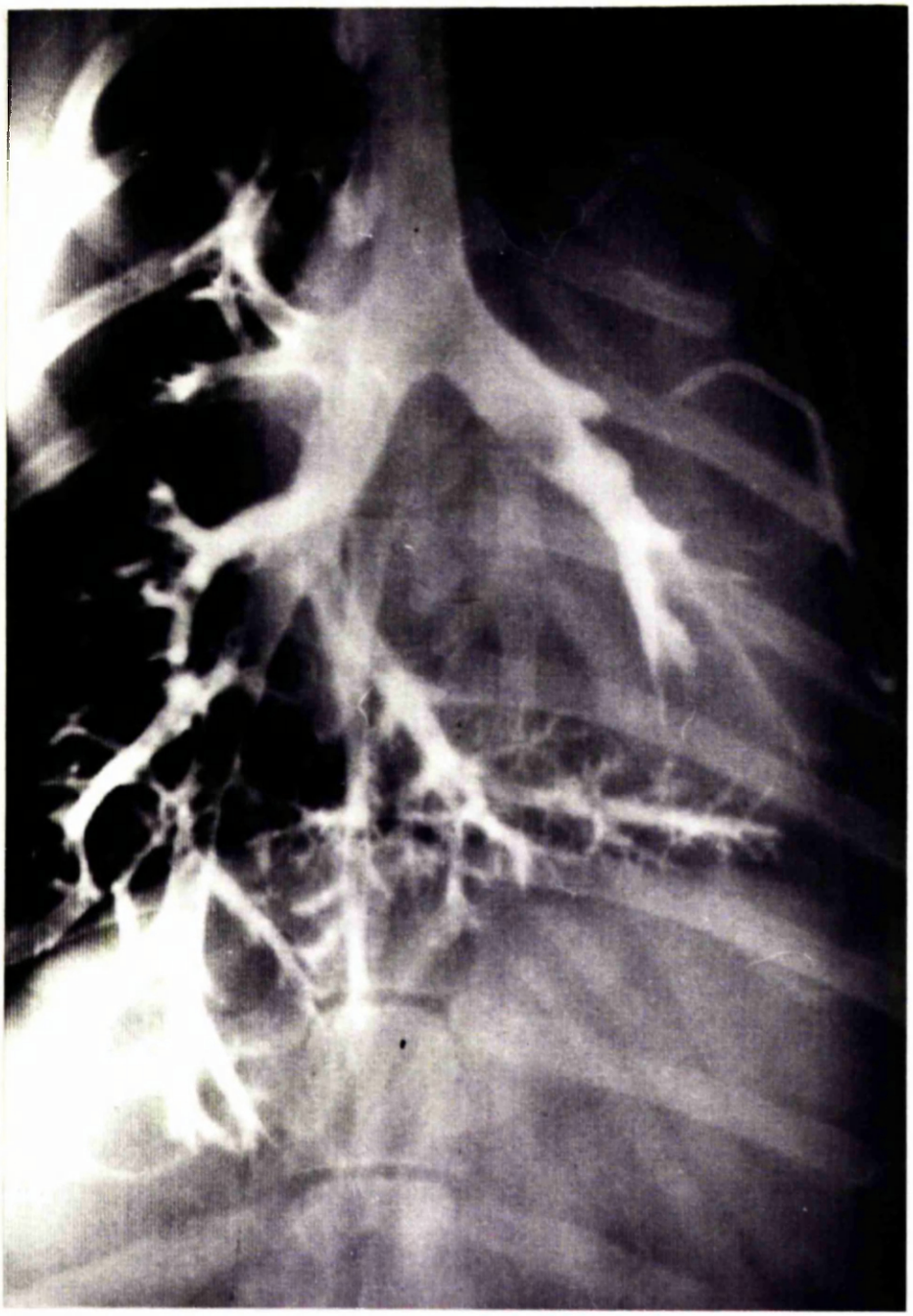

FIGURE 21. Bronchogram. Collapse of left diaphragmatic lobar transplant with mediastinal shift. Right lower lobe bronchus in left pleural cavity.

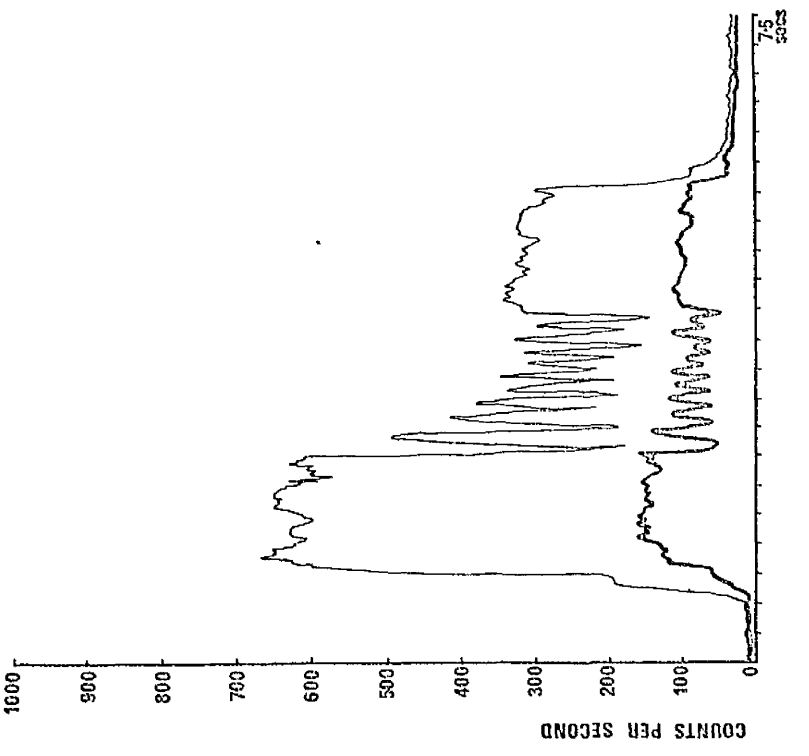

FIGURE 22. Perfusion tracing 40 days after lobar transplant. Effect of Mediastinal shift.

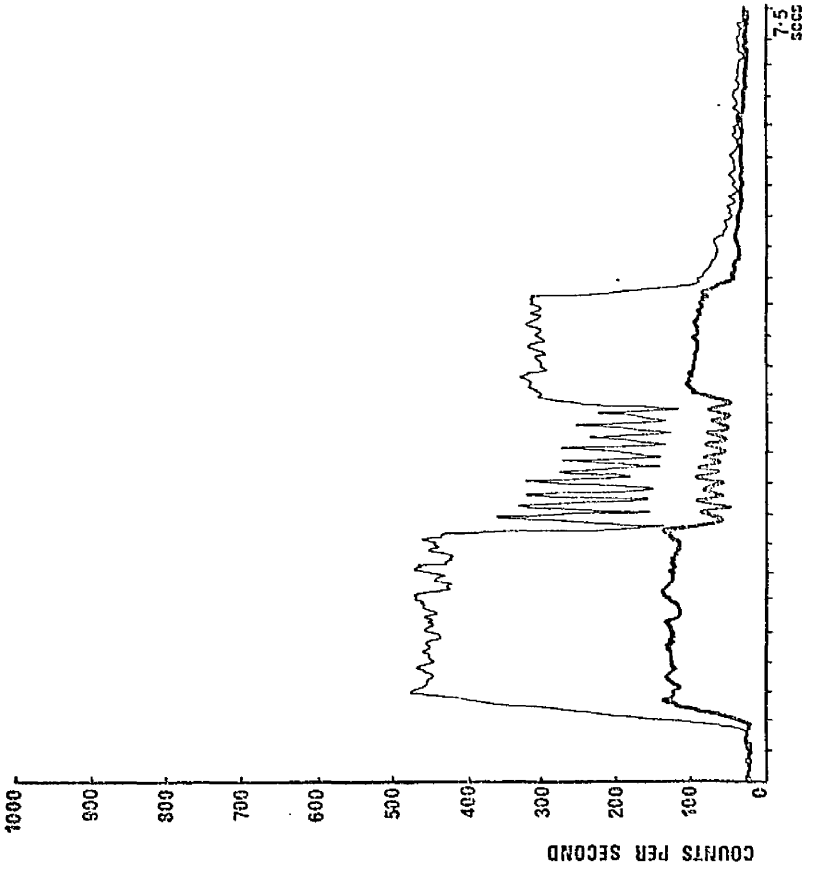

FIGURE 23. Ventilation tracing 40 days after lobar transplant. Effect of mediastinal shift.

which could be attributed to "cross-over", a series of estimations was made on 9 animals who were subjected to left apico-cardiac lobectomy and who had an indwelling catheter inserted into the pulmonary artery supplying the left diaphragmatic lobe. A small aliquot of Xenon^{133} was injected into this catheter and tracings obtained from both sides as before. Figure 24 shows the results obtained. There was a high level of activity on the injected side and a minimal level recorded on the contralateral lung. Calculation of the percentage cross-over from right to left was made in 9 animals in this way. The mean value was 8.54% with standard deviation $\pm$ 5.7%.

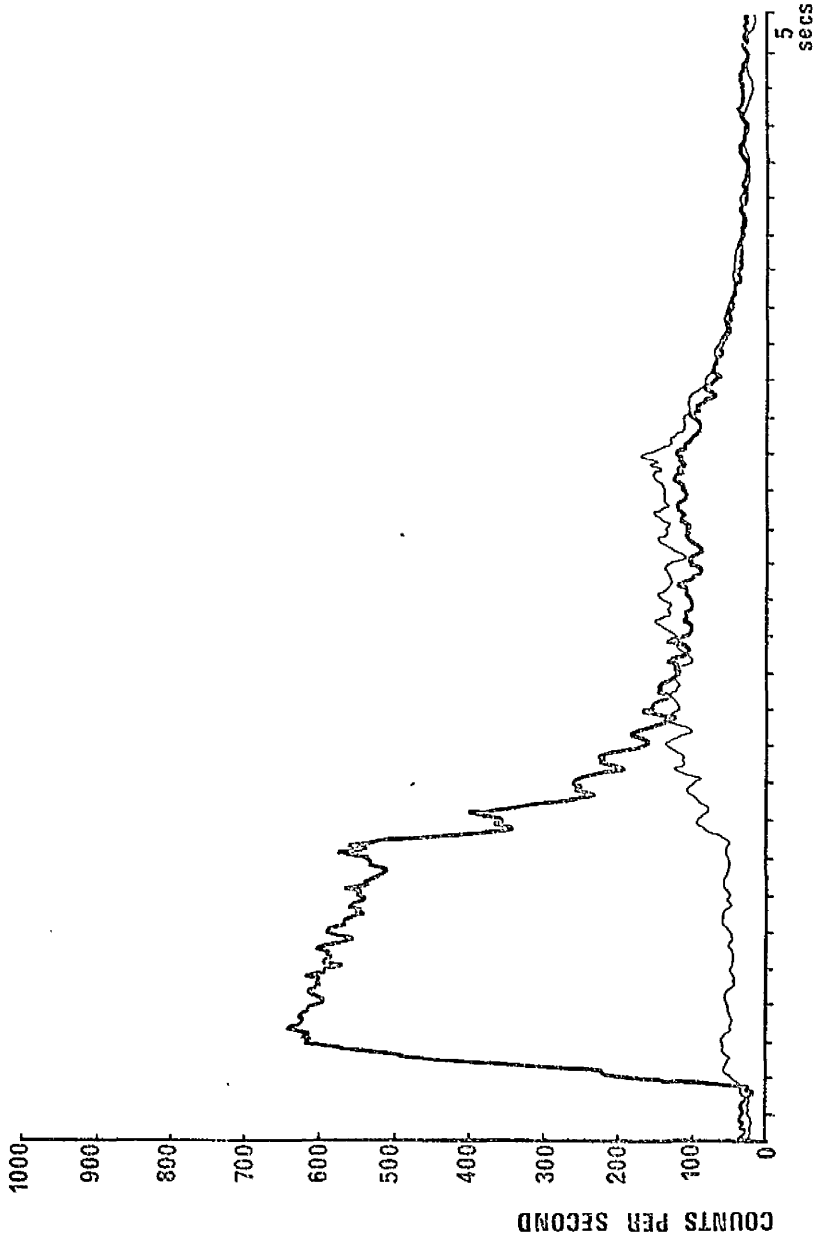

FIGURE 24. Perfusion tracing of diaphragmatic lobe 2 days after apico-cardiac lobectomy. $Xenon^{133}$ injected directly into left P.A. Minimal cross-over of activity from right lung.

PULMONARY FUNCTION IN LUNG TRANSPLANTATION

I. EFFECT ON PERFUSION AND VENTILATION AFTER AUTOTRANSPLANTATIC

Perfusion and ventilation studies using the method described above were performed on 13 dogs who underwent pulmonary auto-transplantation. These studies were undertaken at 2, 9, 16 days and monthly intervals thereafter. One animal died while under anaesthesia while undergoing functional studies; 1 animal with a whole lung graft died following an attempt at contralateral pneumonectomy; 4 animals died of vascular occlusion leaving 7 long term surviving animals for study.

The alterations in function due to the technique of lung transplantation which have been noted can be conveniently classified as early and late.

1. Early Changes

(1) 4 animals died within three weeks of operation and were found to have either venous or arterial occlusion or both. Calculation of the perfusion and ventilation indices in these animals showed that the mean of the blood flow ratio L/R at day 2 was 0.18 and blood flow per unit volume L/R was 0.27 (Table VII). The mean

of alveolar volume L/R was 0.68. The mean of the ratios for the surviving autografts (Table VIII) was determined to be 0.76 (blood flow L/R) and 0.83 (blood flow per unit volume). The alveolar volume indices in the non-surviving animals were however good and these ratios are of no value in predicting survival of either graft or animal. The perfusion ratios in the non-surviving animals on the second day after operation are both below 0.30 and correlate well with the mean of those same indices in animals subjected to apico-cardiac lobectomy, combined with pulmonary arterial or venous occlusion (mean blood flow L/R = 0.14 and 0.12 respectively).

(2) Perfusion and ventilation indices were plotted against time for each animal and the variations occurring in graft function in the early post-transplant period can then be demonstrated. Figure 25 shows the sequential changes in a typical animal alive with a well functioning whole lung graft. There was a slight reduction in ventilation and aeriated volume on the second day but blood flow to the graft was within normal limits, indicating a successful surgical technique. The most noticeable feature thereafter was a constant reduction in

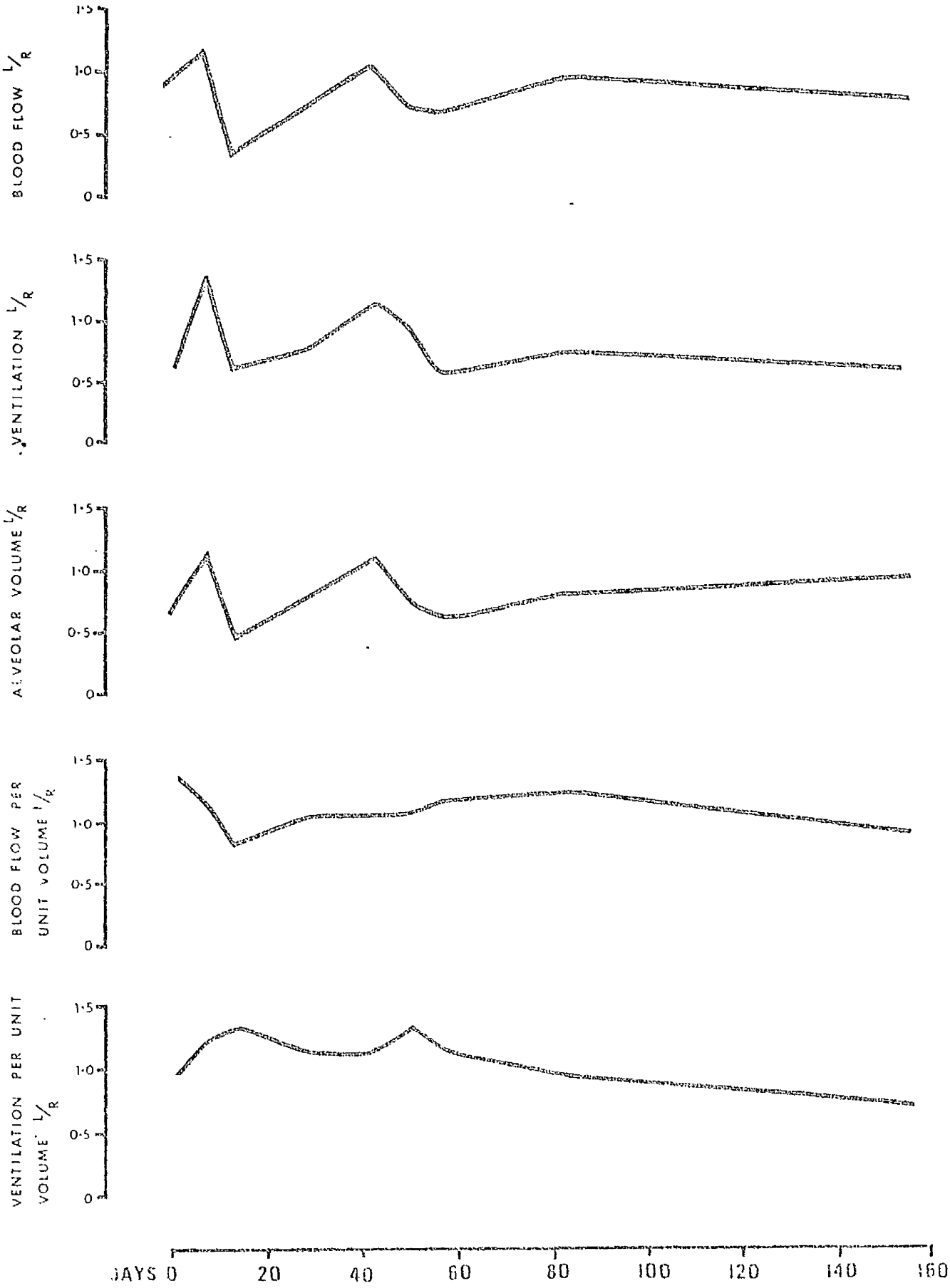
BLOOD FLOW L/R
VENTILATION L/R
ALVEOLAR VOLUME L/R
BLOOD FLOW PER UNIT VOLUME L/R
VENTILATION PER UNIT VOLUME L/R
1·5
1·0
0·5
0
DAYS 0
20
40
60
80
100
120
140
160

FIGURE 25.

blood flow per unit volume of the graft occurring in the second week. At this time ventilation L/R, ventilation per unit volume and alveolar volume tend to vary widely. The alteration in these distribution indices, indicating mild functional impairment, are consistent with the occurrence of intra-alveolar oedema or atelectasis during this period, when the effect of interruption of the lymphatic, nervous and bronchial arterial supply are maximal. There was a gradual recovery of both ventilation and perfusion towards the end of the third week until blood flow, blood flow per unit volume, alveolar volume and ventilation per unit volume of the grafted lung were within normal limits when compared with the contralateral lung.

Thereafter the values remained constant during the period (160 days) under study and confirm the bronchographic appearances in this animal shown in Figure 26. This demonstrates a well ventilated and viable lung with a normal bronchial architecture and no evidence of bronchial stenosis.

2. Late Graft Function

Figures 27 and 28 are tracings of flow and ventilation in an animal 422 days after a lobar autotransplantation; it demonstrates

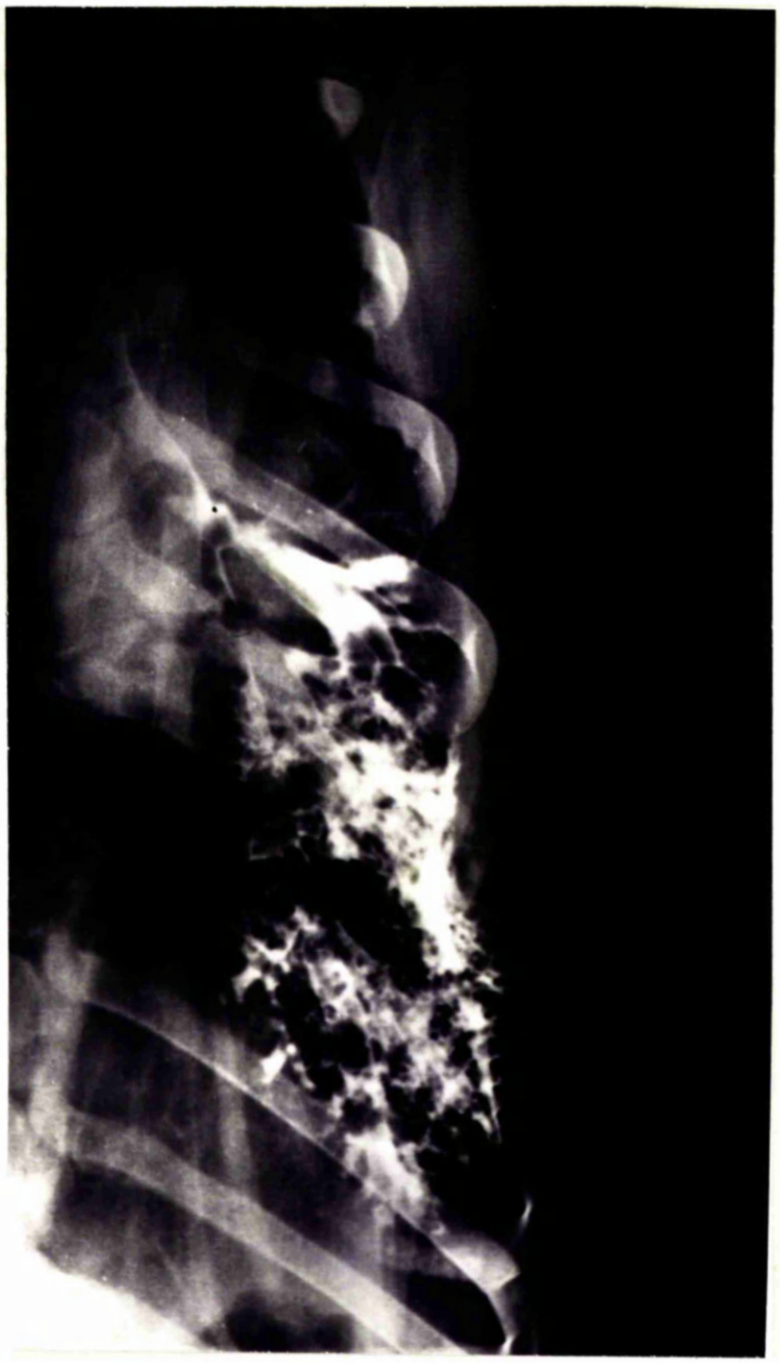

FIGURE 26. Bronchogram. Well ventilated graft with good alveolar filling. No bronchial stenosis.

