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PERFUSION OF THE ISOLATED CANINE LUNG: APPLICATION TO PATHOPHYSIOLOGIC STUDIES.

BY

© ROBERT LEIGHTON FISK

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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THE UNDERSIGNED CERTIFY THAT THEY HAVE READ AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES FOR ACCEPTANCE, A THESIS ENTITLED

"PERFUSION OF THE ISOLATED CANINE LUNG: APPLICATION TO PATHOPHYSIOLOGIC STUDIES"

SUBMITTED BY ROBERT LEIGHTON FISK

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (SURGERY)

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ABSTRACT

The lung is rapidly altered by perfusion under non-physiologic conditions. This problem has prevented the development of techniques for perfusion preservation and complicated many studies which have utilized isolated lungs. The etiology of alterations which take place under conditions of isolation-perfusion are not well understood. Few investigators have endeavored to simulate physiologic conditions for the isolated lung. To do so appeared to offer potential for prolonging functional preservation and providing an improved preparation for pathophysiologic studies.

With the object of providing a more physiologic environment for the isolated lung an attempt was made to simulate normal hemodynamics and ventilation in canine lungs which were perfused with venous blood from supporting animals. A special perfusion apparatus was designed for the purpose of facilitating the control of experimental conditions.

The foregoing approach resulted in reasonably stable function for five to sixteen hours of support dog survival. Morphologically the lungs were not normal but the severity of perivascular edema and hemorrhage which developed were considerably less than has been experienced by others who have perfused isolated lungs.

The pathophysiology and experimental therapy of acute aspiration pneumonitis was amenable to study in isolated autoperfused lobes. Elevated vascular resistance and decreased compliance were the predominant pulmonary effects of acid aspiration. The resultant systemic failure of the supporting dogs appeared to be contributed to by both altered gas exchange in the damaged lung and the absorption of

acid from the aspirate.

Study of the pathophysiology of perfusion lesions was begun in autoperfused isolated lobes. Perivascular edema and associated ventilation-perfusion disturbances contributed to the functional abnormalities which were observed.

In subsequent studies perfusion methods were altered with the object of assessing the role of methodology in the development of perfusion damage to the lung. The isolated lung did not appear to be significantly affected when marked hypotension was induced in the support dog. Severe alterations resulted from the replacement of the support dog by a mechanical gas exchanger. Distinguishable alterations did not result from the use of homologous rather than autologous blood perfusate for up to sixteen hours.

The latter observation was then applied to the homologous perfusion-evaluation of lungs stored by hypothermia and hyperbaria. Lungs were rapidly destroyed by perfusion immediately following storage for sixteen to twenty-four hours under conditions of 4° to 8° C and oxygen at twice atmospheric pressure.

Pilot experiments were carried out to investigate methods of perfusion-preservation. Lungs developed severe edema when they were hypothermically perfused for short periods with ultrafiltrated cryoprecipitated pooled plasma. Although this technique is satisfactory for short term kidney preservation it did not offer promise as a means for lung preservation.

The overall study yielded several general observations. The lung remained intolerant to perfusion under non-physiologic conditions.

Perivascular edema and hemorrhage, which characterize the reaction of the lung to injury, developed in isolated lungs independent of elevation in vascular resistance. The etiology of perfusion damage appeared multifactorial and related to the hemodynamic, hematologic and metabolic effects of isolation-perfusion imposed on the unique and delicate structure of the lung. Techniques involving perfusion do not hold promise as means for prolonged storage of the lung for transplantation.

Elaboration of the mechanisms by which perfusion lesions develop in isolated lungs will provide additional insight into the pathogenesis of similar lesions which develop in various forms of clinical pulmonary failure. Considerations pertinent to future isolated lung studies of this nature are discussed.

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To my wife, MAUREEN

and

children, CHERALEE AND LEIGHTON:

whose patient forebearance and encouragement

have abided throughout my medical school

and post-graduate experiences

and

To my PARENTS

from whom it all began

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"The cultivation of whole organs has already become capable of very extensive application to anatomic, physiological and pathological problems."

-Alexis Carrel, 1938.

CHAPTER I

INTRODUCTION

"Nature, forever ready to defeat an inquiry into her secrets has shown us that slight departures from her own conditions may introduce complications which require special attention."

- I deB. Daly, 1933.

In the history of isolated organ perfusion, the lung has been subjected to a wider range and greater number of experiments than any other organ of the body. Lung perfusion experiments began in 1662 when Malpighi pumped water through the pulmonary artery in order to demonstrate that fluid was normally contained by the pulmonary vasculature. Physiologic inquiry has been carried out in countless numbers and varieties of lung perfusion experiments since that time.

For nearly one hundred years the biologic lung has been used in a variety of ways for extracorporeal oxygenation of perfusates. During the past two decades isolated perfused lungs have been applied to the study of pulmonary insufficiency which occurs following open heart surgery and circulatory shock. Recent interest in transplantation of the lung has stimulated attempts to preserve the lung by perfusion.

When the author initially became interested in lung preservation, satisfactory methods for lung storage were not available. Despite a lengthy history of lung perfusion research, the lung was rapidly destroyed when perfusion-preservation was attempted (Brownlee, 1965). Before embarking on further attempts at perfusion of the lung for preservation it appeared necessary to reassess the overall problem of lung perfusion.

Perfusion Research in Pulmonary Physiology and Pharmacology

These fields have been previously summarized in several excellent reviews (Aviado, 1960; Fishman, 1961; Daly and Hebb, 1966). These authors all stress that confusion remains over many issues as a result of variations in species studies, methods employed and interpretations rendered. The following is therefore limited to milestones and methods, with emphasis on perfusion damage to the lung.

Since vasomotor innervation of the pulmonary vasculature was discovered late in the nineteenth century, investigators have been striving to distinguish and quantitate the roles of neural and humoral mechanisms in the regulation of the pulmonary circulation. During early in situ studies, it was recognized that changes in any of these factors affect cardiac output and thereby prevent objective study of pulmonary vascular response. Investigators turned to isolated lung perfusion in the search for unequivocal evidence of pulmonary vascular reactivity.

The earliest methods consisted of a single passage of perfusate through the lung using isogravimetric arterial pressure and free drainage of venous effluent. From these primitive beginnings, a remarkable variety of methods have been developed. Methods have varied with respect to the means employed for controlling flow, ventilation, temperature and the state of pulmonary innervation. Numerous perfusates have been used.

Fundamental studies which have used isolated lungs can be categorized as either in situ or ex vivo. When investigators have been concerned with the bronchial circulation or pulmonary innervation, in situ

perfusions have been necessary. Many investigators have perfused the lung in situ because the deleterious effects of perfusion are thought to be more pronounced when the lung is removed from the body (Daly and Hebb, 1966). The evolution and sophistication of techniques for in situ perfusion was exemplified by Daly and Daly's (1959) "vasosensory controlled living animal" in which both pulmonary artery and bronchial flow and carotid sinus and body perfusion were controlled.

Daly's work was devoted to elaborating neurovascular control mechanisms and improving techniques for lung perfusion. Regarding the use of the perfused lung for physiologic investigation, Daly stated (1966, p.343): "As originally used, this method of experiment was open to many criticisms of which one of the most serious was the rapid onset of edema, by congestion, alveolar collapse and other structural damage." Daly summarised the factors which he considered important for lung perfusion in his "Techniques for Artificial Perfusion of the Lung" (Daly and Hebb, 1966, pp.350-401). The following factors were stressed: The use of heparinized whole blood; minimal manipulation of the lung; the maintenance of a uniform temperature in the perfusion circuit; the prevention of tissue dessication; the avoidance of hilar distortion and the use of negative pressure ventilation. Regarding the latter, Daly stated (p.370) "It is our experience that perfused lungs cannot usually be maintained for long periods using this method of ventilation (positive pressure ventilation) without the onset of edema." He recognized that ventilation affects the dimensions and physical characteristics of pulmonary blood vessels (p.342).

The early onset of rapidly increasing vascular resistance has been a frequently encountered problem in fundamental investigations.

Daly attributed this to serotonin release as a result of platelet destruction and blood trauma. Heparin "pre-treatment" of the blood donor, avoidance of stagnation of the blood in the perfusion circuit and the use of high flow rates, low priming volume and platelet poor perfusate minimized this problem. He observed that leucocytes, platelets and serotonin gradually decreased in the circulating blood and that mild hypothermia (to 33°C) partially protected the lung from the effects of vasotonins.

Throughout his work, Daly often speculated on the significance of direct bronchopulmonary vascular communication. He felt that simultaneous bronchial and pulmonary perfusion was important for the maintenance of tissue in a healthy state even though the bronchial circulation does not supply the alveolar epithelium directly (p.384). Although he observed that pulmonary vascular resistance was higher when the bronchial system was perfused, a lower net vascular resistance was usually associated with more satisfactory preparations. For most of his work, Daly used isogravimetric arterial flow and cannulation of the left atrium for gravity venous drainage. Despite continual improvement in his methods, Daly frequently observed edema formation as early as 2.5 hours after beginning perfusion.

A number of investigators have carried out ex vivo lung perfusion to determine the effects of inspired gas and altered flows and pressures on the denervated pulmonary vasculature. During the 1950's, Nisell (1950) and Duke (1954, 1957) described the pulmonary vasoconstrictor effect of alveolar hypoxia. The majority of these experiments were carried out using positive pressure ventilation, isogravimetric arterial flow, gravity venous drainage and artificial perfusates. Few investi-

gators have attempted to provide mixed-venous gas composition in the perfusate which entered the pulmonary vasculature and even fewer have used an animal in the perfusion circuit to provide metabolic homeostasis.

In the late 1950's an increasing number of investigators became interested in the physical determinants of pulmonary hemodynamics. In 1956, Carlill and Duke reported that an increase in venous pressure within the physiologic range of left atrial pressures, produced a decrease in pulmonary vascular resistance. It was shown subsequently that diffusion capacity increased with elevation of venous pressure (Rosenberg and Forster, 1960). In 1959, Donald described a method of lung perfusion under conditions of negative pressure ventilation which occasionally delayed the onset of edema for over five hours.

In 1961, Bannister and Torrance described how the alveolar capillaries behave like Starling resistors under the influence of alveolar pressure. Their "Sluice Hypothesis" was independently corroborated by Permutt and co-workers (1962) who described how the pulmonary vasculature behaved like a "waterfall" in which the pressure gradients are controlled by alveolar and venous pressures. Many studies have been carried out during the last decade to advance the understanding of pulmonary pressure-flow relationships.

In 1964, West and co-workers described a sophisticated preparation which utilized negative pressure ventilation, venous pressure control and a support animal in the perfusion circuit. Although satisfactory function was often preserved for up to five hours, some experiments terminated by the development of edema and hemorrhage. These workers stressed the avoidance of excessive venous pressure and scrupulous cleaning of the extracorporeal circuit in order to minimize or delay the onset

of edema. They also suggested that the vasoconstrictor response to alveolar hypoxia was a useful index of the physiologic integrity of the isolated lung.

West's group incorporated perfusion scanning techniques and introduced the concept of "zones" of lung perfusion and an understanding of hydrostatic or gravitational factors which influence flow distribution. Many studies which have been facilitated by their preparation have established West's group as major contributors to new information in the past dacade of investigative pulmonary physiology.

Morphology in Physiologic Studies

Trowell (1943) provided the first comprehensive study of histology in the perfused lung. Trowell studied the alterations in lungs which Daly and co-workers (1941) had perfused with heparinized blood under conditions of negative pressure ventilation. The most common lesions which he observed were: (1) edema (particularly in the alveoli, periarterial lymphatics and arterial walls); (2) periarterial and peribronchial hemorrhage; (3) intravascular aggregation of polymorphonuclear leucocytes; (4) bronchial and bronchiolar dilation and (5) intramural bronchial vascular congestion. His thorough review of existing reports on other forms of pulmonary derangement allowed Trowell to state that "the main pathological features of the perfused lung...seem to represent a common reaction of the lung to injury."

Duke (1951) reported that vascular congestion was a common alteration. Many of her specimens were taken from lungs which had been positive pressure ventilated. Duke may have revealed why morphologic alterations were not a primary concern of physiologic investigators when she

stated that "microscopic examination confirmed the presence of congestion and edema, but also showed that ample functioning tissue was always present."

West's group (1965) demonstrated that dependent zone perivascular edema, to which their preparation was prone, could be partially reversed. It was speculated that this abnormality might be analogous to clinical conditions associated with pulmonary venous hypertension.

The Lung as an Extracorporeal Oxygenator

For reasons of facility, economy and their special properties, isolated perfused lungs have been used as oxygenators for nearly a century. Although Jacobj (1895) is commonly regarded as the father of "biologic" oxygenation, Martin used isolated lungs for heart-lung studies in 1881. Starling used isolated lungs in his classic experiments on mechanical control of mycardial function (Patterson and Starling, 1914), as did Hemingway, Dale and Schuster and Barcroft (Wesolowski et al, 1952).

In 1951, Potts and co-workers observed that "a dog not allowed to breathe with his own lungs can 'breathe' for at least three hours by deflection of part of his blood through homologous lungs." One year later, they showed that the heart could be opened while employing this method (Potts et al, 1952). Their partial bypass oxygenation system allowed some dogs to tolerate nitrogen breathing until the ex vivo lungs developed sanguinous airway edema after three hours of perfusion.

In 1952, Mustard and co-workers demonstrated that brief intracardiac procedures could be undertaken when oxygenation was maintained by homologous lungs for nine to forty-seven minutes. This was an

extension of the seventeen minute perfusion period which they had achieved one year earlier (Mustard and Potts, 1951). Mustard attempted to correct transposition of the great vessels in seven infants using heterologous (monkey) lungs for oxygenation. The lungs became edematous as early as fifty minutes after beginning perfusion (Mustard et al, 1954).

Wesolowski and co-workers (1952) used isolated homologous lungs for two hour oxygenation in experimental cardiopulmonary bypass. Edema developed in most preparations when high positive pressures were used to ventilate the lungs. Lungs were found to tolerate a five hour ischaemic period but were intolerant to heterologous perfusion.

Homologous lobes functioned as well as autologous lobes when partial bypass oxygenation was studied by Campbell et al (1955, 1956) and Crisp et al (1955). Edema developed after thirty minutes of perfusion in either case. Lobes gained an average of 54 grams per 30 minutes when perfused with pulsatile pressure and half as much when arterial flow was "depulsated" (Campbell et al, 1955). These workers advocated open venous drainage to avoid venous distention. Although they did not use negative pressure ventilation, they suspected that this measure would improve blood flow in the isolated lung (Campbell et al, 1956). This latter report dealt with an attempt to correct congenital heart defects in seven severely ill children using canine lungs for oxygenators. Six patients died following fifteen to forty-nine minutes of perfusion.

Waldhausen et al (1956) also demonstrated that heterologous perfusion for thirty minutes at flow rates of 200 millilitres per minute was not tolerated by the canine lung. Histologic studies demonstrated "massive perivascular hemorrhage and edema."

A ten year hiatus in this type of investigation followed the clinical acceptance of improved mechanical oxygenators. Interest in biologic oxygenation has recently been renewed because technologic advances in the field of artificial oxygenators have not been as rapid as was originally expected.

In 1967, Lukin investigated the use of dog lungs for oxygenation of umbilical blood in newborn lambs. In order to attempt removal of "antigenic substances," six litres of six percent dextran were pumped through the lungs over several hours. The lungs were destroyed by edema in less than one hour of subsequent blood perfusion. Lukin speculated that negative pressure ventilation might improve the donor lung.

Veno-venous bypass oxygenation of five dogs using homologous lungs was recently reported by Ratliff (1968). The apneic period of supported dogs lasted three hours unless deterioration of the supporting lung necessitated earlier termination of the experiments. The supporting lungs gained an average of 255 grams. Bryant and co-workers (1968) studied the oxygenating ability of pig lungs which were perfused with autologous or human blood. Low flow rates were maintained for an average perfusion time of 2.1 hours. The course of perfusion was unpredictable and progressive edema and atelectasis was noted during the final hour in most experiments.

Studies related to the use of biologic lungs for clinical cardiorespiratory support span two decades. The lack of significant improvement in the perfusion endurance of the isolated lung has been very disappointing (Eiseman, 1967). Without exception, all studies which have been carried out are identical in several respects. Positive pressure ventilation has been used and attempts have been made to minimize venous

pressure. All preparations developed very severe edema in three hours or less and detailed description of the pathologic alterations in the perfused lungs was uniformly lacking.

The applicability of lung perfusion to pathophysiologic study was demonstrated thirty years ago when Daly (1941) extrapolated his observations on bronchoconstriction in isolated lungs to asthma. Additional avenues of application subsequently became apparent when surgical investigators encountered new problems in patient management.

The Lung in Cardiopulmonary Bypass

Following the wide application of mechanical extracorporeal oxygenation to open heart surgery, clinicians began to observe severe post-operative pulmonary insufficiency in many patients. In the late 1950's a flurry of reports described the pulmonary lesions which accompanied this problem and many suggestions were forwarded regarding their etiology.

Kottmeier and co-workers (1958) observed that pulmonary collapse and peribronchial hemorrhage were partially alleviated by ventilation of the lungs during cardiopulmonary bypass. In the same year, Kolff and associates reported that post-operative pulmonary dysfunction was associated with pre-existing pulmonary disease, oxygen intoxication, fluid overload, air embolism and temporary overloading of the pulmonary circulation during bypass. It was felt that the latter was the most important single factor and could result from forward, retrograde or collateral engorgement of the pulmonary vasculature during intra-cardiac procedures.

Littlefield's group (1958) demonstrated that perivascular, peribronchial and alveolar hemorrhage results from pulmonary hypertension

during cardiopulmonary bypass. They also recommended decompression of the pulmonary vasculature to minimize these alterations.

Baer and Osborne (1960) introduced the term "post perfusion pulmonary congestion syndrome" for this condition. In addition to hemodynamic disturbances, they suggested that other etiologic factors such as anoxia, hypotension, "foreign" proteins and blood denaturation were likely also important.

After 1960, investigation into causes of the post-perfusion syndrome broadened with respect to suspected mechanisms and methods of study. Schramel and co-workers (1963) carried out six-hour periods of veno-venous bypass in dogs. Localized pulmonary collapse, congestion of small vessels in the alveolar walls and extravasation of blood into the pulmonary parenchyma resulted. These lesions were considered to be "indistinguishable from those observed in experimental hypovolemic shock treated by retransfusion and from those in patients who died after cardiopulmonary bypass."

The bio-assay of blood which had been recirculated in a mechanical oxygenator circuit, implicated histamine and serotonin release (Hollenberg et al, 1963). Surfactant alterations were observed in closed-chest veno-arterial bypass oxygenation experiments (Hepps et al, 1963). Platelet and fibrin microemboli were incriminated in veno-venous bypass procedures (Rossi et al, 1964).

Isolated lungs were introduced to this complex field of study in the 1960's. The effects of histamine and serotonin on the isolated lung were among the early reports (Eiseman et al, 1964; Moore et al, 1965).

In 1965, Awad and co-workers reported "A Technique for Perfusion of Pulmonary Lobes for Prolonged Periods." Transfemoral catheterization

of in situ lobes was carried out following thoracotomy. Through a long narrow venous catheter, blood was drained by gravity from the lobes to a sealed venous reservoir. A constant flow pump returned the blood from the reservoir to the lobe artery. Despite the addition of bronchial flow to the blood which left the lobes, effort was made to maintain a constant volume in the venous reservoir. Flow rates were kept very low (an average of 7 millilitres per minute). The lobes were ventilated with positive pressures which occasionally reached 35 cm H_2O. Perfusions lasted one to three hours and averaged about 90 minutes. The quality of lobe perfusion was judged by the post-operative survival of the animals. Of 34 animals, 29 died (85 percent) from two to eighteen hours post-operatively. In their conclusions, these authors stated that "a method for the complete isolation of the pulmonary circulation and the perfusion of a lung or pulmonary lobe for prolonged period of time and with minimal disturbance to the general physiologic processes of the body had been described."

In subsequent experiments this group (Awad et al, 1965-2; 1966-1; 1966-2)studied the effects of various perfusates as judged by mortality rate and histologic studies of specimens obtained at variable times post-mortem. Their investigations led them to state that "more than one factor plays a role in the production of the post-perfusion syndrome."

In 1966 Veith and co-workers reported a perfusion method by which in situ lungs were isogravimetrically perfused under conditions of gravity venous drainage and positive pressure ventilation. A bubble oxygenator was used to deoxygenate the blood before its return to the pulmonary artery. Ten isolated lungs were perfused for 120 to 240 minutes at 25°C with ACD blood. These workers stated that their method

"approximated physiologic conditions more closely because it preserved stable hemodynamics." The preparation was then applied to the study of the effects of homologous versus autologous blood, heparin versus ACD anticoagulation, one day versus twenty-one day stored blood and 25°C versus 38°C perfusion (Veith et al, 1967-1). Perfusions were carried out for 120 minutes. Progressive loss of perfusate into the lungs and the appearance of bloody froth in the respirator forced early termination of several experiments in some groups. Microscopically, periarterial hemorrhages were found even in minimally involved portions of affected lungs in all groups. Despite the "stable hemodynamics" which Veith referred to, vascular resistance doubled by thirty minutes of perfusion and had increased 1,600 percent after seventy-five minutes.

Veith's group (1967-2) reviewed the pulmonary histopathology of patients who died following open heart surgery, dogs following prolonged veno-venous bypass and their isolated perfused lungs and stated that "the occurrence of similar and rather distinctive pulmonary changes in three yet dissimilar situations suggests that they have a common pathogenesis." As a result of their studies, they felt that the following etiologies were satisfactorily ruled out: platelet and fibrin microemboli; homologous blood; oxygen toxicity; alterations in pulmonary ventilation, arterial and venous pressures; bronchial circulation; pulmonary arterial hypoxemia; systemic hypoxia and surfactant changes. Veith concluded that the interaction of blood with an extracorporeal gas exchanger produced a non-filterable factor which acted directly on the vasa vasorum of the small and medium sized arteries and resulted in primary arteriolar constriction.

In 1968 the same investigators demonstrated similar alterations in the lungs of animals which had been subjected to hypovolemic hypotension, but in which no mechanical oxygenators were used. They held to the theory of primary arteriolar constriction and used as substantiating evidence, an observed reduction in the diameter of pulmonary arterioles during the period of systemic hypotension.

Although the opinions of this group have circulated widely, general acceptance of their theories has not yet resulted (discussion of Veith et al, 1968-1).

The Lung in Shock

The immediate survival rate of severely traumatized patients has been gradually improving. A comparable improvement in late survival rate has been abrogated by an increasing incidence of refractory pulmonary insufficiency (Cook and Webb, 1968).

Since Bert observed pulmonary lesions in animals which were subjected to decompression nearly one hundred years ago (Webb, 1969), investigators have been striving to explain the etiology of "acute congestive atelectasis." Many theories of pathogenesis have arisen from in situ investigations which have been carried out (Webb, 1969; Collins, 1969).

The isolated lung was introduced to this field of study because it was felt that greater control of experimental conditions could be achieved (Pomerantz and Eiseman, 1968) and because it was recognized that in many ways, shock lung lesions resemble those inherent in isolated perfused lungs (Veith et al, 1968-1).

Pomerantz and Eiseman (1968) have used a technique of lung perfusion which is similar to Veith's, except the lung is removed from the body. Edema formation has been minimized by avoiding manipulation, limiting flow rates, limiting arterial pressures, periodic hyperinflation, maintaining left atrial pressures less than +10 mm Hg and rotating the lungs to prevent stasis. These methods apparently provide a stable preparation for three hours. Low flow rate, hypoxemia, acidosis and hypercarbia, have not produced shock lung lesions but a few hyperthermic perfusions and one hypothermic perfusion have. All lungs which Pomerantz subjected to oleic acid embolism produced "pathology similar to the pulmonary lesions described in the 'shock-lung syndrome'." Similar lesions developed after three hours of homologous perfusion of the lung.

Pomerantz speculates that the primary problem in the shock-lung is venous constriction. Veith's works have resulted in the "arteriolar constriction" theory. Contrasting theories of pathogenesis have arisen from the study of similar preparations.

Perfusion-Preservation of the Lung for Transplantation

Before 1965, perfusion-ventilation of the lung had not been carried out for the specific purpose of preserving the lung for transplantation. In 1966, Largiader and co-workers isogravimetrically perfused lungs with 150 to 400 millilitres of buffered electrolyte solution per day under conditions of three atmospheres pressure and hypothermia to 3°C. This method was reported to provide "viable" grafts as determined by x-rays and gross and microscopic assessment of necropsy material from allograft recipients. Many perfusion experiments were terminated by the development of excessive vascular resistance despite the very low 24-hour flow rates.

In 1965, Brownlee attempted perfusion-ventilation of lungs at 15°C using homologous plasma or autologous blood and positive or negative pressure ventilation. The lungs became grossly edematous following three hours of perfusion. It was subsequently demonstrated that integrity of the non-perfused lung is adequately preserved for up to six hours by simply ventilating the lung with room air (Homatas et al, 1968; Stevens et al, 1968).

Several investigators have reported the successful 24-hour preservation of lungs which were not perfused but were maintained at 4 to 8°C under conditions of two to four atmospheres of hyperbaric oxygen (Blumenstock et al, 1962; 1965; Garzon et al, 1966; Hino et al, 1968). In these studies the stored lungs were evaluated following unilateral transplantation. Immediate post-operative survival of the recipients was the main index of "successful preservation."

In 1968, Barnes and associates suggested that improvement in lung perfusion techniques might allow the ex vivo evaluation of stored lungs and furthermore that "perfusion may prove to be a means of restoring the lung to a normal state." Barnes' group used positive pressure ventilation, gravity venous drainage, and a disc oxygenator in the perfusion circuit. Their preparations deteriorated rapidly and allowed them little more than speculation regarding the potential for ex vivo evaluation, and functional "restoration" of stored lungs.

Problem Formulation and Objectives

The problem of intolerance of the lung to isolation-perfusion has not been unique to attempts at perfusion-preservation. The propensity of the isolated lung to develop rapidly progressive edema and

hemorrhage has limited the rate of progress in all areas of lung perfusion research.

In various pathologic conditions the lung exhibits "a common reaction to injury." Alterations in perfused lungs resemble those in the "post-perfusion lung" and the "shock-lung". In the attempt to elaborate pathophysiologic mechanisms, investigators have used isolated lung preparations which deteriorate rapidly even under "controlled" conditions. The solution of clinical problems has not been forthcoming from investigations in which severe alterations have been inherent in the study "models."

All forms of lung perfusion represent a departure from normal physiologic conditions of the body. The severity of perfusion damage appears to be related to the degree to which conditions of perfusion differ from the normal environment of the lung. For example, plasma perfusion at 15°C under conditions of positive pressure ventilation produces gross edema in less than three hours (Brownlee, 1965). Negative pressure ventilation and normothermic perfusion with blood from a support animal has resulted in satisfactory function and minimal edema for as long as five hours of perfusion (West et al, 1964).

Without exception, surgical investigators have not made a specific attempt to provide a reasonably physiologic environment for the isolated perfused lung.

The foregoing considerations resulted in the redirecting of our interest from solely perfusion-preservation to perfusion of the isolated functioning lung for the following reasons.

Attempts to provide a more physiologic environment for the

isolated lung would likely enhance the quality and duration of functional preservation. The assessment of factors which are responsible for perfusion damage of the lung might be expedited by first evaluating the isolated lung under reasonably physiologic conditions.

Isolated functioning organs afford certain advantages for the control of experimental conditions. A preparation which eliminated or minimized perfusion damage would possibly facilitate the study of pathophysiology in various types of acute pulmonary insufficiency. Furthermore, such a preparation might facilitate the assessment of lung storage techniques and provide information pertinent to preservation and transplantation of the lung.

Within the context of the foregoing, the experiments which comprise this thesis were carried out with the following major objectives:

1) To attempt to prolong the duration of functional and morphologic integrity of the isolated perfused functioning lung.

2) To devise means for facilitating the perfusion and study of the isolated lung.

3) To apply the isolated functioning lung to the study of acute pulmonary insufficiency.

4) To explore the potential and effects of various lung perfusion methods and attempt to define the role of perfusion methods in damaging the isolated lung.

5) To apply the isolated functioning lung to the evaluation of techniques for lung preservation.

CHAPTER II

THE PERFUSION METHOD

Hemodynamic Considerations

The pulmonary vasculature is sensitive to alterations in both intravascular and extravascular pressures. Alveolar capillaries behave like "Starling Resistors" or sluices" under the influence of alveolar and venous pressure (Bannister and Torrance, 1961; Permutt et al, 1962; West et al, 1964). As a result of the instability of the pulmonary microcirculation, independent changes in alveolar or venous pressure can alter blood flow distribution (West et al, 1964).

Positive pressure ventilation in the closed chest results in concomitant elevation in arterial and left atrial pressures. Under these conditions, blood flow distribution is likely not significantly altered, although intravascular pressures are unphysiologically elevated. Most previous investigators have used positive pressure ventilation of exposed lungs. When venous pressure is not appropriately adjusted, an unphysiologic proportion of blood flow is diverted to dependent zones of the lung. Under conditions of positive pressure ventilation, the control of venous pressure in the exposed lung represents a problem.

Pulmonary vascular resistance is minimum when the lung is inflated to one-half maximum volume and reaches its greatest magnitude at extremes in volume (Thomas et al, 1961). Over-inflation and end-

expiratory collapse are difficult to prevent when exposed lungs are ventilated with positive pressure. End-expiratory collapse also contributes to progressive atelectasis. The aforementioned factors as well as the desire to simulate conditions in the closed chest resulted in the decision to use negative pressure ventilation of the isolated lung. In order to accomplish this, it would be necessary to house the organ in a sealed chamber.

The veins are responsible for nearly two-thirds of the vascular resistance in isolated lungs which are ventilated with negative pressure (Agostini and Piiper, 1962). Elevation of venous pressure lowers vascular resistance by producing passive distention of the post-capillary vessels (Kuramoto and Rodbard, 1962; Niden et al, 1962). The level of venous pressure also influences blood flow distribution (West et al, 1964; West and Dollery, 1965; West and Jones, 1965). Duke and Rouce (1963) demonstrated that elevated pulmonary venous pressure increases diffusion capacity and this was confirmed by Lawson and co-workers (1964) who stated that "transmural pressure in the pulmonary veins is more important than that in the arteries in determining the size of the capillary bed." Rosenberg (1963) observed that diffusion capacity increased under conditions which lowered vascular resistance.