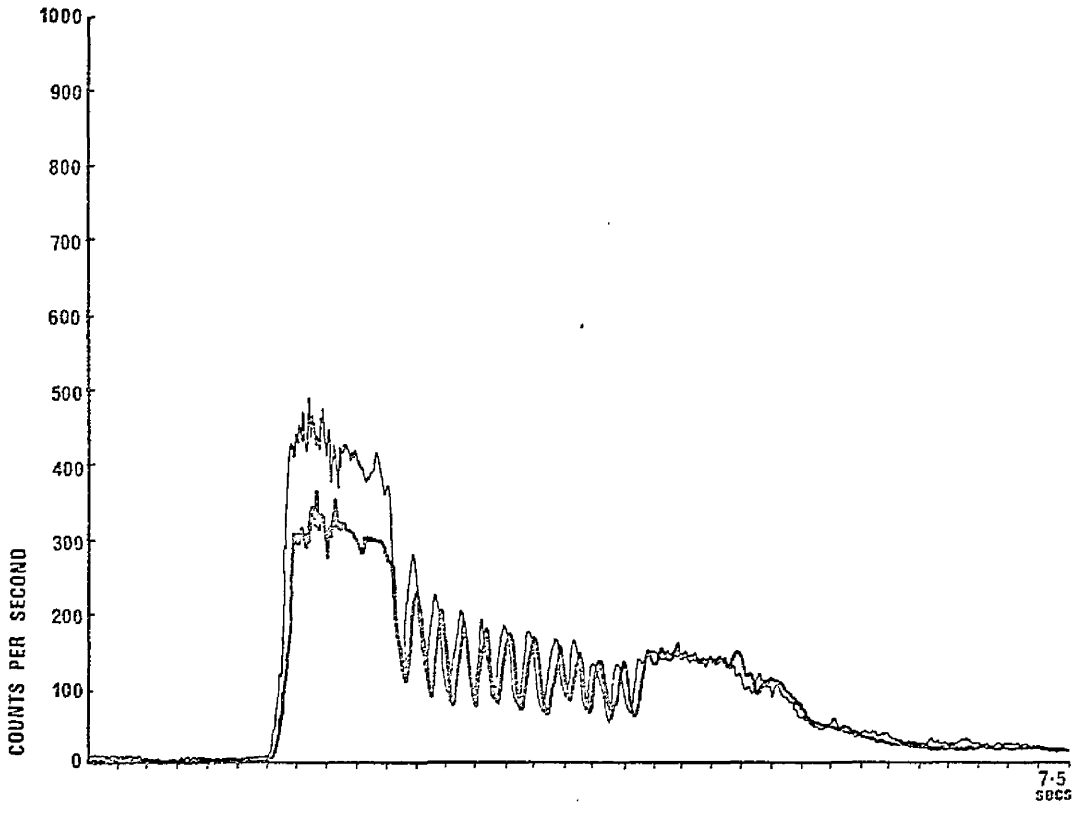

FIGURE 27. Perfusion tracing. Lobar autotransplant 422 days after operation. Perfusion and alveolar volume of graft almost equivalent to normal right lung.

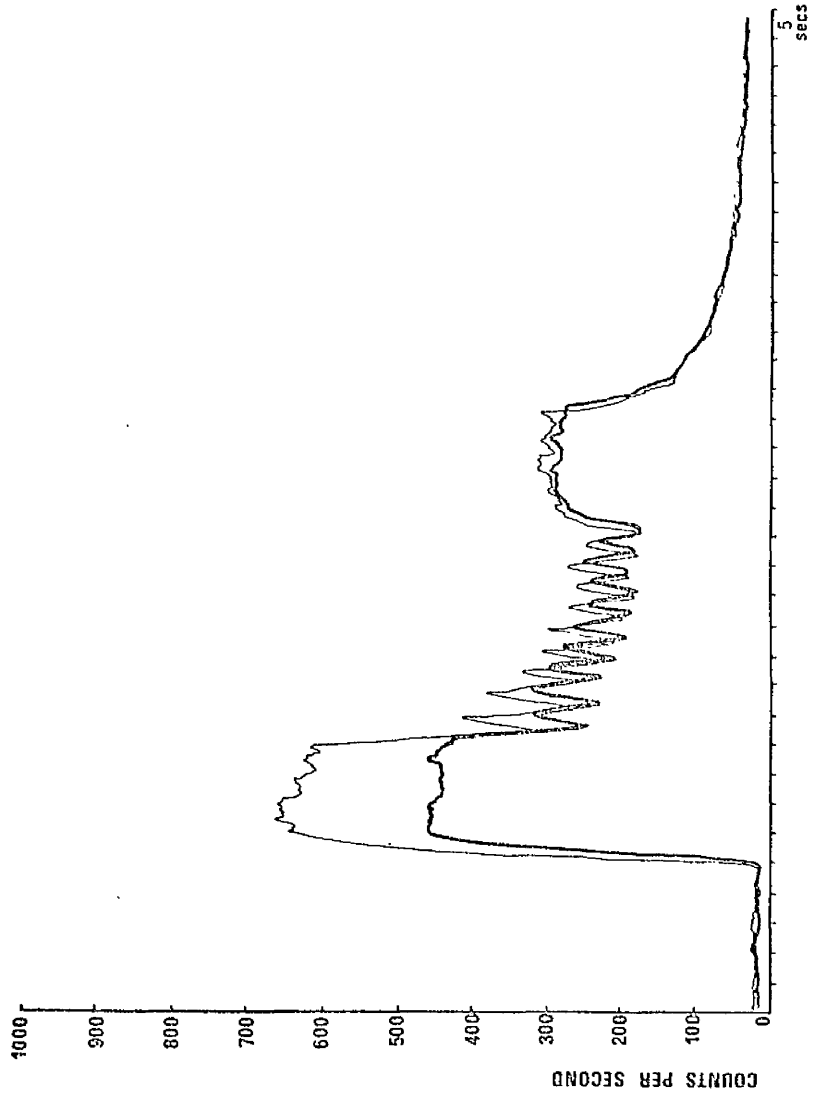

FIGURE 28. Ventilation tracing. Lobar autotransplant 422 days after operation. Ventilation and alveolar volume of graft equivalent to normal right lung.

that the ventilatory capacity of a technically satisfactory graft diminishes very little when compared with the untouched lung on the other side. This suggests that there is no physiological reason why pulmonary grafts should not have useful functional capability despite loss of pulmonary and cough reflexes. Table VIII shows the values of perfusion/ventilation indices of all autograft lungs and their distribution during the period under study. In order to assess the overall sequential function of technically successful autografts the mean values for these indices are shown in graphic form in Figure 29. Blood flow and blood flow per unit volume ratios remained fairly constant during the period of observation although there was overall diminution in perfusion of long term grafts. Ventilation ratios also remained constant and graft ventilation values at 640 days were little altered from the immediate post-operative values. The overall ventilation/ perfusion distribution indices in technically successful transplants demonstrated continued maintenance of blood flow and ventilation in the pulmonary graft. The excellent perfusion shown on arteriography (carried out through the indwelling Teflon catheter) is demonstrated in Figure 30 and confirms the Xenon^{133} studies. The mean blood flow per unit volume L/R and blood flow L/R values were

TABLE VIII

MEAN VENTILATION/PERFUSION INDICES IN FUNCTIONING AUTOGRAFTS

DAYS		0 - 5	6 - 10	11 - 20	21 - 40	41 - 80	81 - 160	161 - 320	321 - 640
BLOOD FLOW L/R	Mean	0.76	0.59	0.36	0.63	0.62	0.73	0.51	0.40
	S.D.	±0.13	±0.24	±0.05	±0.05	±0.17	±0.10	±0.27	±0.18
	Dogs	5	6	3	2	5	2	5	5
	Readings	5	6	3	2	7	3	6	7
ALVEOLAR VOLUME L/R	Mean	1.06	0.77	0.59	0.77	0.86	0.88	0.70	0.62
	S.D.	±0.52	±0.25	±0.17	±0.07	±0.33	±0.11	±0.34	±0.33
	Dogs	5	6	3	2	5	2	5	5
	Readings	5	6	3	2	7	3	6	7
BLOOD FLOW PER UNIT VOLUME L/R	Mean	0.83	0.81	0.64	0.83	0.82	0.86	0.72	0.69
	S.D.	±0.28	±0.25	±0.10	± 0.13	±0.29	±0.22	±0.18	± 0.16
	Dogs	5	6	3	2	5	2	5	5
	Readings	5	6	3	2	7	3	6	7
VENTILATION L/R	Mean	0.68	0.75	0.55	0.67	0.72	0.58	0.83	0.43
	S.D.	±0.08	± 0.33	±0.23	± 0.03	±0.28	± 0.13	±0.30	± 0.27
	Dogs	3	5	3	2	5	3	3	4
	Readings	3	5	3	2	7	4	4	4
VENTILATION PER UNIT VOLUME L/R	Mean	0.86	0.94	1.11	0.98	0.86	0.80	0.93	0.69
	S.D.	±0.06	± 0.16	±0.11	± 0.11	±0.33	±0.11	±0.13	±0.36
	Dogs	3	5	3	2	5	3	3	4
	Readings	3	5	3	2	7	4	4	4

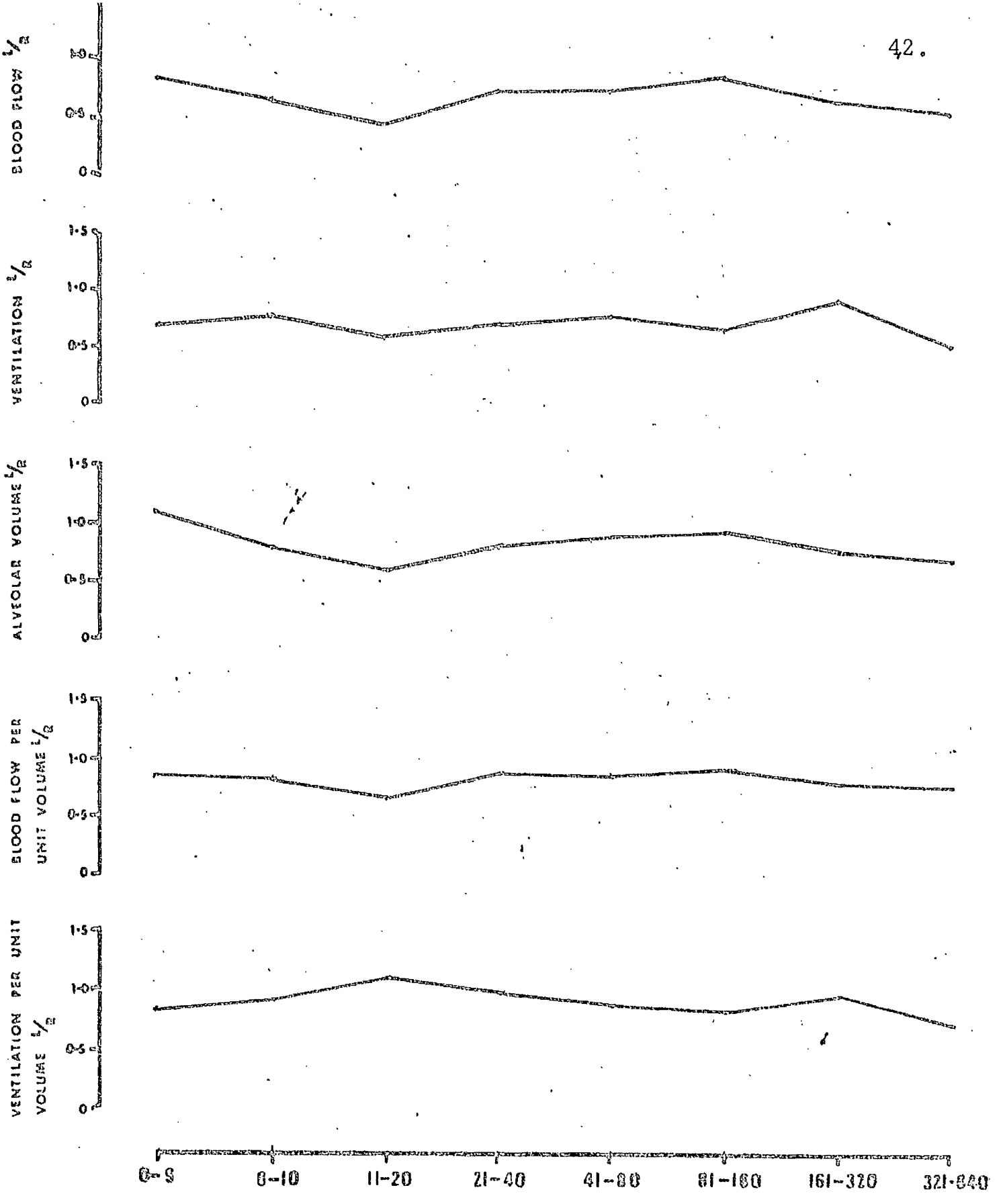
BLOOD FLOW L/m
VENTILATION L/m
ALVEOLAR VOLUME L/m
BLOOD FLOW PER UNIT VOLUME L/m
VENTILATION PER UNIT VOLUME L/m
1·5
1·0
0·5
0
0-9
9-10
11-20
21-40
41-80
81-160
161-320
321-640

FIGURE 29.

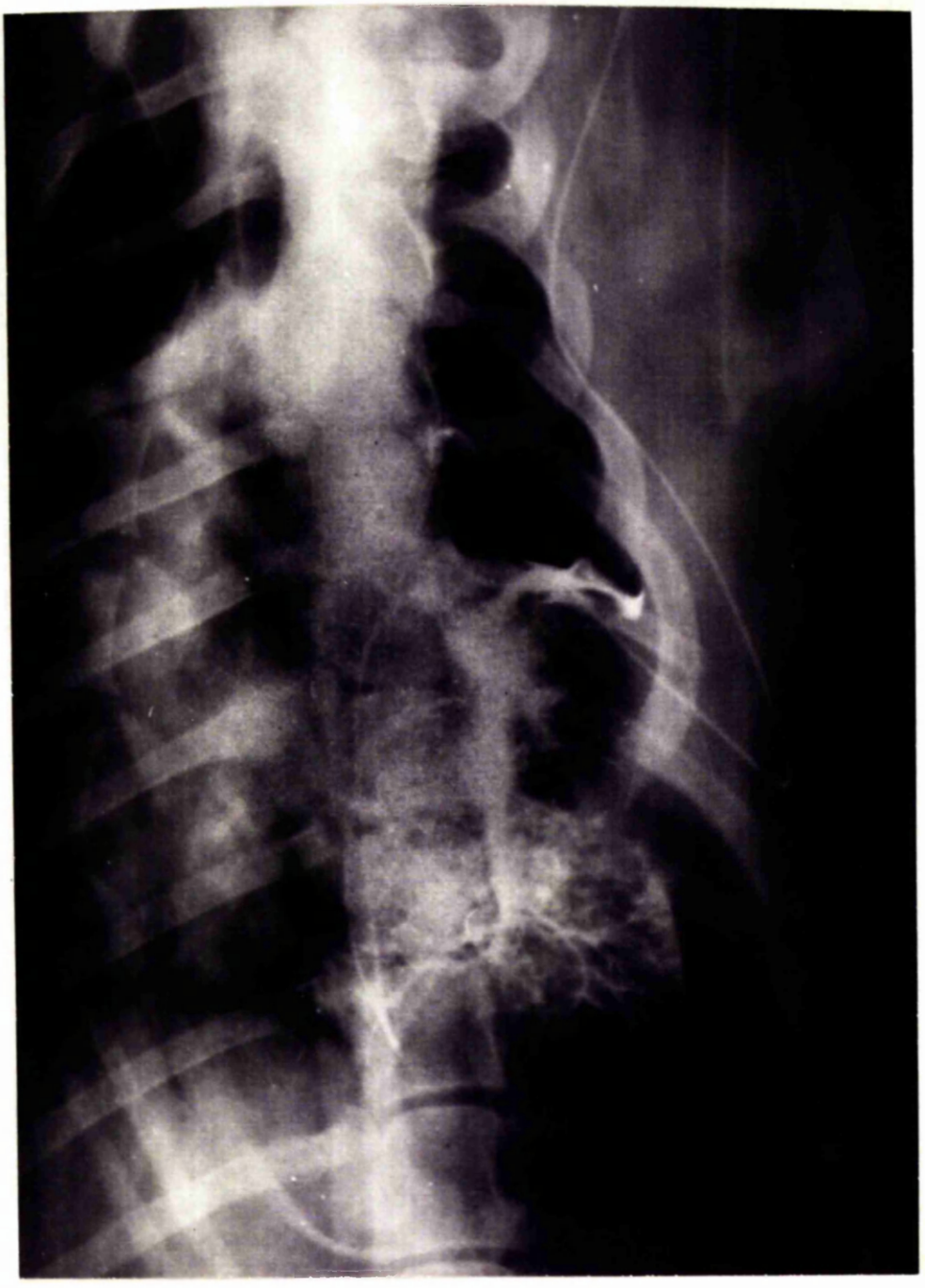

FIGURE 30. Pulmonary angiogram through indwelling Teflon catheter. Lobar autotransplant.

0.69 and 0.40 respectively during the final period of observation between 340-680 days. The mean alveolar volume ratio at this time was 0.62.

The late complication most frequently observed by autopsy studies in all series, has been bronchial stenosis at the anastomotic suture line, due to devascularisation and sloughing of the most vulnerable bronchial mucosa. Figure 31 is a photomicrograph of a bronchial anastomosis and shows the absence of bronchial mucosa in the transplanted bronchus while the host bronchial epithelium (on the left) is still present and viable. Figure 32 shows the macroscopic appearances of a tight stenosis in a lobar autotransplant. The gradually increasing development of this complication is shown radiographically in Figures 33, 34 and 35. At 6 months mild stenosis was present. By one year stenosis is marked and the lobe is reduced in volume. By 1 year 2 months alveolar filling is minimal.

The mean alveolar volume ratios of surviving autografts decreased gradually towards the end of the period under study. This reduction is due to bronchial stenosis in some animals. A comparison of the ventilated alveolar volume ratio in 2 animals is shown in

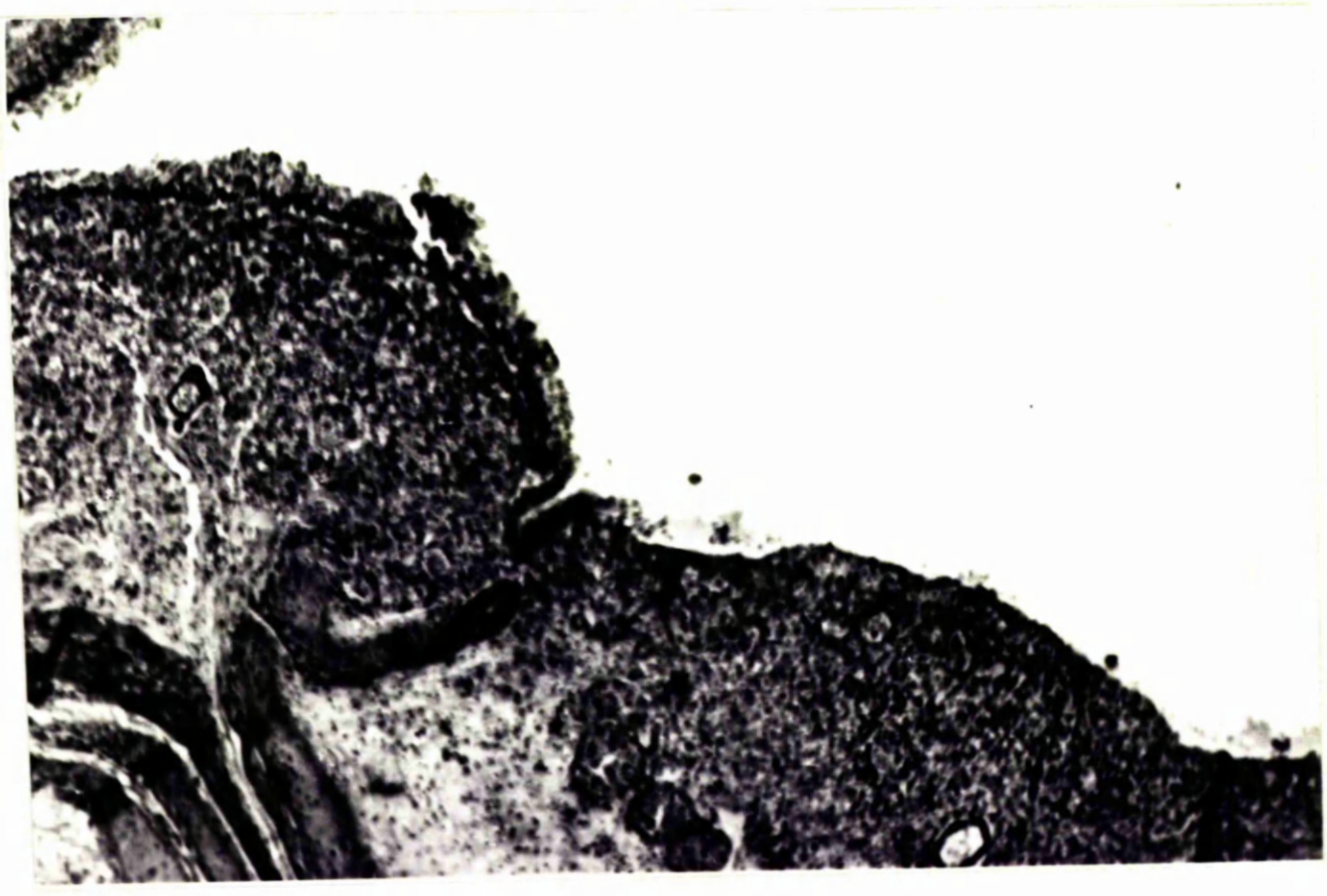

FIGURE 31. Photomicrograph of bronchial anastomosis. Absence of bronchial epithelium in transplant.

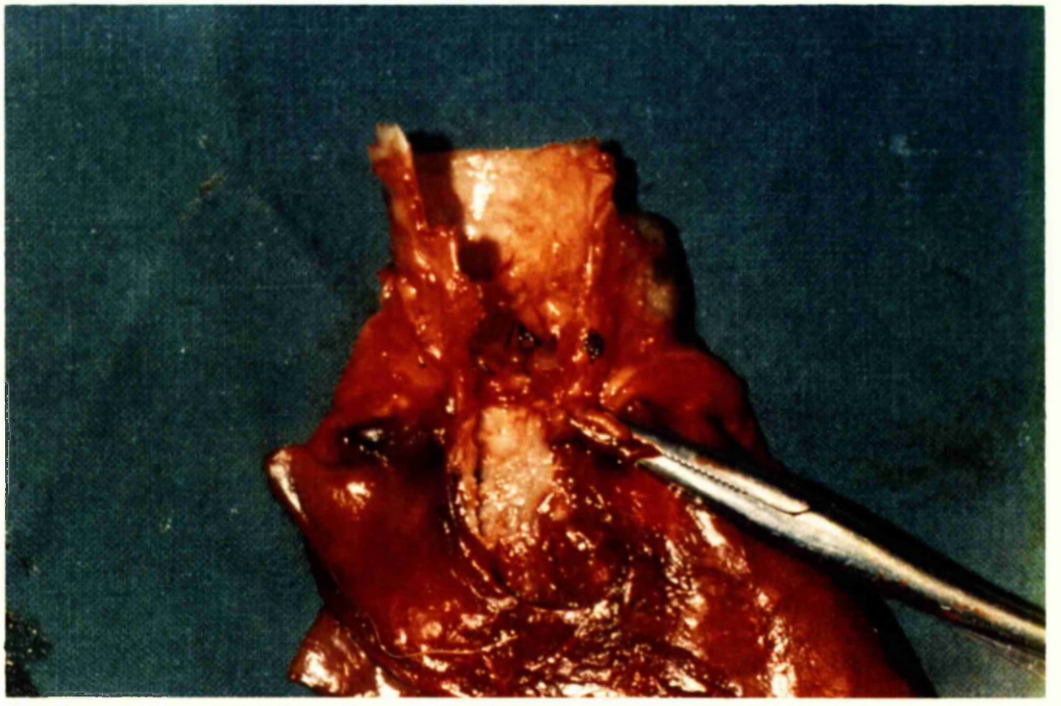

FIGURE 32. Photograph of bronchial anastomosis : tight stenosis present. Graft viable.

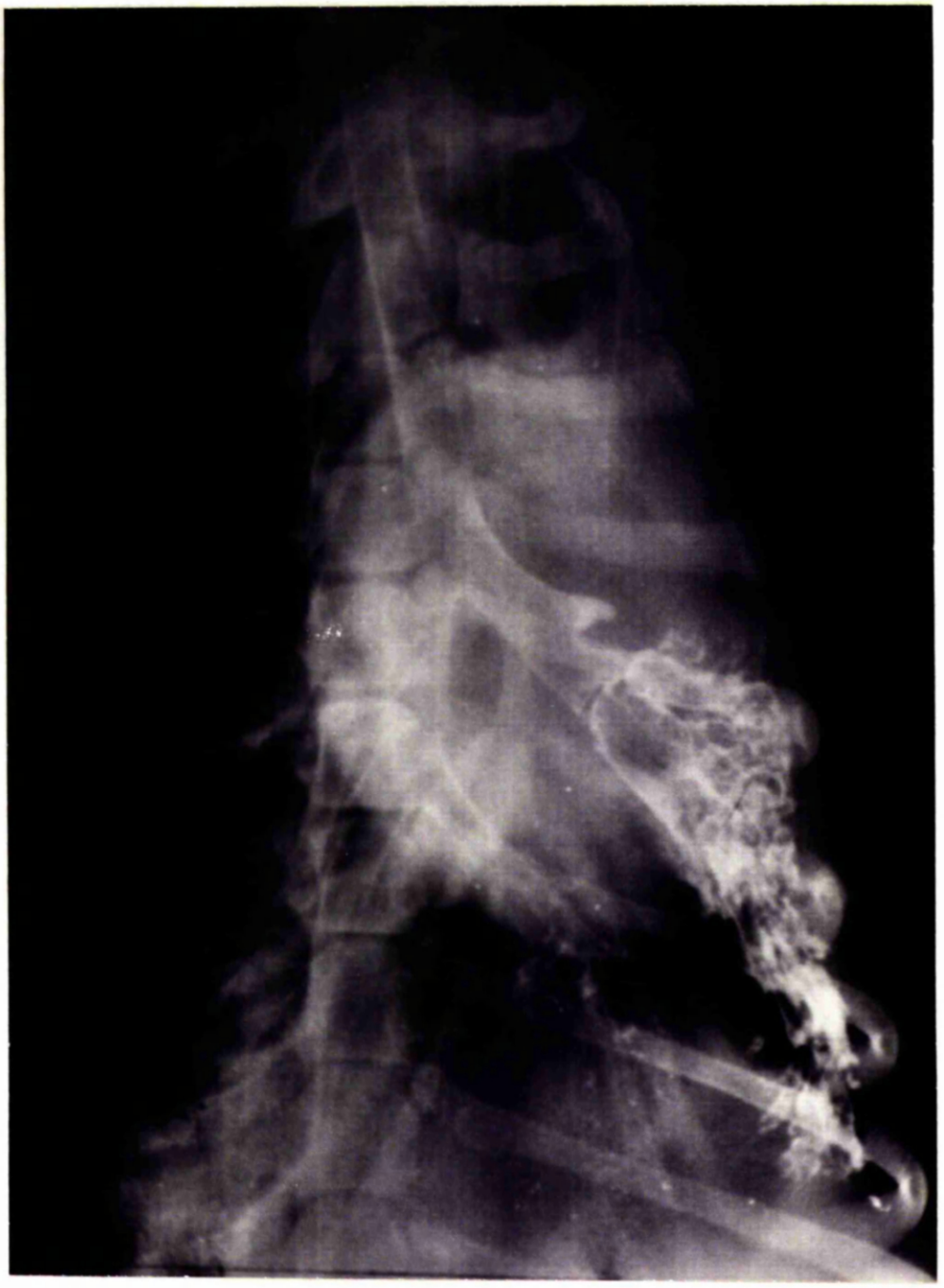

FIGURE 33. Bronchogram. Lobar autotransplant at 6 months.

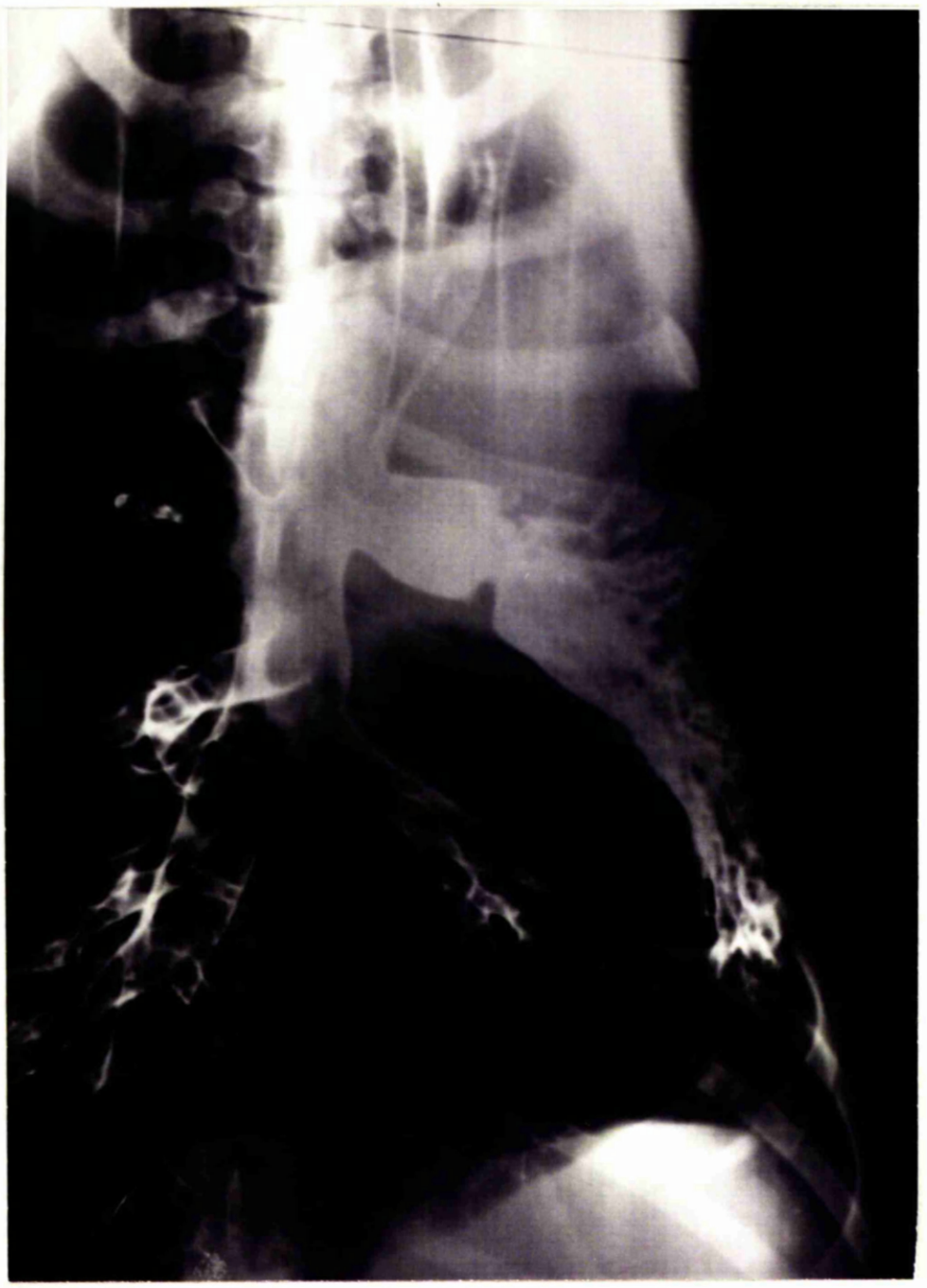

FIGURE 34. Bronchogram at 12 months. Increasing bronchial stenosis.

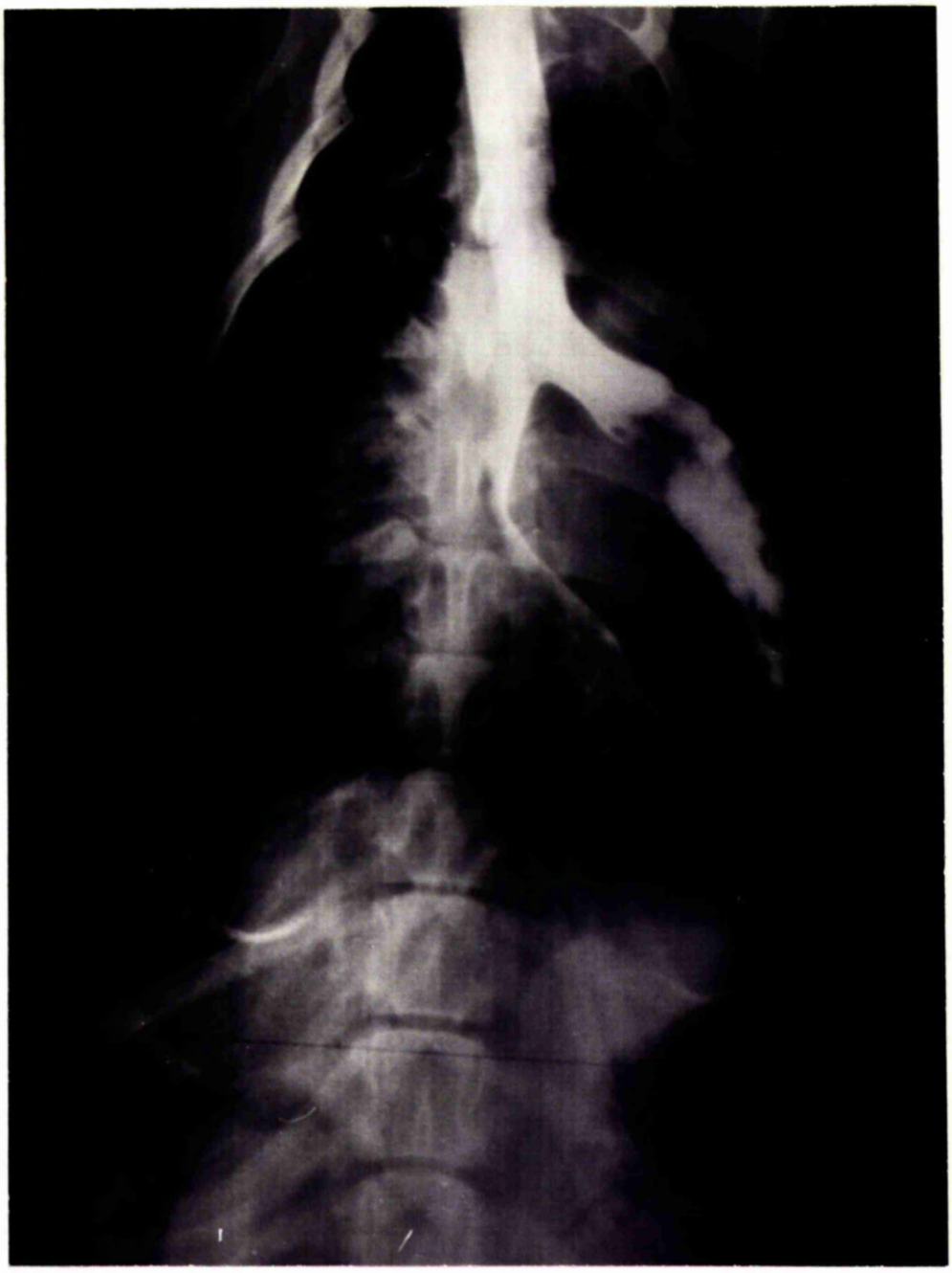

FIGURE 35. Bronchogram at 14 months. Severe bronchial stenosis with poor alveolar filling.

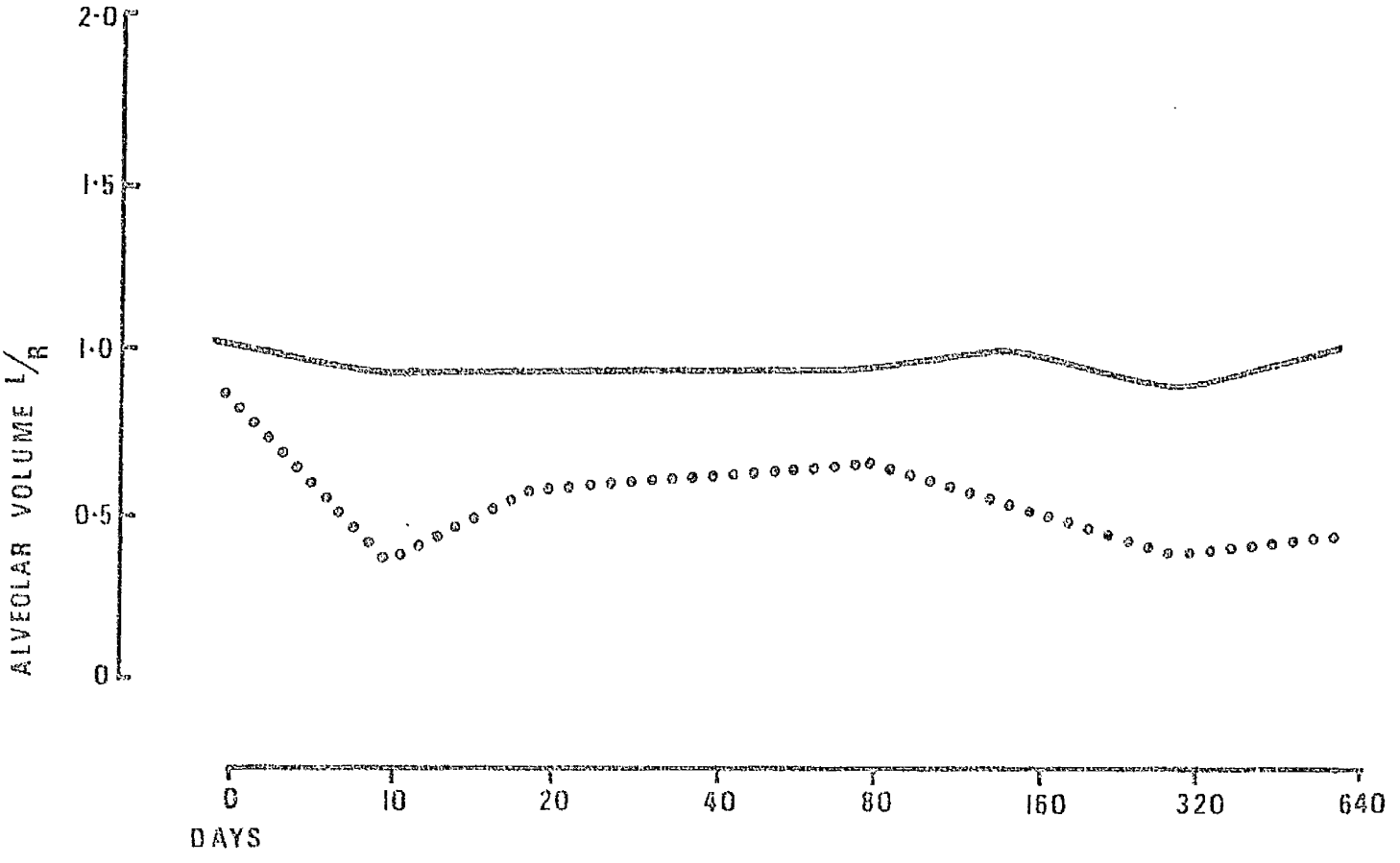

FIGURE 36.

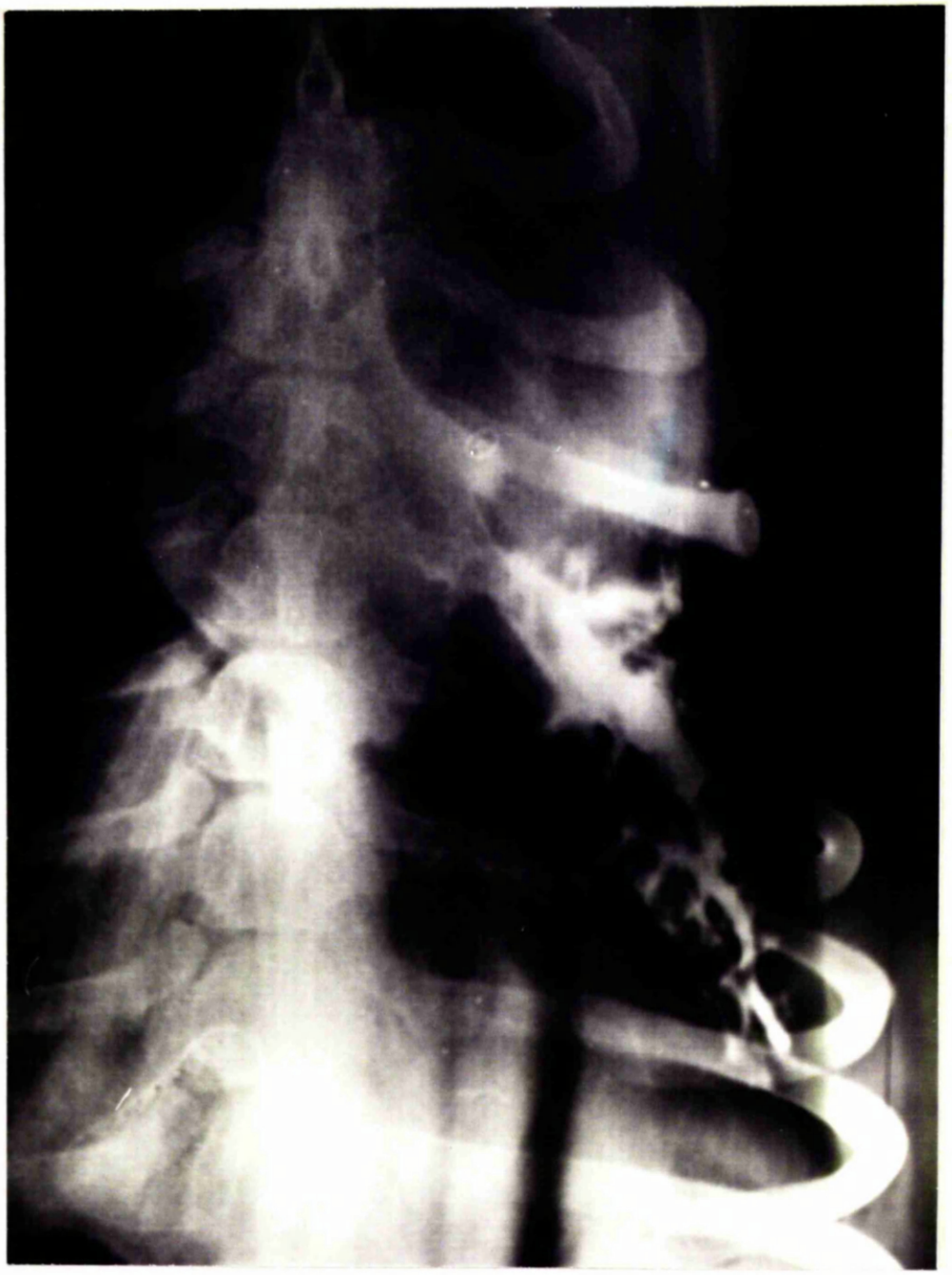

FIGURE 37. Bronchogram. Lobar autotransplant. Good alveolar filling. No stenosis.