Surgical investigators have encountered more severe edema formation when already low venous pressure has been further decreased to produce syphonage in the pulmonary veins (Bryant et al, 1968).

Pulmonary venous pressure varies cyclically with respiration in the closed chest. Means for simulating the dynamic component of venous

pressure appeared to be an important feature in the design of a venous pressure control system. It was felt that a decrease in venous pressure synchronous with inspiration would help to avoid an excessive venous to transpulmonary pressure difference which would otherwise exist during inspiration. West's group (1964) attributed much of the edema which they observed to this factor. The foregoing indicated that the pulmonary veins should be cannulated and means should be devised for simulating left atrial pressure dynamics.

The relationship of arterial flow characteristics to pulmonary function has not been thoroughly studied. The majority of previous experimenters purposely depulsated arterial flow. Maloney et al (1968) have recently shown that kinetic energy which is imparted to the flow during systole improves perfusion in regions of the lung which are hydrostatically above the level of the mean arterial pressure. Occlusive roller blood pumps are dependable and produce pulsations in flow. This means was therefore chosen to propel the perfusate in the extracorporeal circuit.

Metabolic Considerations

In attempt to attenuate perfusion damage, almost every conceivable perfusate and blood-additive combination has been used to perfuse the isolated lung (Daly and Hebb, 1966; Awad et al, 1965-2, 1966-1; Veith et al, 1967-1). A perfusate which eliminates or even substantially minimizes functional and morphologic deterioration has not been discovered. Whole-blood perfusion would be necessary for evaluation of function during perfusion. Fresh, heparinized whole blood is likely the

most satisfactory of any of the perfusates which have been used in the past (Daly and Hebb, 1966, p.355).

The majority of isolated lung perfusions which have been carried out by other investigators have utilized perfusate recirculation with or without mechanical "deoxygenation." The former prevents an assessment of respiratory gas exchange and the latter introduces undesirable hematologic alterations (Galletti and Brecher, 1962, pp. 47-120).

Despite advances in the field of artificial organs, no means are yet available to regulate metabolic homeostasis and venous blood gases as efficiently as in the intact animal. In order to complete the lung perfusion circuit, the use of a support animal would be necessary in order to provide a perfusate with physiologic biochemical composition. This approach would simplify the control of perfusion because the organ support animal would require only the maintenance of anaesthesia, intravenous fluid administration and in certain instances, respiratory assistance.

Technical Considerations

The main component of the perfusion apparatus would be a chamber to house the organ. The chamber should provide means for controlling pressure and temperature in the organ environment. Humidification would be necessary in order to prevent tissue dessication. The isolated organ should be readily accessible for manipulation, constant visualization and continuous monitoring. Steady state control of the organ environment should be automatically regulated. In order to provide versatility in operation and application, the requirements of organs other than the lung

should be anticipated wherever possible and facilities should be provided for hypothermia and hyperbaria studies.

When the foregoing features of the perfusion system had been defined, design and construction of an organ perfusion apparatus was carried out at the University of Alberta under the direction of the author.

THE UNIVERSITY OF ALBERTA ORGAN PERFUSION APPARATUS

(Figure 1)

Organ Chamber

The chamber has an internal capacity of 3.38 cubic feet (Figure 2). Three walls as well as the top and bottom are constructed of two 1/8 inch thickness stainless steel plates which are separated by 5/8 inch to form a water jacket which controls the temperature of the chamber interior. The front of the chamber is attached to a platform which supports the organ. The drawer assembly moves on bearings which are attached to the bottom of the chamber. Handle-locks on each side of the drawer-front permit rapid sealing or entry of the chamber. Five readily removable lucite windows measuring 10 inches in diameter, allow wide visualization of the interior and additional accessibility to the organ.

All weld-joints inside the chamber are polished to facilitate thorough cleansing (Figure 3). Humidification of the interior is accomplished by providing a saline pool below the drawer platform. One hundred percent humidity develops around the organ two minutes following closure of the chamber. Water which accumulates on the inner side of the windows is cleared by externally-controlled wiper blades. Ports through the back of the chamber floor are used to connect pressure-lines

and conduct transducer cables to the chamber interior.

Interchangeable fittings in the drawer window (Figure 1, Chapter III) connect the extracorporeal perfusion circuit with the cannulated vessels of the perfused organ. Special fittings are used to conduct bronchial and organ drainage tubes to the exterior.

Water is pumped through the water jacket of the chamber from a thermostatically controlled water bath, heater-refrigeration system which is located below the chamber (Figure 4). Constant temperature of the interior can be maintained at any desired level in the range 5°C to 50°C.

Pressures within the chamber are controlled by regulating the magnitude of a constant negative pressure (vacuum) or positive pressure (compressed air) from laboratory outlets. This is regulated automatically by a specially modified "Manostat"* (Figure 5). Cyclic pressure changes are produced by a specially designed phase, rate and volume-adjustable, reciprocal respirator pump§ (Figure 6).

Perfusion Circuit

Blood is delivered to the isolated organ by an occlusive roller pump†. Arterial flow rate can be arbitrarily varied by adjusting the pump speed selector or it can be governed automatically for maintenance of a pre-selected constant arterial pressure.

* Cartesian Manostat (Model #8), Manostat Corporation, New York.

§ Harvard Respirator Pump, Special Product #1170, Harvard Apparatus Co. Inc., Dover, Mass.

† Sarns Low Flow Perfusion Pump (#5 M 6050). Travenol Laboratories Ltd., Alliston, Ontario.

Venous return from the organ is conducted to a height-adjustable venous reservoir which is located near the chamber door (Figure 7). The reservoir is surrounded by a water jacket which is connected in series to the water jacket of the chamber wall. Chamber pressures are transmitted to the blood in the venous reservoir by connecting the top of the sealed reservoir to the chamber interior. A second roller pump* returns the blood to the supporting animal at a rate which is automatically controlled by photoelectric sensing of the blood level in the venous reservoir.

Blood loss from the isolated organ is drained by gravity from the organ support tray to an auxillary venous reservoir. A photocell-controlled auxillary venous pump* delivers this blood to the tubing which returns to the support animal.

Pump Controls

Occlusive roller pumps are satisfactory for extracorporeal circulation of blood but they are designed to produce constant flow independent of downstream resistance to flow. Constant surveillance of the pumps is required in order to correct for inherent drift in the pump speed-controls. Not only can net flow change, but an imbalance in arterial and venous flow rates can be disastrous to an experiment. In order to circumvent these problems, electronic feedback arterial and venous pump controls were devised.

*Sarns Modular Pump (# 5 M 6002). Travenol Laboratories Ltd., Alliston, Ontario.

Arterial and venous pressures in the perfused organ are sensed by catheters which are connected by fluid linkage to strain-gauge transducers*, the reponses of which are continuously recorded on a biomedical polygraph recorder§.

The electronic circuit of the arterial pump control receives a signal from the galvanometer output of the recorder which is proportional to arterial pressure. The sensitivity of the pressure monitoring apparatus is adjusted so that the desired perfusion pressure produces approximately one-half of full scale deflection on the recorder. The electrical circuitry of the arterial pump control is shown in Figure 8 and has been described in detail elsewhere (Fisk et al, 1969). The pump control permits a selection of perfusion pressures over a range of plus or minus 50 percent of the "half-scale" pressure in 10 percent increments. The pump control regulates the rotary speed of the pump motor. Variations in the vascular resistance of the perfused organ produce arterial pressure changes which are immediately sensed by the control circuit. The control appropriately alters the power which is applied to the pump motor and arterial flow is adjusted rapidly in order to return pressure to the desired magnitude.

The venous pump-control utilizes a photoelectric cell which senses the blood level in the venous reservoir. The electronic circuitry is similar to that for the arterial pump control (Figure 9). If the

* Statham (P23) series pressure transducers, Bionetics Ltd., Montreal, Quebec.

§ Offner Type R Biomedical Recorder, Beckman Instruments Inc., Palo Alto, California.

blood level in the reservoir rises, the photocell-circuit increases the rotary speed of the venous pump, thereby restoring the reservoir and thus venous pressure to the desired level. If the photocell light should burn out, the pump is stopped and an alarm calls the operator to restore the pump to manual operation. Arterial and venous pumps can be operated manually or simply switched to automatic flow control. The arrangement of the arterial and venous pump controls in the perfusion circuit is shown in Figure 10.

All of the previously described electronic controls are a built-in feature of the perfusion apparatus (Figures 11 and 12). The interrelationship and arrangement of all components and controls of the perfusion apparatus as well as the monitoring system are shown in Figure 13.

Materials in Extracorporeal Circuit

The importance of the selection and care of materials has been emphasized by previous investigators (Daly and Hebb, 1966; West et al, 1964). Blood flow in the extracorporeal circuit was conducted through polyvinyl plastic tubing (Tygon) which was new at the beginning of each experiment.

Catheters, tubing and the venous reservoir were joined with highly polished, stainless steel connectors and fittings which were specially designed to assure minimal turbulance and ease of cleaning and assembly. The steel connectors were constructed of #316 alloy because of the high degree of biochemical stability attributed to this material (Galletti and Brecher, 1962, p.37).

The glass cylinder of the venous reservoir was prepared with biomedical grade silicone spray in order to minimize foaming.

Following each experiment, the catheters, fittings and reservoir components were scrubbed with a concentrated detergent solution (Hemosol) and then left to soak for several hours in a dilute solution of the same. The materials were then thoroughly rinsed with hot tap water to remove the detergent and then placed in an oven to dry prior to re-use. This procedure closely followed methods which have been previously recommended (Daly and Hebb, 1966, p.629).

Prior to each experiment, the extracorporeal circuit was primed with Ringer's solution and "recirculation" through the support dog was carried out.

The priming volume of the extracorporeal circuit was 200 millilitres when 3/16 inch I.D. tubing and a support dog were used. In no experiments were strictly sterile techniques employed.

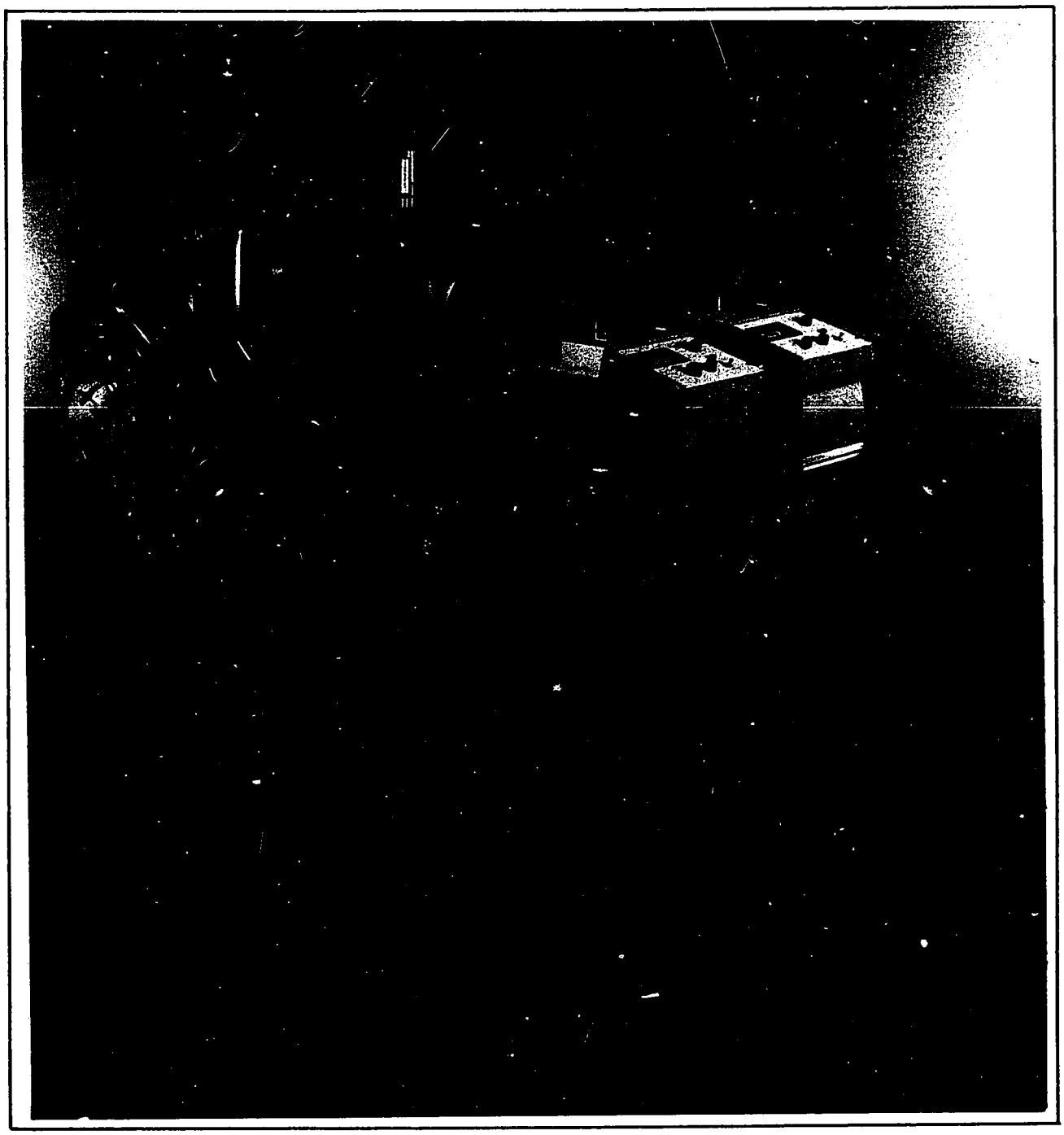
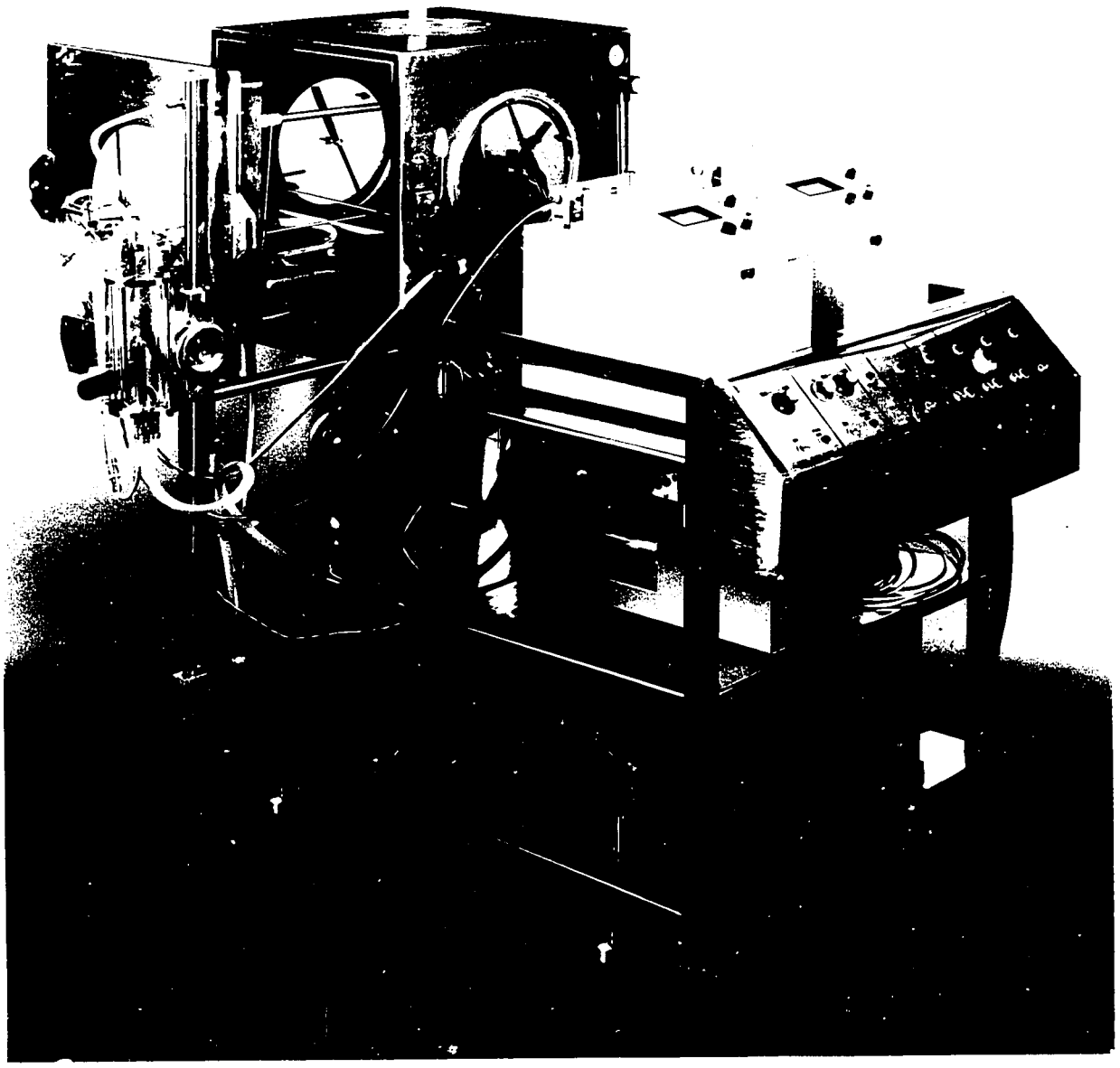


Fig. 1. University of Alberta Organ Perfusion Apparatus.



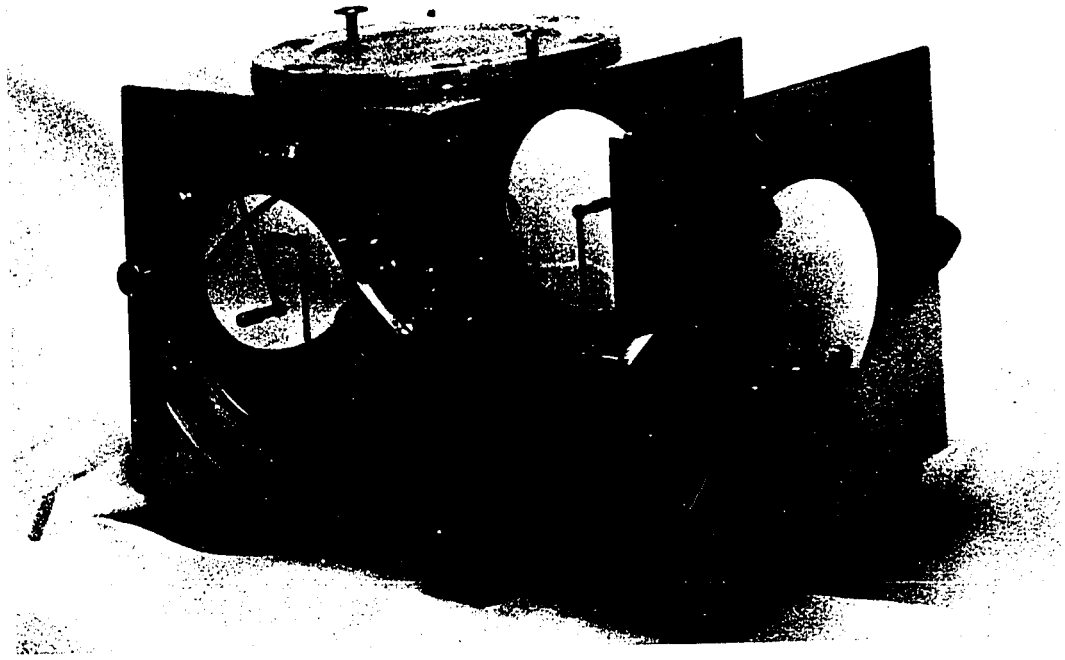


Fig. 2. Organ perfusion chamber

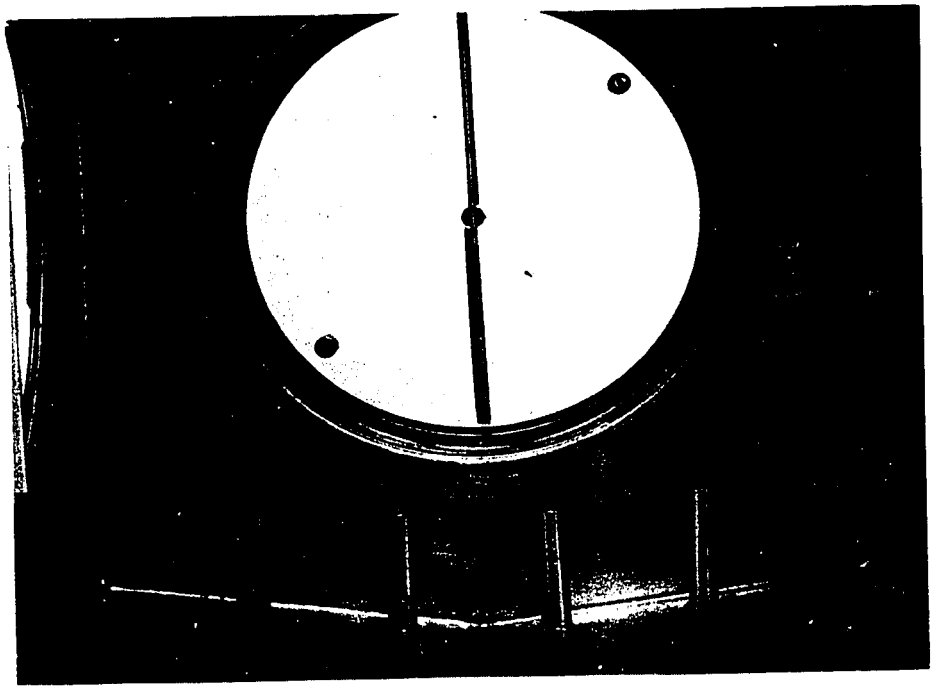


Fig. 3. Organ chamber interior

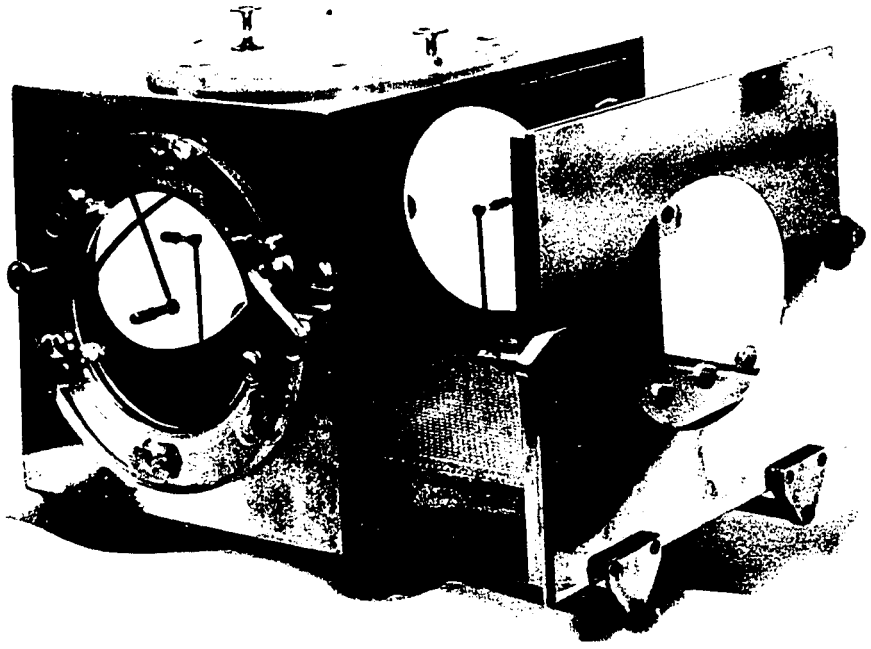


Fig. 2. Organ perfusion chamber

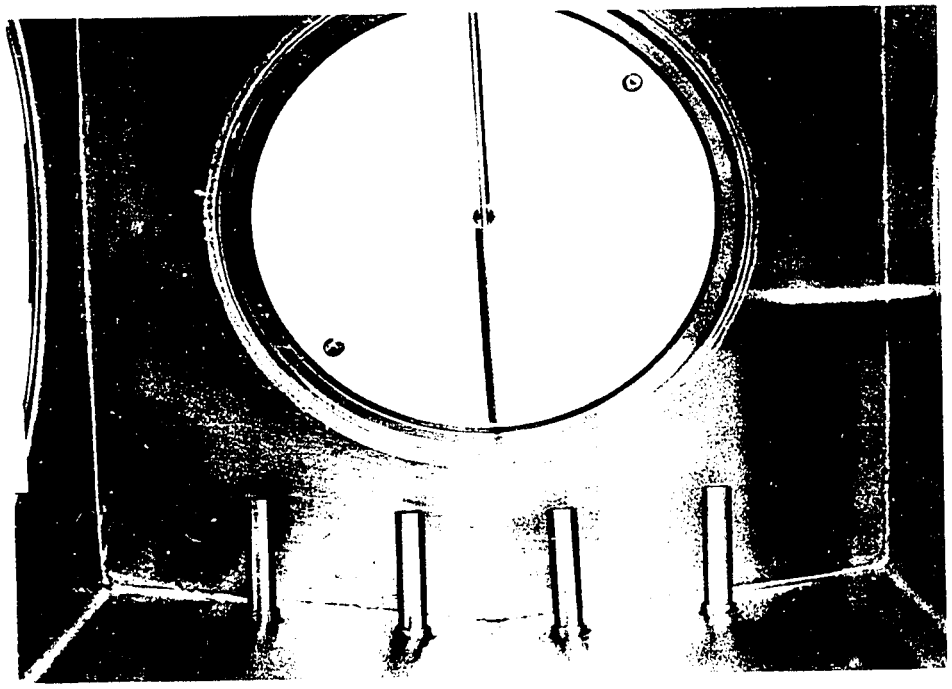


Fig. 3. Organ chamber interior

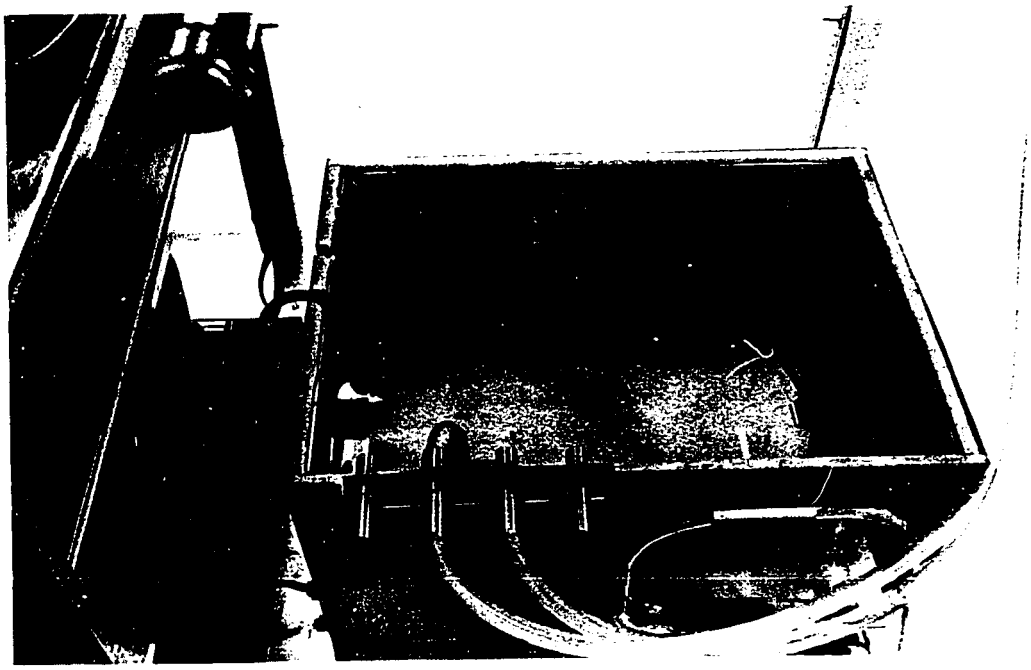


Fig. 4. Water heater-refrigerator for chamber temperature control.

Fig. 5. Manostat for chamber pressure control.

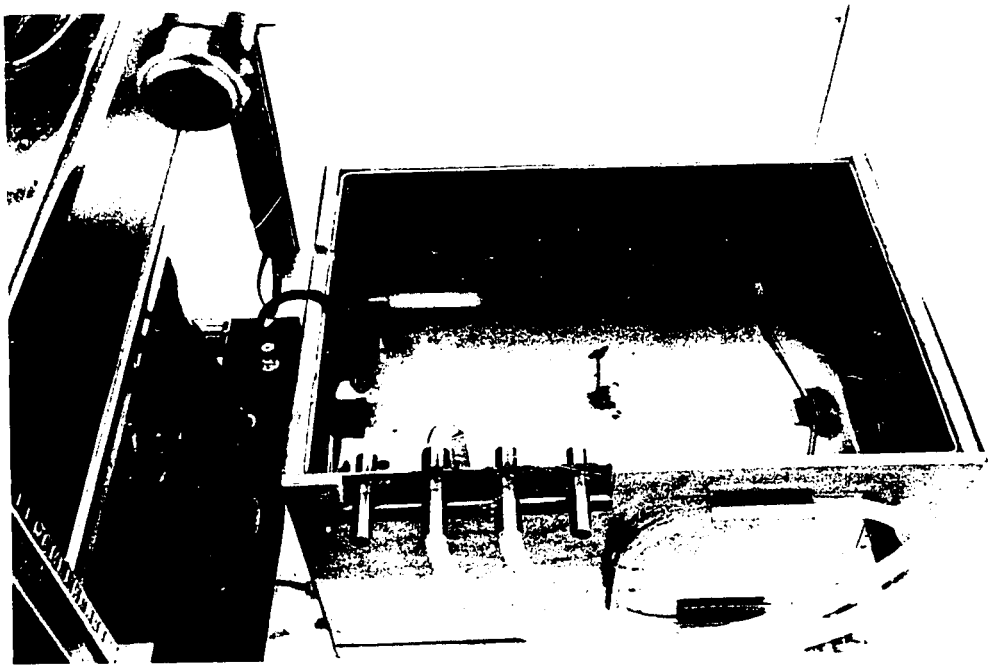


Fig. 4. Water heater-refrigerator for chamber temperature control.