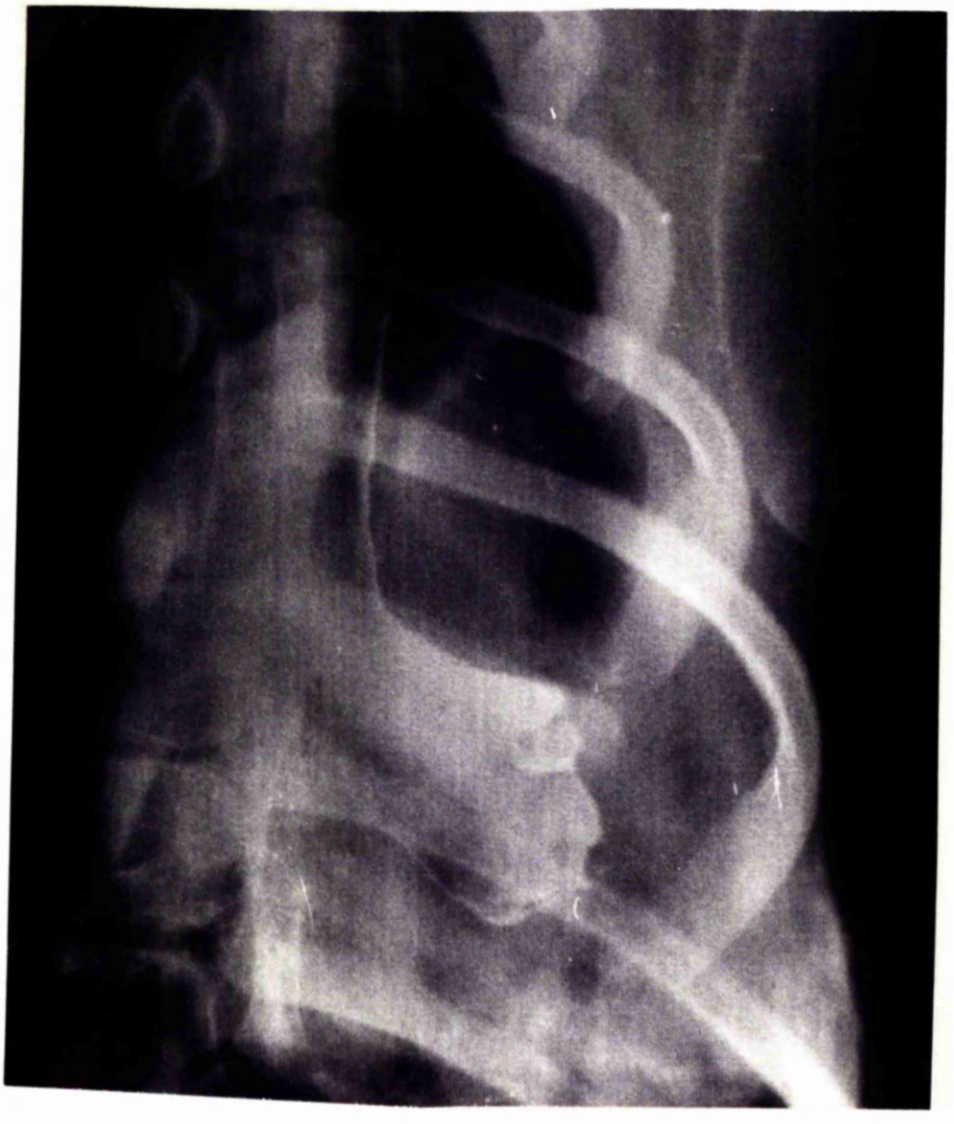

FIGURE 38. Bronchogram. Lobar autotransplant. Complete occlusion.

Figure 36. These values show a constant ratio of 1.00 in one animal where the bronchogram (Figure 37) showed no evidence of stenosis and good alveolar filling. The other animal showed equivalent alveolar volume ratio at 20 days with gradual reduction thereafter to a final ratio of L/R = 0.47 at 640 days. Bronchography (Figure 38) at this time demonstrated that complete occlusion had taken place at the bronchial anastomosis. Measurement of alveolar volume ratios by the Xenon^{133} method does, therefore, accurately predict the insidious onset of bronchial stenosis in vivo without resort to repeated cumbersome radiological studies.

II. EFFECT ON PERFUSION AND VENTILATION AFTER PULMONARY ALLOTRANSPLANTATION

Perfusion and ventilation studies using Xenon^{133} were evaluated in 56 dogs subjected to pulmonary allograft transplantation, treated by local immunosuppression. The functional studies were carried out by the same standard method as in autografts at 2, 9 days and weekly intervals thereafter, till death of the animal from immunological rejection of the graft.

In order to assess those functional changes occurring during

rejection and after adequate immunosuppression, the results obtained in animals who were known to be technical failures (i.e., with blood flow ratios L/R below 0.30 and with confirmation of technical failure at autopsy) are excluded from the data presented. Thus 44 animals were available for determination of the effects on function of pulmonary allografts.

1. Effects on Pulmonary Function during the Rejection Process

Table IX shows the alterations occurring in blood flow L/R, blood flow per unit volume L/R, ventilation L/R, ventilation per unit volume L/R and alveolar volume L/R ratios in 3 animals who died of acute rejection within the first 25 days of operation despite systemic and local immunosuppression. The pattern in each case is similar. Figure 39 is representative of the excellent perfusion in these animals during the immediate post-operative phase with blood flow and alveolar volume equal on both sides. Perfusion and ventilation immediately after transplantation were well maintained, with the mean values of blood flow L/R = 0.64, blood flow per unit volume L/R = 0.70, ventilation L/R = 0.64, alveolar volume L/R = 0.98 and ventilation per unit volume L/R = 0.59 on the second post-operative day. These values indicate technically satisfactory

TABLE IX

MEAN VENTILATION/PERFUSION INDICES DURING REJECTION				
DAYS		0-5 Days	6-10 Days	11-25 Days
Blood Flow L/R.	Mean	0.64	0.15	0.14
	S.D.	± 0.23	± 0.01	±
Blood Flow per Unit Volume L/R.	Mean	0.70	0.62	0.75
	S.D.	± 0.21	± 0.24	± 0.16
Alveolar Volume	Mean	0.98	0.27	0.20
	S.D.	± 0.42	± 0.09	± 0.13
Ventilation L/R	Mean	0.64	0.25	0.31
	S.D.	± 0.45	± 0.07	± 0.12
Ventilation per Unit Volume L/R.	Mean	0.59	0.73	1.22
	S.D.	± 0.19	± 0.09	± 0.28

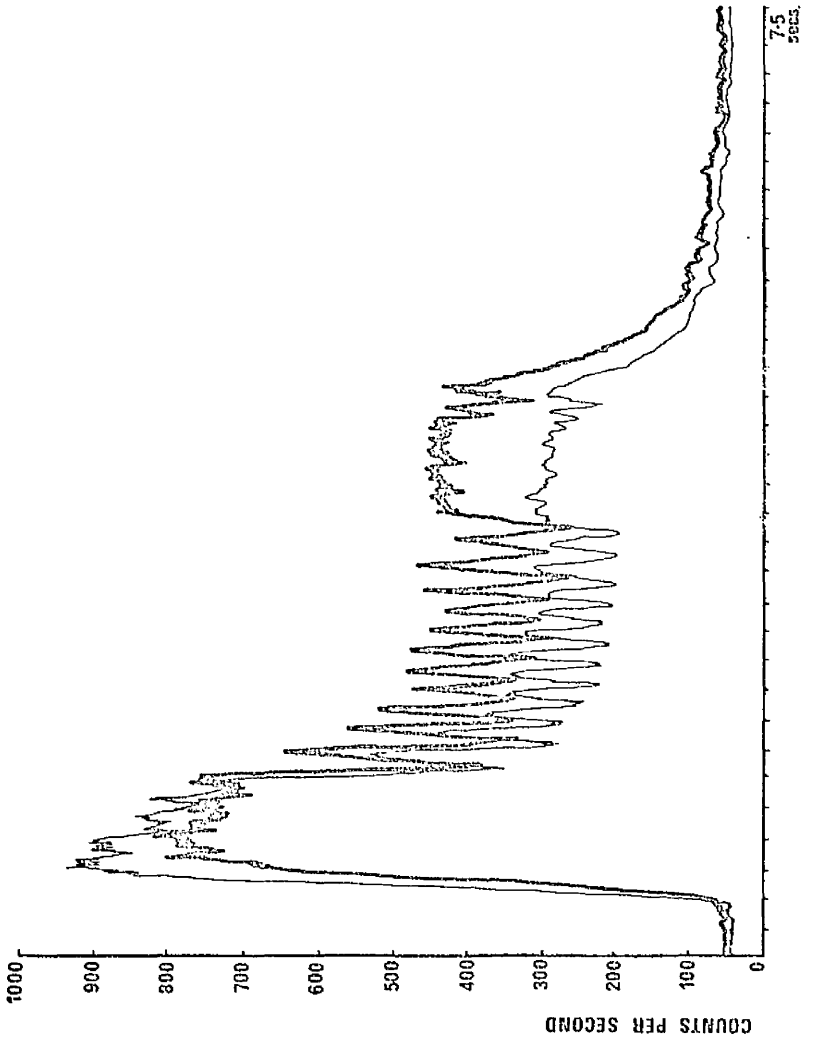

FIGURE 39. Perfusion study in lobar allograft 2 days after operation. Excellent perfusion and alveolar volume in graft.

grafts and are equivalent to the ratios obtained at this time for autografts alive at present. The changes in the perfusion and ventilation indices which take place during rejection despite immunosuppression are shown graphically in Figure 40. The most noticeable and consistent feature was the reduction in blood flow compared with the opposite lung at 6-10 days, followed by a drop in ventilation and alveolar volume. These values remained low until the eventual death of the animal from rejection. The mean values of blood flow per unit volume and ventilation per unit volume of lung remain, however, at a constant high level. The interpretation of these highly significant changes in a technically satisfactory graft is that there is diminution in peripheral perfusion in the graft accompanied by a reduction in ventilated alveolar volume due to accumulation within the graft with oedema and cellular infiltration as a result of the rejection process. The final state determined by $Xenon^{133}$ testing is shown in Figure 41, taken at 23 days. This indicates that blood flow in the graft was minimal compared with the contralateral lung.

Confirmation of these findings was obtained at autopsy. The photograph in Figure 42 shows the typical macroscopic appearances

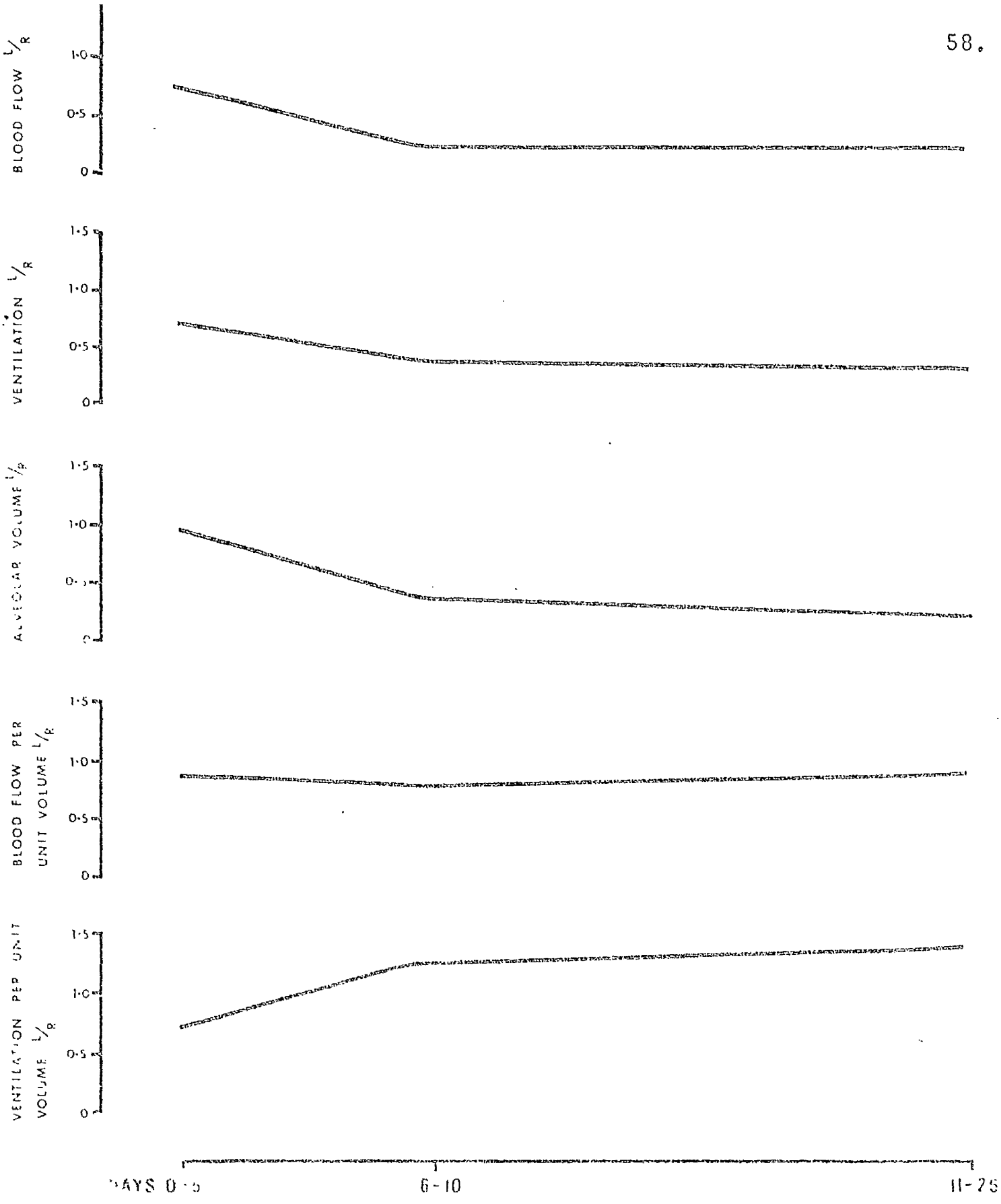
BLOOD FLOW L/R
1·0
0·5
0
VENTILATION L/R
1·5
1·0
0·5
0
1·5
1·0
1·5
1·0
0·5
0
1·5
1·0
0·5
0
6-10

FIGURE 40.

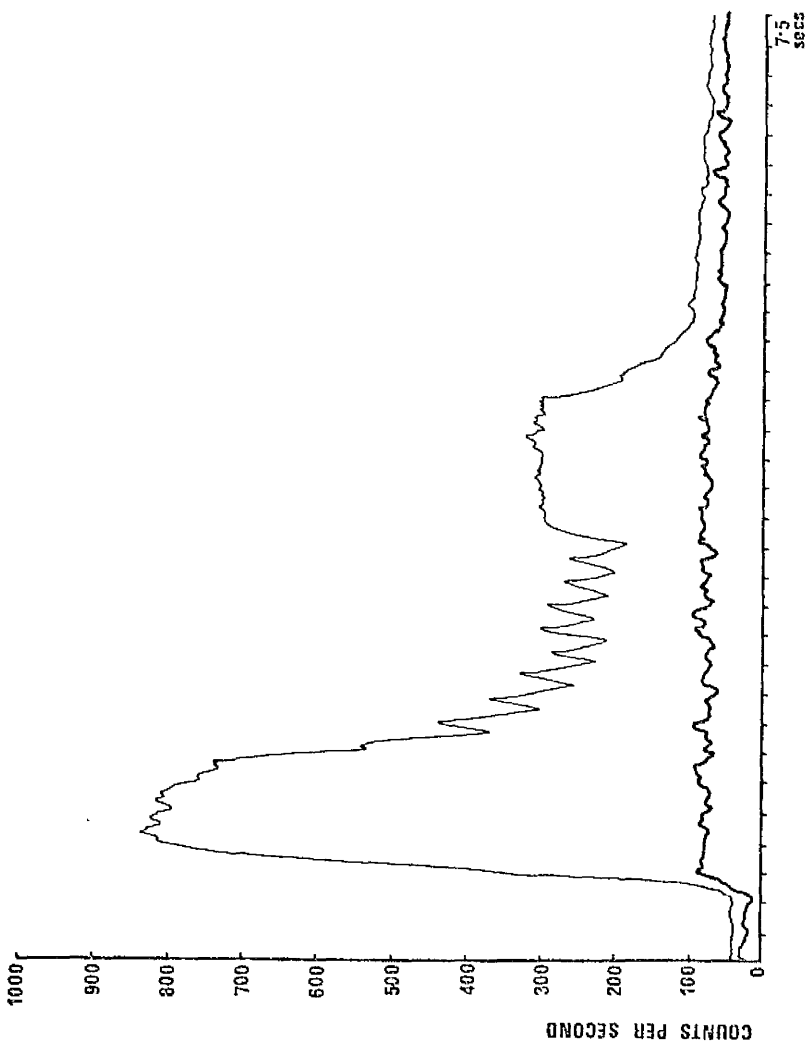

FIGURE 41. Perfusion study in rejecting lobar allograft at 23 days. Blood flow and effectively ventilated alveolar volume absent in graft.

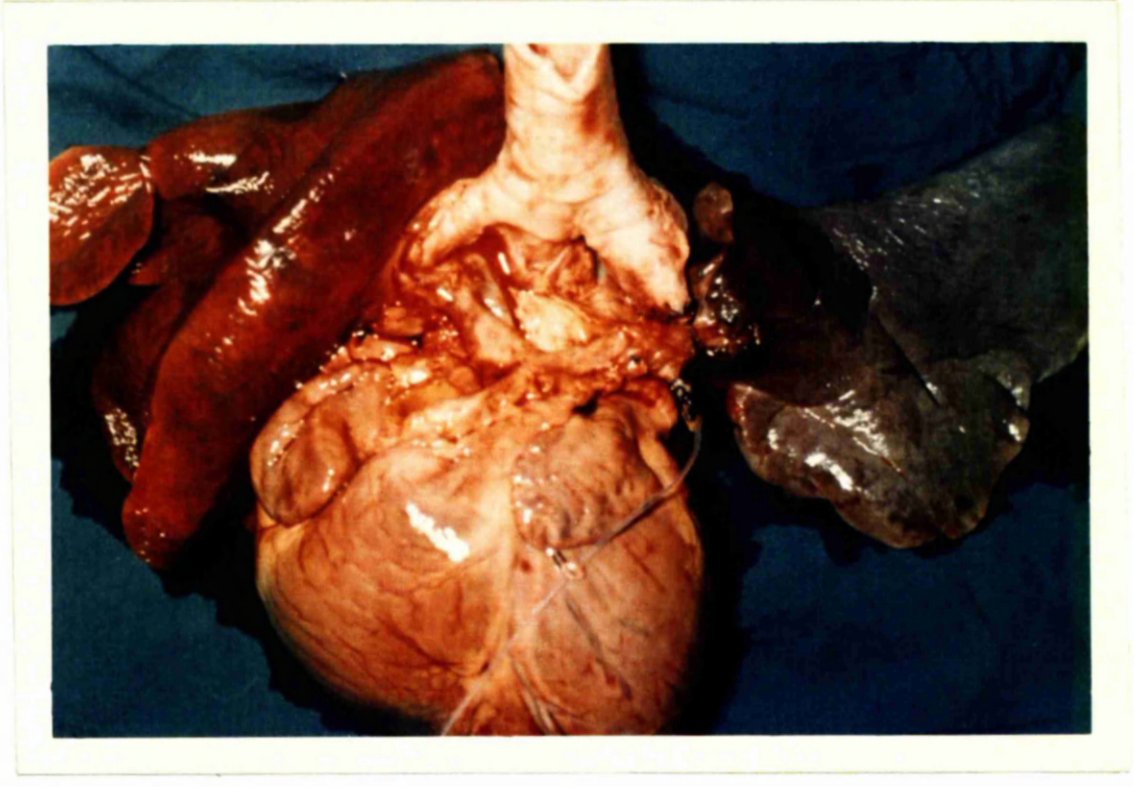

FIGURE 42. Photograph of post-mortem specimen. Rejected lobar allograft.

of a rejected graft. The lung is swollen and consolidated with reduced aeriation. The pleural surface is dull and is covered with a fibrinous exudate. The anstomosis and main vessel show no evidence of thrombus. Post-mortem arteriography (Figure 43) demonstrates the diminished peripheral perfusion; the main vessel is intact, and the small vessels in the graft are apparently diminished in both number and size compared to the contralateral lung due to poor filling with the contrast medium. Within the small vessels in the graft are multiple filling defects.

On microscopy (Figure 44) there is cellular infiltration of the graft, perivascular "cuffing" with mononuclear cells, intra-alveolar infiltration with fluid and cells, and disruption of alveoli. These changes are characteristic of the acute rejection process in pulmonary grafts which have been described by Barnes and Flax (1964) and Lanari et al. (1952).

2. Early Changes in Allograft Function Modified by Adequate Immunosuppression

Table X shows the values of both perfusion and ventilation indices of all allografts which were deemed technically satisfactory and which were treated by local and systemic immunosuppression.

of a rejected graft. The lung is swollen and consolidated with reduced aeriation. The normally pleural surface is dull and is covered with a fibrinous exudate. The anastomosis and main vessel show no evidence of thrombus. Post-mortem arteriography (Figure 43) demonstrates the diminished peripheral perfusion; the main vessel is intact, and the small vessels in the graft are apparently diminished in both number and size compared to the contralateral lung due to poor filling with the contrast medium. Within the small vessels in the graft are multiple filling defects.

On microscopy (Figure 44) there is cellular infiltration of the graft, perivascular "cuffing" with mononuclear cells, intra-alveolar infiltration with fluid and cells, and disruption of alveoli. These changes are characteristic of the acute rejection process in pulmonary grafts which have been described by Barnes and Flax (1964) and Lanari et al. (1952).

2. Early Changes in Allograft Function Modified by Adequate Immunosuppression

Table X shows the values of both perfusion and ventilation indices of all allografts which were deemed technically satisfactory and which were treated by local and systemic immunosuppression.

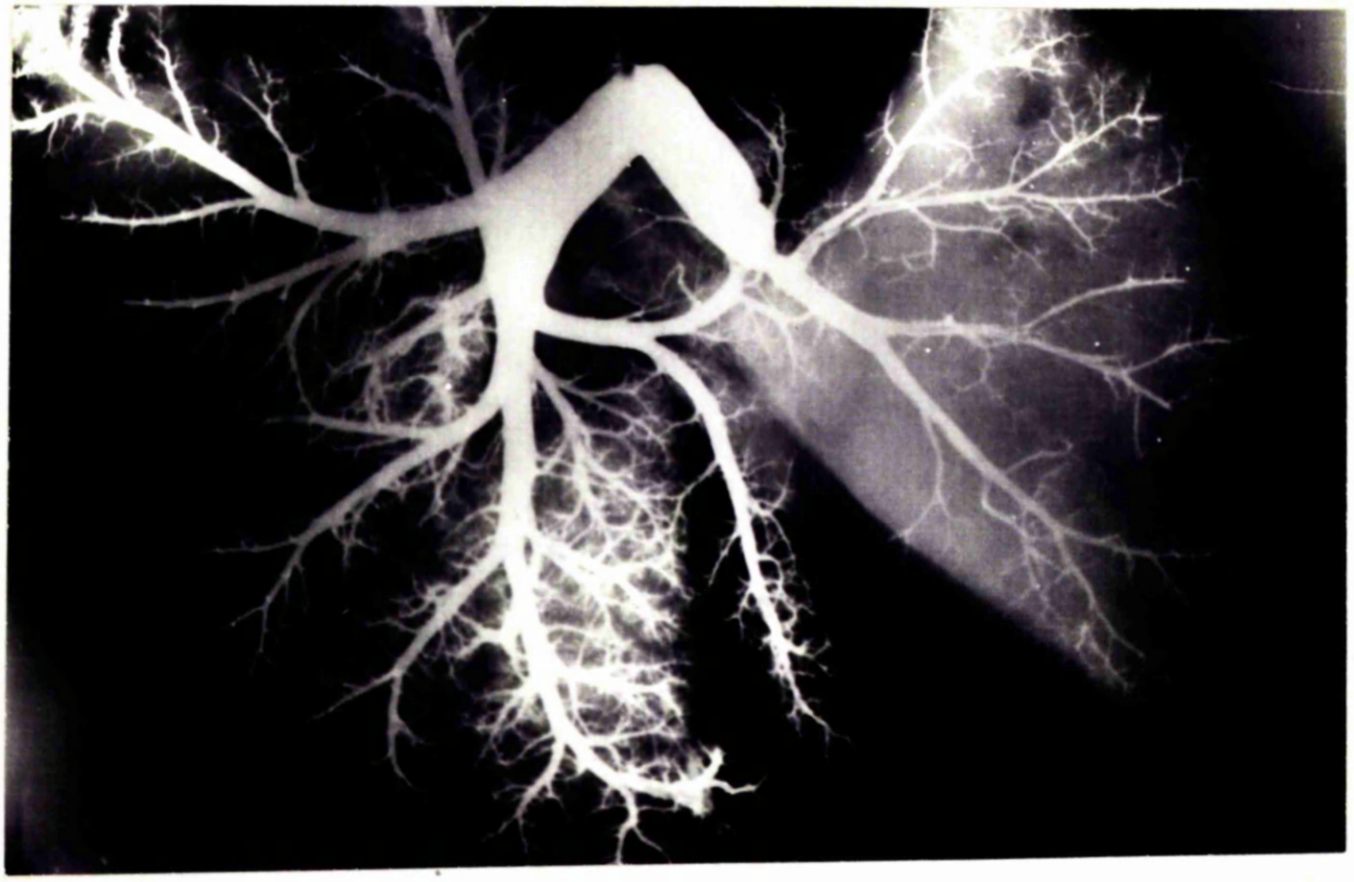

FIGURE 43. Post-mortem pulmonary arteriogram. Rejected graft. Poor peripheral arterial perfusion with filling defects in vessels and graft oedema.

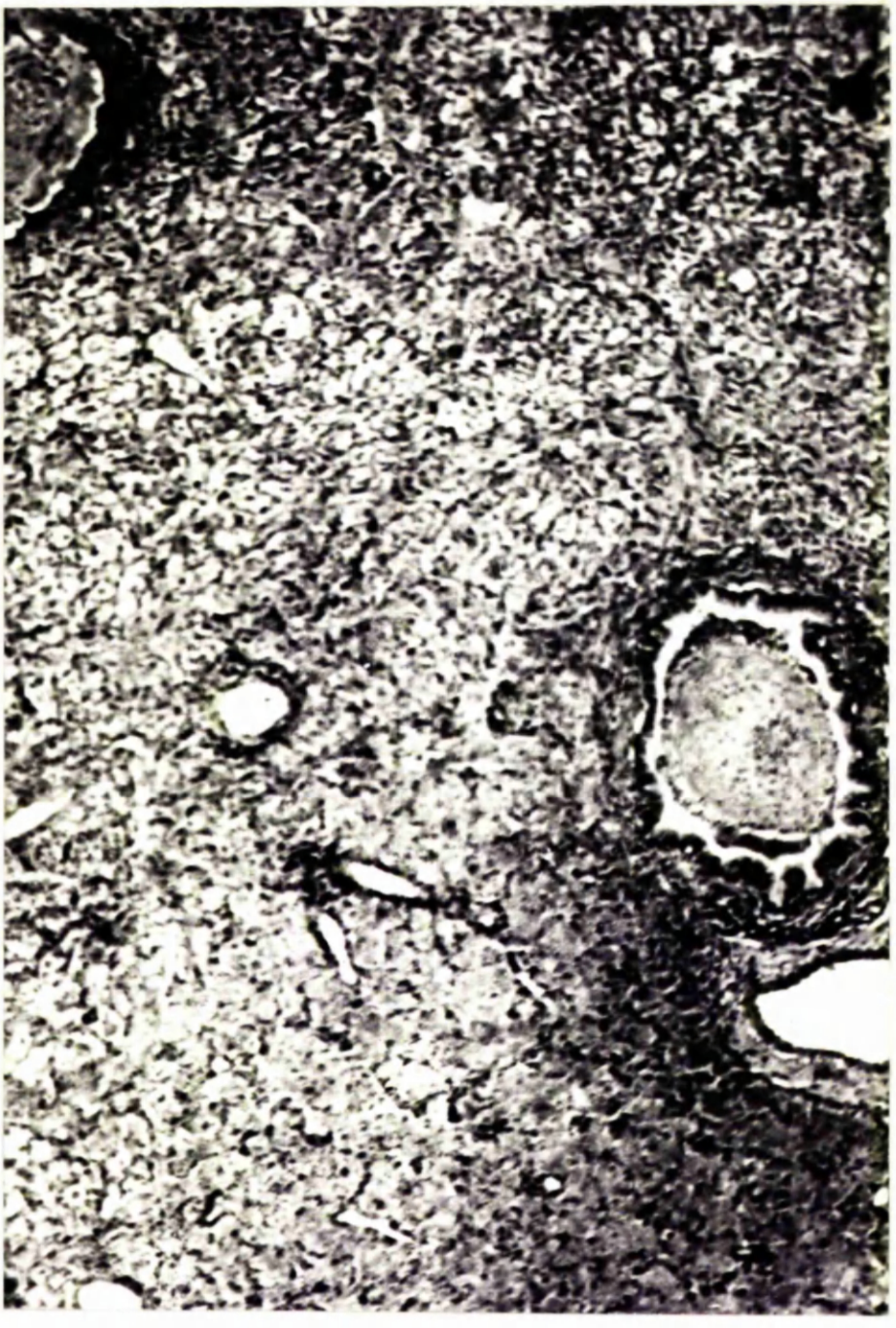

FIGURE 44. Histological features of unmodified rejection in lobar allograft. Widespread cellular and fluid infiltrates; dense peri-vascular cuffing with mononuclear and polymorphonuclear cells.

TABLE X

MEAN VENTILATION/PERFUSION INDICES IN FUNCTIONING ALLOGRAFTS

DAYS		0 - 5	6 - 10	11 - 20	21 - 40	41 - 80	81 - 160	161 - 320	321 - 640
BLOOD FLOW L/R	Mean	0.57	0.48	0.68	0.68	0.55	0.31	0.42	0.34
	S.D.	±0.21	±0.27	±0.64	±0.39	±0.41	±0.03	±0.17	±0.06
	Dogs	24	9	5	5	4	1	1	1
	Readings	24	10	5	7	5	2	3	4
ALVEOLAR VOLUME L/R	Mean	0.76	0.66	0.76	0.73	0.61	0.65	1.27	0.72
	S.D.	±0.32	±0.26	±0.30	±0.33	±0.31	±0.00	±0.49	±0.18
	Dogs	24	9	5	5	4	1	1	1
	Readings	24	10	5	7	5	2	3	4
BLOOD FLOW PER UNIT VOLUME L/R	Mean	0.80	0.80	0.84	0.95	0.85	0.48	0.33	0.48
	S.D.	±0.23	±0.40	±0.36	±0.31	±0.23	±0.05	±0.00	±0.06
	Dogs	24	9	5	5	4	1	1	1
	Readings	24	10	5	7	5	2	3	4
VENTILATION L/R	Mean	0.65	0.49	0.40	0.72	0.48	0.40	0.75	0.39
	S.D.	±0.31	±0.16	±0.06	±0.39	±0.32	±0.00	±0.34	±0.08
	Dogs	20	7	3	3	3	1	1	1
	Readings	20	8	3	5	3	2	3	4
VENTILATION PER UNIT VOLUME L/R	Mean	0.82	1.30	0.84	1.12	0.87	0.54	0.75	0.57
	S.D.	±0.21	±0.61	±0.00	±0.36	±0.10	±0.07	±0.06	±0.08
	Dogs	20	7	3	3	3	1	1	1
	Readings	20	8	3	5	3	2	3	4

Figure 45 demonstrates the progress of these indices in one such graft up to 60 days at which time the animal was killed for confirmation of the functional findings. The immediate post-operative values for perfusion and ventilation indicated a technically satisfactory graft. Thereafter, there was temporary depression of perfusion and ventilation, which was probably of the same aetiology as in the autograft preparation and not due to rejection. Perfusion and ventilation recovered and remained constant until the death of the animal at 60 days. Blood flow per unit volume, ventilation per unit volume and alveolar volume at this time showed equivalence with those values obtained from the contralateral lung. At autopsy the vascular anastomosis showed no evidence of thrombus formation or stenosis and the graft had a similar appearance macroscopically to the normal lung.

Figure 46 shows the macroscopic appearances of a well functioning allograft, at autopsy. The transplant was of a normal consistency, well aeriated and shows minimal induration. There was little pleural reaction but no other sign of acute rejection. The post mortem arteriogram (Figure 47) showed a normal vascular pattern in the graft when compared with contralateral lung. Histological

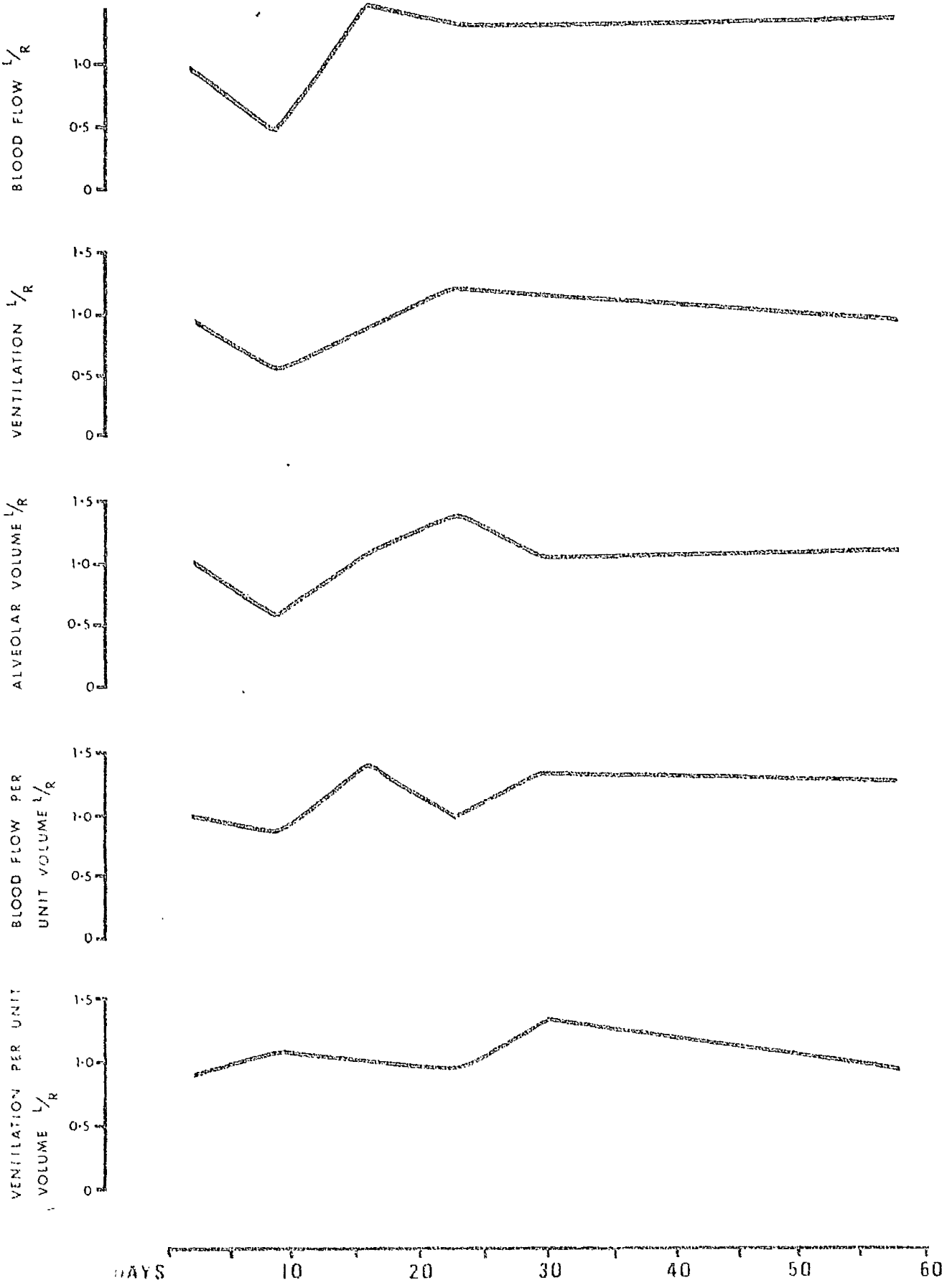
BLOOD FLOW L/R
VENTILATION L/R
ALVEOLAR VOLUME L/R
BLOOD FLOW PER UNIT VOLUME L/R
VENTILATION PER UNIT VOLUME L/R
0
0·5
1·0
1·5
DAYS
10
20
30
40
50
60

FIGURE 45.

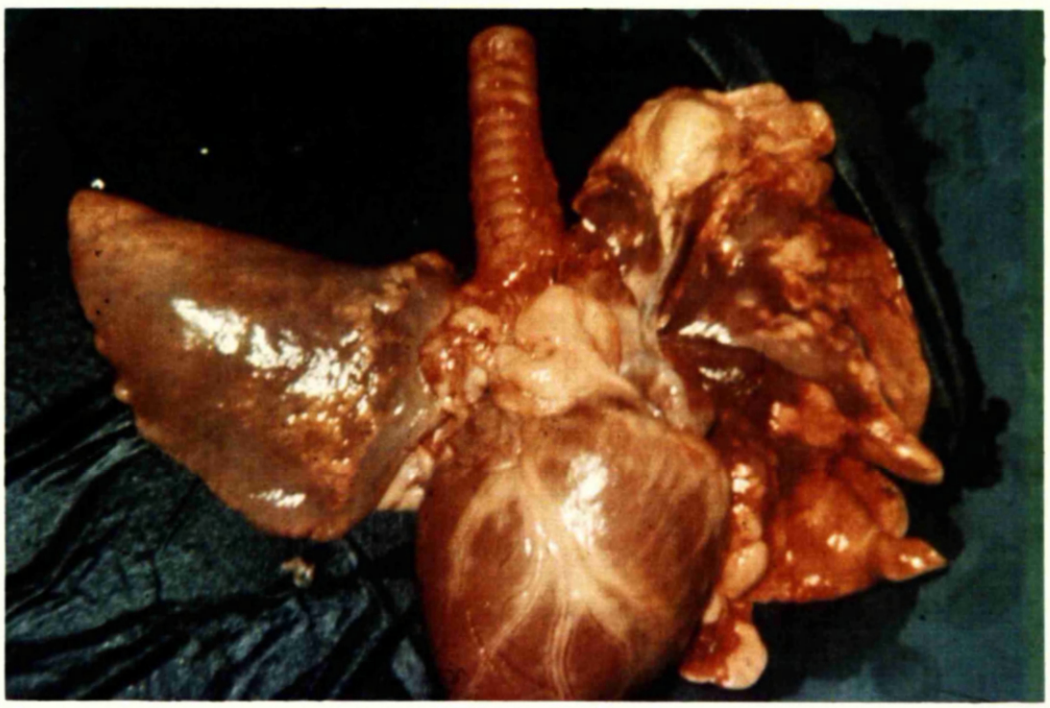

FIGURE 46. Photograph of post-mortem specimen. Lobar allograft (on left) modified by immunosuppression. No graft oedema, well ventilated, same consistency as the normal lung.

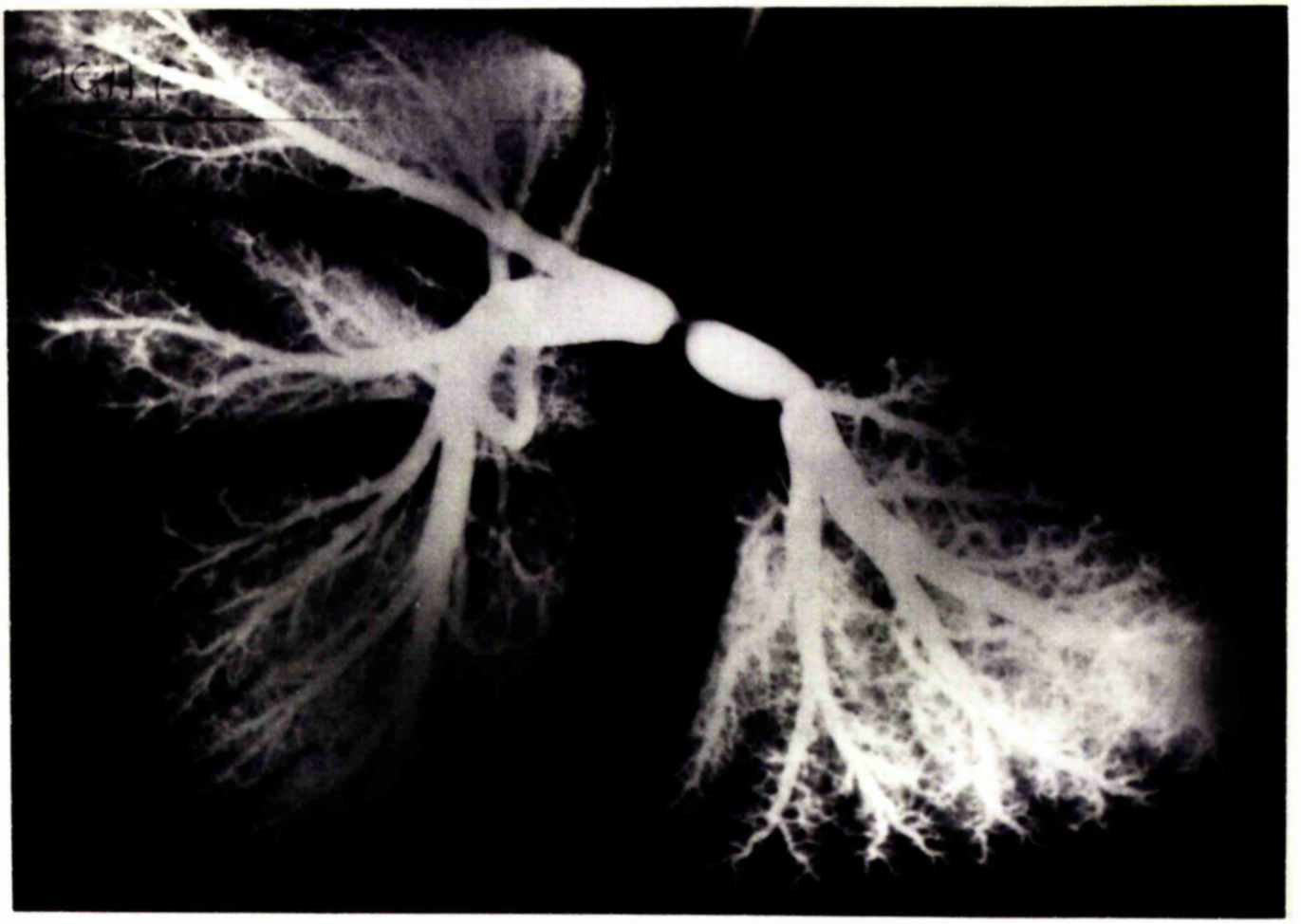

FIGURE 47. Post-mortem pulmonary arteriogram. Lobar allograft on the right. Good filling of vessels and no graft oedema. (The constriction at the bifurcation of the main pulmonary trunk is due to a ligature around the cannula used for injecting contrast medium).

examination of such specimens (Figure 48) shows that the pulmonary tissue can be of almost normal appearance with no evidence of cellular or fluid infiltrates and with no perivascular cuffing.