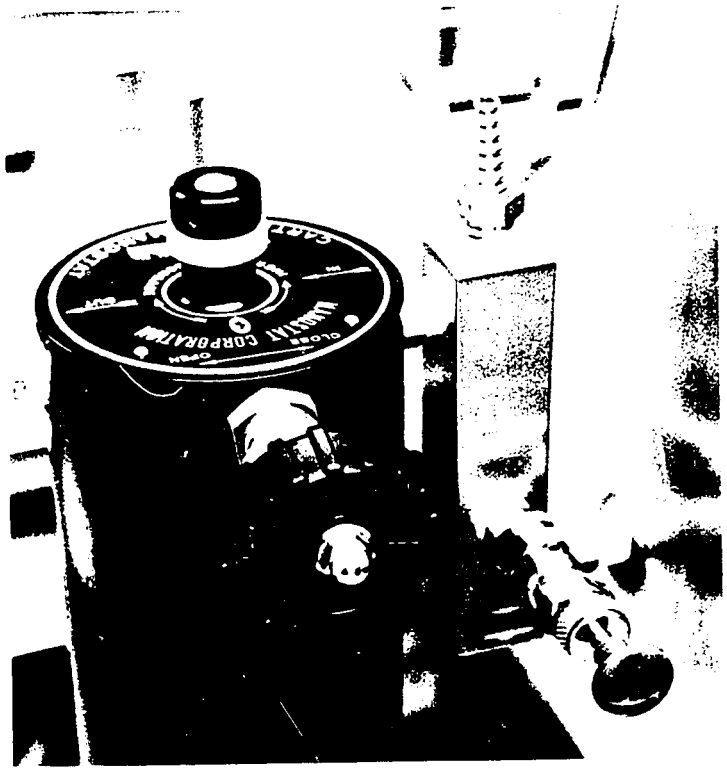


Fig. 5. Manostat for chamber pressure control.

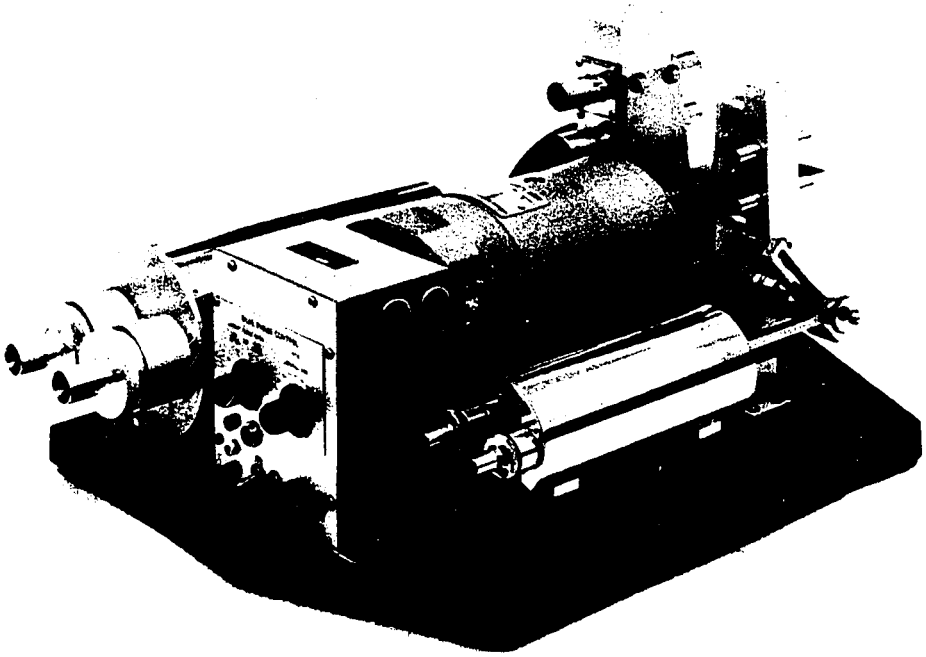


Fig. 6. Respirator pump for controlling chamber pressure fluctuations.

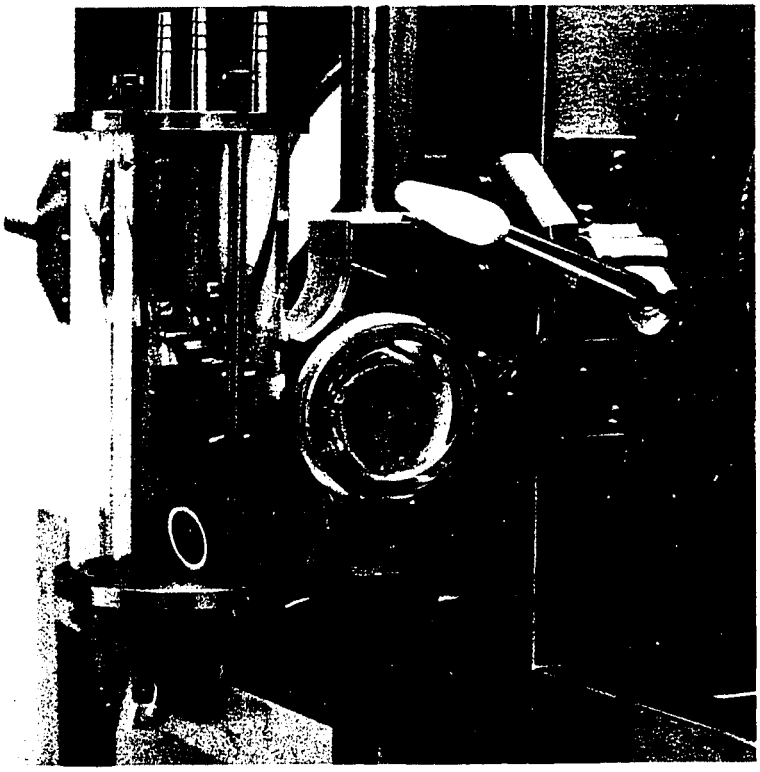


Fig. 7. Venous reservoir

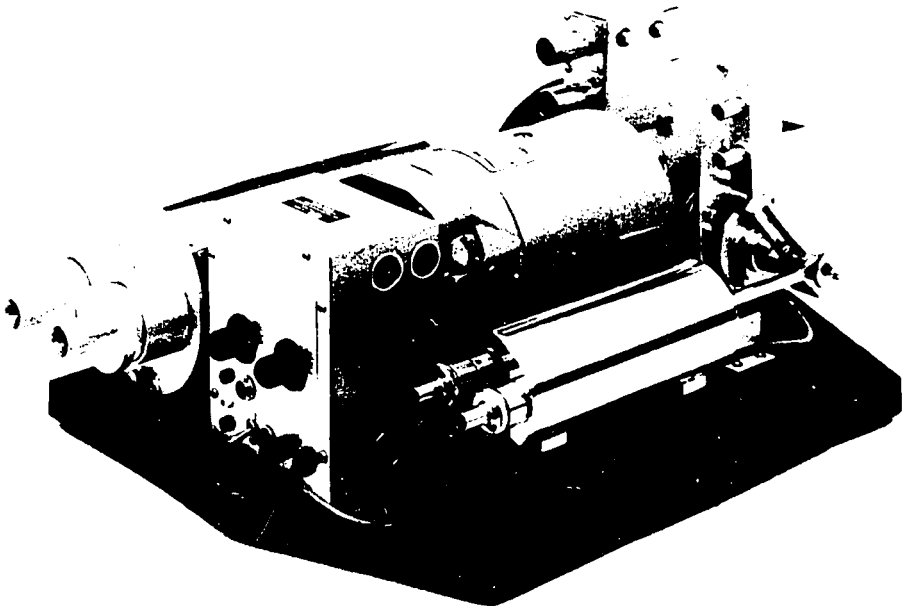


Fig. 6. Respirator pump for controlling chamber pressure fluctuations.

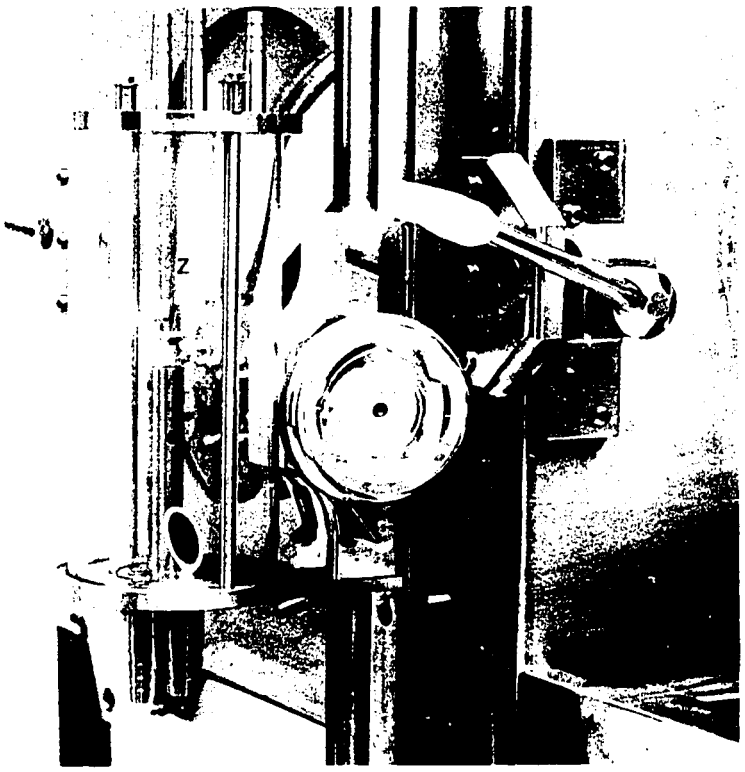


Fig. 7. Venous reservoir

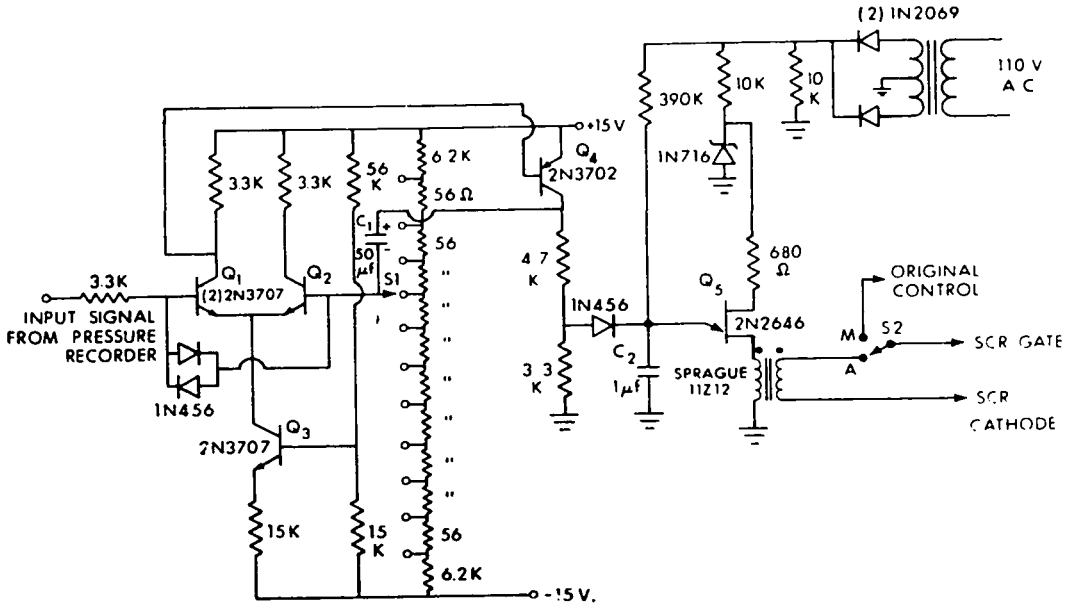


Fig. 8. Electronic circuit for arterial pump control.

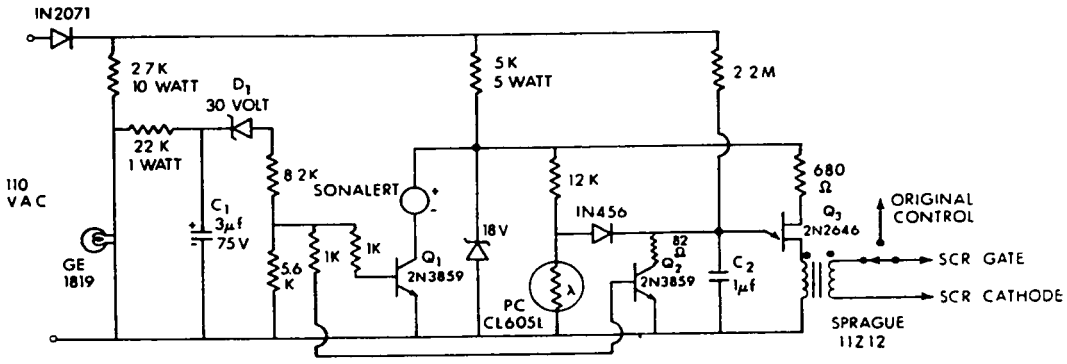


Fig. 9. Electronic circuit for venous pump control.

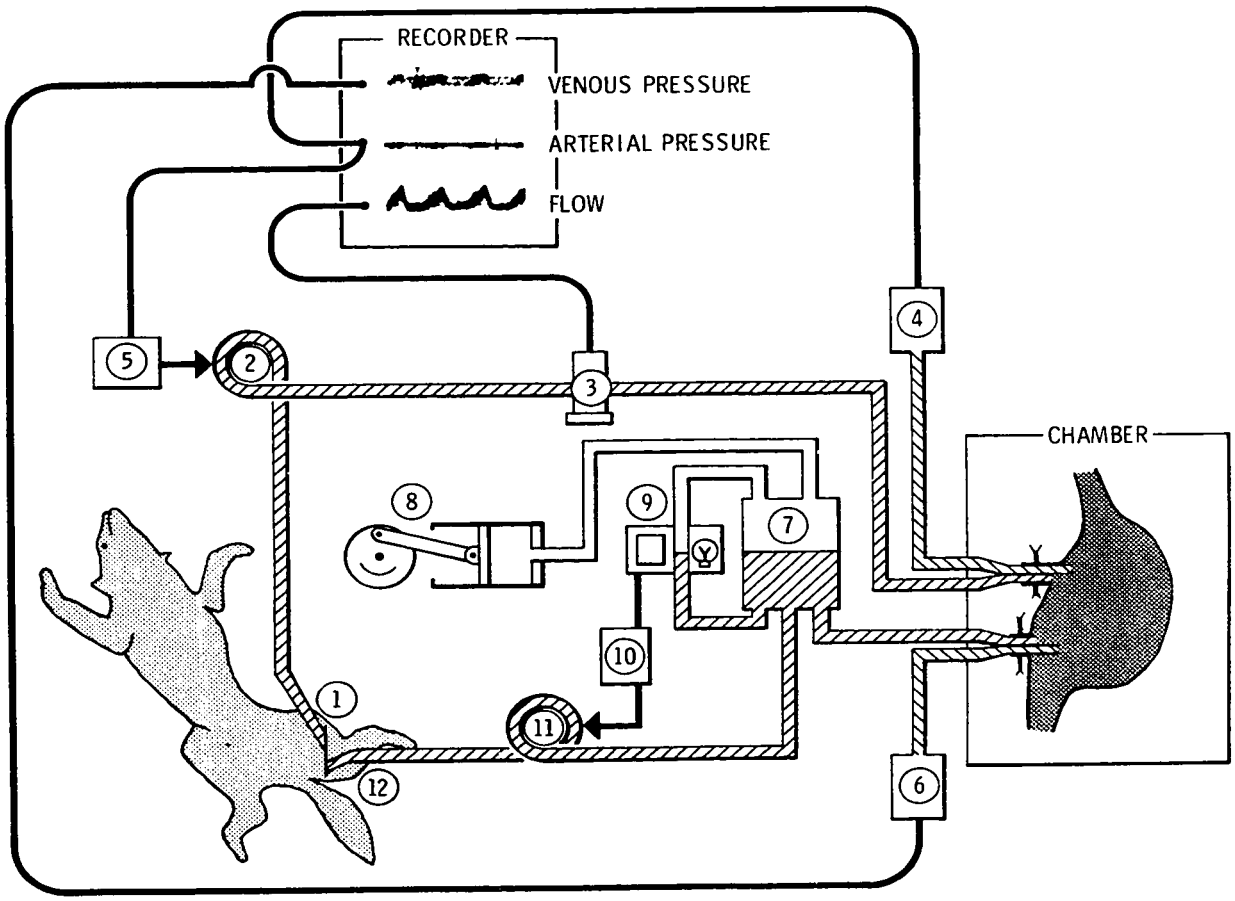


Fig. 10. Blood pump controls in perfusion circuit: 1, artery; 2, arterial pump; 3, flow probe; 4, arterial pressure transducer; 5, arterial pump control; 6, venous pressure transducer; 7, venous reservoir; 8, respirator pump; 9, photo-electric cell; 10, venous pump control; 11, venous pump; 12, femoral artery.

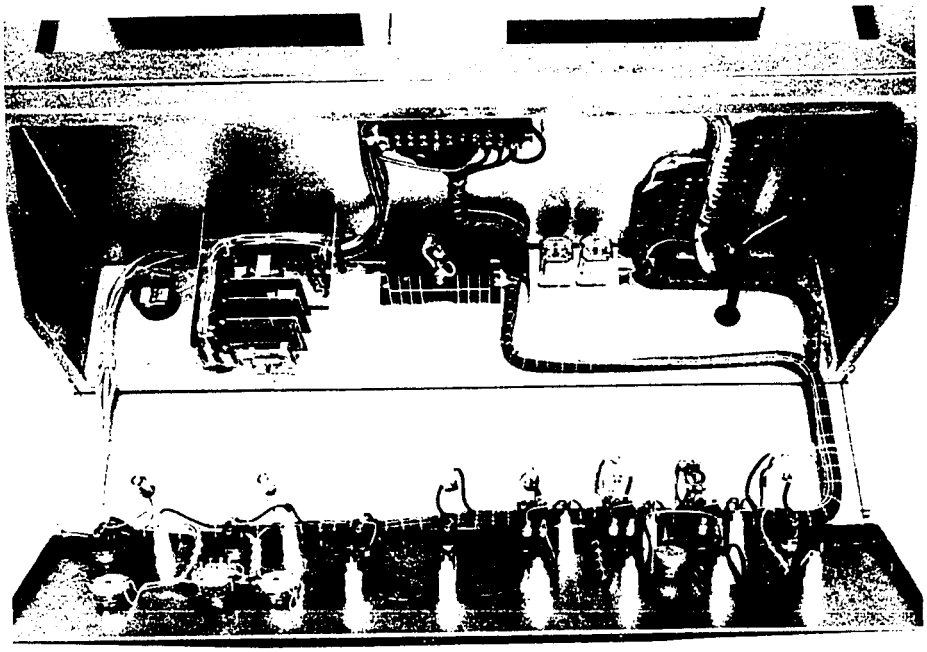


Fig. 11. Electronics of perfusion apparatus.

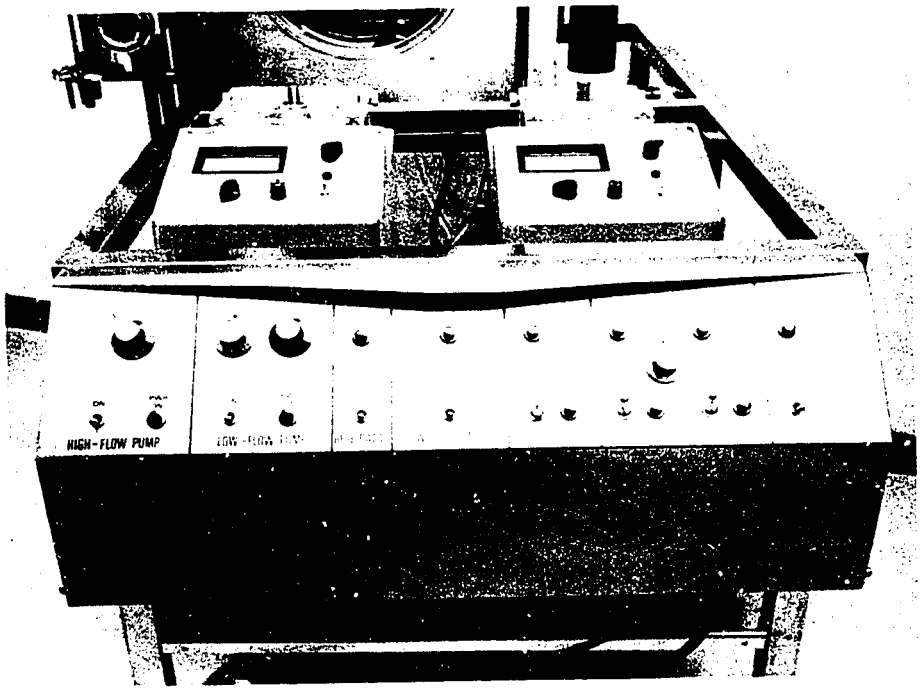


Fig. 12. Perfusion control panel.

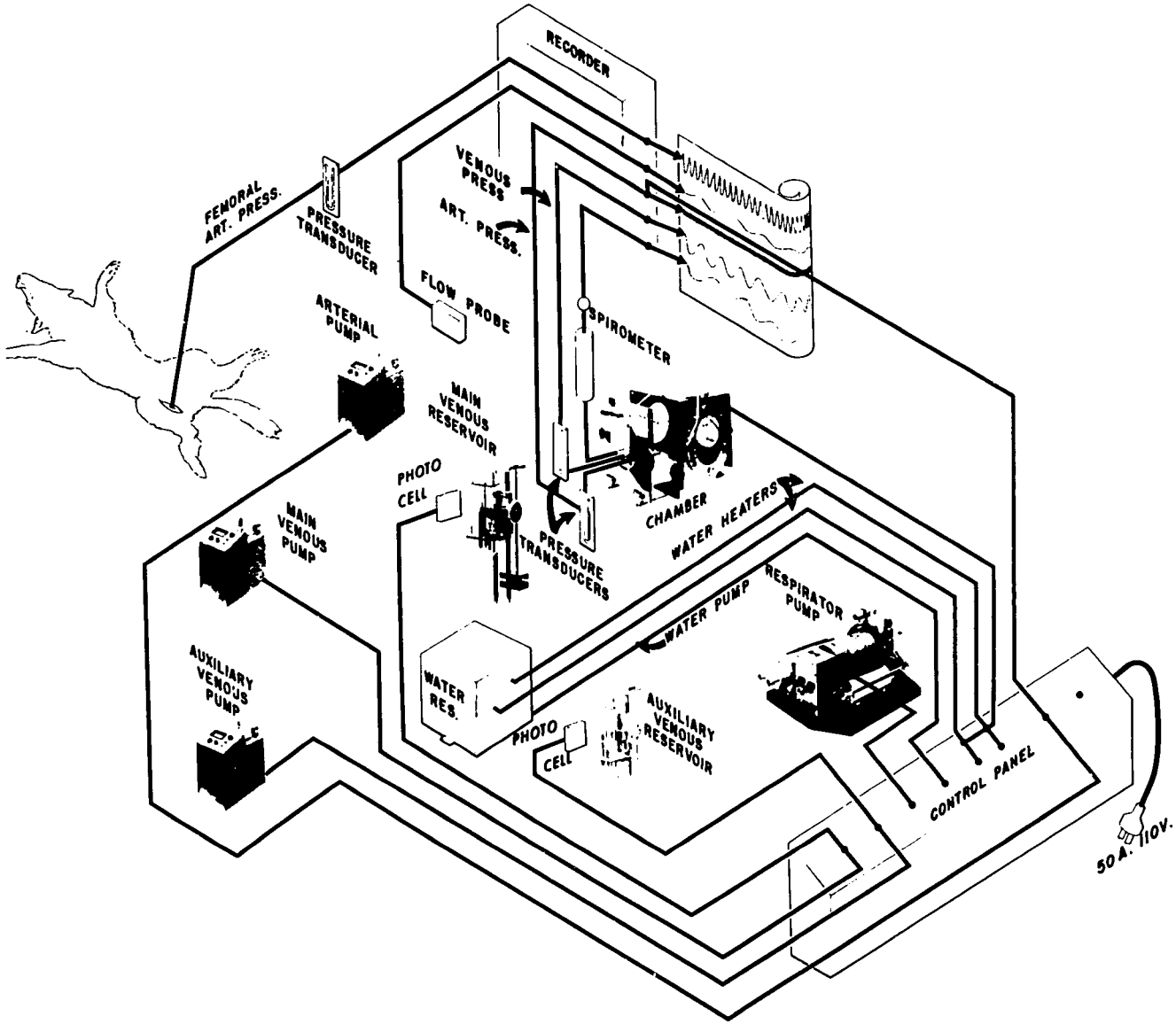


Fig. 13. Monitor and control systems for isolated organ perfusion.

CHAPTER III

STUDY METHODS

SURGICAL TECHNIQUES

Left lower (diaphragmatic) lobes or lungs from healthy mongrel dogs weighing 15 to 35 kilograms were used in all experiments. The surgical technique for excision of either lobe or lung was similar. Lobe excision will be described in detail and departures for lung excision will be outlined.

Lobe donors were anaesthetized by administering 30 milligrams per kilogram sodium pentobarbital intravenously. Additional dosages of 15 milligrams per kilogram were administered at intervals of one to two hours throughout the remainder of the period of donor survival in order to maintain a level of surgical anaesthesia. Following induction, the animal was intubated with a cuffed endotracheal tube and placed in the right semidecubitus position. Positive pressure ventilation was controlled* using compressed air or 97 percent oxygen and 3 percent carbon dioxide.

A left fifth intercostal space thoracotomy was performed. Left upper lobectomy was carried out by ligation and division of artery and vein in continuity. The upper lobe bronchus was divided between clamps and the proximal stump was closed with a continuous locking suture of 000 silk.

*Bird Mark 7 Respirator, Bird Corporation, Palm Springs, California.

The left lower lobe was carefully mobilized by division of the inferior pleural ligament and maximum lengths of the lower lobe vessels and bronchus were cleared. A tourniquet tape was then placed proximally around the left main pulmonary artery. Three hundred units per kilogram of sodium heparin were administered to the donor and the return line from the extracorporeal circuit was connected to the femoral artery using a #16-22 arterial catheter.

The left pulmonary artery was divided between vascular clamps and the bronchus was divided distal to a bronchial clamp which was placed near the carina. Gentle traction was placed on the lobe vein(s) and an atraumatic curved vascular clamp was placed on the left atrium in such a way that the ostia of the lobe veins were circumscribed by a cuff of left atrium which facilitated subsequent venous cannulation. During this manoeuver, care was exercised not to compromise the right pulmonary veins. The lobe was then removed following division of the atrium on the lobe-side of the atrial clamp.

The atrium and proximal bronchial stump in the donor were sutured in several early experiments. For most experiments, occluding clamps were simply left in place for the duration of perfusion.

Following removal of the lobe, the arterial line to the perfusion circuit was connected to the proximal pulmonary artery segment in the donor using a #18-22 vena caval catheter which was secured by a ligature tape. The lobe was then weighed and placed on the organ platform where vessels and bronchus were connected to the chamber door fittings by appropriate cannulas.

The lobe donor served also as the "autologous" support animal in most experiments.

When the entire left lung was perfused it was desirable to obtain a large cuff of left atrial tissue in order to allow venous cannulation without obstruction of venous return. It was necessary to sacrifice the lung donor for whole lung perfusion experiments.

In preparation for lung excision, the hilar structures were cleared by dissection, with care being exercised to spare the lung unnecessary manipulation. In addition to the left hilar structures, the right inferior pulmonary vein was dissected. Following division of the left pulmonary artery and bronchus, the left atrium was divided in such a way that the atrial cuff included all left pulmonary veins and the right inferior pulmonary vein. Prior to division, the atrial cuff was sealed by an atraumatic vascular clamp which remained on the lung during perfusion. The right inferior pulmonary vein was cannulated for venous return.

A second dog was used as a support animal when "homologous" ex vivo lung perfusion was undertaken. Blood from the extracorporeal circuit was returned to the femoral artery of the support dogs. Mixed venous blood for the arterial line in the circuit was obtained from the right atrium using transjugular catheters.

PERFUSION CONTROL

Support Dog

A transfemoral catheter was placed in the proximal aorta of the support animals for pressure recording and blood sampling. The ipsilateral femoral vein was cannulated for continuous administration of Ringer's solution. The fluid infusion rate was periodically adjusted in

an attempt to maintain relatively constant values for hematocrit which was measured hourly. Seventy-five to 125 millilitres of fluid was usually administered per hour. Blood which had collected in the chest or other wounds was not returned to the perfusion circuit, nor was stored blood administered when the support animal developed hypotension.

When the lower lobe donor also served as the support animal, the respirator was adjusted to produce an end-inspiratory pressure of approximately 10 cm H_2O and the respiratory rate was adjusted to just exceed the rate of spontaneous respiratory efforts. Homologous support dogs were allowed to breathe spontaneously but were intermittently hyperinflated with positive pressure.

Support animals were placed on an electric blanket and covered with surgical drapes in an attempt to maintain normothermia.

Isolated Lung (Lobe)

The rates of perfusion and ventilation of the isolated lung were based upon established values for the normal dog which were extrapolated to the weights of the lung donors (Dittmer and Grebe, 1958; Daly and Hebb, 1966). Conditions were occasionally modified on the basis of gross appearance and function of the lung during the first thirty minutes of perfusion.

After the lung had been cannulated and the chamber sealed, a negative pressure was established in the chamber to maintain an end-expiratory volume. Tidal volume respirations were then instituted by the

respirator pump which cycled sixteen to twenty times per minute and produced negative pressure fluctuations of -11/-4 to -9/-6 cm H_2O in the chamber. During the first few respirations, gentle positive pressure was introduced into the bronchial cannula in order to expand atelectatic areas of the lung.

The tip of the bronchial cannula was placed in the exhaust line from a vessel in which inspired gas bubbled through heated water. In this way, an attempt was made to provide normally humidified inspiratory gases.

The venous reservoir was adjusted to produce a mean pressure of +3 to +7 mm Hg in the pulmonary veins. Blood flow was initiated slowly and gradually increased during the first five to ten minutes of perfusion to a final rate of 15 to 20 millilitres per donor kilogram per minute for lobes and 25 to 40 millilitres per donor kilogram per minute for lungs.