3. Long Term Effects on Allograft Function

The mean values and their standard deviation of all measurements are listed in Table X. Figure 49 is a graphic representation of those values in treated allografts up to 640 days. After an initial depression of perfusion there was recovery of blood flow and blood flow per unit volume to near normal values within two to three weeks after operation, and this continued until the end of the period under observation. It would seem, therefore, that given an adequate method of immunosuppression (in this case local or regional immunosuppression) the characteristic effects of chronic rejection on the vascular system can be abrogated. Similarly, ventilation per unit volume, ventilation L/R and alveolar volume remain markedly constant and approach those values obtained in the functioning autografts. Figure 50 shows the comparison in the functional parameters between a long term surviving autografts and allografts, and demonstrates effectively that the long term function in both in both is similar. The histological studies carried out in long

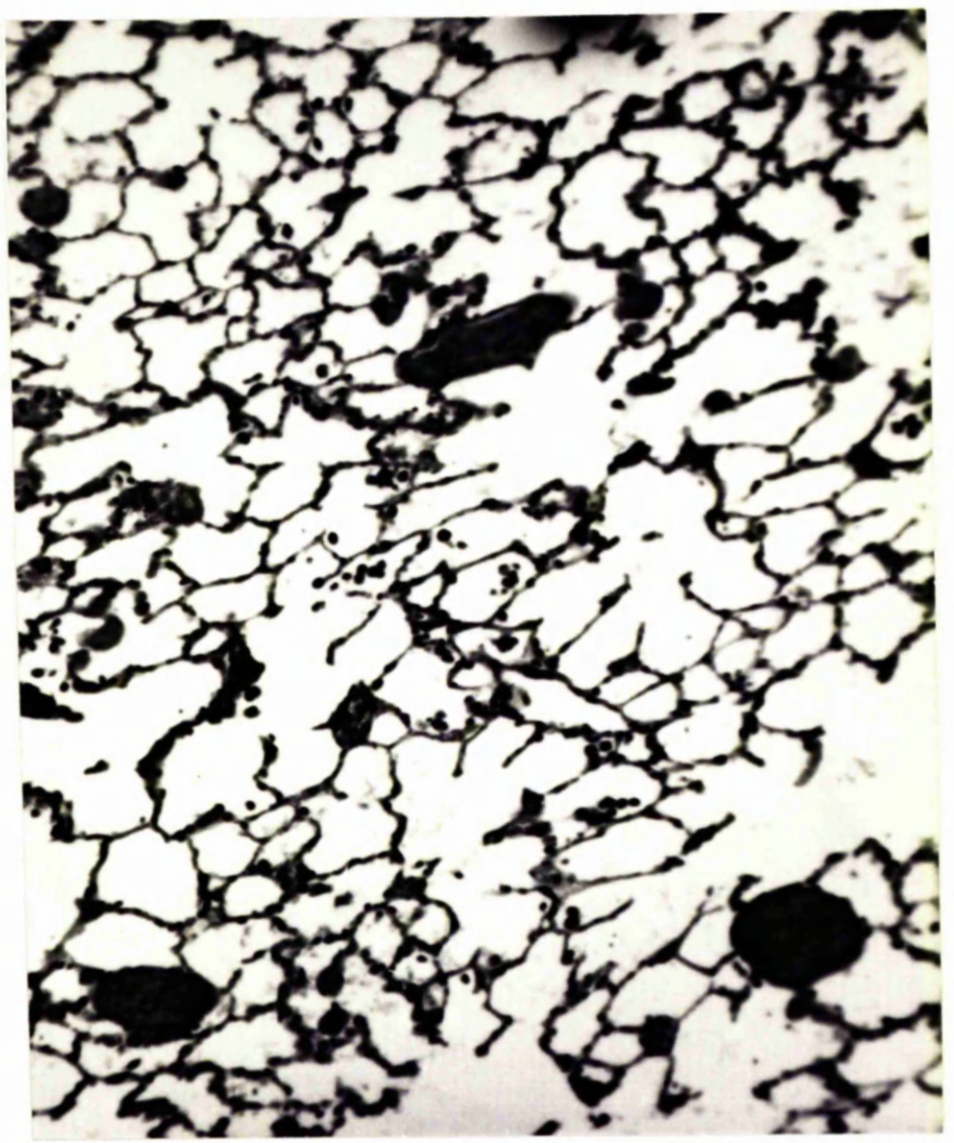

FIGURE 48. Histological appearances of allograft modified by local immunosuppression. Normal architecture. No evidence of rejection.

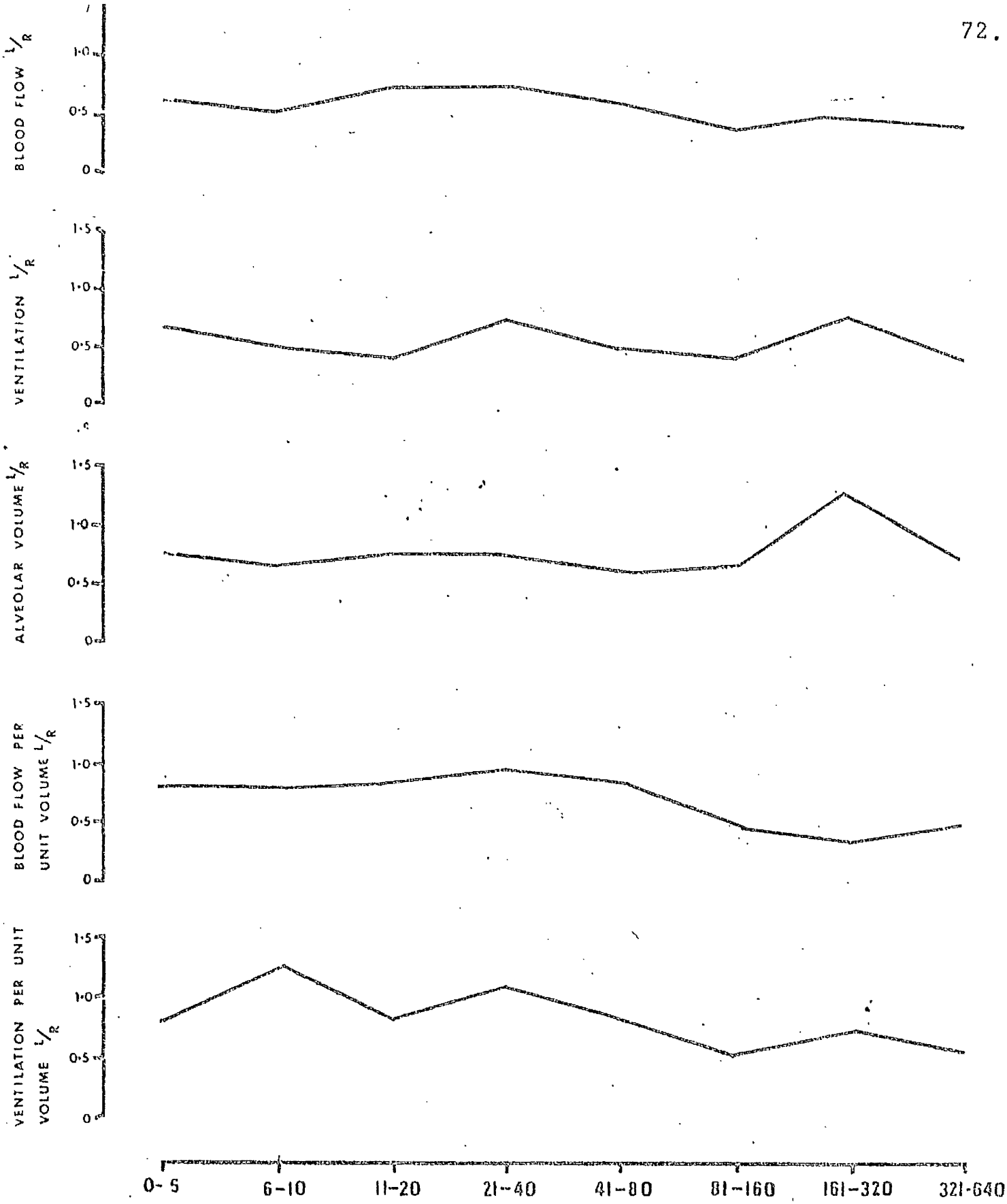
BLOOD FLOW L/R
1·0
0·5
0
VENTILATION L/R
1·5
1·0
0·5
0
ALVEOLAR VOLUME L/R
1·5
1·0
0·5
0
BLOOD FLOW PER UNIT VOLUME L/R
1·5
1·0
0·5
0
VENTILATION PER UNIT VOLUME L/R
1·5
1·0
0·5
0
0-5
6-10
11-20
21-40
41-80
81-160
161-320
321-640

FIGURE 49.

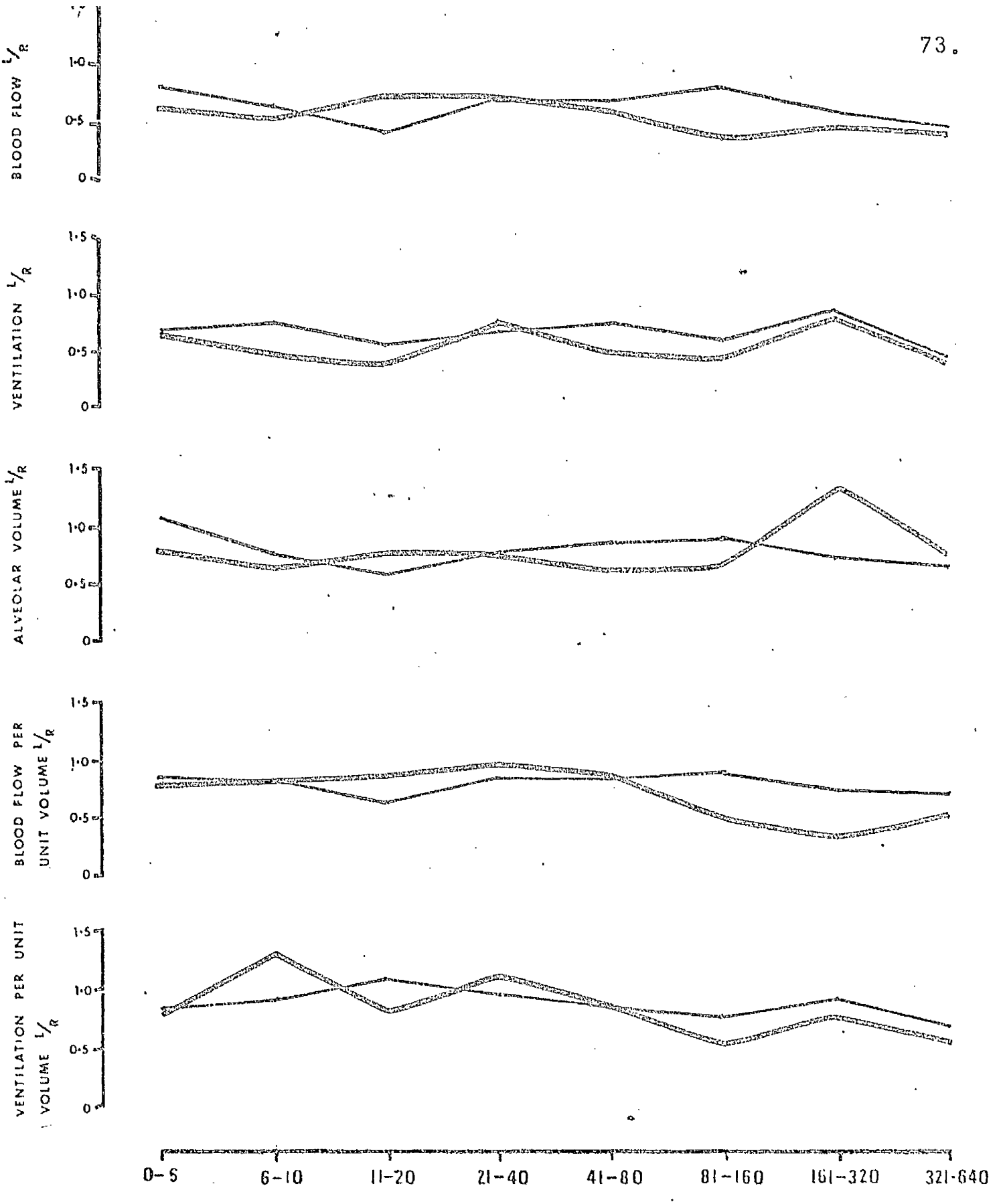

FIGURE 50.

(Allografts in heavier lines)

term surviving allografts by lung biopsy confirm this finding. The photomicrograph in Figure 51 is a biopsy taken at open thoracotomy at 540 days on a surviving allograft treated by local immunosuppression. There are minimal changes of rejection. The alveoli are well aeriated, there is little evidence of round cell or mononuclear infiltration. There was no evidence of atelectasis on histological examination or at operation and the graft was obviously functioning well. Bronchography at this time demonstrates good alveolar filling with no evidence of bronchial stenosis (Figure 52). Figures 53 and 54 are perfusion and ventilation studies in this animal at 506 days and show sustained function in respect of blood flow, ventilation and alveolar volume.

CONCLUSIONS

1. The Xenon^{133} test is a reliable technique in the measurement of function in a transplanted lung, and gives more extensive information than can be obtained by other methods. The most serious inaccuracy in the method lies in the transposition effect from the contralateral lung if there is widespread collapse of the transplant. This can be surmised from sequential analysis of the readings and may be confirmed or excluded by bronchography. It can, however, more readily be determined by direct measurement of perfusion and ventilation.

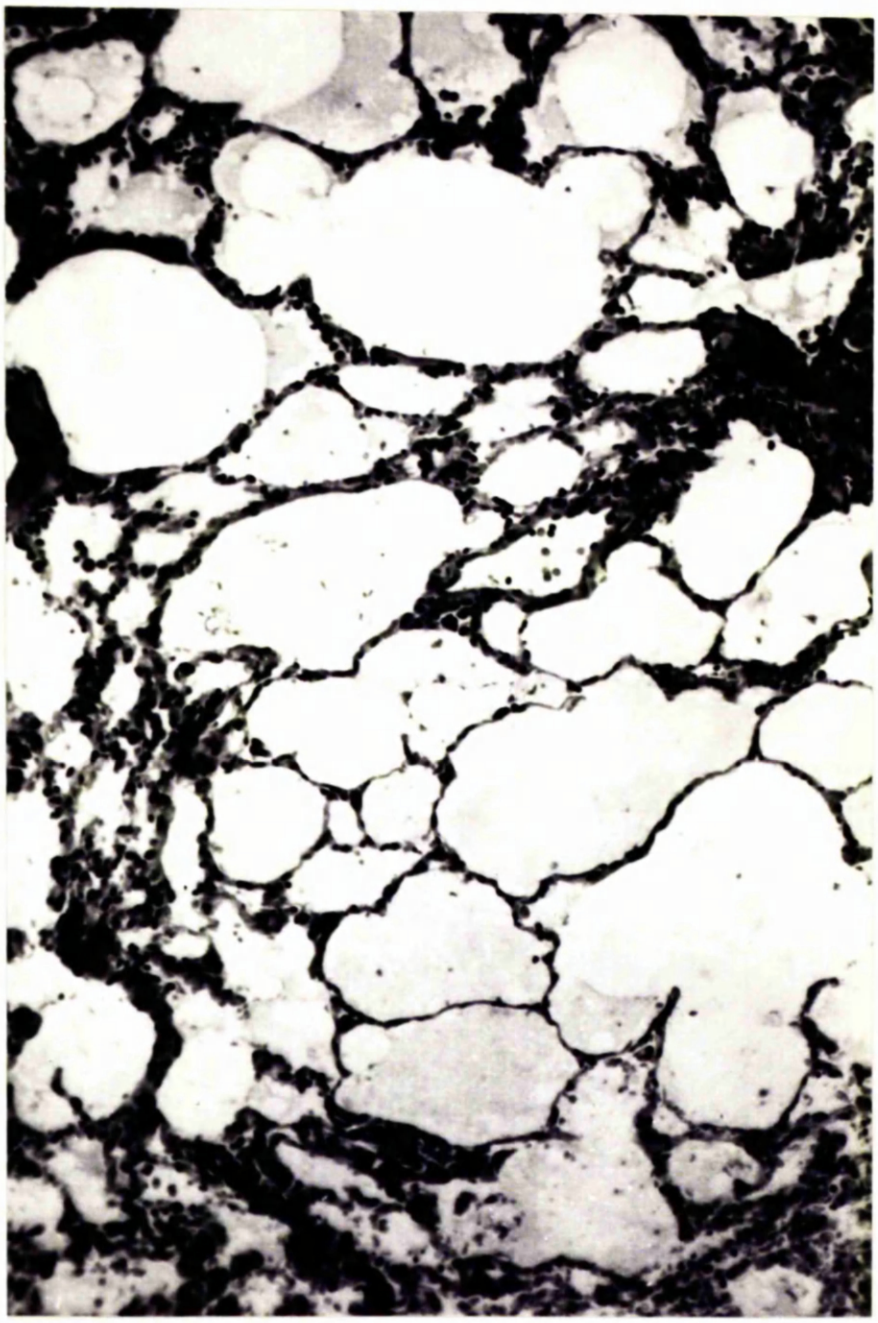

FIGURE 51. Lung biopsy at 540 days. Lobar allograft treated by local immunosuppression. Alveolar architecture good. Little cellular or fluid infiltrates. (H & E x 10).

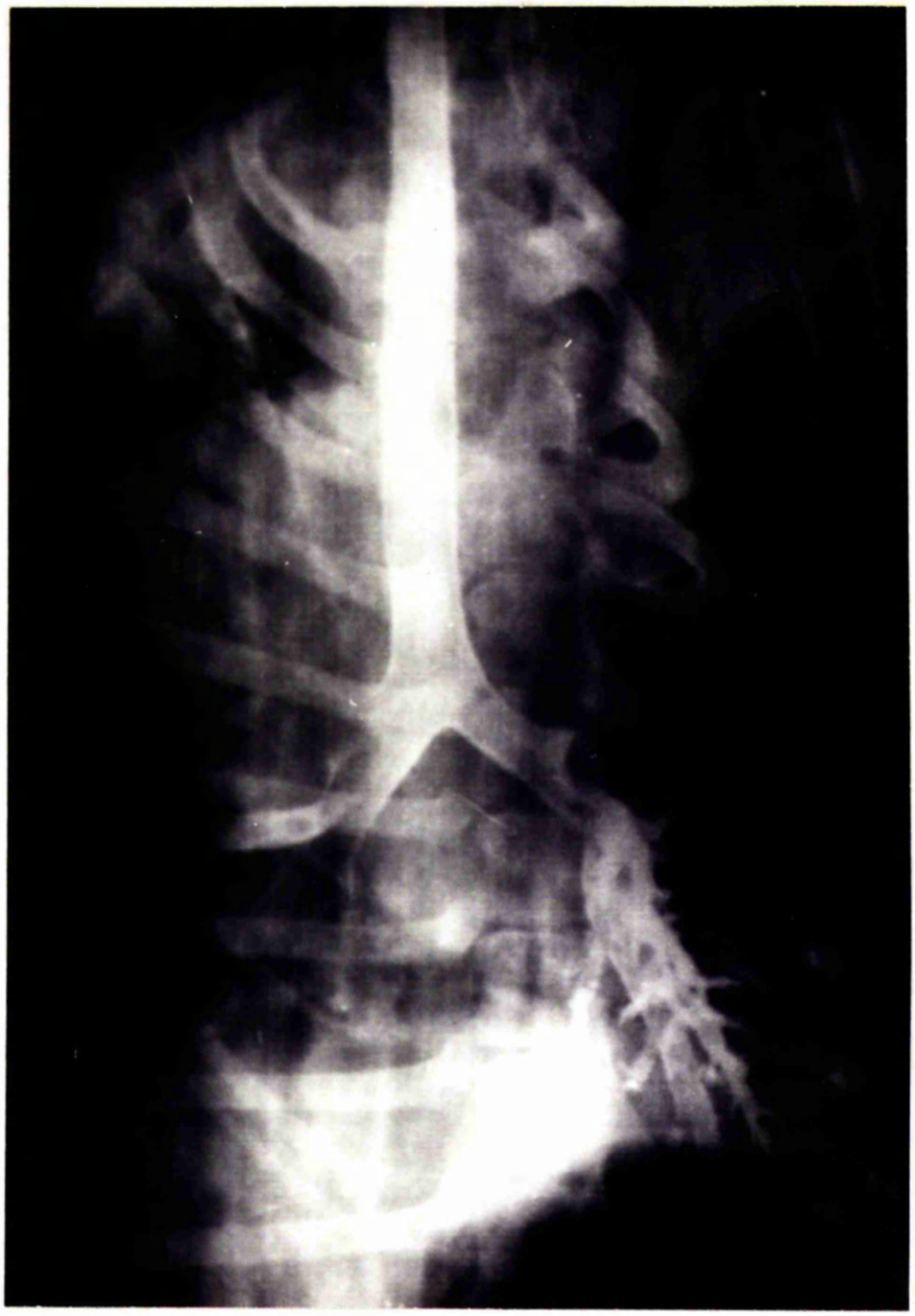

FIGURE 52. Bronchogram at 500 days. Lobar allograft.

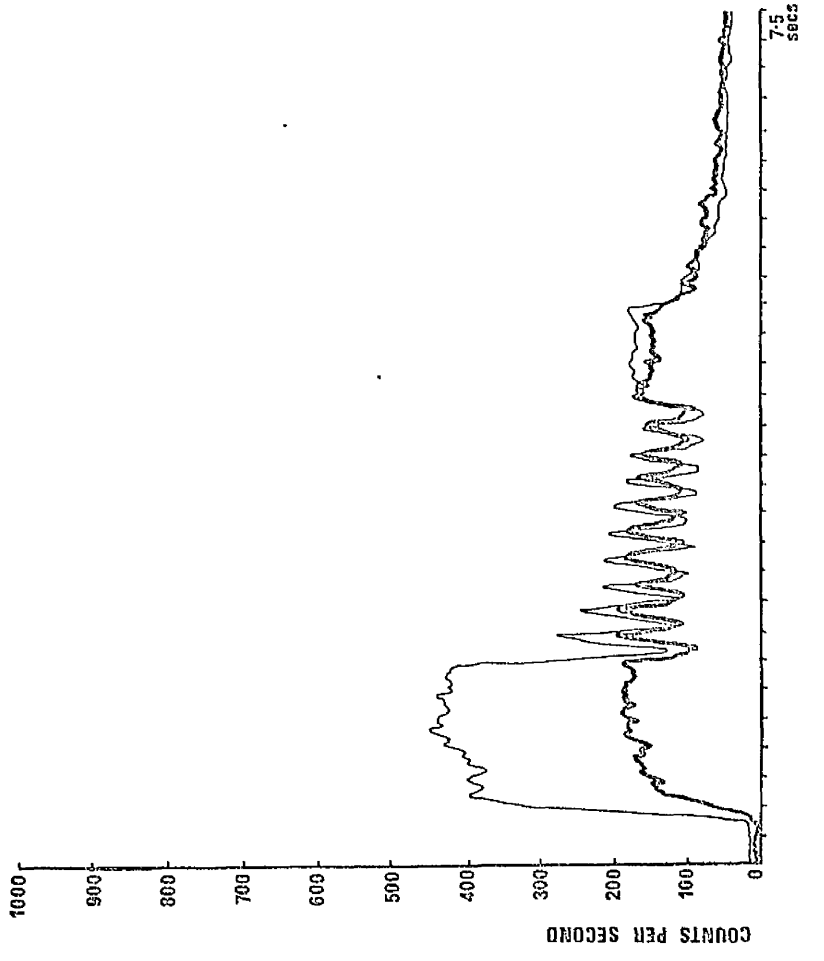

FIGURE 53. Perfusion study in lobar allograft at 506 days. Blood flow reduced but alveolar volume normal.