In most experiments, the first hour of perfusion represented a stabilization period during which "control" measurements were obtained. Following control measurements, the respiratory and venous pressures and arterial flow rates were not altered for the duration of the experiment unless the specific effects of alterations in these was the subject of study. Perfusion was continued until the objective of the experiment was satisfied, the lung was severely damaged, or the support dog died.

PHYSIOLOGIC MEASUREMENTS AND CALCULATIONS

Hemodynamics

Vascular Resistance (PVR)

Arterial and venous pressures were continuously recorded on the polygraph using "fluid linkage" of tubing which connected the arterial and venous catheters to strain gauge transducers. Arterial and venous port fittings on the chamber drawer were designed to adapt large Foley catheters to the perfusion and pressure sensing tubing (Figure 1). The ends of the Foley catheters were modified in order to sense lateral pressure in the cannulated vessels beyond the end of the flow channel. The polygraph recorder was equipped with a signal attenuator which critically damped the recording galvanometer and provided direct mean pressure readout.

The arterial pump rate occasionally drifted by a maximum of ten percent over periods of one hour. The pump was always returned to the "control" rate prior to the hourly recording of flows and pressures.

The difference between mean arterial and venous pressure was divided by the flow rate to obtain vascular resistance units (R):

$$R = \frac{\overline{pPA} - \overline{pPV}}{FLOW\ (ml/min)} \qquad (i)$$

Calculated values for vascular resistance were usually expressed in percent of control in order to simplify comparison of resistance at

various times during perfusion. Absolute values for vascular resistance, expressed in dynes. sec. cm^{-5} were derived from Aperia's Formula (Warren and Gorlin, 1958):

$$PVR = \frac{(pPA - pPV) \times 1332 \times 60}{FLOW\ (ml/min)} \qquad (ii)$$

Potential Sources of Error: A pressure recording can drift as a result of variations in the main power supply or inherent instability in the carrier amplifier or bridge circuitry of the strain gauge transducers. Bridge balance and pressure calibrations were checked before, after, and often several times during every experiment. Correction for errors which occurred were extrapolated on the basis of the time between calibration checks. The zero reference point in the pressure monitoring system, i.e. the lung hilum, was checked and the fluid linkage in the pressure catheters was flushed with heparinized saline before each pressure recording.

Blood loss from the organ could lead to erroneous pressure and flow determinations. In none of the lung perfusion experiments was this loss greater than 150 millilitres per hour. The error which this factor introduced was therefore always less than 1%. At least once every five experiments the flow rate - pump speed calibration was checked. The pump rate was accurate to within 5%. In several longer experiments, the pump flow setting was compared to the continuous recording of an electromagnetic square wave flowmeter probe.* These values also corresponded to

*Zepda EPD - 2RD flowmeter, Zepda Instruments, Inc., Seattle, Washington.

within 5%. The effect of pump variation between experiments was minimized because in each experiment an initial period of measurement provided "control" values.

Intrapulmonary Blood Volume (PBV)

Organ blood volume has been defined as the product of the mean time for blood (or dye) to flow through the organ (mean transit time) and the flow rate (Zierler, 1962; Grodins, 1962). The lack of a single inflow and outflow orifice, variable flow rate, and systemic recirculation all limit the accuracy of dye dilution measurement of in situ organ blood volume (Zierler, 1962). The isolated lung avoided these problems and appeared amenable to relatively accurate dye dilution measurement of intravascular blood volume (PBV). In order to investigate the usefulness of this technique and to provide supplementary information, dye curve recording was carried out in many experiments.

One milligram of Cardio-green* dye was injected by bolus into the pulmonary artery catheter for each determination. A venous blood-dye sample was withdrawn at a rate of 50 millilitres per minute through a densitometer which was connected to the pulmonary venous catheter. The densitometer§ was equipped with an automatically recording dye-curve area-integrator.

* Hynson, Westcott and Dunning Inc., Baltimore, Maryland: brand of Indocyanine-green.

§ Beckman Cardiodensitometer, Beckman Instruments Inc., Palo Alto, California.

The time taken for blood flow in the non-pulmonary channels equalled the volume of tubing divided by flow rate. The mean transit time ($\overline{MTT}$) was then directly measured (Figure 2). The intravascular blood volume was calculated as follows:

$$PBV(ml) = \overline{MTT} \times FLOW\ (ml/sec) \qquad (iii)$$

The results of several experiments in the same group were compiled and the blood volume was expressed as "percent of control."

Potential Sources of Error: Initial measurements revealed that mean transit time was influenced by the time in the respiratory cycle when the dye was injected. This was apparently related to cyclic variation in pulmonary blood volume with respiration. The dye was always injected at the moment of end-expiration to obtain the data which are presented.

The effect of dye-curve variation which results from a slight variation in bolus injection time was minimized by obtaining triplicate dye curves under any given study condition. This study did not require flow measurement because this value was known. On several occasions flow rate was calculated from the dye curve and flow rate as indicated by the pump setting coincided to within five percent. Accurate quantitation of average dye concentration was not required for $\overline{MTT}$ determination. Variations in optical properties of the dye which occur with time and variation in densitometer calibration would have no effect on the measured mean transit time.

Gas Exchange

Arteriovenous Oxygen Difference ($[a-\bar{v}]\ pO_2$) and Oxygen Uptake ($\dot{V}O_2$)

Hourly measurements of pO_2, pCO_2 and pH were obtained for pulmonary arterial (mixed venous) and pulmonary venous (arterialized) blood in the lung perfusion circuit and aortic blood from the support dog. Blood gas determinations were carried out using direct reading microelectrodes.* Arteriovenous oxygen tension and content differences were calculated using the following formulae:

$$(a-\bar{v})\ O_2\ (\text{mm Hg}) = PVpO_2 - PApO_2 \qquad \text{(iv)}$$

$$\dot{V}O_2\ (\text{ml/min}) = CaO_2 - C\bar{v}O_2 \qquad \text{(v)}$$

CaO_2 and $C\bar{v}O_2$ are the calculated oxygen contents in arterialized and mixed venous blood respectively. These values were obtained in the following way: Oxygen saturation was first calculated using the following formula for the oxyhemoglobin dissociation curve of the dog (Rossing and Cain, 1965):

$$\log \frac{s}{1-s} = 2.5198 \log pO_2 - 1.1804\ (pH - 7.40) - 0.047234\ T - 2.3621 \qquad \text{(vi)}$$

* Radiometer, Type PHA 927 Blood gas monitor, Bach Simpson Ltd., London, Ontario.

Oxygen content was then derived as follows (Kelman, 1966):

$$CxO_2 = pO_2 \times \alpha + 1.34 \times Hgb \times \frac{S}{100} \qquad \text{(vii)}$$

$$\text{Where } \alpha = 0.0059519 - 0.0001266T = 0.0000013T^2 \qquad \text{(viii)}$$

(T = 38° C was used in all calculations.)

Potential Sources of Error: Assessment of gas exchange using $(a-\bar{v})\ pO_2$ and $\dot{V}O_2$ is subject to possible error in both measurement technique and interpretation. In several early experiments, blood gas determinations were carried out on the laboratory instrument as well as an IA blood gas analyser at the University Hospital and found to coincide to $\pm$ 2 mm Hg. Microelectrode readings were taken in duplicate or triplicate for most samples. pO_2, pCO_2 and pH usually corresponded to $\pm$ 2 mm Hg and $\pm$ .02 respectively. The gas analyser was recalibrated every one to two hours in order to correct a variation in sensitivity of up to 5%.

The temperature at the source of the sample and the temperature of the measurement device must be identical in order to obtain quantitatively accurate blood gas determinations (Bradley et al, 1956). Effort was made to control the temperature of the components of the perfusion apparatus which would affect the temperature of the lung and the blood. Temperature discrepancy among the blood gas analyser, support dog, blood entering the perfusion chamber and the lung parenchyma was nevertheless encountered. The analyser temperature was constant at 38°C. The temperature of the support dog and the chamber interior occasionally differed by as much as 4°C. In none of the experiments were the temperature of

the lung or the pulmonary arterial blood accurately measured.

The $(a-\bar{v})$ O_2 tension and content differences depend to a large extent on the region of the oxyhemoglobin dissociation curve about which oxygen exchange is taking place in the lung. The pO_2 in the mixed venous blood was not constant in most experiments. In later experiments, more sophisticated indices of gas exchange were used to assess gas exchange in the isolated lung.

Alveolar-arterial Gradient $[A-a]O_2$ and Arteriovenous "Shunt" $(\dot{Q}s/\dot{Q})$

Pulmonary gas exchange is impaired by three forms of abnormality in function (Comroe et al, 1962, p.358):

1. Uneven ventilation-perfusion relationships; "physiologic" shunting.
2. Anatomic arterio-venous shunts.
3. Alveolar capillary diffusion abnormalities.

All of the foregoing contribute to an increase in the difference between the calculated Alveolar pO_2 (pAO_2) and the measured arterial pO_2 (paO_2) when the lung is inspiring gas of room-air composition (20.93% O_2); the so-called alveolar-arterial oxygen gradient ($[A-a]O_2{}^{20}$). The gradient when the lung is breathing twelve to fourteen percent oxygen ($[A-a]O_2{}^{14}$) reveals the efficiency of alveolar capillary oxygen diffusion. Under these conditions, the effects of the anatomic and physiologic shunts are minimized although they are not eliminated (Comroe et al, 1962, p. 132). The equilibration of the pulmonary alveoli with 100 percent oxygen in the inspired gas, virtually

eliminates all but the "anatomic" veno-arterial shunts. The $[A\text{-}a]O_2^{100}$ serves as an index of the "true" arteriovenous shunt.

A more quantitative assessment of the gas exchange is obtained by calculating the percentage of the total pulmonary blood flow ($\dot{Q}$) which is shunted ($\dot{Q}s$) under each of the three inspiratory oxygen conditions ($\dot{Q}s/\dot{Q}^{20}$, $\dot{Q}s/\dot{Q}^{14}$, $\dot{Q}s/\dot{Q}^{100}$).

In order to calculate oxygen gradients and "shunts," several additional measurements were obtained simultaneous with arterial and venous blood sampling.

A two-way, non-rebreathing low resistance valve was attached to the bronchial cannula port on the perfusion chamber. The inspiratory side of the valve was connected to a low resistance weather balloon which provided humidified gas of desired oxygen concentration (room air, 12 to 14 percent oxygen in nitrogen, or 100 percent oxygen). When blood gas samples were being withdrawn, another low resistance balloon collected expired gases for a period of 90 to 150 seconds. Endobronchial CO_2 was continuously measured by an infrared carbon dioxide analyser* which withdrew 150 millilitres of gas per minute from a fine polyethylene catheter which was located in the bronchial cannula. The percentage of CO_2 at end-expiration was recorded for each breath. The average of 20 to 30 of these values was considered representative of the concentration of CO_2 in the alveoli ($FACO_2$).

The fraction of CO_2 in mixed expiratory gas ($FECO_2$) was measured with the CO_2 analyser. pEO_2 (partial pressure of oxygen in mixed expira-

*Godart Type KK Capnograph, Instrumentation Associates Inc., New York.

tory gas) was measured by expressing gas from the bag into the pO_2 microelectrode.

$[A\text{-}a]O_2$ under conditions of room air and fourteen percent oxygen breathing were calculated using a modification of the alveolar air equation (Comroe et al, 1962, p.353):

$$[A\text{-}a]O_2^{20,14} = [pIO_2 - \frac{FACO_2}{FECO_2} (pIO_2 - pEO_2)] - paO_2 \qquad (ix)$$

$[A\text{-}a]O_2$ under conditions of 100 percent oxygen breathing was calculated using the following formula (Comroe et al, 1962, p.344):

$$[A\text{-}a]O_2^{100} = (pBAR - pAH_2O - pACO_2) - paO_2 \qquad (x)$$

(where pBAR = barometric pressure, pAH_2O = calculated water vapor pressure at 38°C and pBAR and $pACO_2 = FACO_2 \times p\ BAR$)

$\dot{Q}s/\dot{Q}$ under conditions of room air and fourteen percent oxygen breathing were calculated using the following equation (Comroe et al, 1962, p.344):

$$\dot{Q}s/\dot{Q}^{20,14} = \frac{CaO_2 - C\acute{c}O_2}{C\bar{v}O_2 - C\acute{c}O_2} \qquad (xi)$$

(where $C\acute{c}O_2$ is the calculated oxygen content in end-pulmonary capillary blood.)

The accuracy of the shunt equation (formula xi) is dependent on accurate oxygen content derivation. The use of this equation for the calculation of shunt when pIO_2 is 100 percent would be subject to considerable error. The accuracy of $C\acute{c}O_2$ relies on accurate pAO_2 (which is used as an approximation of end-capillary pO_2) as well as α (the temperature factor) which appears in equation (viii). In the range of 150 to 700 mm Hg, the oxygen tension in blood samples and the oxygen electrode are both unstable. When arterialized blood and end-capillary blood are "supersaturated," their content differences are small. Small error in the $C\acute{c}O_2$ or CaO_2 will therefore produce a relatively large error in the numerator of the equation.

In order to circumvent the foregoing problems, $\dot{Q}s/\dot{Q}^{100}$ was calculated using a modified form of the "venous shunt calculation" as proposed by Chiang (1968):

$$\dot{Q}s/\dot{Q}^{100} = \frac{(pAO_2 - paO_2)}{[(pAO_2 - paO_2) + \frac{(CaO_2 - C\bar{v}O_2)}{0.003}]} \qquad \text{(xii)}$$

All [A-a] gradients and shunts are expressed in absolute terms, i.e. mm Hg and percentage in the presentation of results.

Potential Sources of Error: Discrepancy in the temperature of the lung parenchyma and inspired and expired gas can introduce error in the measurement of gas concentrations. Correction could not be made because the aforementioned temperatures were not measured.

Several additional factors which could introduce error in the measurement of the ventilation gases were recognized and care was exercised to minimize these by: frequently checking the pO_2 electrode and the pIO_2 as pressures in the inspiratory gas tank decreased; attempting to assure equilibration of the humidifying and warming appratus with the inspiratory gases; repeatedly flushing the inspiratory-gas balloon before sampling times; using the same gas for calibration of both the CO_2 analyser and the pCO_2 electrode and twice flushing the expiratory gas bag before test collections. For uniformity of derivation, all calculations were standardized at normothermia and all samples were assumed to be saturated (BTPS).

The assumption that the partial pressures of alveolar and end-capillary oxygen equilibrate and therefore that pAO_2 can be used with reasonable confidence to obtain $C\acute{c}O_2$ for the "room-air shunt" calculation, is considered to be valid (Comroe et al, 1962, p.132). The calculation of shunt for <u>low</u> pIO_2 conditions using this assumption is subject to error when there is a diffusion abnormality. Under these conditions, the $C\acute{c}O_2{}^{14}$ which is used is erroneously high ($p\acute{c}O_2$ will, in fact, be less than pAO_2). The effects of this incorrect assumption are partially cancelled by the appearance of $C\acute{c}O_2{}^{14}$ in both numerator and denominator of the equation. The error which remains, however, is that of a higher calculated $\dot{Q}s/\dot{Q}^{14}$ than really exists.

Methods are not available for measuring or accurately calculating end-capillary pO_2. The Bohr "trial and error method" which was an alternative, is at best cumbersome, subject to errors in the many additional measurements which are required and still relies on certain assumptions

(Comroe et al, 1962, p. 357).

A diffusion abnormality would also be reflected in the $[A\text{-}a]O_2$[14] and this supplementary information was obtained.

Computer Calculations

The foregoing calculations were facilitated by the use of a computer* which was programmed using a modification of techniques which Kelman (1966) reported for calculation of pulmonary function indices. The following data were provided for the calculations:

TIME, FIO_2, pEO_2, $FACO_2$, $FECO_2$, paO_2, $paCO_2$, paH, $p\bar{v}O_2$, $p\bar{v}CO_2$, $p\bar{v}H$, Hgb, pBAR, BLOOD FLOW RATE and TEMPERATURE

These data were processed by the program which is shown schematically in Figure 3. The following results were then printed out:

TIME, FIO_2, pAO_2, saO_2, $s\bar{v}O_2$, $s\acute{c}O_2$, CaO_2, $C\bar{v}O_2$, $C\acute{c}O_2$, $p(a\text{-}\bar{v})O_2$, $\dot{V}O_2$, $\dot{Q}s/\dot{Q}$, and $[A\text{-}a]O_2$. (Figure 4)

Mechanics

Compliance (C_L)

The method which was used for controlling ventilation of the isolated lung facilitated compliance measurements. The stroke volume of the respirator pump was adjusted to superimpose pressure excursions

*University of Alberta IBM 360 Computer

of -4 to -6 cm H_2O on a constant -3 to -5 cm H_2O end-expiratory pressure in the chamber. The chamber pressure and respiratory rate remained unchanged for the duration of each experiment.

Following the measurement of $FECO_2$ and pEO_2 in the expired gas, the volume of gas which remained in the collection bag ($\dot{V}_E$) was measured using a balanced-spirometer.* Using $\dot{V}_E$ and the recorded number of respirations for the collection (N_R), tidal volume ($\dot{V}_T$) was calculated using the formula:

$$\dot{V}_T = \frac{\dot{V}_E}{N_R} \qquad \text{(xiii)}$$

From $\dot{V}_T$ and the chamber pressure excursion (P), lung compliance was calculated:

$$C_L = \frac{\dot{V}_T}{P} \qquad \text{(xiv)}$$

The foregoing provided an index of dynamic compliance which reflected changes in airway calibre and parenchymal tissue properties. In the compiled results of experimental groups, compliance was expressed as "percent of control."

Potential Sources of Error: The measured volume of expired gas did not include the volume of gas which was diverted through the CO_2 analyser or the volume which was used to measure pEO_2 with the oxygen

*Collins P-600 Recording Vitalometer, W.E. Collins Inc., Braintree, Mass.

electrode. The CO_2 analyser withdrew gas from the airway at a rate of 150 millilitres per minute. The expiratory phase of respiration was controlled at 60 percent of the respiratory cycle time. Approximately 180 millilitres of expired gas was therefore lost to the CO_2 analyser during a two-minute collection time. An estimated 20 to 40 millilitres of gas was expressed from the collection bag for pEO_2 determinations. Two hundred millilitres was added to the measured $\dot{V}_E$ to approximate correction for the total volume which was lost from the gas collection bag in the course of sampling. The error which was introduced by the foregoing would understandably be relatively greater for small lobes than for large lungs.

The temperatures of the expired gas and the airway were not measured. Measured volumes were used in the calculation of $\dot{V}_T$. The error which was introduced by temperature discrepancies would be smaller for small lobes than for large lungs. Emphasis on the relative changes in serial measurements during individual experiments should have minimized the effects of these errors on the overall interpretation of the results.

Dead space - Tidal volume Ratio ($\dot{V}_D/\dot{V}_T$)

$\dot{V}_D/\dot{V}_T$ was calculated using a modification of Bohr's equation (Comroe et al, 1962, p. 335):

$$\dot{V}_D/\dot{V}_T = 1 - \frac{FECO_2}{FACO_2} \times 100 \qquad \text{(xv)}$$

This value represents the proportion of tidal volume which is wasted on anatomic dead space and areas of the lung which are ventilated but grossly under-perfused.

Potential Sources of Error: For purposes of calculation, end-tidal CO_2 was used for $FACO_2$ in the equation. An end-tidal sample is not necessarily representative of average alveolar gas composition. At best it is an approximation of alveolar gas in the absence of major defects in ventilation. In the absence of significant venous to arterial shunting, $paCO_2$ is more closely representative of $pACO_2$. The use of the end-tidal CO_2 in the calculations for the experiments which were carried out appeared acceptable for several reasons. The difference between $pACO_2$ and $paCO_2$ was seldom greater than the combined error of the methods for determining $paCO_2$ (2 mm Hg) and $pACO_2$ (2 - 3 mm Hg). Both $FACO_2$ and $FECO_2$ were measured at the same analyser calibration and proportionate errors in their absolute values were cancelled by the fraction in the $\dot{V}_D/\dot{V}_T$ equation.

Presentation of Function Results

In the experiments which were carried out, emphasis was placed on the relative change in the function indices during the period of perfusion. Most of the studies were ideally suited for this because individual experiments usually provided their own "control" periods. By this approach, errors in measurements and calculations were likely expressed relatively equally in the series of results which were obtained in each experiment.

In every group of identical experiments, the hourly values for individual indices of function were averaged. Where three or more experiments provided data for a given hour of perfusion, the standard deviation (SD) for the values was calculated. For the majority of experimental groups the results are presented graphically. Hourly mean values are joined by a solid line and $\pm$ 1 SD is represented by vertical bars.

When only one or two values were obtained for a given hour, the value or average of two is joined by a broken line. In several pilot experiments, values were obtained at variable times before or after an alteration in the conditions of the experiment. These were averaged and presented in vertical bars resting on the abscissa or perfusion-time axis.

MORPHOLOGIC STUDIES

Weight Change

All lungs were weighed before and after perfusion. Clamps or other materials which were attached to the lung before perfusion were separately weighed after perfusion and appropriate correction was made in the recorded weight. Weight change was expressed in terms of percentage gain or loss.

A gain in weight reflected an increase in intracellular, interstitial and intra-alveolar fluid and blood. These values did not permit distinction between the relative changes and distribution of the various "fluid" components.

Potential Sources of Error: The volume of blood which was present in the vasculature at the time of lung excision was undoubtedly influenced by manipulation, respiration and vascular reflexes prior to excision. Post-perfusion weights were subject to error because variable amounts of fluid escaped from the airways and vasculature when the lungs were collapsed and disconnected from the perfusion circuit. Most lungs weighed more following perfusion than following excision. Intravascular blood volume was likely greater immediately following perfusion because the lung was spared manipulative surgery prior to the second weight determination.

Gross Examination

Following every perfusion the gross appearance of the organ was recorded. The degree of atelectasis, congestion, airway edema and/or hemorrhage and parenchymal hemorrhage was graded. Colour photographs were obtained following most experiments to further document the gross appearances.

Histology

After weighing the lung, the bronchus was cannulated and the airway was filled with ten percent formaldehyde to a hydrostatic pressure of 4 to 6 cm H_2O above the hilum. The bronchus was then tied and the organ was immersed in ten percent formaldehyde. Seven to fourteen days later, specimens were taken from regions which appeared to be the most abnormal, usually the dependent zones. The specimens were embedded in paraffin for histologic section.

All sections were stained by the routine hematoxylin-eosin method (H & E) and many were stained by Verhoeff's method for elastic tissue (VER) in order to facilitate the identification of small arteries and veins.

All preparations were evaluated independently by the author and another microscopist. Attention was focused on the following abnormalities: perivascular edema, interstitial edema, perivascular, interstitial, and alveolar hemorrhage, vascular congestion and abnormal aeration (atelectasis, alveolar irregularity). Other abnormalities such as lymphatic distention, vascular occlusion and thrombotic material were also noted.

In an attempt to quantitate these admittedly subjective gross and histologic observations, grades of nil, mild, moderate and marked degrees of alteration in the individual features were ascribed the numbers 0, 1, 2, and 3 respectively. The summation of these values for each group of lungs was averaged in an attempt to obtain indices which would allow approximation of the magnitude and pattern of alterations under the conditions of perfusion for each group (Appendix Figure 1).

Potential Sources of Error: Diffuse bronchiolar dilation was a common finding. Whether this resulted from the instillation of formalin or from devitalization of bronchial wall as Trowell (1944) has suggested, was impossible to determine. The introduction of fluid into the airway also prevented the estimation of alveolar edema.

The location from which the histologic specimens were obtained varied somewhat. The assumption that the specimens were fully representative is tenuous because perivascular edema varies regionally (West et al, 1965).

The duration of individual perfusions varied in most groups of experiments. The severity and pattern of morphologic alteration were likely influenced by this factor.

The grades which were ascribed to alterations in individual histologic specimens were arbitrary and could not, with confidence, be considered representative of an arithmetic progression in degrees of morphologic change. The histologic results for the experiments which follow are summarized in Appendix Figure 1.

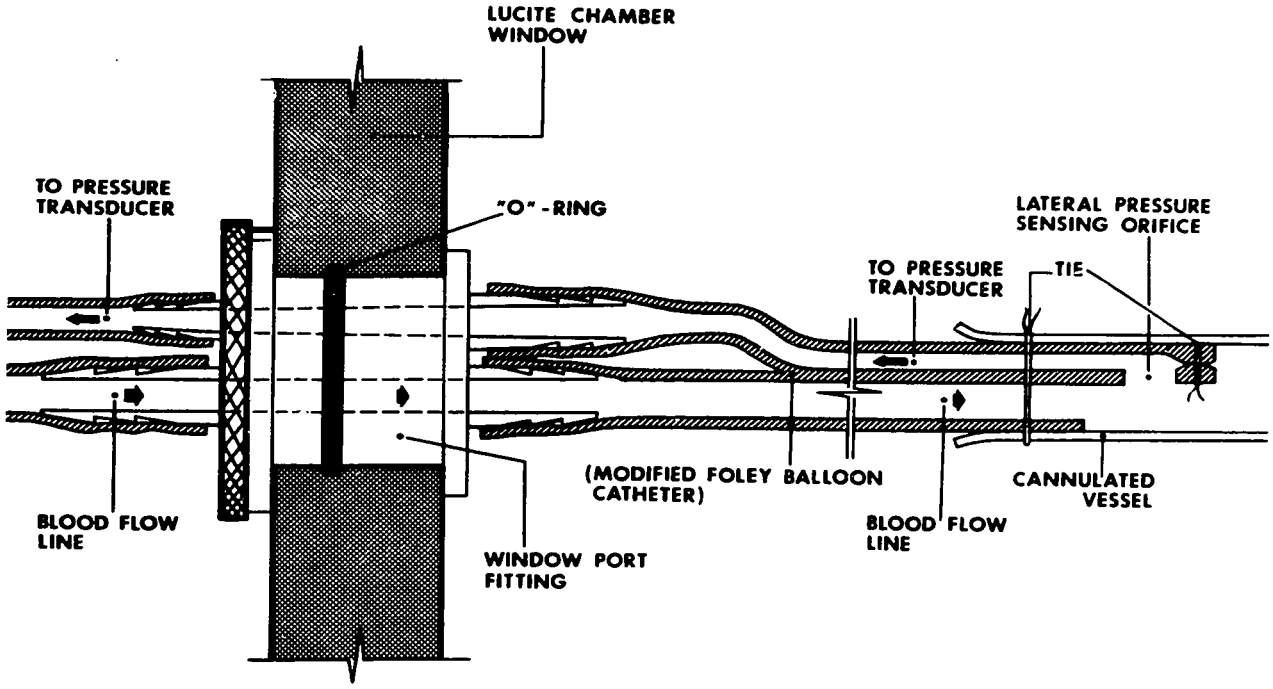


Fig. 1. Blood flow-pressure catheter and chamber port design.

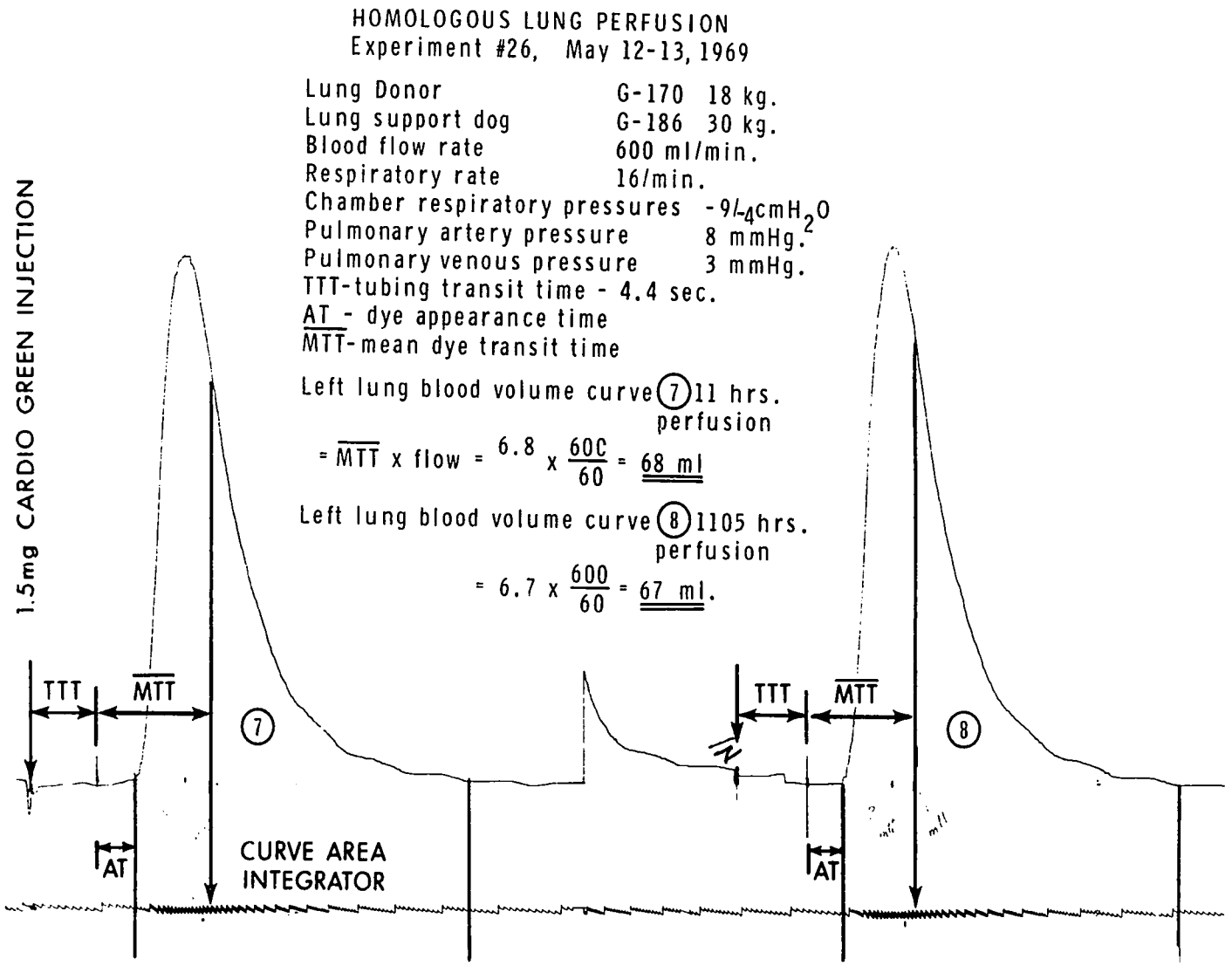


Fig. 2. Dye-dilution curves for intrapulmonary blood volume determination.

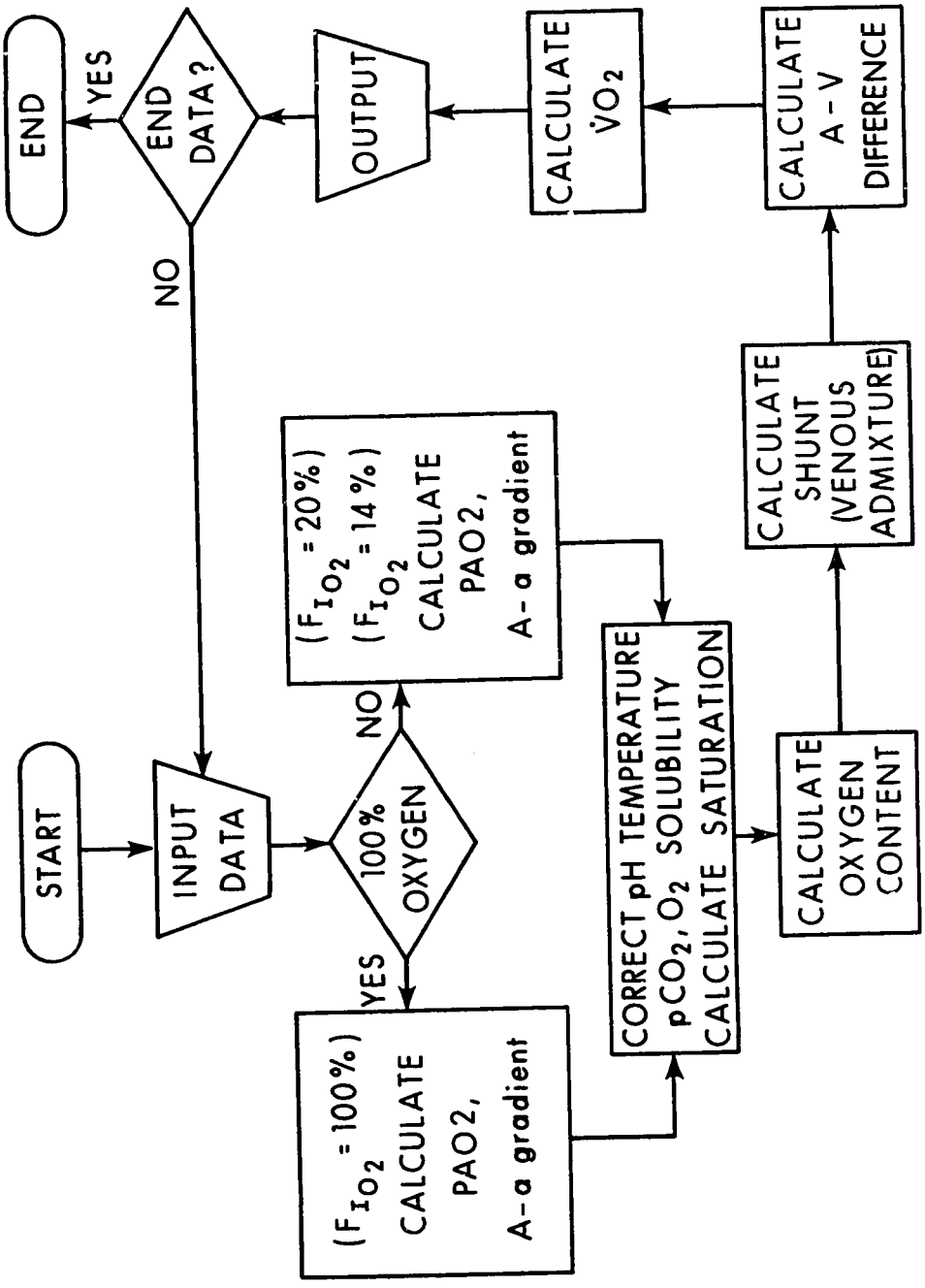


Fig. 3. Flow diagram of gas exchange computer program.

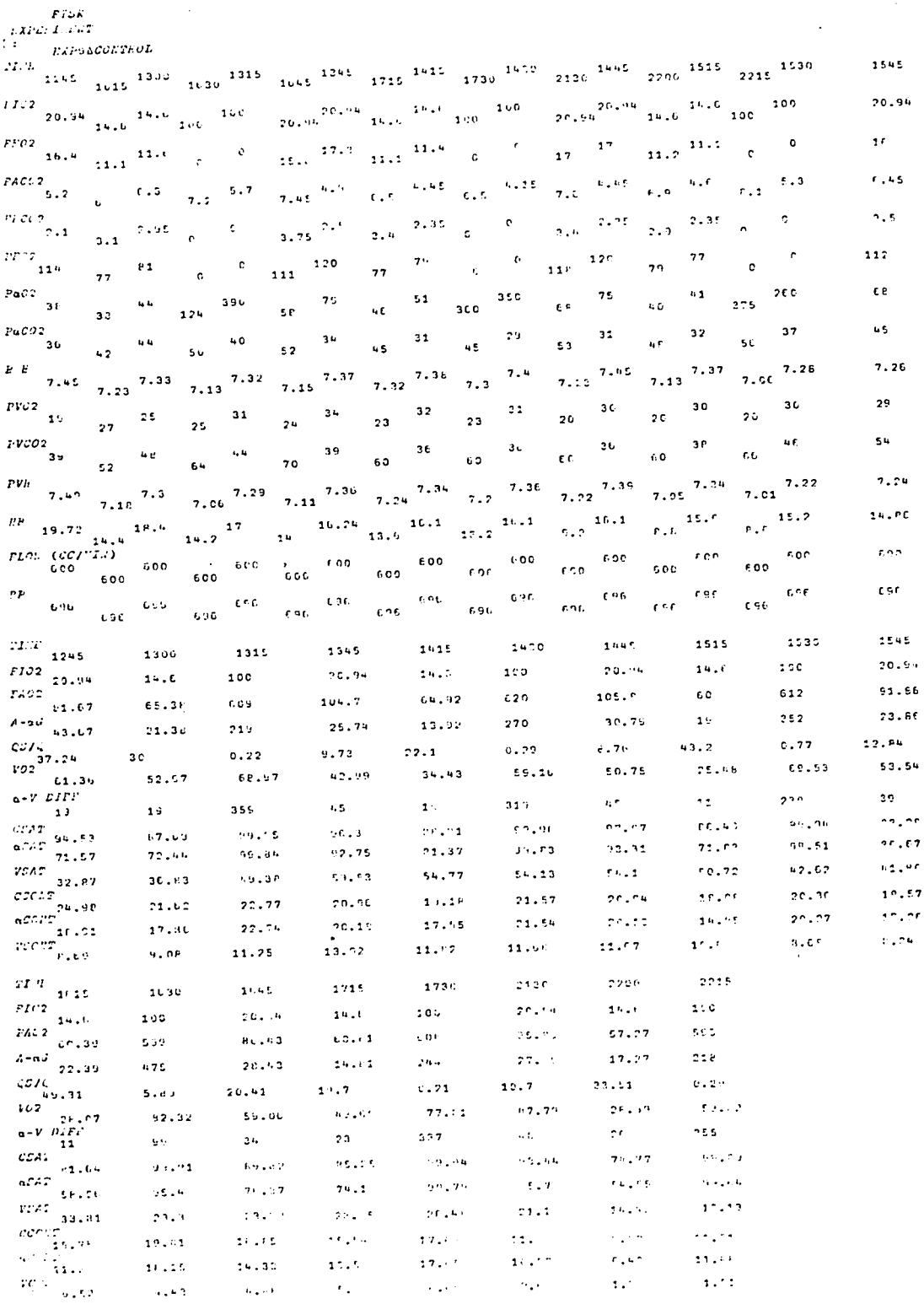


Fig. 4. Print out of gas exchange data and calculations.

CHAPTER IV

EVALUATION OF THE PERFUSION METHOD

The selection of methods and design of the perfusion apparatus were based on the assumption that attempts to simulate a physiologic environment would enhance functional preservation in the isolated perfused lung.

When the apparatus became available a series of lung perfusion experiments were carried out to test the method. In this initial series emphasis was placed on the duration of perfusion and stability of function. The duration of satisfactory function should reflect the adequacy of the method to provide for the requirements of the lung. Reasonably stable function of the lung under control conditions was a prerequisite for pathophysiologic studies.

METHODS

Eight left lower lobes were autologously perfused at a rate of 15 millilitres per kilogram per minute. The lobes were ventilated using negative pressures which were necessary to produce an initial tidal volume of 5 millilitres per kilogram (Ditmer and Grebe, 1958). Venous pressures were maintained at a mean of +7 mm Hg. Positive pressure ventilation with 97 percent oxygen and 3 percent CO_2 was used to support respiration in the donors.

The perfusion circuit is presented schematically in Figure 1.

RESULTS

None of the first eight experiments failed because of technical problems with the perfusion apparatus. Three of the experiments terminated between the fifth and sixth hours when the supporting dogs died. The remaining five experiments were discontinued during the sixth hour when the donors became severely acidotic and hypotensive.

Figure 2 presents the average hourly indices of function in the eight lobes. At five hours, the vascular resistance was 27 percent higher than at the beginning of perfusion. During the first two hours, the vascular resistance was lower than when perfusion began.

The compliance of the lobes decreased to 85 percent of the initial value over the five-hour period, but the arteriovenous pO_2 difference and the $\dot{V}O_2$ increased. These increases were accompanied by a decrease in the pO_2 of the mixed venous blood which was pumped to the lobes from the support animals.

Gross edema did not occur in any of the lobes in this series. The lobes gained an average of 20 percent in weight. Throughout perfusion, most of the lobes exhibited mild plethora which was usually more marked in the dependent regions (Figure 3).

A photomicrograph of the five-hour lobe is shown in Figure 4. Perivascular and interstitial edema and leucocytic infiltration was a frequent finding. Vascular congestion, perivascular hemorrhage and alveolar edema and hemorrhage were rarely seen.

Figure 5 presents the hourly values for arterial pressure and mixed venous pO_2 and pH in the support animals. All three values

progressively decreased despite satisfactory function of the isolated lobes and ventilation of the in situ right lungs with 97 percent oxygen and 3 percent CO_2.

Following many of the experiments, the right lungs of the donors were grossly congested and atelectatic, and in three, tracheobronchial edema and hemorrhage were present. Moderate interstitial edema and alveolar septal thickening, congestion and alveolar collapse were commonly seen histologically (Figure 6). Perivascular hemorrhage was also noted in several histologic specimens.

DISCUSSION

Many attempts have been made to prolong functional and morphologic integrity of isolated lungs under non-physiologic conditions of perfusion. In all of these, major derangements in function have been evident early in the period of perfusion and lungs have usually been destroyed following one to five hours of perfusion (Awad et al, 1965-1; Veith et al, 1966; Eiseman, 1967).

Progressively increasing pulmonary vascular resistance is a common observation. A two-fold increase in resistance has developed during the first hour of perfusion in preparations which have used positive pressure ventilation and gravity venous drainage (Veith, 1966; Thelmo et al, 1970). A considerable increase in vascular resistance has been observed after two to four hours in negative pressure ventilated lungs when lower than normal flow rates have been used (Fowler et al, 1966).

Major increases in vascular resistance did not develop over a

five-hour period of perfusion in this initial series of experiments. The average resistance decreased during the first hour of perfusion. This possibly reflected vasomotor relaxation following the anoxic period which was incurred during the pre-perfusion period of lobe ischaemia.

Many possible causes for increased vascular resistance in perfused organs have been forwarded. These include: (1) erythrocyte, leucocyte and platelet aggregation; (2) denaturation of plasma proteins; (3) embolism of fat and lipid; (4) liberation of vasoactive materials; (5) metabolic derangements, and (6) abnormal hemodynamics resulting from non-pulsatile arterial flow and abnormal venous pressures, (Humphries, 1967; Robertson and Jacob, 1968; Belzer et al, 1968-1). These factors are exaggerated in totally mechanical circuits where artificial oxygenators are used or under hypothermic conditions (Robertson and Jacob, 1968; Belzer et al, 1968-1).

The lobes in this study were perfused at normothermia. The mechanical components of the extracorporeal circuit likely produced minimal blood trauma. The systemic circulation of the support dogs provided a "physiologic" filter for the blood. These factors undoubtedly contributed to minimizing hematologic and metabolic alterations in the perfusion circuit. The attempt to simulate physiologic ventilation, flows and venous pressures likely also contributed to attenuating perfusion damage to the isolated lobes.

Progressive edema, congestion, atelectasis and loss of compliance have been associated with rapidly increasing vascular resistance in other preparations. In this series, compliance also diminished, but not enough to impair oxygen uptake by the isolated lobes.

Causes for the alterations which did develop were not apparent

from these experiments. The lobes were subjected to a progressively decreasing pH and pO_2 in the mixed venous blood. Vasoactive materials may have been released during the development of hypotension in the donors. These factors, in addition to the limitation of perfusion-duration by the "support" dogs represent disadvantages of the preparation. Despite these disadvantages, the preparation was superior to those which have been previously used in pathophysiologic studies. It appeared that the preparation might be useful for studies which required less than five hours of perfusion.

Attempted simulation of the normal environment appeared important to the functional integrity of the isolated perfused lung. When the perfusion apparatus was constructed, it was hoped that this approach would be useful for the study of other isolated organs. Following the foregoing series of lobe perfusions, isolated canine stomachs and brains were perfused. The perfusion methods were modified in order to provide known conditions for these organs in situ. By utilizing the perfusion apparatus to simulate a normal hemodynamic and physical environment for the organs and incorporating a support dog in the perfusion circuit stomachs functioned satisfactorily for twelve to twenty-five hours and brains for up to eight hours (Fisk et al, 1968, 1970; Yates et al, 1970.

SUMMARY

1. Eight left lower canine pulmonary lobes were normothermically perfused with venous blood from a support dog under conditions of negative pressure ventilation and positive venous pressure.

2. Perfusions were limited to five hours duration by cardiorespiratory deterioration in the supporting dogs.
3. At five hours the lobes were functioning satisfactorily, although they had undergone mild changes in vascular resistance and compliance.
4. Attempt to simulate a normal environment improves the functional preservation of the isolated perfused lung and other organs.

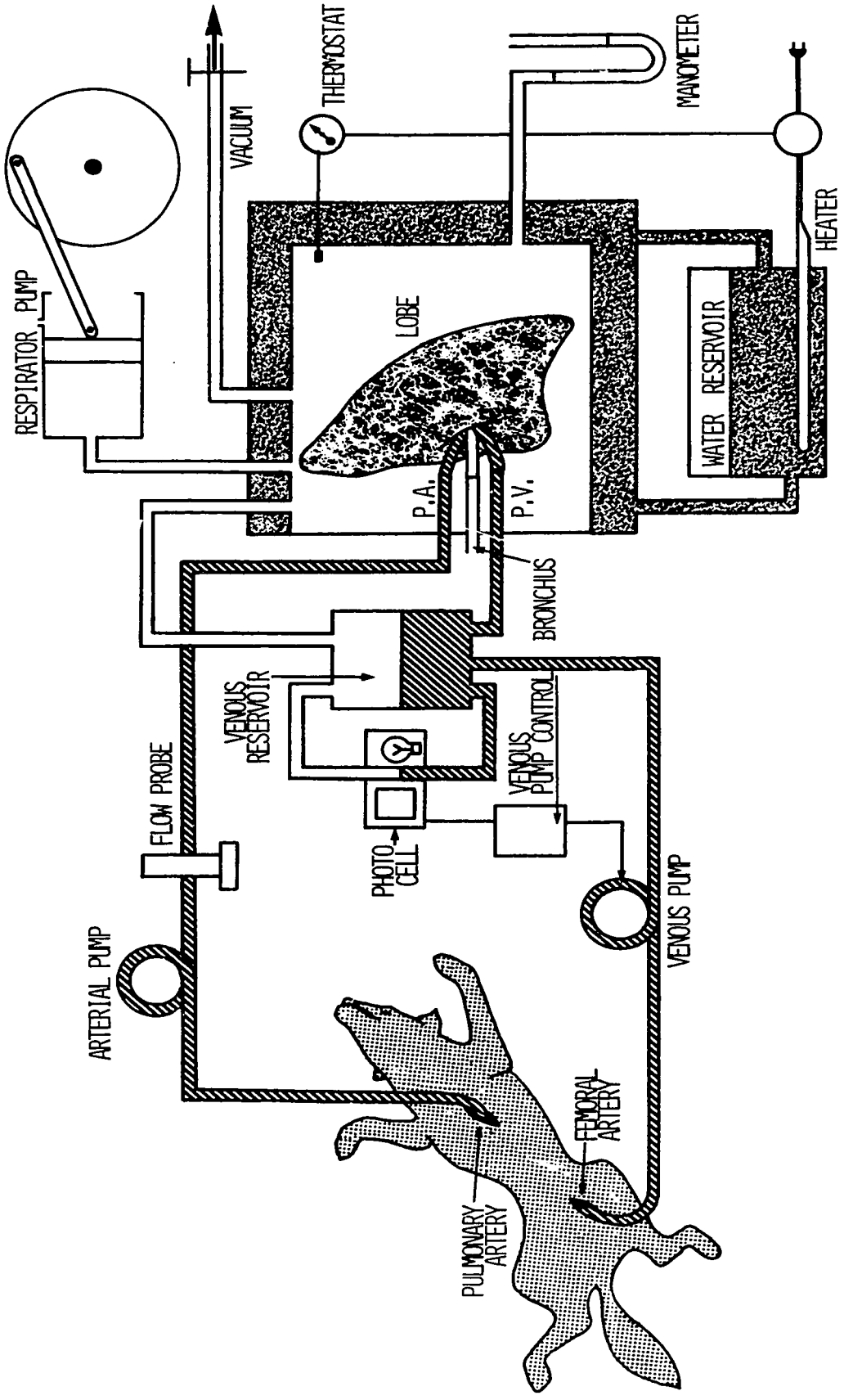


Fig. 1. Flow circuit for autologous perfusion of isolated pulmonary lobes.

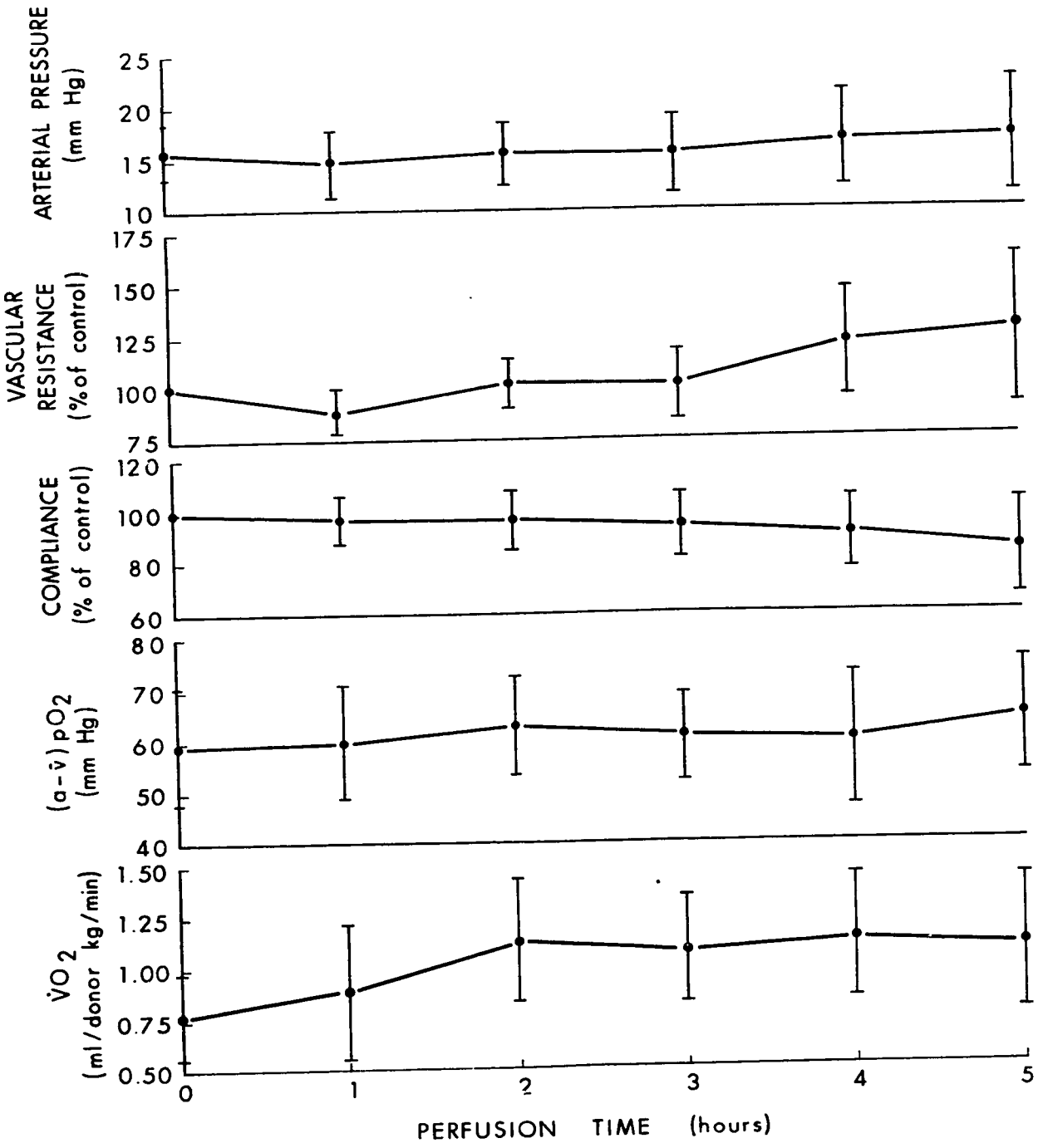


Fig. 2. Function of 8 lobes autologously perfused for 5 hours.

Fig. 3. Lobe after 5 hours of autologous perfusion.

Fig. 4. Lobe after 5 hours of perfusion (H & E x 150) mild interstitial edema; leukocytic infiltrati[illegible].

Fig. 3. Lobe after 5 hours of autologous perfusion.

Fig. 4. Lobe after 5 hours of perfusion (H & E x 150) mild interstitial edema; leukocytic infiltration.

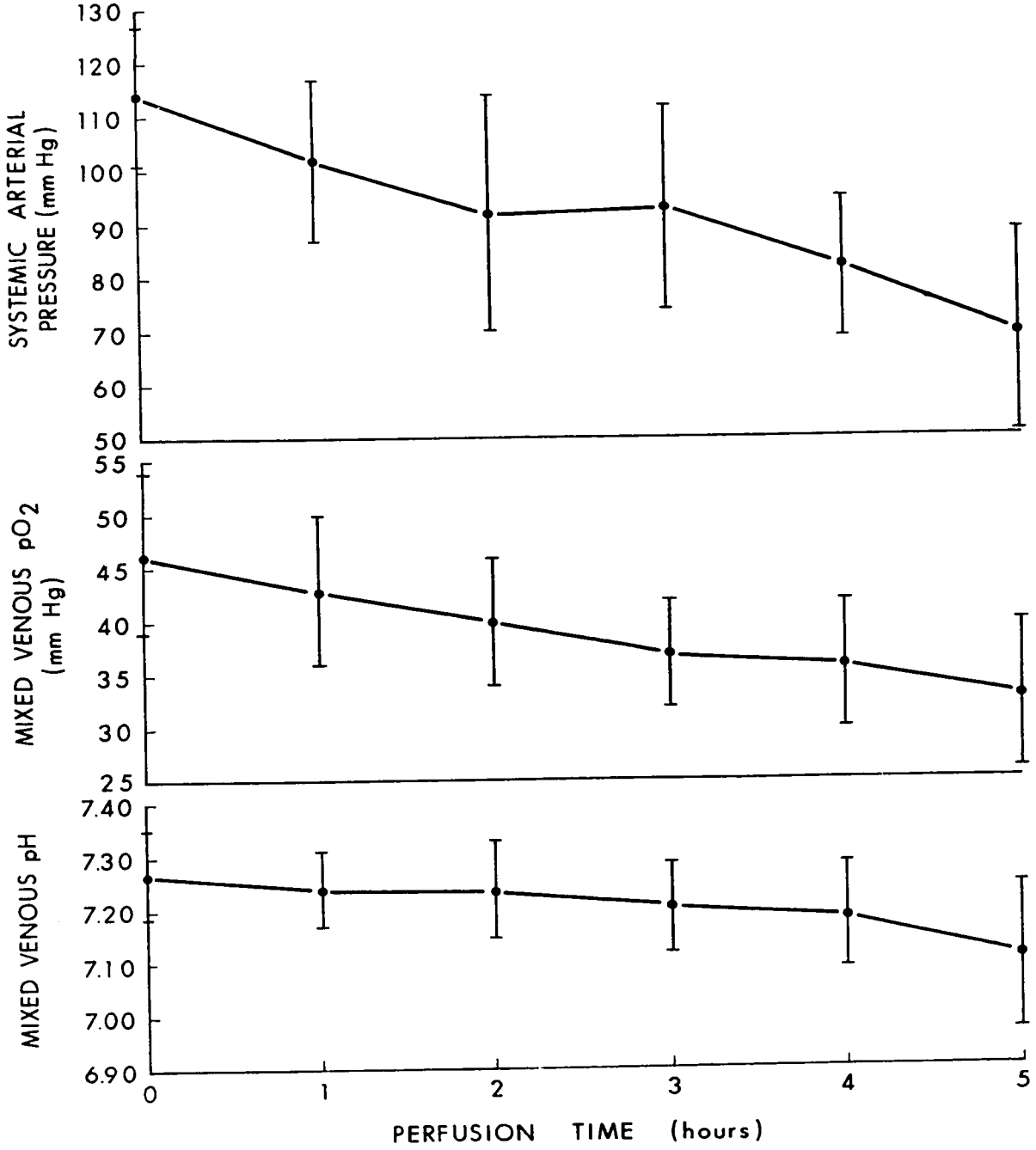


Fig. 5. Cardicrespiratory indices of 8 dogs autoperfusing isolated lobes.

Fig. 6. In situ lung following 5 hours of isolated lobe perfusion. (H & E x 150): Vascular congestion microalveolar collapse, inter-alveolar thickening.

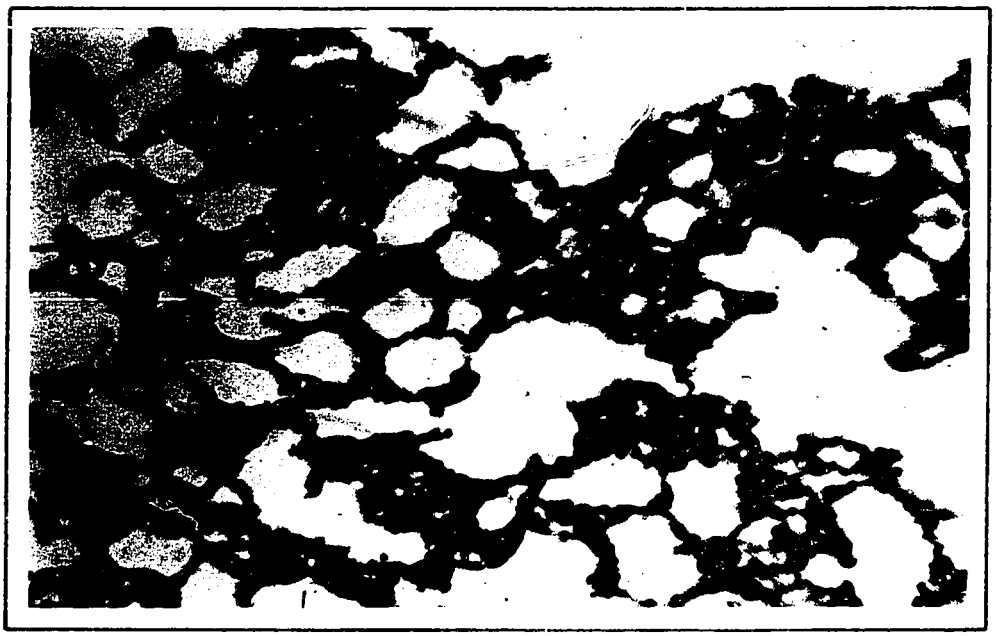


Fig. 6. In situ lung following 5 hours of isolated lobe perfusion. (H & E x 150): Vascular congestion microalveolar collapse, inter-alveolar thickening.

CHAPTER V

ASPIRATION PNEUMONITIS IN THE ISOLATED LUNG: PATHOPHYSIOLOGY AND EXPERIMENTAL THERAPY

The development of an isolated lung preparation which would facilitate the study of acute pulmonary disease was one of the author's major objectives. When five hours of satisfactory function had been achieved by the preceding experiments, it was felt that the potential of the preparation for the investigation of pathologic conditions warranted study.

The instability of the support dogs was a major concern at this point. It seemed that the introduction of factors which would directly compromise the support dog would further complicate the interpretation of events in the isolated lung. For this reason, it was decided to study the effects of direct pulmonary insult.

Aspiration pneumonitis was chosen because the pathophysiology of this condition is poorly understood. Furthermore, investigators who have studied experimental pneumonitis in situ, have experienced difficulty standardizing the degrees of pulmonary insult and distinguishing primary alterations in the lung from their systemic sequelae. Regarding the latter, it was felt that the apparent "sensitivity" of the support animals might become advantageous.

The feasibility of investigating acute acid pneumonitis in isolated lobes was investigated in a preliminary series of experiments. The pathophysiologic effects of acid damage were then studied and an inquiry into the cause of systemic failure in aspiration pneumonitis was

begun. One form of therapy was assessed with the object of determining the usefulness of the model for evaluating treatment methods.

METHODS

General

All lobes were autologously perfused using methods which were described in Chapter IV. In all pneumonitis experiments, 0.5 millilitres per donor kilogram of "aspirate" was sprayed into the endobronchial tube during several tidal volume respirations (Exharos et al, 1965). An attempt was made to distribute the aspirate diffusely to all segments of the perfused lobes.

The study is comprised of four groups of experiments.