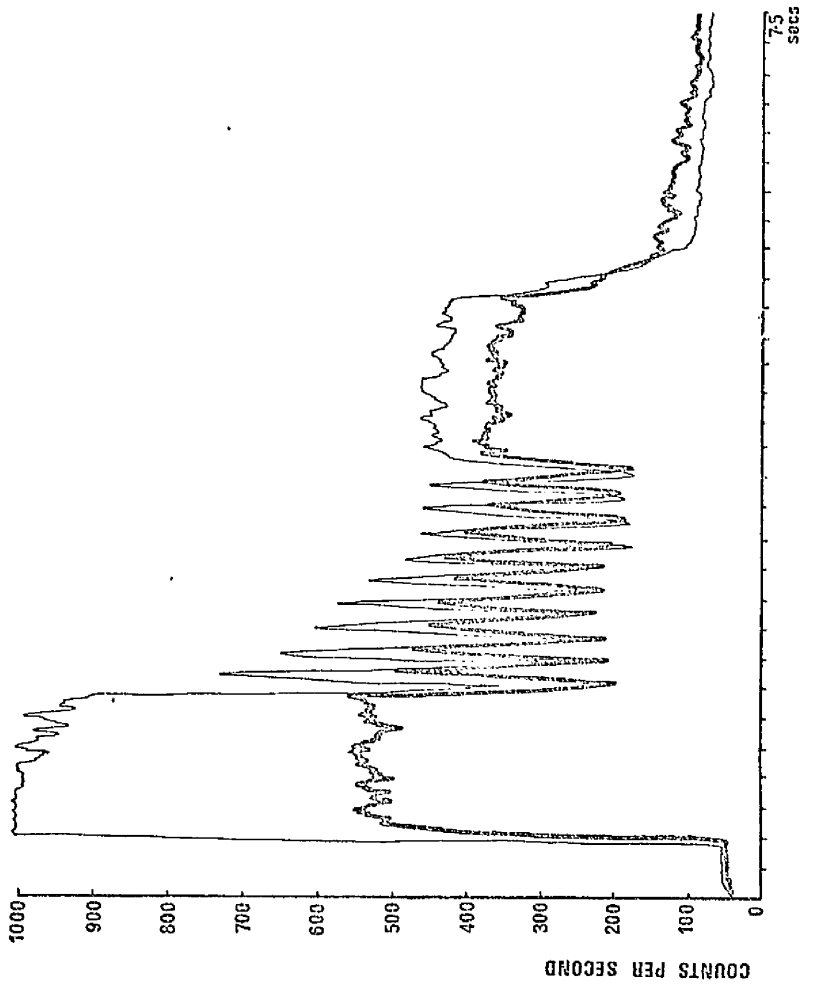

FIGURE 54. Ventilation study in lobar allograft at 506 days. Ventilation and alveolar volume of graft maintained.

2. Both perfusion and ventilation can be maintained adequately in satisfactory autograft preparations indefinitely. Pulmonary allograft function behaves in an exactly similar manner if immunosuppression is successful.

3. Errors in surgical techniques leading to vascular occlusion can be predicted in the immediate post-operative period by the Xenon method. If blood flow L/R ratios are less than 0.30 it is certain that occlusion has taken place and non-survival of the animal is likely.

4. Early prediction of rejection can be made by sequential analysis of the perfusion and ventilation indices. The most sensitive parameters in this respect are blood flow L/R, ventilation L/R and alveolar volume L/R.

5. The most frequent late complication in pulmonary transplantation is bronchial stenosis. This can be surmised in vivo by measurements of the reduction in ventilated alveolar volume of the graft without resort to the cumbersome method of bronchography previously necessary.

6. Frequent assessment of blood flow, ventilation and alveolar volume by this method is practicable in the human pulmonary transplant situation, where co-operation of the patient and voluntary breath-holding would obviate the need for general anaesthesia.

CHAPTER V

DISCUSSION

Experimental work on lung transplantation has delineated those difficulties which might be expected to arise in the clinical application of lung transplantation in man. The biological processes of the immune response common to all organ transplants and the problem of monitoring transplant function require detailed study in this field.

The success in experimental pulmonary allotransplantation using conventional general immunosuppression is sporadic. The data presented here shows that the results obtained by local infusion of immuno-suppressants bear favourable comparison with those obtained by generalised immunosuppression (including anti-lympho-cytic serum) and confirm the beneficial effects reported by Kountz and Cohn in human renal allotransplantation. From this study it seems that recirculation of the infusate is not responsible for the observed effects of local immuno-suppression and that some other mechanism is responsible.

Despite extensive investigation, our knowledge of the processes of rejection is incomplete and the agents used to depress the immune response to tissue allografts are employed in a somewhat empirical fashion.

Interference with those phases of the immune response other than the

central mechanism has been suggested by other workers. Pre-treatment of murine donors of grafts of lymphoid cells with antigen diminishes the graft-versus-host reactivity of these cells (Liacopoulos, Merchant and Harrell, 1967). Local irradiation of canine renal grafts in situ diminishes the intensity of rejection (Dempster, 1953, Kauffman et al., 1965, and Wolf et al., 1969) and has been used clinically in human renal transplants (Hume et al., 1966) and liver transplants (Starzl et al., 1968). Wolf et al. (1969) has shown that the locally irradiated canine kidney of a pair from the same donor survived longer and the report of the human kidney transplant register (Murray, Barnes and Atkinson, 1967) has clearly demonstrated that local irradiation had a statistically significant effect in prolonging survival.

It is also claimed that local application of the naturally occurring polypeptide "trasylol" to human skin allografts delays rejection (Cassiano, Beccario and Troncare, 1967). Dukes and Blocher (1962) similarly reported prolonged survival of skin grafts when pre-treated with streptokinase and streptodornase. It seems likely therefore, that agents used in immunosuppressive treatments may have a local effect in depression of the immune response.

The possibilities to be considered for such a mechanism are the

alteration of graft antigenicity, interference with antigen release or uptake by "antigen-reactive" lymphocytes, diminution in the efferent or afferent arcs of the immune response and non-specific depression of inflammation.

Modification of graft antigenicity to a mild degree has been shown to occur with both drugs, such as thalidomide (Hellman and Duke, 1965) and urethrane (Bonmassar et al., 1966), and by biological means, such as incubation of the graft with allogeneic skin (Hellman, 1967) or with ribonucleic acid (Mannick, 1962, 1964, Fishman, 1963, Cohn, 1965, and Lemperle, 1967). There is, however, no evidence that this can occur with heparin or actinomycin D, although treating skin homografts with steroids directly has been shown to prolong their survival by Billingham, Krohn and Medawar (1951). Although the action of actinomycin D is unknown, Geller and Spiers (1968) have suggested that it prevents ribonucleic acid synthesis and differentiation by antigen.

In this respect, Elliot and Sinclair (1968) in their studies with steroids, have suggested that a defect in antigen trapping, processing and retention is present and that this could be due to a direct action of the steroids.

Ada and Byrt (1969) have described "antigen-reactive" cells similar

to small lymphocytes which proliferate in response to antigen but do not themselves form antibody. Dwyer and MacKay (1970), using autoradiographic techniques and labelling lymphocytes with ^{125}I flagellin found similar "antigen binding" cells which responded by an eight-fold increase in their number to immunisation with foreign antigen. This increase was depressed by the immunosuppressive drug cyclophosphamide and it seems possible that other drugs have a similar action.

Cleveland, Kauffman and Hume (1965) have concluded from their studies that azathioprine acts upon the afferent arc and that it may interfere with the mechanism whereby antigen gains access to the host and initiates the efferent arc of the immune response. Guttman (1967) using antilymphocytic globulin found that pretreatment of the donor decreased the rate of rejection of grafts, and Cerilli (1967) suggested that it formed complexes with donor antigen in his renal transplants. It has been shown however, by Diethelm and Murray that prior intra-arterial infusion of antilymphocytic serum of the donor fails to prevent rejection in renal transplants and that the action of this substance is on the efferent arc, and that the 'coating' effect is probably unstable and of short duration. Similarly, Wilson et al. (1965) have demonstrated that azathioprine has a cytocidal effect on specifically sensitised lymphoid

cells on homologous target cells in culture and that this may be the mechanism of its peripheral action.

Most experimental work has established that the vascular system is the primary site of the immunological interaction between the host and an allograft. Kountz, Williams, Kapros and Dempster (1963) and Porter (1967) demonstrated that the acute first set rejection response is associated with progressive vascular disruption accompanied by adherence of platelets and fibrin to the endothelial wall and escape of fluid and immunoblasts into the interstitial spaces. Knudsen et al. (1967) found that the filling defectsin renal allografts were due to peri-vascular infiltration of host cells and the defects on the pulmonary angiograms presented here are of a similar origin. Porter (1965) has furthermore shown that treated human renal allografts, rejected after some months, may have little evidence of cellular infiltrate whilst damage to arteries and arterioles is present.

The maintainance of perfusion in the pulmonary graft along with graft survival and the absence of any marked histological changes in those grafts treated by local infusion suggests that the usual sequence of vascular changes can be eliminated and implies that blockage of contact between the vectors of immunity in the host and the vascular endothelium

of the graft is the most likely patho-physiological expression of this effect.

Determination of the effect of the individual components of the infusate requires further study. Dougherty, Nehlsen, Minick and Veith (1968) treated two groups of renal canine allografts by intra-arterial infusion of heparin in one group and one of three difference conticosteroids in another without recording the infusion period and had little success in prolonging graft survival or function. They did not, however, test the effect of the soluble corticosteroid methyl-prednisolone, a constituent of the infusate used in this series and that of Kountz and Cohn who have reported striking prolongations of graft survival and a marked increased glomerular blood flow and effective renal plasma flow. Retik, Dubernard, Hester and Murray (1966) have found prolonged survival of canine renal allografts with local infusions of actinomycin C, azathioprine alone and azathioprine with azaserine.

From the experiments described here, it is clear that local immuno-suppression achieves prolongation of graft survival and has application to the specific challenge of chronic survival of pulmonary allografts in the present circumstances. Longer term intra-arterial therapy combined with tissue typing and administration of antilymphocytic serum can be expected

to give better results within the context of the human pulmonary transplantation and may reduce those unwanted side effects of generalised chemical immunosuppression.

Given adequate control of the biological process of rejection in its initial stages, the problems of surgical technique, of the functional capability of a pulmonary transplant to maintain life in the absence of a suitable means of mechanical assistance, the difficulty in monitoring function and detecting rejection, require solution.

The low rate of operative mortality, venous occlusion and bronchial disruption in this series suggests that with care and attention to detail, the immediate problems related to surgical technique can be surmounted.

The main late complication in this and all other series has been the development of bronchial stenosis. The fact that a few long term surviving autografts and allografts do not develop this complication suggest that the pulmonary arterial circulation, although containing venous blood, may be sufficient to prevent this occurring, provided that the anastomoses are adequate, the lung is well oxygenated and the donor bronchial stump is divided close to the parenchyma.

The most pragmatic test of the function of organ grafts to support

life is the removal of the other organ (if it is paired) or bilateral reimplantation. In the case of pulmonary transplants this has been proven by survival after bilateral reimplantations (Haglin, 1964) auto-transplantation of one lung followed by contralateral pneumonectomy (Nigro et al., 1963, and Duvoisin et al., 1964) and by ligation of the opposite pulmonary artery after transplantation of a lung (Veith and Richards, 1969).

The introduction of radioactive gas techniques allows greater discrimination of the assessment and continuous monitoring of transplant function. The results show that measurement by Xenon^{133} gives reliable differential measurements with certain precautions and that more information can be obtained by this method than by the previous methods of repeated biopsy, differential bronchospirometry or measurement of peripheral arterial oxygen saturation, although these have provided in the past invaluable information on the behaviour of pulmonary grafts.

The infrequent interval at which these functional studies tend to be made has given rise to considerable variability in the results and their interpretation. Nigro (1961) claimed that the function of a reimplanted lung was depressed two months after surgery, that oxygen uptake ventilation and carbon dioxide excretion were markedly reduced, and that

pulmonary arterial pressures were elevated. Yeh and Ellison (1962) claimed that the arterial pressure was normal in 9 dogs subjected to pulmonary autograft procedures and that ventilation and elimination of carbon dioxide were unimpaired 6 to 24 weeks after operation. Christiansen, Buck, Fanfera, Gross, Pinch, Stainback and Trummer (1966) have suggested that there is some impairment of ventilation secondary to a deficient transport of oxygen across the alveolar capillary membrane following homologous transplantation of the lung. Shaw and Burton (1964) found that the oxygen uptake and the tidal ventilation were considerably reduced although both became increased to near normal values on clamping the contralateral bronchus. More recently, however, oxygen uptake after transplantation has been assessed by Zajtchuk, Rostik, Gago and Adams (1967) to approach 75% of the expected values, whereas Kottmeir, Cheng and Fitzgerald (1969) in a study of 10 re-implanted lungs reported that oxygen consumption was diminished to 45%.

The considerable variation in the reported series suggests either that the methods used are open to error or that there are sequential changes in transplant function after operation. This investigation shows that sequential changes do occur. The mechanical effects of the operation can be most conveniently studied on the autotransplant procedures. There is

a temporary depression of function with diminution of perfusion which returns to normal within a few days after operation. Transient depression of oxygenation and ventilation has been noted by others (Richter and Bucherl, 1960, Faber, 1961, Reemsta et al., 1963, Bucherl, 1964, and Daly and Waldhausen, 1966). This present investigation confirms this finding.

Strieder, Barnes, Aronow, Russell and Kazemi (1967) studied the ventilation and perfusion of left lung autografts in 4 dogs up to 40 days after operation using $Xenon^{133}$. They found diminution in both ventilation and perfusion during this period with reduction of the aeriated volume of the graft to 39% of the total volume. In contradistinction to both the present investigation and that of Pain, De Bono, Glazier, Maloney and West (1967) however they did not find any significant variation in perfusion or ventilation with time. The difference between these findings probably lie in the fact that their estimations were of an intermittent nature (only one autograft preparation having more than three consecutive readings) and also that they did not record whether structural abnormalities were present in their few grafts examined. The pattern of function in the immediate post-transplant period has been attributed to a variety of causes such as a diffusion defect in the capillary membrane (Reemsta, 196

transient decrease in pulmonary alveolar surfactant (Trimble, 1966) spasm of pulmonary arteries (Nigro, 1964) ischaemia during the transplant procedure (Davies and West, 1965) and interruption of lymphatic supply (Hardy, 1963, and Stone, 1966). These speculations, however, do not take into account similar effects which take place after operation not involving pulmonary transplant procedures. Bryant and his colleagues using scintiscanning techniques (1967) have demonstrated in 97 patients that there was an abnormal distribution in flow after abdominal operations in 61% of those studied and that this returned to normal within 7 days. This effect was noted to be more marked after thoracic operations.

The present experimental studies using Xenon^{133} on lung transplants show that there is diminution in perfusion per unit volume of effectively ventilation lung tissue and suggest that the early depression of pulmonary function in the post-transplant period is primarily due to abnormal perfusion in underventilated lung tissue as a consequence of segmental atelectasis.

The biological potential of long term pulmonary transplants has been under considerable discussion and the significance of denervation in relation to the late deterioration in function disputed. Pain and his colleagues using Xenon^{133} studied 2 canine lung autografts, 1 at 2 months

and 1 at 9 months following the procedure. They reported that blood flow was reduced in the 9 months survivor and that both perfusion and ventilation were reduced in the other animals, whose graft was subsequently found to be collapsed at post-mortem. Strieder, however, reported that there was normal function as measured by Xenon^{133} in the single 6 month autograft survivor in their series.

The present study demonstrates clearly that long term autograft survivors may have good perfusion and ventilation in the absence of structural abnormalities; persistently diminished blood flow ratios correlates well with vascular occlusion and late reduction in alveolar volume is due to bronchial stenosis. In addition the functional assessment of a graft by Xenon^{133} perfusion and ventilation obviates the necessity of determining these abnormalities by direct methods involving complicated radiographic techniques.

The continued absence of the Hering-Breuer reflex, which has been noted by both Duvoisin and Portin (1960) at 8 months and 35 months after transplantation respectively, bears little relationship to the functional capability of pulmonary grafts. Numerous investigators (Nigro, 1963, Bucherl, 1964, Faber, 1965, Christiansen, 1965, Trummer, 1965, Sharma, 19[illegible] Pain, 1967, Allgood, 1968) have reported elevations in pulmonary vascular

resistance which were ascribed to denervation. The findings of normal perfusion, ventilation and alveolar volume by Xenon^{133} in technically satisfactory autografts, however, confirms the recent observations of Benfield (1968) and Veith (1969) that the alteration in pulmonary haemodynamics is more related to the technical complications of the vascular anastomoses. Benfield's work incriminates the venous anastomosis and Veith has shown that the indistensible arterial anastomosis can give rise to pulmonary arterial hypertension. This can be eliminated by attention to surgical technique in the prevention of vascular stenosis.

The situation with regard to denervation has not been completely clarified in respect of other facets of pulmonary physiology, such as ciliary movement, bronchial secretion and muscle function, compliance and pulmonary surfactant activity. Waldhausen, Giamoona and Kilman (1965) reported an initial fall in compliance, lung stability and surfactant activity which returned to normal 15 days after transplantation. Utilising the relatively recent procedure of tantalum bronchography, Edmunds and his colleagues (1969) described delay in mucous clearance in transplanted lungs which lasted for 80 days after transplantation. Little note has again been taken in the transplant literature of the human experiments in

this field. Klassen, Morton and Douglas (1951) described the effects of vagal and sympathetic nerve section in patients with bronchogenic carcinomata. They found that despite the absence of a cough reflex in the homolateral bronchial tree, there were little effect on the normal bronchial movement, no change in amount or quality of bronchial secretions and no impairment of tracheo-bronchial clearance. It seems, therefore, unlikely that there will be any deleterious effects in this respect following pulmonary transplantation especially in the human situation where adequate physiotherapy and postural drainage can be timeously efficacious.

Determination of function and the effects of rejection has given rise to considerable difficulty in the past due to rapidity with which rejection takes place and to the sporadic nature of chronic survival beyond a few weeks in treated animals. Some series show a gradual loss of function despite treatment. Gago (1964) found that only 1 dog out of 4 long term survivors had a functioning allograft at the end of 6 months. Yeh and his colleagues as well as Reemsta's group found similar diminution in oxygen uptake after homotransplantation although Sharma reported normal oxygen uptake and ventilation in 2 chronic survivors out of 16 animals.

Blumenstock (1967) using differential bronchospirometry reported

that the oxygen consumption of one long term survivor was 40% of the total function; 2 animals, alive at 3 and 5 years were shown to have normal gross and microscopic architecture. Evaluation of perfusion and ventilation indices by Xenon^{133} show that the long term function of an allotransplant is altered very little in the absence of rejection, compared with a similar autograft preparation and tends to confirm Blumenstock's work.

The histological features of rejection in pulmonary transplants are well documented but the physiological sequence of functional alteration is less well determined in relation to transplant rejection. Waiting for signs of rejection almost certainly allows the development of irreversible necrosis. Thus, the early recognition of these feature is essential to proper control of therapy. Strieder and her colleagues determined that the aeriated volume of the graft fell as rejection progressed in their study of 6 untreated pulmonary allografts up to 10 days after operation. Pain measured ventilation and perfusion by Xenon^{133} in 1 homograft survivor up to 20 days. They stated that blood flow per unit volume was not decreased with rejection although alveolar volume was reduced. Xenon^{133} measurements in this investigation, hoever, show conclusively that there is diminution in perfusion and sequential reduction in

effectively ventilated alveolar volume as one might reasonably expect from the known effects of rejection. The difference between this conclusion and that of Pain's group can be clarified by the observation of their graphs. There is in fact marked reduction in the blood flow ratios as well as alveolar volume with the result that blood flow per unit volume of the graft is only apparently undiminished during rejection.

It is clear, therefore, that pulmonary transplantation in man is feasible, practicable and worthy of cautious clinical application given suitable methods of immunosuppression. Xenon distribution studies can be used to permit rapid evaluation of the changes during rejection and late technical failure in the human subject, where voluntary breathholding will obviate the need for anaesthesia and the presence of an indwelling catheter in the pulmonary arterial circulation will allow not only intermittent local immunosuppression but also more frequent determination of perfusion in the graft.

REFERENCES

1. Ada, G.L. and Bryt, P. (1969): Nature (Lond.) 222: 1291.

2. Alican, F. and Hardy, J.D. (1963): J.A.M.A. 183: 849.

3. Allgood, R.J., Ebert, P.A. and Sabiston, D.C. (1968): Ann. Surg. 167: 354.

4. Anderson, J.M. and Hutchison, J. (1968): Dis. Chest 53: 551.

5. Ball, W.C.Jr., Stewart, P.B., Newsham, L.G.S. and Bates, D.V. (1962): J. clin. Invest. 41: 519.

6. Barnes, B.A., Flax, M.H., Burke, J.F. and Barr, G. (1963): Transplantation 1: 351.

7. Barnes, B.A. and Flax, M.H. (1964): Transplantation 2: 343.

8. Benfield, J.R., Gago, O., Nigro, S.L., Fry, W.A. and Adams, W.E. (1963): Surg. Forum 14: 198.

9. Benfield, J.R. and Coon, R. (1967): J. thorac. cardiovasc. Surg. 53: 676.

10. Benfield, J.R., Coon, R. and Cree, E.M. (1967): Dis. Chest 52: 321

11. Billingham, R.E., Krohn, P.L. and Medawar, P.B. (1951): Brit. med. J. 2: 1049.

12. Blades, B., Beattie, E.J., Hill, R.P. and Thistlewaite, R. (1952): Ann. Surg. 136: 56.

13. Blumenstock, D.A., Collins, J.A., Thomas, E.D. and Ferrebee, J.W. (1961): Surg. Forum 12: 121.

14. Blumenstock, D.A. and Kahn, D.R. (1961): J. surg. Res. 1: 40.

15. Blumenstock, D.A., Collins, J.A., Hechtman, H.B., Hosbein, D.J., Lempert, N., Thomas, E.D. and Ferrebee, J.W. (1964): Ann. N.Y. Acad. Sci. 120: 677.

16. Blumenstock, D.A. (1967): Transplantation 5: 917.

17. Bogardus, G.M. (1958): Surgery 43: 849.

18. Bonmassar, E., Francesconi, G., Manzoni, S.C. and Perelli-Ercolini, M. (1966): Nature 209: 1141.

19. Borrie, J. and Montgomery, J.L. (1958): Proc. Univ. Otago.med. Sch. 36: 9.

20. Borrie, J. and Montgomery, J.L. (1958): Proc. Univ. Otago.med. Sch. 36: 10.

21. Borrie, J., Campbell, G.D. and Fulton, I.B. (1958): Proc. Univ. Otago. med. Sch. 36: 31.

22. Borrie, J. and Lichter, I. (1964): Thorax 19: 383.

23. Bryan, A.C., Bentivoglio, L.G., Beerel, F., Macleish, H., Zidulka, A. and Bates, D.V. (1964): J. appl. Physiol. 19: 395.