Specific

In Group 1 (12 lobes) the effect of various aspirates on gas exchange was assessed. Two lobes served as controls for this group and in these no instillate was introduced. In two lobes, saline was instilled. In three lobes, the gastric juice instillate had a pH of 3.0. In five lobes, gastric juice having a pH of 1.7 was instilled. In three of the latter, the gastric juice did not contain a measurable amount of pepsin.

Group 2 consists of eight lobes from the initial series of lobe perfusions. These served as controls for the following two groups of experiments.

In Group 3 (10 lobes), the lobes were subjected to the instilla-

tion of pH 1.7 gastric juice after the initial measurements had been obtained. The instillate for these and the following group of experiments were obtained from the same canine gastric juice collection.

In five experiments (Group 4), the lobes were subjected to the same insult as Group 3 lobes, but one hour following gastric juice instillation, 0.5 millilitres of Prodecadron* was sprayed into the bronchus during several tidal volume respirations.

Lobes in Groups 3 and 4 were perfused for four hours.

RESULTS

Group 1: The relationships between the composition of the aspirate and the arteriovenous blood gas differences two hours following instillation are shown in Figure 1. Endobronchial saline did not appreciably alter gas exchange or gross appearance of the lobes. Severe changes in gross appearance (Figure 2) and gas exchange were produced by the pH 1.7 gastric juice, but not by the instillate having a pH of 3.0. The effect of highly acid gastric juice did not appear to be dependent on the presence or absence of pepsin.

Figure 3 compares the indices of function in the last three groups of experiments. During four hours of perfusion, the vascular resistance in the control lobes (Group 2) did not change significantly. The resistance in the pneumonitis lobes (Group 3) tripled. Prodecadron did not modify the progressive increase in vascular resistance in the Group 4 lobes.

*Prodecadron - Merck, Sharp and Dohme. (West Point, Virginia). Aerosol preparation containing 0.6 mg. dexamethasone and 0.7 mg. isoproterenol per 0.5 ml.

Compliance steadily diminished in the Group 3 lobes. Prodecadron alleviated the effect of gastric juice on compliance in the Group 4 lobes.

Arteriovenous pO_2 difference increased markedly in the Group 3 lobes. This index changed little during the four-hour period of study in the control group. The administration of Prodecadron appeared to modify gas exchange but there was considerable variation in response among these lobes.

Systemic arterial pressure averaged 72 percent of the control value in the Group 2 dogs at four hours. More severe hypotension developed in all Group 3 experiments after the instillation of gastric juice. Between the third and fourth hours, two of the eight supporting dogs in Group 3 died. None of the supporting dogs in Group 4 died.

The pneumonitis lobes gained six times as much weight as the control lobes. The lobes which were"treated" with Prodecadron, gained only one-half as much weight as the non-treated pneumonitis lobes.

Severe diffuse congestion and parenchymal hemorrhage developed in the pneumonitis lobes within one hour of gastric juice instillation and sanguinous edema fluid poured from the bronchial cannula during the subsequent hour. Histologically the pneumonitis lobes demonstrated perivascular edema, severe peribronchial and endobronchial hemorrhage and interstitial and intra-alveolar edema and hemorrhage three hours following instillation of gastric juice (Figures 4 and 5).

The arteriovenous pCO_2 difference (ΔpCO_2) across the lobes which were subjected to gastric juice, increased progressively following aspiration whereas the arteriovenous pH difference (ΔpH) did not change

correspondingly. ΔpH was consistently related to ΔpCO_2 in the Group 2 lobes. Acid-base analysis was carried out in order to ascertain whether acid was absorbed from the pneumonitis lobes.

Hourly arterial and venous base excess (or deficit) (BE) was calculated using the respective pH and pCO_2 values and the nomogram of Siggaard-Andersen (1963). The pulmonary venous BE minus the pulmonary arterial BE was considered an index of the net metabolic acid-base alteration which was occurring across the lobes. Figure 6 graphically presents the results of this analysis. The data indicate that base deficit was continuously present across the lobes in which gastric juice was present (Group 3). The apparent, though smaller accumulation of base excess in the Group 2 lobes is unexplained.

DISCUSSION

The relationship between the acidity of aspirated fluid and the resultant damage to the lung was first demonstrated by Teabeaut (1952) who studied in situ lungs. This observation was subsequently corroborated by other investigaors (Exharos et al, 1965; Cameron et al, 1967). Ultrastructural changes occur in lungs which are subjected to water or saline but damage is more severe when highly acidic fluid is aspirated (Alexander, 1968). The Group 1 observations and the histologic abnormalities which were produced in the isolated lobes correspond to the in situ results which have been obtained by others. These findings indicate that acute acid pneumonitis can be produced in the isolated perfused lung.

Pulmonary edema which accompanies aspiration pneumonitis has been attributed to direct lung damage. The possibility that pulmonary venous hypertension contributes to the edema formation has not previously been ruled out by in situ studies. Venous pressures were constant in the isolated lobe experiments. The severe edema which occurred in the pneumonitis lobes was clearly a result of local pulmonary damage. This lends additional support to the theory that microcirculatory derangement is produced by altered osmotic gradients at the alveolar capillary level (Alexander, 1968).

The pulmonary vascular response to aspiration pneumonitis has been debated by various authors (Hamelberg and Bosomworth, 1964; Lawson et al, 1966, Awe et al, 1966; Cameron et al, 1967). The role of reflexes and compensatory flow through unaffected areas of the in situ lung are difficult to assess. Under conditions of constant arterial flow rate, lobes which were subjected to acid developed a threefold increase in resistance to flow. The relative role of vascular spasm and perivascular alteration cannot be determined from these results to date.

In the two Group 3 dogs which died, death was preceded by profound acidosis and marked hypotension. Decreased compliance and oxygenating ability by the isolated lobes undoubtedly contributed to systemic hypoxia and acidosis in this group. The in situ right lungs should have compensated considerably for this. Hypovolemia and hemoconcentration occur in animals which are subjected to acid pneumonitis (Cameron et al, 1967). Transudation of plasma into the damaged lung is thought to contribute to shock in aspiration pneumonitis (Awe et al,

1966). In these perfusion experiments, fluids were administered to prevent hemoconcentration.

Base excess (deficit) data for the lobes which were subjected to gastric juice, significantly contrasted with the data for the control lobes. The persistent base deficit suggests that acid was absorbed from the aspirate. Quantitation of the contribution of acid absorption to systemic failure requires additional investigation. This type of study would be facilitated in future by using labelled gastric juice.

It appears that in addition to respiratory failure in the isolated lobes, absorption of metabolic acid and possible changes in total circulating blood volume, other factors may have contributed to hypotension and acidosis in the support animals. The possibility that the lungs release or absorb vasoactive or biochemically potent materials has not been adequately investigated. Future investigations of this nature should be possible using the isolated lung. A sterile extracorporeal circuit will be required for such studies (Daly and Hebb, 1966).

Figure 7 presents a summary of the pathophysiology which was observed in these acid pneumonitis experiments.

The endobronchial administration of Prodecadron resulted in better compliance and arteriovenous oxygen difference than in the non-treated lobes. Tissue damage appeared to be considerably less in the treated lobes. The donor dogs tolerated the insult to the isolated lobes better in the treated than in the non-treated group. The individual roles of isoproterenol and dexamethasone which are contained in the combined drug which was used are unfortunately difficult to assess.

In these experiments, the Prodecadron was instilled one hour following instillation of acid when appreciable damage had already occurred. The role of topical steroids in the treatment of aspiration pneumonitis remains controversial. Lewinski (1965) feels that this is the most effective form of treatment. Other investigators have observed little benefit from topical steroids and indeed, damage has been produced in normal lungs by steroid instillation (Taylor and Pryse-Davies, 1968).

Better function of the lobes in the treated group may have contributed to better support of the donors. The possibility that some of the drug may have entered the donor circulation requires additional study.

SUMMARY

1. The alterations which are produced by aspiration of gastric acid were studied in 23 autologously perfused isolated pulmonary lobes.
2. Acute acid pneumonitis can be produced in the isolated lung.
3. The functional alterations were characterized by a threefold increase in resistance to blood flow and a substantial decrease in compliance and oxygenating ability.
4. A significant amount of acid was absorbed from the lungs which contained gastric juice.
5. The absorption of additional materials may have contributed to systemic failure in the lung donor - support dogs.

6. The study of pathophysiologic as well as treatment methods for acid pneumonitis can be carried out objectively in the isolated functioning lung.

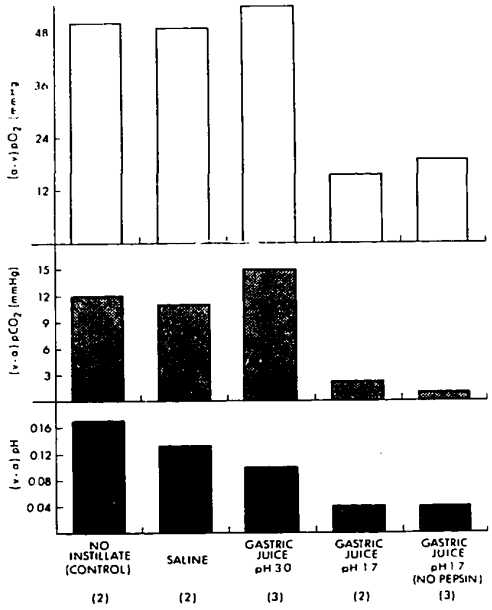
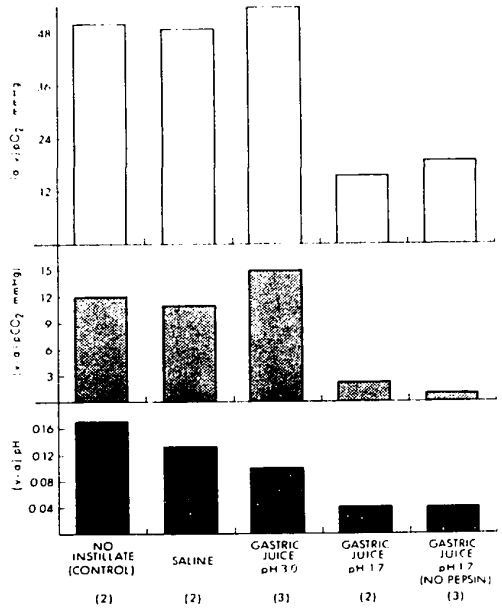


Fig. 1. Arteriovenous pO_2, pCO_2 and pH difference 2 hours following endobronchial instillation of various solutions.

Fig. 2. Lobe 1 hour after endobronchial gastric juice instillation.



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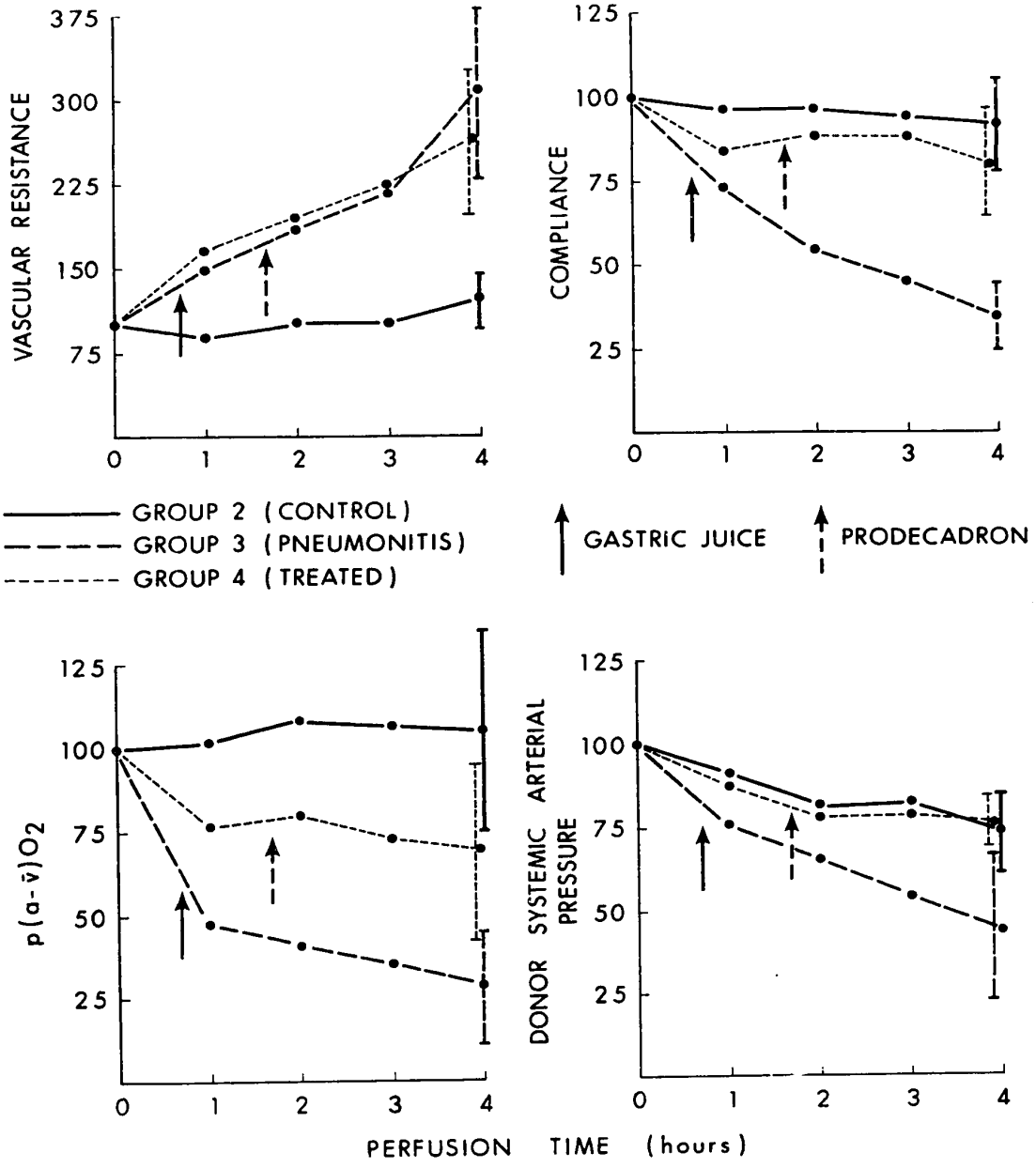

Fig. 3. Function of 8 control, 10 pneumonitis and 5 "treated" pneumonitis lobes (all values-- percent of control).

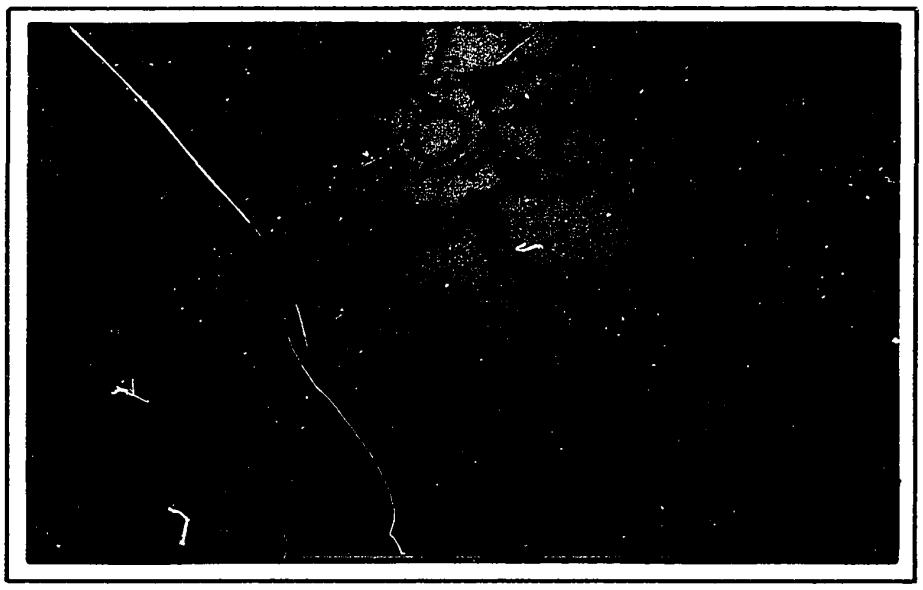

Fig. 4. Lobe 3 hours after gastric juice instillation (H & E x 55)

Fig. 5. Lobe 3 hours after gastric juice instillation (H & E x 125).

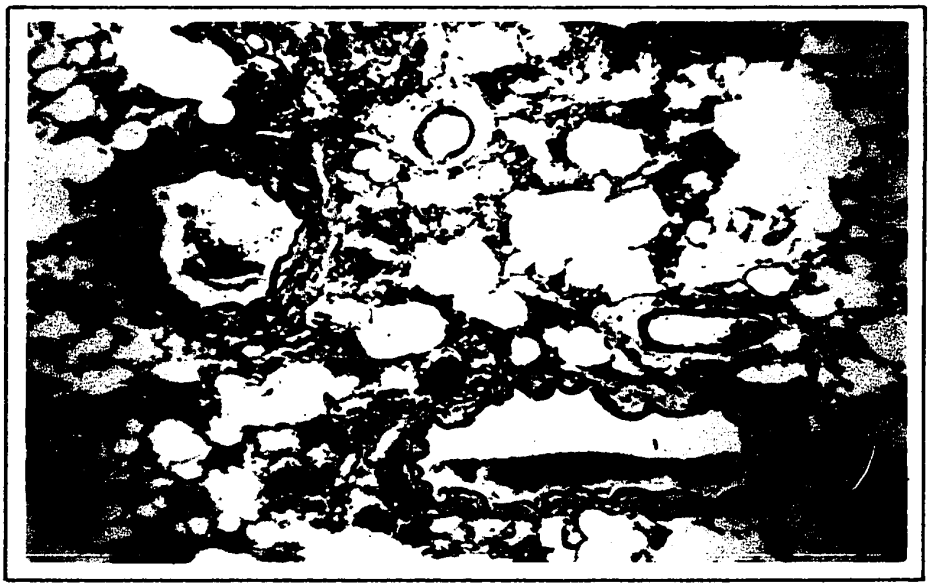

Fig. 4. Lobe 3 hours after gastric juice instillation (H & E x 55)

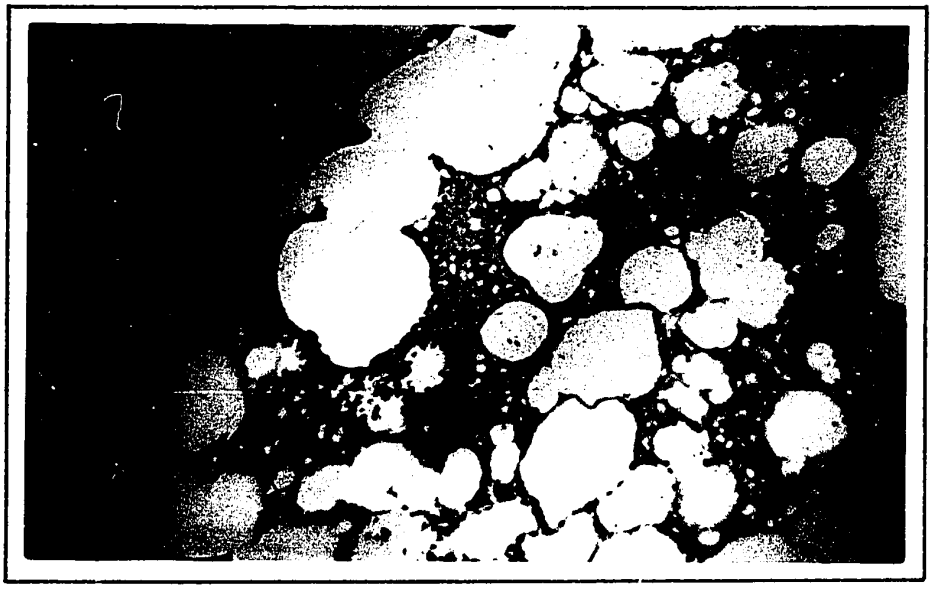

Fig. 5. Lobe 3 hours after gastric juice instillation (H & E x 125).

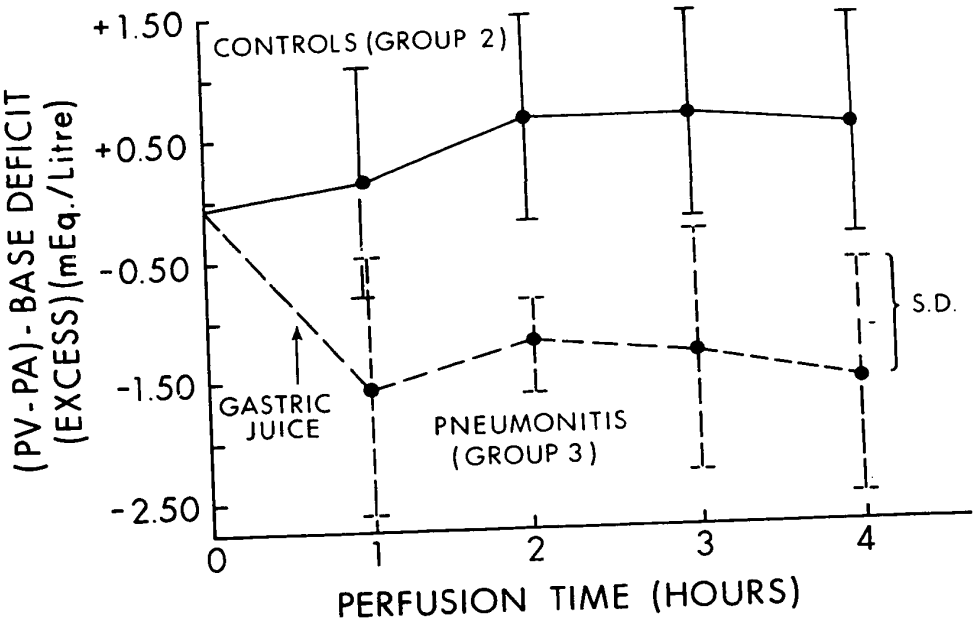

Fig. 6. Pulmonary venous minus pulmonary arterial base excess (deficit) in control and pneumonitis lobes.

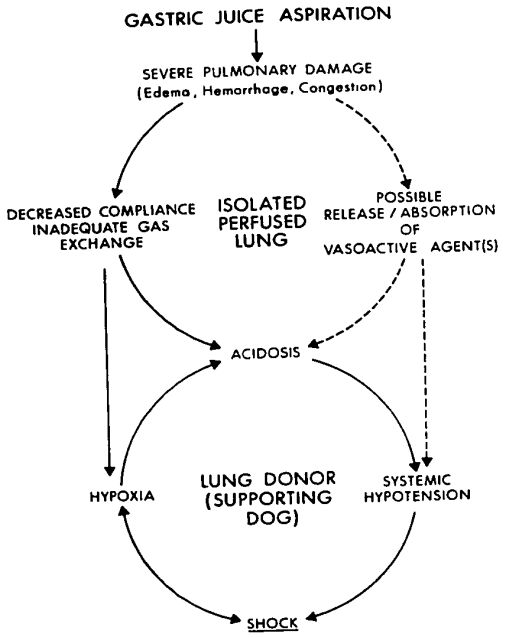

Fig. 6. Pathophysiology of aspiration pneumonitis in the isolated lung.

CHAPTER VI

PATHOPHYSIOLOGY OF THE AUTO-PERFUSED ISOLATED LUNG

The preceding experiments demonstrated that direct pulmonary injury could be studied in the isolated lung. The "control" lungs were not ideal however, because they developed functional and morphologic alterations during five hour periods of perfusion. Furthermore, the nature and magnitude of these abnormalities were not clearly apparent.

Most of the previous lobes exhibited a "congested" appearance which diminished when the venous pressure was lowered. The long axis of the lobes was usually vertical and a venous pressure of +7 mm Hg was necessary in order to evenly perfuse the highest areas of the lobes. This venous pressure may have been excessive. It appeared that venous pressure could be lowered and still contribute to optimal flow distribution if the long axis of the lobes was horizontal. This would minimize hydrostatic differences between various regions of the lung (Fowler et al, 1966).

The previous lobes were not periodically hyperinflated. This measure would likely improve compliance by minimizing focal atelectasis.

Deterioration of the support dogs had been associated with morphologic alterations in the in situ lungs and circulatory failure. Blood was continually lost from atrial purse strings and "central" flow likely diminished as a result of purse string encroachment on the left atrium. Progressively increasing respiratory pressures undoubtedly contributed to the circulatory failure. These factors and possibly

prolonged oxygen administration may have played a role in damaging the in situ lungs.

An attempt was made to further minimize perfusion damage and to more comprehensively evaluate function in the following series of lobe perfusions. The perfusion methods were modified on the basis of the foregoing considerations and additional study methods were introduced in order to more comprehensively evaluate the function of the isolated lobes.

METHODS

Lung Environment

Ten left lower lobes from dogs weighing an average of 23 kilograms were autologously perfused at a rate of 15 to 22 (average 18) millilitres per donor kilogram per minute. The perfusion circuit was the same as in previous experiments. Mean venous pressures were maintained at +3 $\pm$ 1 mm Hg.

Chamber pressures were controlled at -10/-4 $\pm$ -1/-1 cm H_2O. The respiratory rates were controlled at 16 $\pm$ 1 per minute. The respirator pump which controlled the chamber pressure was adjusted to produce inspiratory and expiratory phases of 40 percent and 60 percent of the respiratory cycle respectively. Every fifteen minutes, the lobes were hyperinflated two or three times using chamber pressures of -14 to -18 cm H_2O.

The lobes lay on their medial or lateral surface during perfusion. Pre-perfusion ischemia time ranged from 15 to 30 minutes (average 23 minutes).

Support Dog

The time for surgical preparation of the lobes was minimized and rangedfrom 30 to 60 minutes. The atraumatic vascular clamp which was used on the atrium for left atrial division was left on the atrium for the duration of the experiments.

The support dogs were ventilated with compressed air and also subjected to intermittent hyperinflation. Respiratory rates and pressures were not increased as the experiments progressed. Additional anaesthetic agent was administered when the animals became tachypneic.

Ringer's solution was administered intravenously at rates up to 200 millilitres per hour in attempt to decrease the rate of systemic pressure decline. Sodium bicarbonate was administered when arterial pH was below 7.25. Several support animals were digitalized at five hours.

The experiments were continued until the support dog died, the isolated lung was obviously deteriorating, or until a perfusion duration of ten hours had been achieved.

RESULTS

General

The duration of perfusions ranged from 4.75 to 10 hours (average 6.5 hours). Five experiments lasted longer than five hours and two were continued for ten hours. In both of the latter, moderate hypotension developed in the support dogs during the second five hour period.

Technical problems resulted in the termination of five

experiments after 4.75 to 5 hours. In two of these, marked hypotension developed in the support dog following major intrathoracic hemorrhage. Main pulmonary artery collapse around the arterial catheter killed one support dog at five hours. One lung was grossly abnormal from the beginning of perfusion. One lung was inadvertently subjected to venous occlusion when the experiment was begun and this lobe remained plethoric during the 4.75 hour experiment. In three of the foregoing experiments, the isolated lung was grossly normal at the termination of the study period. Data from all ten experiments are included in the result presentation which follows.

Support System

Figure 1 presents hourly indices which relate to the support system in these experiments. Donor arterial pressures averaged 125 mm Hg at the beginning of the perfusions and 97 mm Hg at five hours (a pressure change of 20 percent over the five hour period). The range of pressures at each hour of perfusion remained fairly constant.

The paO_2 of individual donors varied considerably, but in general was maintained adequately during the first five hours of perfusion. In contrast, the average arterial pH in the donors decreased and demonstrated slightly greater variability as the experiments progressed.

Blood hemoglobin was quite stable during the first five hours despite wide variation at the beginning of the perfusions. The donor rectal temperatures averaged 36.5° C when preparatory surgery had been completed and decreased only 1° C on the average, over the five hour period.

The chamber temperature was somewhat unstable, particularly during the first two hours of perfusion. The thermostat was manually adjusted in the early period of several perfusions in attempt to rapidly correct the cooling of the interior which occurred during lobe cannulation.

LOBE FUNCTION

Hemodynamics (Figure 2)

At five hours, the vascular resistance of the lobes averaged 119 percent of the initial value, although during the final three hours the resistance in individual lobes varied considerably. The vascular resistance was lowest following one hour of perfusion. Thereafter, resistance slowly increased. The main pulmonary artery minus pulmonary venous pressure increased only 1 mm Hg from an average of 7.5 mm Hg (SD 3) to 8.5 mm Hg (SD 3) at five hours.

Vascular resistance was calculated for each condition of inspiratory gas composition. Resistance increased appreciably on 85 percent of the occasions when the inspired gas was altered from room air to 14 percent oxygen. In seven of the ten experiments, the magnitude of the pulmonary vasoconstrictor response to low pIO_2 progressively increased during perfusion. The responses averaged 110 percent, 125 percent, and 140 percent in the first, fourth, and sixth hours of perfusion respectively.

In the tenth experiment, the intrapulmonary blood volume was 32 millilitres ($\pm$ 1 ml) during the first hour and 27 millilitres $\pm$ 1 ml

during the sixth hour.

Mechanics (Figure 2)

Compliance decreased slightly during the initial two hours of perfusion and then stabilized at 95 percent of the control value. The compliance of the lobes was 9.0 litres per centimetre H_2O per kilogram (SD 1.5) during the first hour of perfusion and 8.6 litres per centimetre H_2O per kilogram (SD 1.4) in the fifth hour.

Dead space-tidal volume ratio exhibited considerable individual variation, but on the average was quite stable for the five hour period. $\dot{V}_D/\dot{V}_T$ averaged 48 percent (SD 8%) at the beginning of perfusion and 48 percent (SD 11%) at five hours.

Gas Exchange (Figures 2 and 3)

$(a-\bar{v})pO_2$ averaged 37 mm Hg at the beginning of perfusion and 46 mm Hg at five hours. Oxygen uptake averaged 34 millilitres per minute and 51 millilitres per minute at one and five hours respectively. Mixed venous oxygen saturation decreased from 62 percent at the beginning of perfusion to 38 percent at five hours.

$\dot{Q}s/\dot{Q}^{20}$ decreased an average of eight percent (from nineteen percent to eleven percent) over the five hour study period although this change was not significant. The $[A-a]O_2^{20}$ however, decreased from 44 mm Hg (SD 6) to 30 mm Hg (SD 7) and this change appeared to be significant.

$\dot{Q}s/\dot{Q}^{14}$ was more variable than $\dot{Q}s/\dot{Q}^{20}$ but did not increase during

the five hour period of perfusion. The $[A\text{-}a]O_2^{14}$ demonstrated a progressive and significant improvement during the second to fifth hour period (27 mm Hg, SD 3 to 18 mm Hg, SD 3).

$\dot{Q}s/\dot{Q}^{100}$ ranged from nine to five percent and $[A\text{-}a]O_2^{100}$ from 290 to 200 mm Hg, but changes in these values were not significant.

Ten Hour Lobe

The results of one of the ten hour lobes are shown in Figure 4. Vascular resistance was 80 percent of the original level at ten hours. Compliance decreased by sixteen percent over the ten hour period. $\dot{V}_D/\dot{V}_T$ increased from 52 to 58 percent. $\dot{Q}s/\dot{Q}^{20}$ decreased from 12 percent to 11 percent during the last nine hours. $\dot{Q}s/\dot{Q}^{14}$ was 20 percent at one hour and 34 percent at ten hours. The "true shunt" increased very little (5.4 to 6.2%). $\dot{Q}s/\dot{Q}^{14}$ and $\dot{Q}s/\dot{Q}^{100}$ increased during the third and fourth hours but subsequently returned toward the initial values.

Morphology

The lobes in this series gained in weight an average of 22 percent. The measured weight changes ranged from a loss of 7 percent to a gain of 42 percent. The two ten-hour lobes both appeared to lose weight (7 and 4 percent respectively).

Four of the isolated lobes were grossly normal. Each of the remaining lobes demonstrated several small focal subpleural hemorrhages, most of which appeared during the early period of perfusion. Four of

these lobes, including the second ten-hour lobe were otherwise normal (Figure 5). The first lobe in this series developed a moderate amount of sanguinous endobronchial edema fluid. One lobe was atelectatic and congested. These abnormalities were present when that experiment was begun and were also observed in the right lung of the donor prior to the beginning of perfusion.

The latter two lobes demonstrated perivascular and interstitial hemorrhage. Perivascular edema was present to a mild to moderate degree in all lobes. Seven lobes demonstrated mild alveolar irregularity. Three developed mild perivascular hemorrhage in association with moderate perivascular edema in the peribronchial areas. Interstitial and alveolar hemorrhage developed in only one lobe. Three lobes demonstrated mild degrees of vascular and lymphatic aggregation of leucocytes and cellular debris. Aside from perivascular edema and slight alveolar irregularity, seven of the ten lobes were otherwise normal histologically (Figure 6).

Most of the in situ lungs demonstrated moderate to marked atelectasis and vascular congestion grossly. Tracheobronchial edema and hemorrhage did not occur, but half of the lungs developed focal and confluent subpleural ecchymoses (Figure 7). More than half (four out of the seven which were studied) demonstrated intraparenchymal hemorrhage microscopically. Most of the right lungs demonstrated perivascular edema (Figure 8).

The morphologic abnormalities in the donor lungs were comparable to those in the perfused lungs in overall degree of alteration, although the changes were somewhat different in pattern. Atelectasis, congestion

and subpleural ecchymoses were present in the donor lungs. Focal subpleural hemorrhage was present in many isolated lobes. Perivascular edema was present in both, but more severe in the isolated lobes. Intraparenchymal hemorrhage was more severe in the in situ lungs.

DISCUSSION

Attempts have been made to demonstrate that "physiologic function was relatively normal" in preparations which have been used by other investigators to study pulmonary hemodynamics and function (West et al, 1964; Daly and Hebb, 1966; Fowler et al, 1966). Vascular resistance has increased and sections of the lungs have shown areas of perivascular and peribronchial edema and hemorrhage after two to four hours (West and Jones, 1965).

The resistance of the lobes in this series remained within the physiologic range for the normal dog (Daly, 1961). Vascular resistance increased however, during perfusion.

Perivascular edema was observed in histologic specimens from all of the perfused lobes. Perivascular edema compromises the net cross-sectional area of the pulmonary vasculature (West et al, 1965). The intrapulmonary blood volume in the tenth lobe of the series decreased over the period of perfusion. This supports the assumption that vascular cross-sectional area decreased during perfusion.

On many occasions, the resistance increase which attended alveolar hypoxia (pIO_2 [14]) was not completely reversed when inspired gas was subsequently changed to room air or 100 percent oxygen. Subjecting

the lobes to recurrent acute vasospasm may have altered vascular integrity and contributed to the development of perivascular edema. Specific information in this regard could not be derived from these experiments. Increased vasomotor tone is associated with an increase in perivascular edema in the dependent zones of isolated lungs (West et al, 1965). The two ten-hour experiments were not frequently subjected to alveolar hypoxia during the second five-hour period. The lobes lost weight and perivascular edema in these was minimal.

The pulmonary vascular response to alveolar hypoxia has been used as a test of functional integrity and physiologic performance of the isolated lung (West et al, 1964; Daly, 1961). In this group of experiments, resistance increased appreciably when inspired gas was altered from room air to 14 percent oxygen. In more than half of the preparations, the magnitude of this response increased as the experiments progressed. This series did not provide an explanation for the increase in response to alveolar hypoxia which was observed in several experiments.

Compliance was well maintained in these experiments. Dead space - tidal volume ratio varied among the experiments, but the average $\dot{V}_D/\dot{V}_T$ was reasonably stable throughout the five-hour periods of study. The volume of the "anatomic" dead space from the non-rebreathing valve to the lung parenchyma was estimated at 40 to 60 millilitres. This volume, which included the bronchial cannula, was similar for each of the lobes although the lobe sizes varied. Tidal volumes in different experiments ranged from 95 to 140 millilitres. Differences in the "anatomic" components of $\dot{V}_D/\dot{V}_T$ among the different lobes partially ex-

plains the range of $\dot{V}_D/\dot{V}_T$ values which were obtained.

Alveolar dead space is minimal when arterial pressure is greater than both alveolar pressure and the hydrostatic pressure difference between the top of the lung and the hilum (West and Jones, 1965). These pressure relationships were maintained in the perfused lobes. In the absence of gross airway edema, it can be assumed that the anatomic dead space changed little. The stability of the $\dot{V}_D/\dot{V}_T$ values indicates that alveolar dead space did not increase significantly in these experiments.

The perfused lobes demonstrated a rather high initial venous shunt after one hour of perfusion ($\dot{Q}s/\dot{Q}^{20}$ - 14% and $[A\text{-}a]O_2^{20}$ - 44 mm Hg). These values are somewhat higher than those reported for the normal anaesthetized dog (10% and 24 mm Hg respectively - Finley et al, 1960). The venous shunt was substantially contributed to by "true shunt" ($\dot{Q}s/\dot{Q}^{100}$ - 8%, SD 3). These true shunt values were very similar to those found in the intact dog (5% - Finley et al, 1960).

In the anaesthetized dog, the true shunt has been attributed to blood flow through areas of atelectatic lung (Finley et al, 1960). The isolated lobes were intermittently hyperinflated, but their weight was supported by the dependent zones. This factor would compromise ventilation in the lower zones of the lobes. Blood flow rates are greater in the dependent zones of the lung (West et al, 1964) and this additionally contributes to low $\dot{V}_A/\dot{Q}$ in these regions. The foregoing partially explains the elevated venous shunt which was observed in the lobes. Areas of high $\dot{V}_A/\dot{Q}$ contribute little to venous shunt in the

isolated lung (West and Jones, 1965).

West and Jones (1965) varied venous pressures and flow rates in isolated lungs in order to evaluate the effect of gravitational factors on the "venous admixture." Care was exercised to prevent collapse of dependent alveoli. The calculated venous admixtures averaged five percent but ranged from minus five percent to plus ten percent. Alveolar-arterial oxygen gradients ranged from minus twelve to +22 mm Hg. The presence of many physiologically impossible negative values in these data leave them subject to question.

Barnes and co-workers (1968) studied serial "true shunt" ($\dot{Q}s/\dot{Q}^{100}$) in two isolated lungs. The shunt was initially ten percent and increased rapidly to twenty-five percent after three hours of perfusion. In our isolated lobes, the true shunt improved from an average of eight percent at one hour to five percent at five hours. Our true shunt values at five hours are similar to the 2.5 percent which was obtained by West and Jones (1965) during the first two hours in their preparation.

A consideration of events in the dependent zone of the lobes offers partial explanation for the apparent improvement in the shunt values. Perfused, non-ventilated or poorly ventilated dependent areas of the lung represent low $\dot{V}_A/\dot{Q}$ areas. Perivascular edema predominates in the more dependent zones of the isolated lung. The resistance in the dependent vessels is disproportionately increased (West et al, 1965). As perivascular edema developed in the lobes, blood flow may have been gradually diverted from low $\dot{V}_A/\dot{Q}$ areas to the higher $\dot{V}_A/\dot{Q}$ regions with resultant improvement in the gradients and shunts.

The inter-relationship of vascular resistance and shunting is further illustrated by the data for the ten-hour lobe (Figure 4). During the first five hours, when resistance was low, shunting increased. As resistance increased during the second five hours, the shunt values improved.

Alveolar arterial oxygen gradient and shunt values under conditions of low pIO_2 in isolated lungs have not been previously reported. The 14-percent gradient and shunt data which were obtained in these isolated lobes indicated that a diffusion abnormality was present. The magnitude of the diffusion abnormality was likely not so large as the values suggested. Anatomic and "physiologic" shunts contribute to the calculated "low oxygen shunt," particularly when the former are larger than ten percent (Farhi, 1966). Secondly, the existence of a diffusion abnormality exaggerates the calculated shunt when paO_2 is substituted for $p\acute{c}O_2$ in the $\dot{Q}s/\dot{Q}^{14}$ calculation (see Study Methods).

The improvement in the average $[A\text{-}a]O_2{}^{14}$ during the course of perfusion suggested that diffusion abnormalities may have improved. Small improvement in the anatomic and physiologic shunts may have contributed to the improvement in the $[A\text{-}a]O_2{}^{14}$ values. Nevertheless, the "low oxygen" data indicated that diffusion properties of the lobes did not significantly deteriorate during the course of perfusion.

The calculated weight changes did not always parallel degrees of morphologic change. West and co-workers (1964) recorded a weight gain of only 30 percent in association with frank endobronchial edema. Their measurements were obtained continuously during perfusion, thereby eliminating the effect of manipulation on fluid volume; a factor which undoubtedly affected our measurements.

It seems unlikely that these lobes gained 22 percent or more in weight in view of the stability of compliance and $\dot{V}_D/\dot{V}_T$. It is interesting, however, that the two ten-hour lungs appeared to have lost weight in comparison to the other eight lobes. The reasons for this are not clear. Early edema formation may be partially reversible in perfusions which last longer than five hours.

The support animals survived longer periods of perfusion in this group of experiments. Most indices of donor condition were reasonably satisfactory during the first five hours of study although metabolic acidosis still developed. Systemic pressure decreased 22 percent in this group compared with 40 percent in the initial group of experiments. The avoidance of excessive alveolar pressures and prevention of atrial obstruction may have helped to maintain cardiac output in the support dogs. The mixed venous pO_2 in this group decreased to a degree comparable with the previous group of experiments (from an average of 45 mm Hg initially to 30 mm Hg at five hours.)

The in situ lungs were not as abnormal morphologically as they had been in the previous experiments. Nevertheless, in more than half of the experiments, the in situ lungs seemed more severely altered than the isolated perfused lungs. This was certainly the case in both of the ten-hour experiments. Many of the indices of function in the isolated lobes were quite well preserved and some appeared to have improved during perfusion. In contrast, the in situ lungs, which were spared the initial manipulative surgery, appeared to have progressively deteriorated.

The isolated lungs in this series appeared to function better than in the initial series, but technical problems in several experiments and variability in results did not allow conclusion in this regard.

Attention to conditions in the supporting dogs permitted several perfusions of longer duration than had previously been achieved.

The results of attempts to minimize perfusion damage had to this point been encouraging, but the isolated lungs continued to develop alterations during the course of perfusion.

SUMMARY

1. Ten isolated canine pulmonary lobes were autologously perfused for 4.75 to ten hours.
2. The hemodynamic characteristics of the lobes were satisfactory as judged by previously reported criteria and in comparison with the results of others.
3. The gas exchange properties of the lobes were reasonably stable, although not normal when compared to known values for the canine lung.
4. Many of the functional alterations which were observed in the isolated lobes appeared to be attributable to the development of perivascular edema.

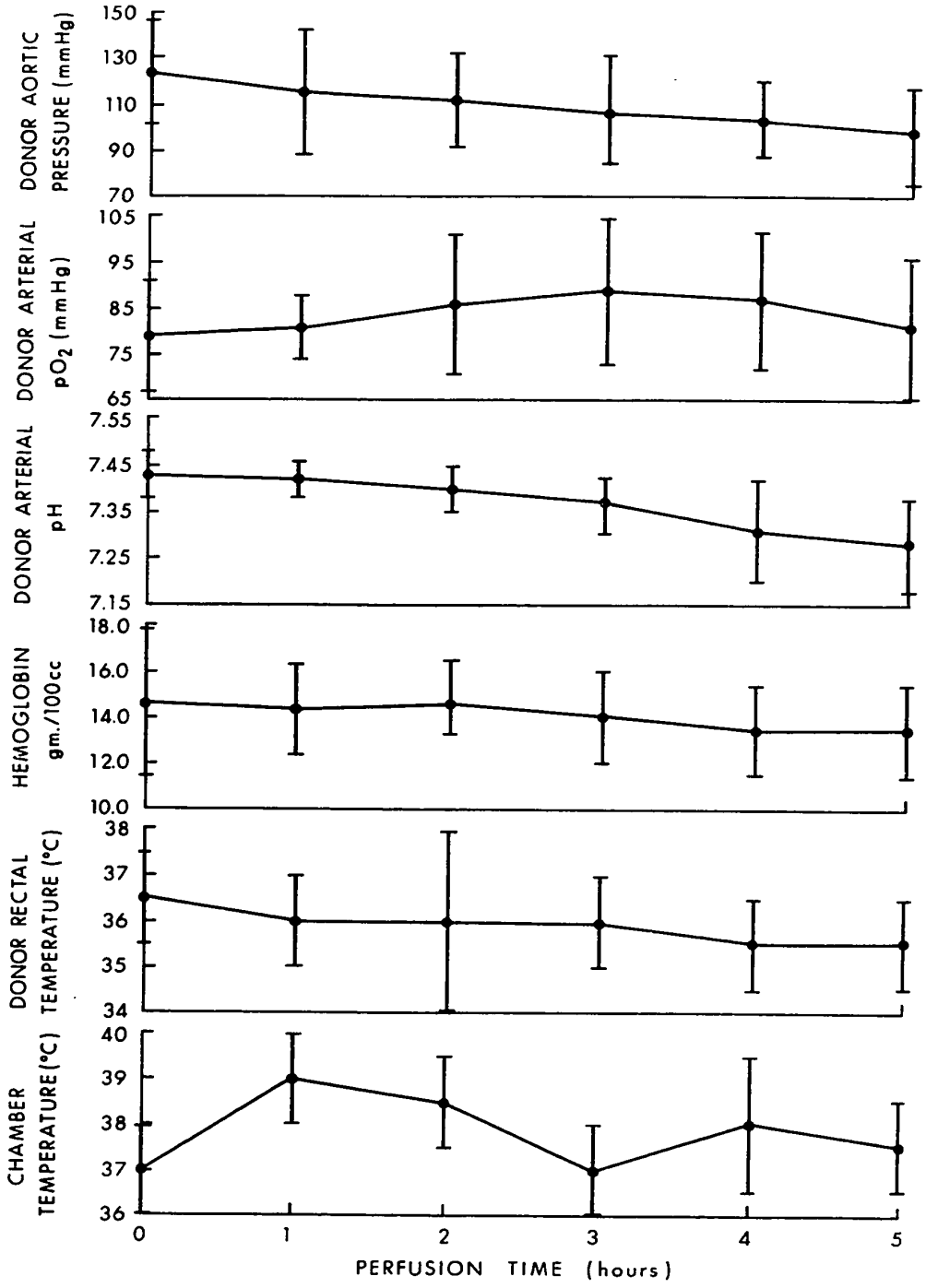

Fig. 1. Indices reflecting conditions in support dogs and perfusion system.

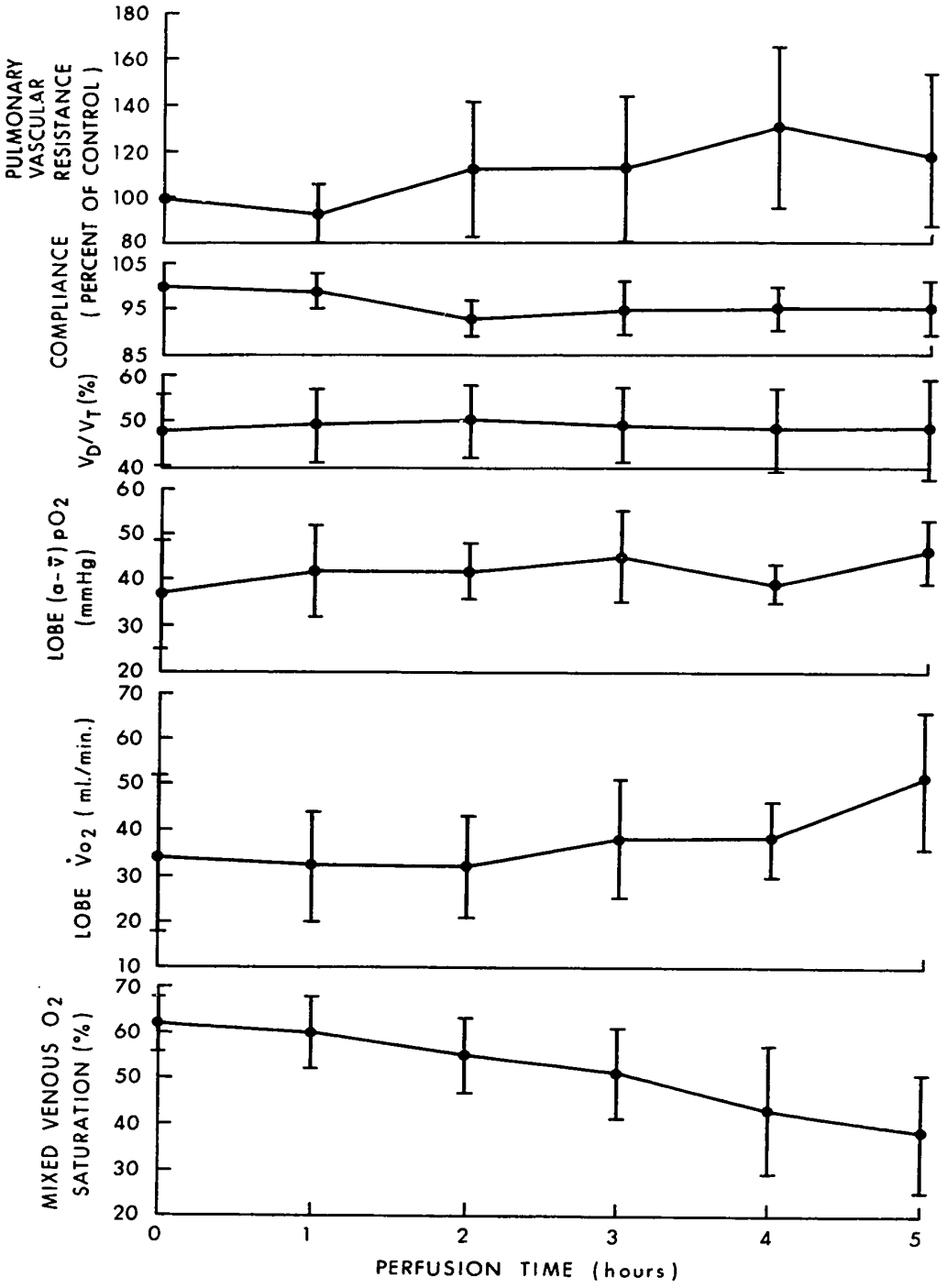

Fig. 2. Hemodynamics, mechanics and gas exchange of 10 autologously perfused lobes.

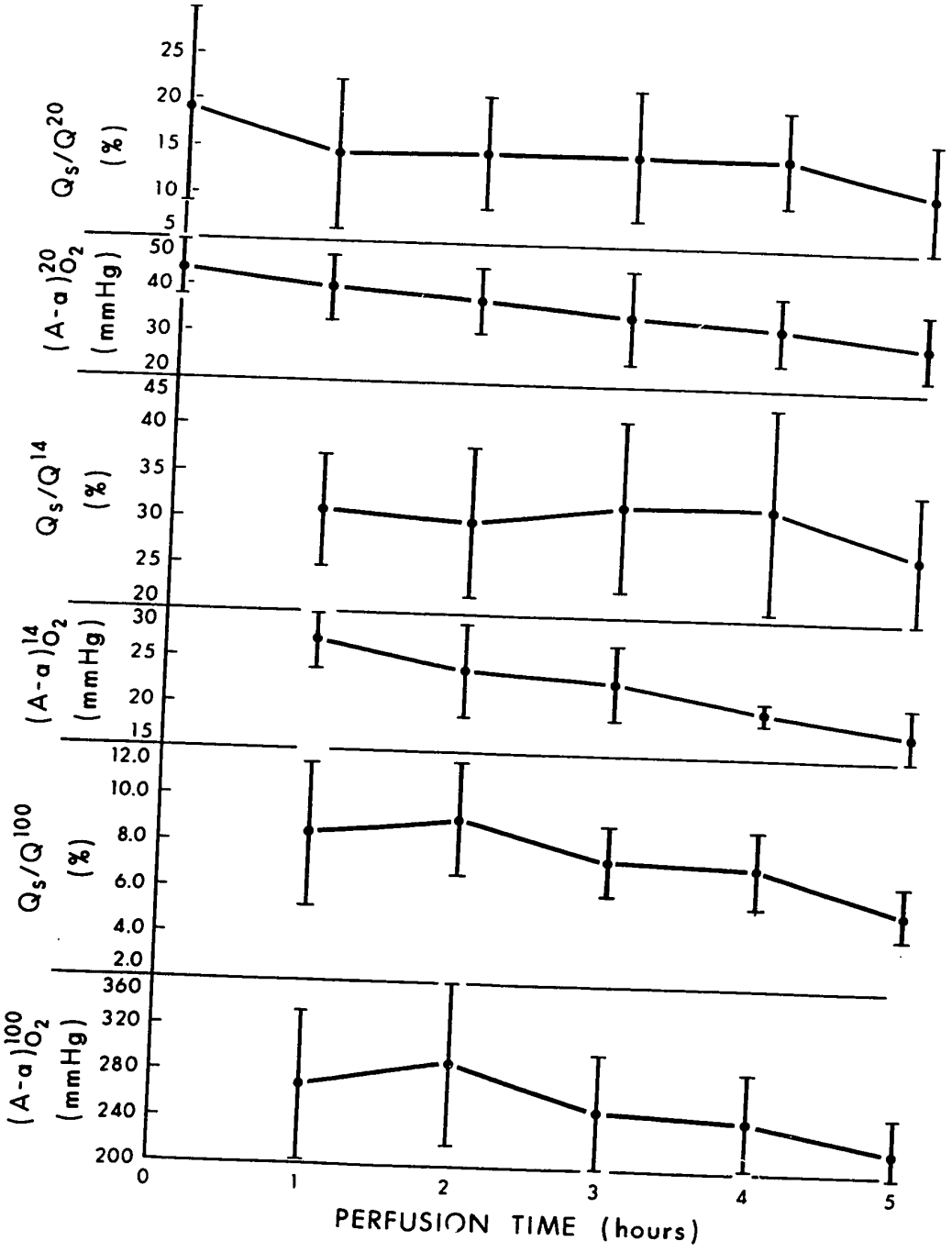

Fig. 3. Shunts ($\dot{Q}_S/\dot{Q}$) and alveolar-arterial oxygen gradients ($[A-a]O_2$) under conditions of room air (20), low oxygen (14), and 100 percent oxygen-breathing (100) of 10 autologously perfused lobes.

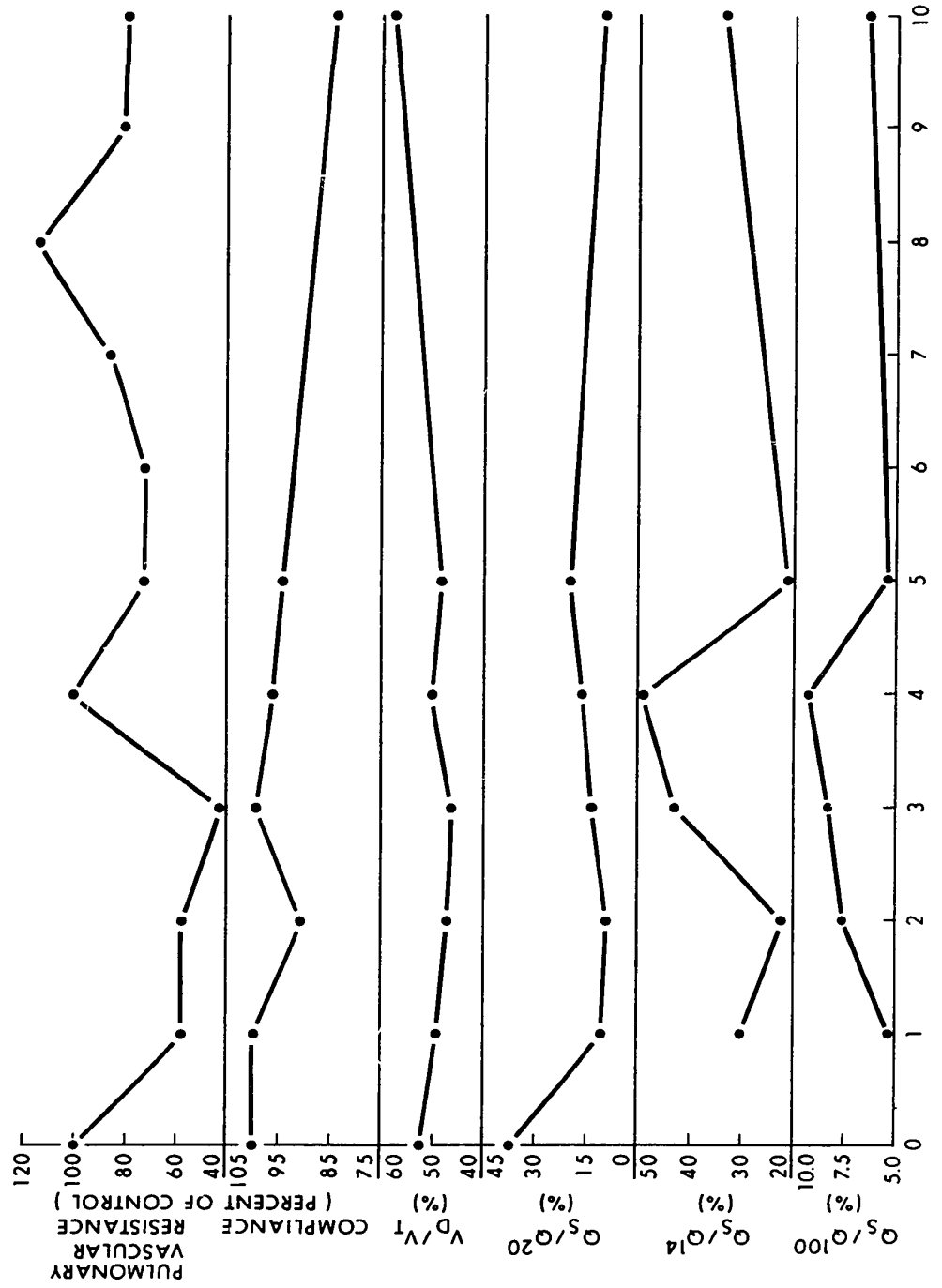

Fig. 4. Function of 1 lobe autologously perfused for 10 hours.

Fig. 5. Lobe after 10 hours of autologous perfusion.

Fig. 6. Lobe after 10 hours of perfusion (H & E x 40); mild perivascular edema, alveolar irregularity.

Fig. 5. Lobe after 10 hours of autologous perfusion.

Fig. 6. Lobe after 10 hours of perfusion (H & E x 40); mild perivascular edema, alveolar irregularity.

Fig. 7. In situ lobe after 10 hours of isolated lobe perfusion (same experiment as Fig. 5).

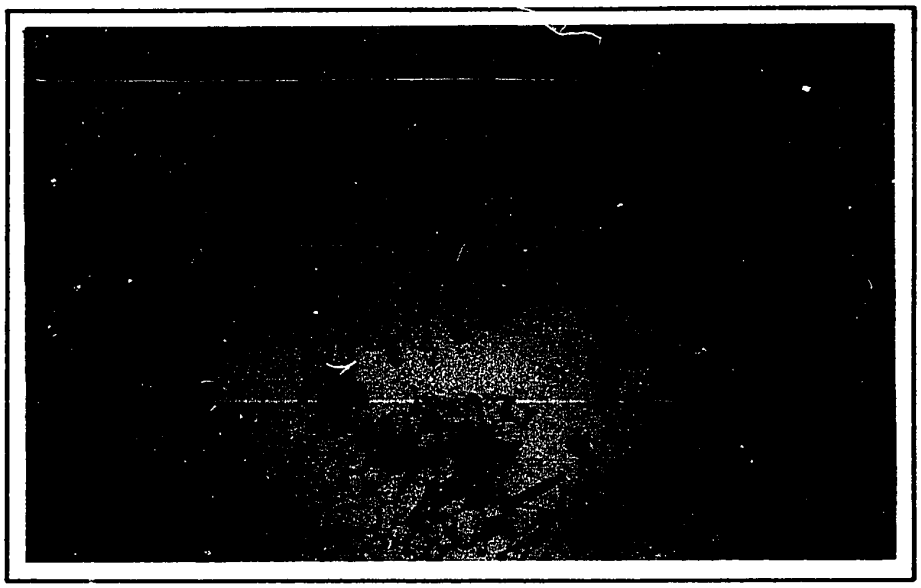

Fig. 8. In situ lobe after 10 hours of isolated lobe perfusion (H & E x 40) (same experiment as Fig. 5, 7); moderate perivascular edema, alveolar irregularity, intraparenchymal hemorrhage.

Fig. 7. In situ lobe after 10 hours of isolated lobe perfusion (same experiment as Fig. 5).