24. Bryant, L., Spencer, F.C., Greenlaw, R.H., Prathnadi, P. and Bowlin, J.W. (1967): J. thorac. cardiovasc. Surg. 53: 64.

25. Bucherl, E.S., Nasseri, M. and Von Prodzynski, B. (1964): J. thorac. cardiovasc. Surg. 47: 455.

26. Burrows, B. and Earle, R.H. (1969): Amer. Rev. resp. Dis. 99: 865.

27. Calne, R.Y. (1961): Plast. reconstr. Surg. 28: 445.

28. Calne, R.Y. (1963): Renal transplantation (Publishers: Arnold, London)

29. Carrel, A. (1907): Johns Hopk. Hosp. Bull. 190: 18.

30. Cassiano, O., Beccaria, A. and Troncare, S. (1967): Minerva chir. 22: 702.

31. Cerilli, J., Groth, C., Taylor, P. and Daloze, P. (1967): Transplantation 5: 1334.

32. Christiansen, K.H., Buck, A.S., Fanfera, F., Gross, R., Pinch, L.W. Stainback, W.C. and Trummer, M.J. (1965): Arch. Surg. 90: 38.

33. Cleveland, R.J., Kauffman, H.M. and Hume, D.M. (1965): Surg. Forum 16: 269.

34. Cohen, E.P., Newcomb, R.W. and Crosby, L.K. (1965): J. Immunology 95: 583.

35. Connaughton, P.S., Bahuth, J.J. and Lewis, F.J. (1962): Dis. Chest 41: 404.

36. Daly, W.J. and Waldhausen, J.A. (1966): Surg. Forum 17: 214.

37. Davies, L.G., Rosser, T.H.L. and West, L.R. (1965): Thorax 20: 481.

38. De Bono, A.H.C. and Brock, R.C. (1964): Brit. J. Surg. 51: 72.

39. Dec Astello, V. (1902): Wien. klin. Wschr. 5: 317.

40. Demikov, V.P. (1962): Experimental Transplantation of Vital Organs. (translation by Basil Haigh). Consultants Bureau Enterprises Inc. New York. p130.

41. Dempster, W.J. (1953): Brit. J. Surg. 40: 447.

42. Derom, F. (1969): J. thorac. cardiovasc. Surg. 58: 340.

43. Dollery, C.T., Hugh-Jones, P. and Matthews, C.M.E. (1962): Brit. med. J. 2: 1006.

44. Dougherty, J.C., Nehlsen, S.L., Minick, R. and Veith, F.J. (1968): Transplantation 6: 554.

45. Diethelm, A.G., Busch, G.T., Dubernard, H.M., Orr, W.N., Glassoch, R.J., Birtch, A.G. and Murray, J.E. (1969): Ann. Surg. 169: 569.

46. Dukes, C.D. and Blocker, T.G. (1962): Ann. Surg. 136: 999.

47. Duvoisin, G.E., Fowler, W.S., Payne, W.S. and Ellis, F.H. (1964): Surg. Forum 15: 173.

48. Duvoisin, G.E., Fowler, W.S., Payne, W.S. and Ellis, F.H. (1968): Arch. Path. 85: 51.

49. Dwyer, J.M. and Mackay, I. (1970): Lancet 1: 164.

50. Ebert, P.A., Allgood, R.J., Jones, H.W.III. and Sabiston, D.C. (1967): Surgery 62: 18.

51. Edmunds, L.H., Stallone, R.J., Graf, P.D., Sagel, S.S. and Greenspan, R.H. (1969): Surgery 66: 15.

52. Elliott, E.V. and Sinclair, N.R. St.C. (1968): Immunology 5: 643.

53. Ellis, F.H. Jr., Grindlay, J.H. and Edwards, F. (1951): Surgery 30: 81C

54. Faber, L.P. and Beattie, E.J. (1958): Surg. Forum 9: 383.

55. Faber, L.P., Kenwell, J.M. and Beattie, E.J. (1961): Arch. Surg. 83: 491.

56. Faber, L.P., Perreira, A.L.S., Pevsner, P.H. and Beattie, E.J.Jr. (1965): J. thorac. cardiovasc. Surg. 50: 761.

57. Fegiz, G., Pedreira, A.L.S., Peusner, P.H. and Beattie, E.J.Jr. (1959): Surg. 50: 761.

58. Fishman, M., Hammerstrom, R.A. and Bond, V.P. (1963): Nature 198: 549.

59. Floresco, (1905): J. Physiol. Path. gen. 27.

60. Fonkalsrud, E.W., Ono, H., Shafey, O.H. and Longmire, W.P.Jr. (1967): Surg. Gynec. Obstet. 125: 319.

61. Fonkalsrud, E.W., Stevens, G.H., Rangel, D.M., Joseph, W.L., Yakeishi, Y. and Tocornal, J.A. (1969): J. thoracic. cardiovasc. Surg. 57: 607.

62. Gago, O., Zajtchuk, R., Nigro, S.L. and Adams, W.E. (1950): J. thorac. cardiovasc. Surg. 50: 775.

63. Gago, O., Dalgado, E., Archer, F.L., Schoenfield, F.G., Ranniger, K., Nigro, S.L. and Adams, W.E. (1964): J. thorac. cardiovasc. Surg. 48: 726.

64. Gago, O., Benfield, J.R., Nigro, S.L. and Adams, W.E. (1965): J.A.M.A. 191: 306.

65. Garzon, A.A., Cheng, C., Lichenstein, S. and Karlson, K.E. (1966): Surg. Forum 17: 208.

66. Geller, B.D. and Spiers, R.S. (1968): Immunology 15: 707.

67. Goodrich, E.D., Welch, H.F., Nelson, J.A., Beecher, T.S. and Welch, C.S. (1956): Surgery 39: 244.

68. Guthrie, C.C. (1907): Johns Hopk. Hosp. Bull. 18: 25.

69. Guttman, R.B., Carpenter, G.B., Lindquist, R.R. and Merrill, J.P. (1967): Lancet 1: 248.

70. Guz, A., Noble, M.M., Trenchard, D., Cochrane, H.L. and Makey, A.R. (1964): Clin. Sci. 27: 293.

71. Haglin, J., Telander, R.L., Muzzall, R.E., Kiser, J.C. and Strobel, C.J. (1963): Surg. Forum 14: 196.

72. Haglin, J. and Orn, A. (1964): Surg. Forum 15: 175.

73. Hallmann, G.L., Leatherman, L.L., Leachman, R.D., Rochelle, D.G., Bricker, D.L., Bloodwell, R.D. and Cooley, D.H. (1969): J. thorac. cardiovasc. Surg. 58: 318.

74. Hardin, C.A. and Kittle, C.F. (1954): Science 119: 97.

75. Hardin, C.A., Kittle, C.F. and Schafer, P.W. (1952): Surg. Forum 3: 374.

76. Hardin, C.A. (1955): Surg. Forum 6: 565.

77. Hardin, C.A. (1956): Transplant. Bull. 3: 45.

78. Hardy, J.D., Eraslan, S., Dalton, M.Jr., Alican, F. and Turner, M. (1963): Ann. Surg. 157: 706.

79. Hardy, J.D., Webb, W.R., Dalton, M.L.Jr., and Walker, G.R. (1963): J.A.M.A. 186: 1065.

80. Hardy, J.D., Eraslan, S. and Dalton, M.L.Jr. (1963): J. thorac. cardiovasc. Surg. 46: 606.

81. Hardy, J.D., Eraslan, S. and Webb, W.R. (1964): Ann. Surg. 160: 440.

82. Hellmann, K., Duke, D.I. and Tucker, D.F. (1965): Brit. med.J. 2: 687.

83. Hellmann, K. and Duke, D.I. (1967): Transplantation 5: 184.

84. Hendry, W.F., Struthers, N.W., Duguid, W.P. and Hopkinson, W.I. (1968): Brit. J. Surg. 55: 431.

85. Hill, R.B.Jr., Dahrling, B.E., Starzl, T.E. and Rifkind, D. (1967): Amer. J. Med. 42: 327.

86. Hino, K., Grogan, J.B. and Hardy, J.D. (1968): Transplantation 6: 25.

87. Hirsch, E.F., Kaiser, G.C., Barner, H.B., Cooper, T. and Rams, J.J. (1968): Arch. Path. 85: 51.

88. Huggins, C.E. (1959): Lancet 1: 59.

89. Hume, D.M., Lee, H.M., Williams, G.M., White, H.J.O., Ferre, J., Wolf, J.S., Prort, G.R., Slapak, M., O'Brien, J., Kilpatrick, S.J., Kauffman, H.M. and Cleveland, R.J. (1966): Ann. Surg. 144: 352.

90. Jones, W.H. (1966): Thorax 21: 405.

91. Jones, W.H. (1967): Brit. J. Surg. 54: 70.

92. Juvenelle, A.A., Citret, C., Wiles, C.E.Jr. and Stewart, J.D. (1951): J. thorac. Surg. 21: 111.

93. Kahn, D.R., Wilson, W.S., Tolbert, J.H. and Sloan, H. (1965): Ann. Thorac. Surg. 1: 97.

94. Karsner, H.T. and Ash, J.E. (1912): J. med. Res. 27: 205.

95. Kauffman, H.M., Cleveland, R.J., Dwyer, J.J., Lee, H.M. and Hume, D.M. (1965): Surg. Gynec. Obstet. 120: 149.

96. Klassen, K.P., Morton, D.R. and Curtis, G.M. (1951): Surgery 29: 483.

97. Knipping, H.W., Bolt, W., Venrath, H., Valentin, H., Ludes, H. and Endler, P. (1955): Dtsch. med. Wschr. 80: 1146.

98. Knudsen, D.F., Davidson, A.J., Kountz, S.L. and Cohn, R. (1967): Transplantation 5: 256.

99. Kottmeir, P.K., Cheng, C. and Fitzgerald, J. (1969): J. Pediat. Surg. 4: 66.

100. Kountz, S.C., Williams, M.A., Williams, P.L., Kapros, C. and Dempster, W.J. (1963): Nature 199: 251.

101. Kountz, S.L., Laub, D. and Cohn, R. (1965): J.A.M.A. 191: 997.

102. Kountz, S.L. and Cohn, R. (1967): Advance in Transplantation: Proceedings of the First International Congress of the Transplantation Society.

103. Kountz, S.L. and Cohn, R. (1969): Lancet i: 338.

104. Kramer, M.S. and Nakamoto, S. (1968): Abstr. Amer. Soc. Nephrology. Washington D.C. p 34.

105. Lanari, A., Molins, M. and Croxatto, O. (1950): Ass. Cat. Pat. Tuberc. 12: 5.

106. Lanari, A., Croxatto, O. and Molins, M. (1951): Medicina (B. Aires) 11: 12.

107. Lanari, A., Croxatto, O. and Molins, M. (1956): Poumon. 1: 67.

108. Lanari, A., Croxatto, O. and Molins, M. (1956): Medicina (B. Aires) 15: 83.

109. Largiader, F., Manax, W.G., Lyons, G.W. and Lillehei, R.C. (1965): Schweiz med. Wschr. 95: 1151.

110. Largiader, F., Manax, W.G., Lyons, G.W. and Lillehei, R.C. (1965): Trans. amer. Soc. artif. Organs XI: 197.

111. Largiader, F., Manax, W.G., Lyons, G.W. and Lillehei, R.C. (1966): Dis. Chest 49: 1.

112. Lemperle, G. (1967): Second Congress of the European Society for Experimental Surgery, Belgium.

113. Lempert, N. and Blumenstock, D.A. (1964): Surg. Forum 15: 179.

114. Liacopoulos, P., Merchant, B. and Harrell, B.E. (1967): Transplantation 5: 1423.

115. Linberg, E.J., Demetriades, A., Armstrong, B.W. and Konsuwan, N. (1961): J.A.M.A. 178: 486.

116. MacGovern, G.J. and Yates, A.J. (1964): Ann. N.Y. Acad. Sci. 120: 710.

117. MacPhee, I.W. and Wright, E.S. (1964): Lancet 1: 192.

118. Mannick, J.A. and Egdahl, R.H. (1962): Science 137: 976.

119. Mannick, J.A. and Egdahl, R.H. (1964): J. clin. Invest. 3: 2166.

120. Matthew, H., Logan, A., Woodruff, M.F.A. and Heard, B. (1968): Brit. med. J. 3: 759.

121. Meshalkin, E.N. and Feofilov, G.L. (1964): Eksp. Khir. Anest. 9: 26.

122. Meshalkin, E.W., Sergievsky, V.S., Arkipova, G.F., Okuvena, G.F Savinsky, G.A., Vlasov, Y.A. and Didenko, V.I. (1964): Eksp. Khir. Anest. 9: 34.

123. Metras, H. (1950): C.R. Acad. Sci. (Paris) 231: 1176.

124. Milhaud, A., Leandri, J., Pariente, R. and Laurent, D. (1969): Europ. surg. Res. 1: 126.

125. Miller, W.S. (1925): Amer. Rev. Tuberc. 12: 87.

126. Mouritzen, C., Boye, E., Lyager, S. and Ottosen, P. (1967): Scand. J. thorac. cardiovasc. Surg. 1: 87.

127. Murray, J.E., Barnes, B.A. and Atkinson, J. (1967): Transplantation 5: 752.

128. Neptune, W.B., Redondo, H. and Bailey, C.P. (1952): Surg. Forum 3: 379.

129. Neptune, W.B., Weller, R. and Bailey, C.P. (1953): J. thorac. Surg. 26: 275.

130. Nettlebad, S.C., Soroff, H.S., Sachs, B., Sharma, A.N., Bellas, A.F., Sharman, J., Harrison, H.N. and Deterling, R.A. Jr. (1964): Ann. N.Y. Acad. Sci. 120: 689.

131. Nigro, S.L., Reimann, A.F., Fry, W.A., Mock, L.F. and Adams, W.E. (1961): Surg. Forum 12: 56.

132. Nigro, S.L., Reimann, A.F., Mock, L.F., Benfield, J.R. and Adams, W.E. (1963): J.A.M.A. 183: 854.

133. Nigro, S.L., Evans, R.H., Benfield, J.R., Gago, O., Fry, W.A. and Adams, W.E. (1963): J. thorac. cardiovasc. Surg. 46: 598.

134. Nigro, S.L., Evans, R.H., Gago, O. and Adams, W.E. (1964): Dis. Chest 46: 318.

135. Nigro, S.L., Hirsch, E.F., Rams, J.J., Hamouda, F. and Adams, W.E. (1968): Arch. Surg. 96: 138.

136. Otto, D., Honore, D. and Blumenstock, D. (1967): Acta. chir. Belg. 8: 747.

137. Pain, M.C.F., De Bono, A.H., Glazier, J.B., Maloney, J.E. and West, J.B. (1967): J. thorac. cardiovasc. Surg.

138. Parsa, P., Faber, L.P., Staub, E.W. and Beattie, E.J.Jr. (1964): Dis. Chest 45: 365.

139. Pegg, D.E., Calne, R.Y., Pryse-Davies, J. and Brown, F.L. (1964): Ann. N.Y. Acad. Sci. 120: 506.

140. Porter, K.A., Calne, R.Y. and Zukoski, C.F. (1964): Lab. Invest. 13: 809.

141. Porter, K.A., Marchioro, T.L. and Starzl, T.E. (1965): Brit. J. Urol. 37: 250.

142. Porter, K.A. (1965): Brit. med. Bull. 21: 171.

143. Porter, K.A., Dosseter, J.B., Marchioro, T.L., Peart, W.S., Rendall, J.M., Starzl, T.E. and Terasaki, P.I. (1967): Lab. Invest. 16: 153.

144. Portin, B.A., Rasmussen, G.I., Stewart, J.D. and Andersen, M.N. (1960): J. thorac. cardiovasc. Surg. 39: 380.

145. Rahn, H., Sadoul, P., Farhi, L.E. and Shapiro, J. (1956): J. appl. Physiol. 8: 417.

146. Rangel, D.M., Stevens, G.H., Yakeishi, Y., Sanchez, M. and Fonkalsrud, E.W. (1969): Surgery 66: 863.

147. Reemsta, K., Lucas, J.F., Schmidt, F.E. and Davies, F.H.Jr. (1963): J. thorac. cardiovasc. Surg. 46: 589.

148. Retik, A.B., Dubernard, J.M., Hester, W.J. and Murray, J.E. (1966): Surgery, St. Louis 60: 1242.

149. Richards, V. and Asway-Pinto, D. (1964): Amer. J. Surg. 110: 218.

150. Richter, M. and Bucherl, E.S. (1960): Verh. dtsch. Ges. Path. 44: 178.

151. Shackman, R., Dempster, W.J. and Wrong, D.M. (1963): Brit. J. Urol. 35: 222.

152. Sharma, A.N., Soroff, H.S., Bellas, A.E., Harrison, H.N., Sherman, J. and Deterling, R.A.Jr. (1966): Surg. Gynec.Obstet. 123: 295.

153. Sharma, A.N., Soroff, H.S., Bellas, A.E., Sachs, B.F., Nettleblad, S.C. and Deterling, R.A.Jr. (1966): Surg. Gynec. Obstet. 123: 1001.

154. Sharma, A.N., Soroff, H.S., Bellas, A.E., Giron, F. and Deterling, R.A.Jr. (1966): J. thorac. Surg. 52: 635.

155. Shaw, K.M. and Burton, N.A. (1964): Thorax 19: 180.

156. Shaw, K.M. and Hill, P.McN. (1968): Thorax 23: 408.

157. Shinoi, K., Hayata, Y., Aoki, H., Kosaki, M., Yoshioka, L., Shinoda, A., Inahashi, H., Ito, M. and Endo, M. (1966): Amer. J. Surg. 112: 617.

158. Slim, M.S., Yacoubian, H.D., Wilson, J.L., Rubeiz, G.A. and Ghandur-Manymneh, L. (1964): Surgery 55: 676.

159. Slim, M.S., Yacoubian, H.D., Simonian, M.B. and Sahyoun, P. (1965): Ann. Thorac. Surg. 1: 755.

160. Starzl, T.E., Kaupp, H.A., Brock, D.R. and Linman, J.W. (1961): Surg. Gynec. Obstet. 112: 135.

161. Starzl, T.E., Groth, C.G., Brettschneider, L., Moon, J.B., Fulginiti, V.A., Cotton, E.R. and Porter, K.A. (1968): Surgery 63: 549.

162. Staudacher, V.E., Bellinazzo, P. and Pulin, A. (1950): Chir. Pat. sper. 5: 223.

163. Staudacher, V.E., Bellinazzo, P. and Pulin, A. (1950): Chirurgia 5: 211.

164. Staudacher, V.E., Bellinazzo, P. and Pulin, A. (1951): Surg. Gynec. Obstet. 93: 236.

165. Stinson, E.B., Dong, E.Jr., Bieber, C.P., Schroeder, J.G. and Shumway, N.E. (1969): J.A.M.A. 207: 2233.

166. Stone, R.M., Ginsberg, R.J., Colapinto, R.F. and Pearson, F.O. (1966): Surg. Forum 17: 109.

167. Strieder, D.J., Barnes, B.A., Aronow, S., Russell, P.S. and Kazemi, H. (1967): J. appl. Physiol. 23: 359.

168. Susuki, C., Nakada, T., Watanabe, A., Fujimura, S. and Kawakami, M. (1968): J. thorac. cardiovasc. Surg. 55: 200.

169. Trimble, A.S., Kim, J. and Bharadwaj, B. (1966): Surg. Forum 17: 210.

170. Trimble, A.S., Kim, J., Bharadwaj, B., Bedard, B. and Wells, C. (1966): J. thorac. cardiovasc. Surg. 52: 271.

171. Trummer, M.J. and Christiansen, K.H. (1965): J. thorac. cardiovasc. Surg. 49: 1006.

172. Trummer, M.J. (1965): Ann. Thorac. Surg. 1: 203.

173. Ullmann, (1902): Wien. klin. Wschr. S. 281.

174. Ullmann, (1902): Wien. klin. Wschr. S. 707.

175. Veith, F.J., Hagstrom, J.W.C., Nehlsen, S.L., Karl, R.C. and Deysine, M. (1967): Ann. Surg. 165: 267.

176. Veith, F.J., Panossian, A., Bogartz, L. and Koerner, S. (1969): Surg. Gynec. Obstet. 493.

177. Veith, F.J. and Richards, K. (1969): Surg. Gynec. Obstet. 129: 768.

178. Veith, F.J., Richards, K. and Lalezari, P. (1969): J. thorac. cardiovasc. Surg. 58: 829.

179. Vuillard, P., Gadot, P. and Ravier, M.A. (1969): Ann. chir. thorac. cardiovasc. 8: 117.

180. Waldhausen, J.A., Kilman, J.W. and Giammona, S.T. (1964): J.A.M.A. 188: 440.

181. Waldhausen, J.A., Giammona, S.T., Kilman, J.W. and Daly, W.J. (1965): J.A.M.A. 191: 1002.

182. Waldhausen, J.A., Daly, W.J., Baez, M. and Giammona, S.T. (1967): Ann. Surg. 165: 580.

183. West, J.B. (1962): J. appl. Physiol. 17: 893.

184. White, J.J., Tanser, P.H., Anthonisen, N.R., Wynands, J.E., Pare, J.A.P., Becklake, M.R., Munro, D.D. and Maclean, L.D. (1966): Canad. med. Ass. J. 94: 1199.

185. Wildevuur, C.H. and Benfield, J.R. (1970): Sixth Annual Meeting of the Society of Thoracic Surgeons, Altanta. (A Review of 21 Clinical Lung Transplants).

186. Wilson, D.B. (1965): J. exp. Med. 122: 143.

187. Wolf, J.S., McGavie, J.D. and Hume, D.M. (1969): Surg. Gynec. Obstet. 128: 584.

188. Yeh, T.J., Ellison, L.T. and Ellison, R.G. (1962): Amer. Rev. resp. Dis. 86: 791.

189. Yeh, T.J., Ellison, L.T. and Ellison, R.G. (1964): Surg. Forum 15: 191.

190. Yeh, T.J., Toyohara, A., Ellison, L.T., Parker, J.L. and Ellison, R.G. (1965): Ann. Thorac. Surg. 2: 195.

191. Yeh, T.J., Manning, H. and Ellison, R.G. (1966): Surg. Forum 17: 211.

192. Zajtchuk, R., Gago, O. and Adams, W.E. (1967): J. thorac. cardiovasc. Surg. 53: 109.