Fig. 8. In situ lobe after 10 hours of isolated lobe perfusion (H & E x 40) (same experiment as Fig. 5, 7); moderate perivascular edema, alveolar irregularity, intraparenchymal hemorrhage.

CHAPTER VII

THE EFFECTS OF THE SUPPORT DOG ON THE ISOLATED LUNG: THE ROLE OF HYPOVOLEMIC SHOCK

Morphologic abnormalities and functional disturbances in the preceding experiments indicated that the physiologic requirements of the lung were not yet being adequately provided. The previous experiments had not revealed the cause for these alterations but they appeared to be related to abnormal hemodynamics in the isolated lung, metabolic or humoral disturbances in the supporting dog, or a combination of these factors. Practical ways in which hemodynamics might be improved were not apparent. Despite all available measures, the supporting animals still became hypotensive and acidotic.

The lungs in severely traumatized patients and in shocked animals have demonstrated congestion, perivascular, interstitial and alveolar edema, hemorrhage and atelectasis (Webb, 1969). The abnormalities which were observed in the preceding isolated lobes and in the in situ lungs were in many ways similar to "shock-lung" lesions. The perfused lungs may have been exhibiting a response to humoral or metabolic consequences of altered hemodynamics in the supporting animals. If this was the case the isolated lung should undergo more severe alteration if the donor was intentionally subjected to a more severe degree of hypotension. In order to investigate this possibility and the feasibility of studying shock in the isolated lung, the following experiments were carried out. For these and all subsequent experiments, the immediately preceding series of lobes (Chapter VI) serves as the CONTROL.

METHODS

Left lower lobes from 20 to 28 kilogram dogs were autologously perfused using methods identical to the CONTROL series. Following one hour of perfusion, hypovolemic hypotension was produced in the donor dogs by the Wiggers' reservoir technique. The arterial reservoir maintained systemic pressure at 40 to 55 mm Hg for the duration of the experiments. Experiments were continued until the supporting dogs died.

RESULTS

General

One support dog died after three hours of hypotension and one after four hours when the main pulmonary artery collapsed around the outflow catheter. Similar events resulted in air embolism to the third lobe after six hours. The fourth experiment lasted through nine hours of severe hypotension which eventually killed the donor. All of the blood in the reservoir had returned isogravimetrically to the circulation before the end of the last two perfusions. Blood was returning to the donors when the first two experiments were terminated.

The average duration of perfusion was 6.25 hours. Throughout the entire period of hypotension, the arterial pressure in the supporting dogs was less than it had been at any time in the CONTROL series of experiments. Figure 1 presents the data for the donors and the hemodynamics and mechanics for the lobes in this group.

Hemodynamics

Despite early hypotension and acidosis in the supporting dogs, the vascular resistance and compliance of the lobes at five hours was not significantly different from those values in the CONTROL lobes. The data which were obtained for blood volume demonstrated changes similar to the last experiment in the CONTROL group.

Mechanics

Dead space-tidal volume ratio was somewhat higher in this group and compliance decreased sooner than in the CONTROL lobes. Compliance and $\dot{V}_D/\dot{V}_T$ in the ten-hour lobe compared favourably with the CONTROL lobes.

Gas Exchange

The rate of oxygen uptake increased commensurate with progressive mixed-venous hypoxemia in the support dogs. The venous shunts and oxygen gradients for this group are summarized in Figure 2. These values did not change significantly during the first five hours of perfusion. The average values were lower than in the CONTROL group during the same period. The differences between the two groups were not significant. In the ten-hour lobe, gas exchange deteriorated during the final three hours of perfusion.

Morphology

The lobes in this group gained an average of 21 percent in

weight. Weight changes ranged from a loss of 15 percent in one lobe to a gain of 77 percent in the ten-hour lobe. The gross appearance of these lobes was comparable to the lobes in the CONTROL group. A small amount of sanguinous fluid was present in the bronchial cannula of the ten-hour lobe. A few subpleural ecchymoses developed in the ten-hour lobe and one other. In both of these lobes, focal subpleural hemorrhages were present prior to the period of hypotension in the donor dog. The in situ right lungs were all moderately congested and atelectatic.

Three of the isolated lobes were almost normal histologically. In the ten-hour lobe, perivascular and alveolar hemorrhage accompanied more severe perivascular edema than was observed in the other lobes. The overall degree of histologic abnormality in this group was comparable to the lobes in the CONTROL group.

DISCUSSION

The pathogenesis of the "shock-lung syndrome" has not been clearly defined. Among the etiologic factors which have been proposed are: pulmonary neurovascular reflexes; direct action of circulating vasoactive agents; vascular occlusion by microembolism of thrombi, platelets, lipids or fibrin; local effects of tissue metabolites as a result of systemic or pulmonary hypoperfusion; oxygen toxicity; fluid overload; direct toxic effects of circulating endotoxin, and altered pulmonary hemodynamics (Webb, 1969; Collins, 1969). The variety of etiologic factors which have been forwarded suggests that no single factor is entirely responsible for the pulmonary derangement in shock.

The role of pulmonary neurovascular reflexes remains a matter

of dispute (Folkerth et al, 1970). The denervated state of the isolated lobes may be one of the reasons why the isolated lungs were not more severely damaged in these experiments. In future studies of this nature, greater attention should be focused on the differences between the in situ and ex vivo lung.

The flow rate through the isolated lobes was not decreased during the period of hypotension in the support dogs. The technical difficulty which was experienced in maintaining outflow to the isolated lobes, suggested that the majority of venous return in the donor was being diverted to the isolated lobes. Histologic study of the isolated lobes did not reveal the presence of embolic material. The lobes were similarly subjected in an exaggerated way to circulating metabolites from the donor dogs. The foregoing casts some doubt on the roles of these factors in the pathogenesis of the "shock-lung."

Pulmonary arterial and venous pressures can vary independently during systemic hypotension and alveolar pressures are certainly altered under conditions of respirator therapy. Diminished pulmonary flow increases alveolar dead-space because the majority of existing flow preferentially courses through the more dependent zones of the lung (Naimark et al, 1968). The dependent zones are more susceptible to the development of perivascular edema (West et al, 1965). The low $\dot{V}_A/\dot{Q}$ in these areas contributes to deficiency in oxygen uptake. If positive pressure ventilation is imposed on the already acidotic, hypoxic and tachypneic patient, all of these hemodynamic factors can be further aggravated beginning with cardiac output.

Sealy and co-workers (1966) observed a better survival rate among dogs in shock which were not ventilated with positive pressures.

This parallels unpublished experiences of the author. The survival rate in dogs which were subjected to four-hour periods of reservoir-hypotension to 40 mm Hg was better under conditions of spontaneous respiration or negative pressure assisted ventilation than when positive pressure ventilation was employed. In 1949, Gerst and co-workers stated that "with intermittent positive pressure ventilation, reduction of pulmonary blood flow may lead to complete closure of portions of the pulmonary bed." In the isolated lungs negative pressure ventilation and venous pressures were maintained as they had been in the CONTROL series. The role of altered pulmonary hemodynamics in the shock-lung deserves further investigation.

The group of experiments in this series was small, but abnormalities which differed significantly from the CONTROL group of experiments were not observed. This suggests that the milder cardiorespiratory disturbance which occurred in the support dogs of the CONTROL series was not primarily responsible for the abnormalities which developed in the isolated lobes. These experiments, however, do not provide further insight into the etiology of the abnormalities which developed in these and the preceding isolated lobes.

SUMMARY

1. Four isolated canine pulmonary lobes were autologously perfused during three to nine hour periods of hypovolemic hypotension in the donor dogs.
2. The morphologic and functional alterations in these lobes were not significantly different from those in previous experiments.

3. These findings suggest that hypotension in the support dogs was not primarily responsible for perfusion damage in the previous isolated lobes.
4. The isolated, autologously perfused lung may be applicable to carefully selected studies related to the "shock-lung" syndrome.

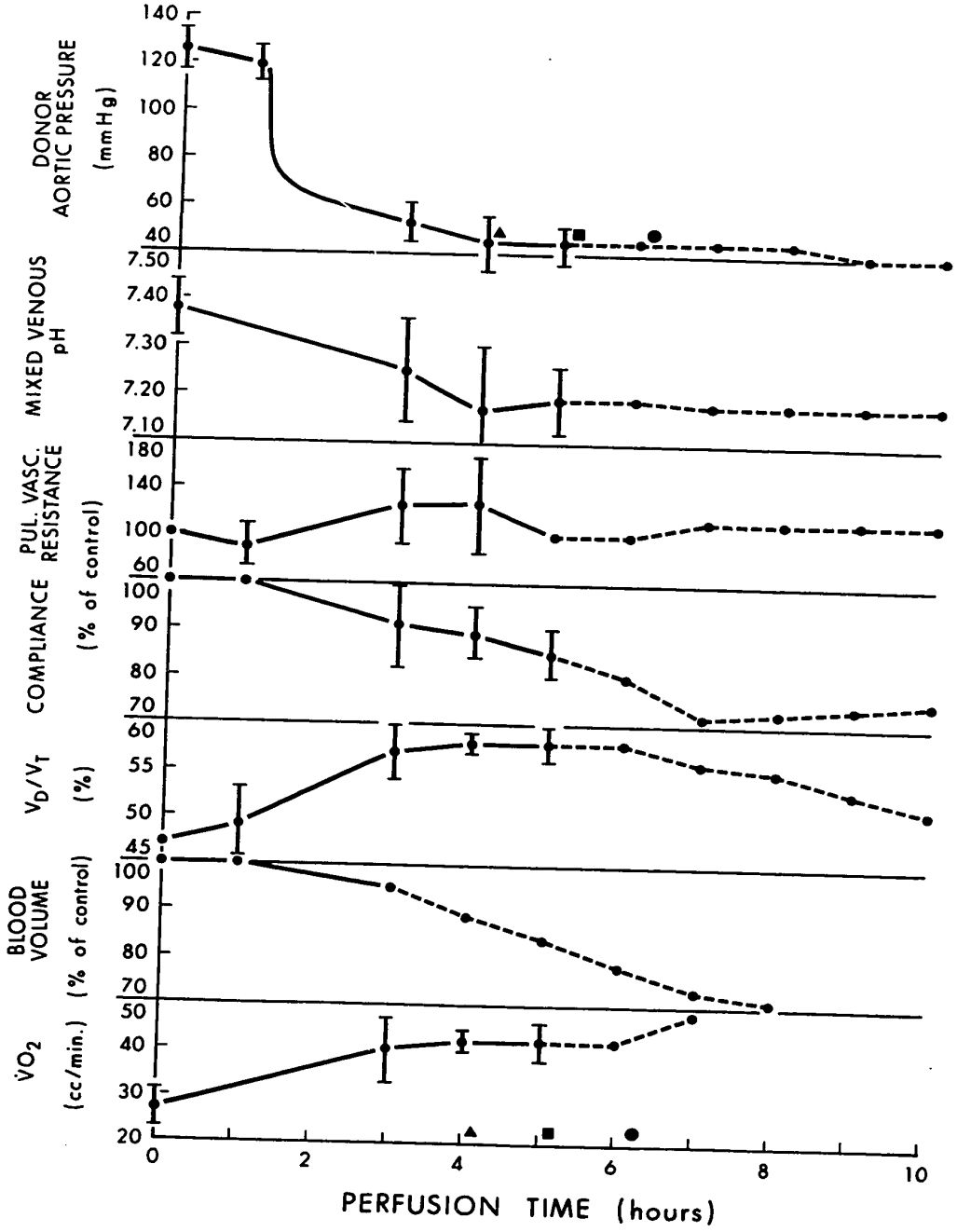

Fig. 1. Lobe hemodynamics and mechanics during hypotension in 4 support dogs (▲, ■ - support dogs died; ● experiment discontinued; --- 10 hour survivor).

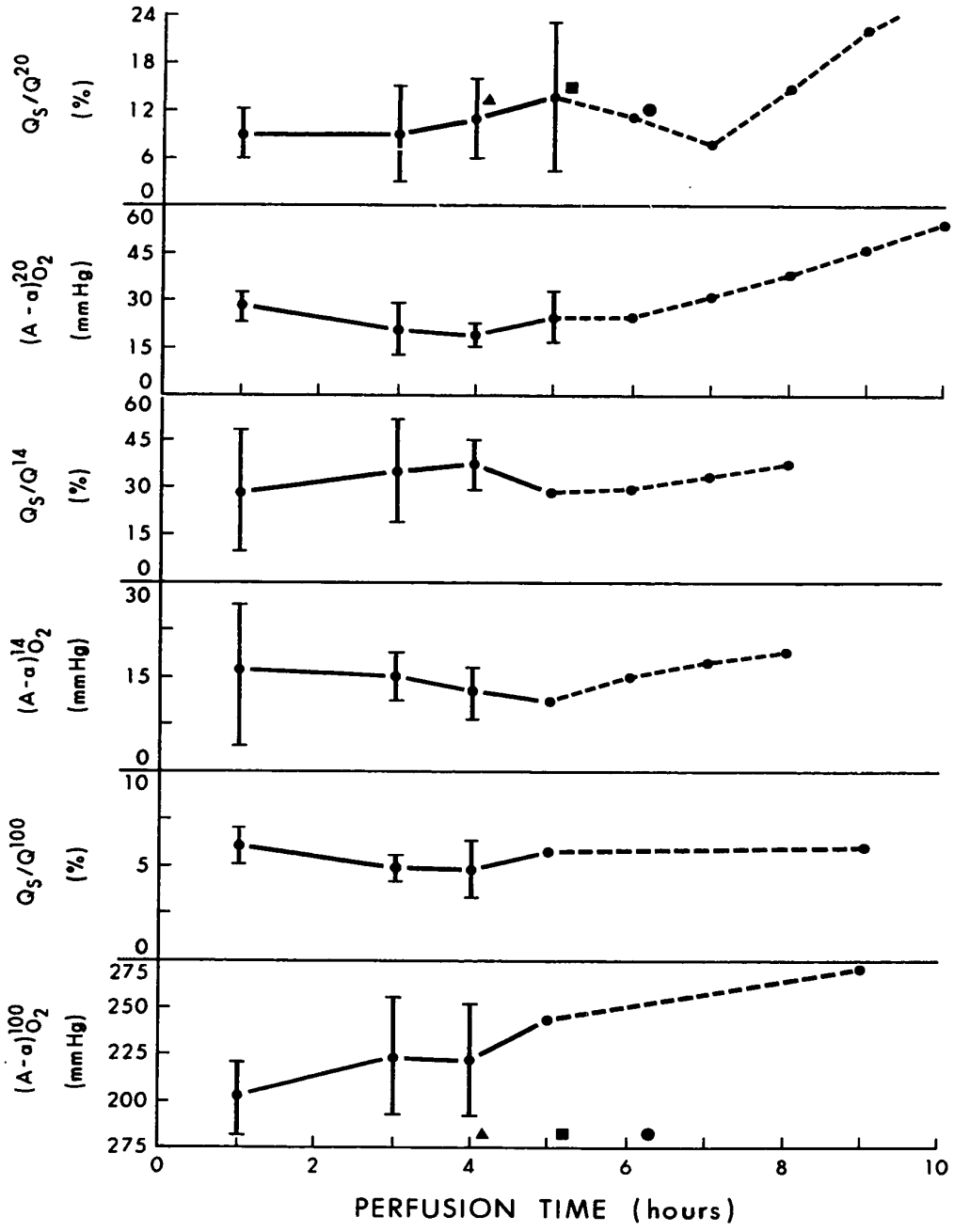

Fig. 2. Lobe gas exchange during hypotension in 4 support dogs.

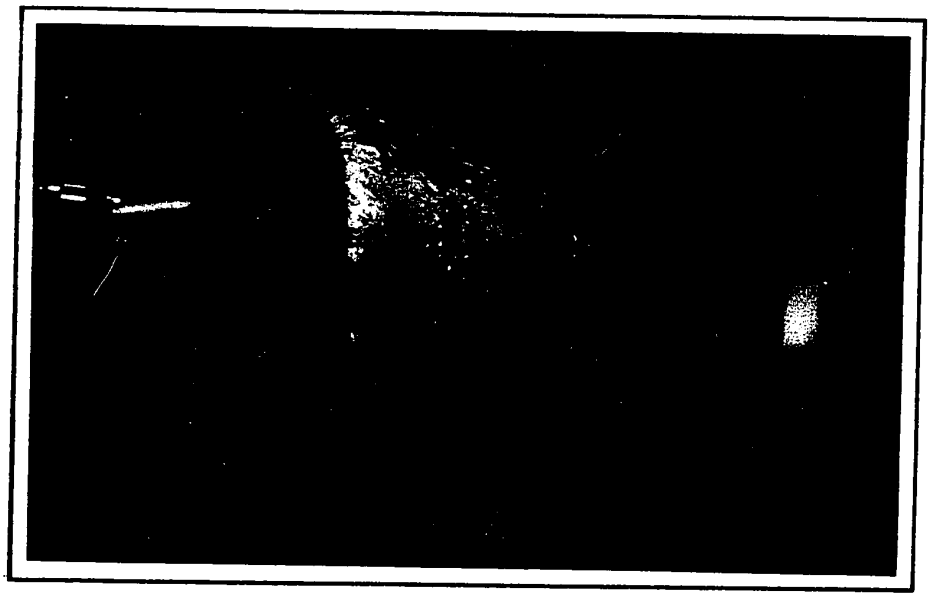

Fig. 3. Isolated lobe after 9 hours of donor hypotension.

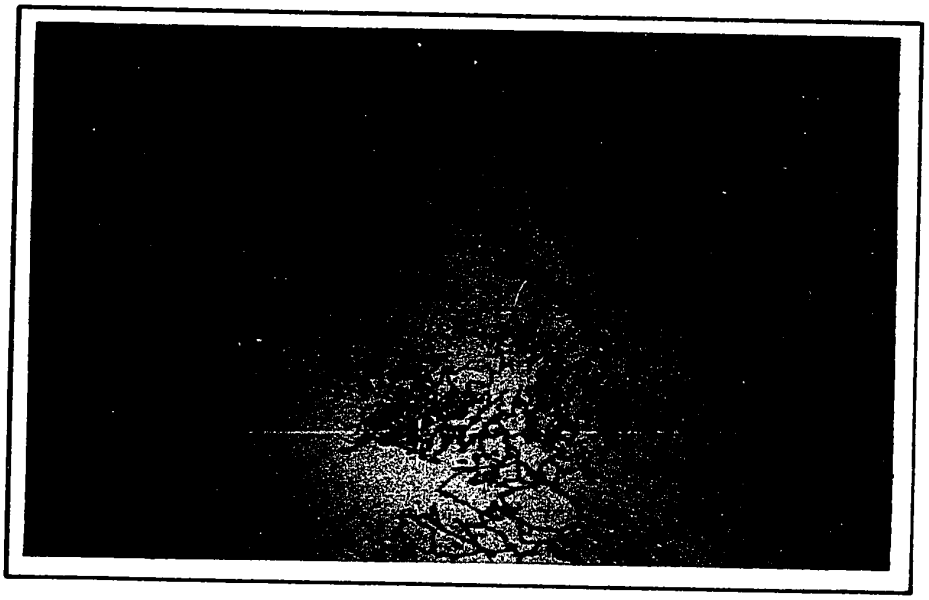

Fig. 4. Isolated lobe after 9 hours of donor hypotension (H & E x 40); peribronchial-perivascular edema, parenchymal hemorrhage, alveolar irregularity.

Fig. 3. Isolated lobe after 9 hours of donor hypotension.

Fig. 4. Isolated lobe after 9 hours of donor hypotension (H & E x 40); peribronchial-perivascular edema, parenchymal hemorrhage, alveolar irregularity.

CHAPTER VIII

THE EFFECTS OF REPLACING THE SUPPORT DOG WITH MECHANICAL GAS EXCHANGERS

In preceding experiments, it was assumed that the support dog provided an essential degree of "homeostasis" in the extracorporeal circuit. Progressive deterioration of the support dogs not only limited the duration of perfusions but produced variability and instability in experimental conditions. Alterations in the mixed-venous blood gases were always observed. It is likely that other metabolic factors were also varying considerably in the support dogs.

In isolated lung studies which have been carried out by other surgical investigators, mechanical devices have been used to "deoxygenate" blood in the perfusion circuit. This approach can provide the advantage of constancy in venous blood gases. In order to evaluate the effect on the ex vivo lung of replacing the support dog with a mechanical deoxygenating system, a series of experiments was undertaken.

The "bubble oxygenator" is the simplest and most commonly used mechanical gas exchanger. In four experiments, a device of this type was used in place of the support dog. The direct exposure of blood to gas is held responsible for many of the hematologic alterations which complicate bubble gas exchange (Lee et al, 1961-1; Peirce II, 1967, Peirce II et al, 1969). Membrane oxygenators eliminate the raw blood-gas interface. In five experiments, a membrane device was used to "deoxygenate" the blood in the perfusion circuit.

METHODS

General

The methods for controlling respiration and hemodynamics in the isolated lung were identical to those which had been used in the CONTROL series. The lung donors were exsanguinated into the extracorporeal circuit simultaneous with excision of the organ. Perfusion was continued in all experiments until function and morphology had grossly deteriorated.

Bubble Gas Exchanger

For these experiments, a pediatric bubble oxygenator* was incorporated in the circuit. Oxygenated blood was exposed to five percent CO_2 and 95 percent N_2 in the bubble column of the device. The gas mixture passed through a water bath to become prewarmed and humidified before entering the bubble column. A gas flow rate of 3 litres per minute was necessary in order to produce mixed-venous pO_2 and pCO_2 in the blood leaving the oxygenator.

Membrane Gas Exchanger

In these experiments, an experimental type of membrane oxygenator§ (Bramson et al, 1965) was used. An integral heat exchanger eliminated the need to prewarm the "degassing" mixture. Water mattresses in

* 3LF Travenol Bubble Oxygenator, Travenol Laboratories, Alliston, Ont.

§ Bramson Model 1432 Membrane Lung, Hallikainen Instruments Inc., Richmond, California.

the oxygenator were maintained at a pressure of 50 to 75 mm Hg in order to distribute blood flow over layers of silastic membrane (Gerbode et al, 1967). It was necessary to drive 10 percent CO_2 in N_2 at a rate of 20 litres per minute through the device in order to adequately "dearterialize" the blood.

The perfusion circuits are shown in Figure 1.

RESULTS

The function indices for these two groups of experiments are presented in Figure 2.

Bubble Gas Exchanger

All of the experiments in this group were terminated after 2.5 to 3.5 hours of perfusion. Vascular resistance rose to only 111 percent (SD 14%) of the initial value after 3 hours. This change was similar to that observed in the CONTROL lobes at three hours (112% SD 32%). Compliance remained at 93 percent (SD 5%) of the initial level at two hours, but during the third hour, compliance decreased to 69 percent (SD 14%). The compliance in the CONTROL lobes was 95 percent (SD 6%) of the initial value at three hours. Dead space - tidal volume ratio rose from an average of 41 percent to 53 percent at three hours. The data for venous shunt and oxygen gradients was incomplete. The few values which were obtained demonstrated deterioration of gas exchange during the first to third hours. All values were appreciably greater than those in the CONTROL lobes.

Membrane Gas Exchanger

In contrast to all previous experiments, the vascular resistance in this group decreased during the first three hours of perfusion. At five hours, vascular resistance was 56 percent (SD 11%) of the initial value.

Compliance decreased at a slower rate than in the previous group but more than in the CONTROL group. The difference in average values was not statistically significant. In this group, the dead space - tidal volume ratio was constant throughout the five-hour period of study.

For the first three hours of perfusion, the room air shunts and oxygen gradients for this group compared with values obtained in the CONTROL series. At five hours, the shunts were significantly higher (44% SD 19%) than in the CONTROL lobes (11% SD 5%) as were the gradients (59 mm Hg, SD 11 mm Hg compared to 30 mm Hg, SD 7 mm Hg).

Under conditions of alveolar hypoxia, the shunts were also higher (56%, SD 22% compared with 27%, SD 7%) as were the gradients (27 mm Hg, SD 6 mm Hg compared with 18 mm Hg, SD 7 mm Hg). From the second to fifth hours of perfusion, the average shunt and gradient under conditions of room air and low oxygen breathing progressively increased. These values in the CONTROL lobes appeared to progressively improve.

All of the "true shunt" values in this group were significantly higher than in the CONTROL lobes. The 100 percent oxygen gradients were not significantly higher than in the CONTROL lobes. This discrepancy in the gradient and shunt values was likely a result of higher oxygen saturations in the venous blood which entered the lobes in this group (75%) in comparison to the CONTROL group (50%).

Morphologic Observations

The gross alterations in these two groups were comparable, but the rate of morphologic change contrasted. At two hours in the first group and three to four hours in the second, the lungs appeared nearly normal except for patchy areas of pallor. During the final hour in the first group, compliance decreased and areas of congestion became apparent. Focal hemorrhages developed in the terminal period of perfusion (Figure 3).

Normal appearance remained until the final moments of perfusion in the second group. Severe intraparenchymal and alveolar edema suddenly developed in all lobes following a routine hyperinflation. Focal and confluent hemorrhages appeared soon after (Figure 4). In both groups the fluid volume in the extracorporeal circuit decreased during the final hour of perfusion. In most experiments, the measured weight gain was much greater than 100 percent. In one lung from the first group, the measured gain was 20 percent and two from the second group gained a measured 77 and 92 percent respectively. In all of these experiments, unmeasured volumes of edema fluid drained from the bronchial cannulas following collapse of the organs at the end of perfusion.

The histologic abnormalities were similar in the two groups and appeared to represent exaggerated forms of the alterations which had been observed in all previous experiments. Although patchy congestion was apparent grossly, microvascular congestion was not a predominant histologic abnormality.

A marked degree of diffuse perivascular edema and hemorrhage and interstitial edema were the most common histologic abnormalities (Figures

5 and 6). These abnormalities were more pronounced in the second group. Functional indices in the second group indicated that alterations were taking place over a more protracted period and during the period when gross pallor was observed.

DISCUSSION

Functional and morphologic deterioration developed in the "bubble deoxygenator" group in the absence of a significant elevation in vascular resistance. This observation contrasts markedly with the observations of others who have attributed the pathophysiologic sequence of events to primary arteriolar constriction (Barnes et al, 1968; Veith et al, 1968-1; 1968-2; Thelmo et al, 1970). These investigators have postulated that arteriolar constriction is a response to humoral factors which are activated by mechanical trauma to the blood in an extracorporeal circuit. Morphologic and functional derangements similar to the first group developed in the second group despite an appreciable <u>decrease</u> in vascular resistance.

Investigators who have observed marked increased vascular resistance have used positive pressure ventilation and gravity or syphon venous drainage. Their preparations deteriorated much more rapidly than the lobes in this study. Vascular derangements may be exaggerated under conditions of unphysiologic hemodynamics. This postulation must remain speculative until the combined effects of mechanical deoxygenation and abnormal hemodynamics are investigated.

The progressive decrease in resistance in the lobes in circuit with the membrane device is not yet explainable. This phenomena deserves further attention. The sudden and fulminate deterioration of the lobes

in this group is equally puzzling.

Numerous theories have been forwarded in an attempt to explain the cause of perfusion damage of the isolated lung and the lung in cardiopulmonary bypass (Awad et al, 1966; Veith et al, 1968). Many proposals have followed upon observations made in grossly unphysiologic isolated lung preparations. The experiments which have been carried out to this point permit comment on several of the existing theories.

Hemodynamic disturbances such as impediment of venous return, overloading of the pulmonary circulation (Awad et al, 1965), sludging as a result of hypothermia (Sakai and Lewis, 1965) and low perfusion pressures were prevented in the foregoing experiments. Embolic material such as fibrin and silicone (Schramel et al, 1963) were not seen in the histologic specimens. Blood incompatibility (Awad et al, 1966) was not introduced in these experiments. Nevertheless, the lungs deteriorated more rapidly than in previous experiments.

Denaturation of plasma proteins is a well recognized effect of extracorporeal circulation of blood (Lee et al, 1961-2; Peirce II, 1967; Belzer et al, 1968). These effects are exaggerated by the use of a bubble or disc oxygenator and less in oxygenator circuits which employ a membrane gas exchanger (Lee et al, 1961-2; Peirce II et al, 1969). This helps to explain the longer duration of function in the membrane deoxygenator than the bubble oxygenator group.

In both of the foregoing groups of experiments, alveolar irregularity and atelectasis developed earlier and more severely than in the preceding experiments. Surfactant is altered during extracorporeal circulation (Gardner et al, 1962). The maintenance of surfactant is a

dynamic metabolic process. Precursors of surfactant are possibly depleted in the absence of a support dog. This must remain an important area for further study.

Platelets progressively disappear in a lung perfusion circuit (Hauge et al, 1966). Platelets are important to the maintenance of microvascular integrity (Zweifach, 1961). Unfortunately, platelet determinations were not carried out in any of the author's studies. It is very likely that this factor plays a role in microvascular deterioration of the perfused lung, particularly in circuits devoid of a source of platelet replenishment.

Glucose utilization continues in a totally isolated lung (Daly and Hebb, 1966). In the foregoing experiments and in the experiments which others have carried out, the glucose level in the circulating blood was neither measured nor supplemented. In an extension of these studies in our laboratory, the glucose level in the perfusate was found to rapidly decrease. The addition of glucose and insulin attenuated perfusion damage and allowed longer perfusions (Modry et al, 1971).

The metabolic and hematologic factors which contribute to the integrity of the isolated perfused lung are undoubtedly numerous and are certainly poorly understood. Within the present limitations of our knowledge, the use of a support dog appears to be essential for the optimal support of an isolated functioning lung.

Elimination of the support dog provides a "closed" circuit free of"exogenous" metabolic alterations. In this respect, this preparation should be useful in the future study of the metabolic requirements of the isolated lung. Extrapolation of observations in this type of

preparation to the clinical problems of the shock lung and the lung in extracorporeal circulation is subject to considerable misinterpretation.

SUMMARY

1. Nine canine lobes were perfused with autologous blood in a closed extracorporeal circuit which included a bubble or membrane gas exchanger.
2. The lobes were destroyed after three to five hours of perfusion.
3. The membrane oxygenator delayed, but did not minimize the undesirable effects of replacing the support dog.
4. Under conditions of negative pressure ventilation and positive venous pressure, the severe morphologic alterations which were produced in these experiments were not associated with a significant elevation in vascular resistance.

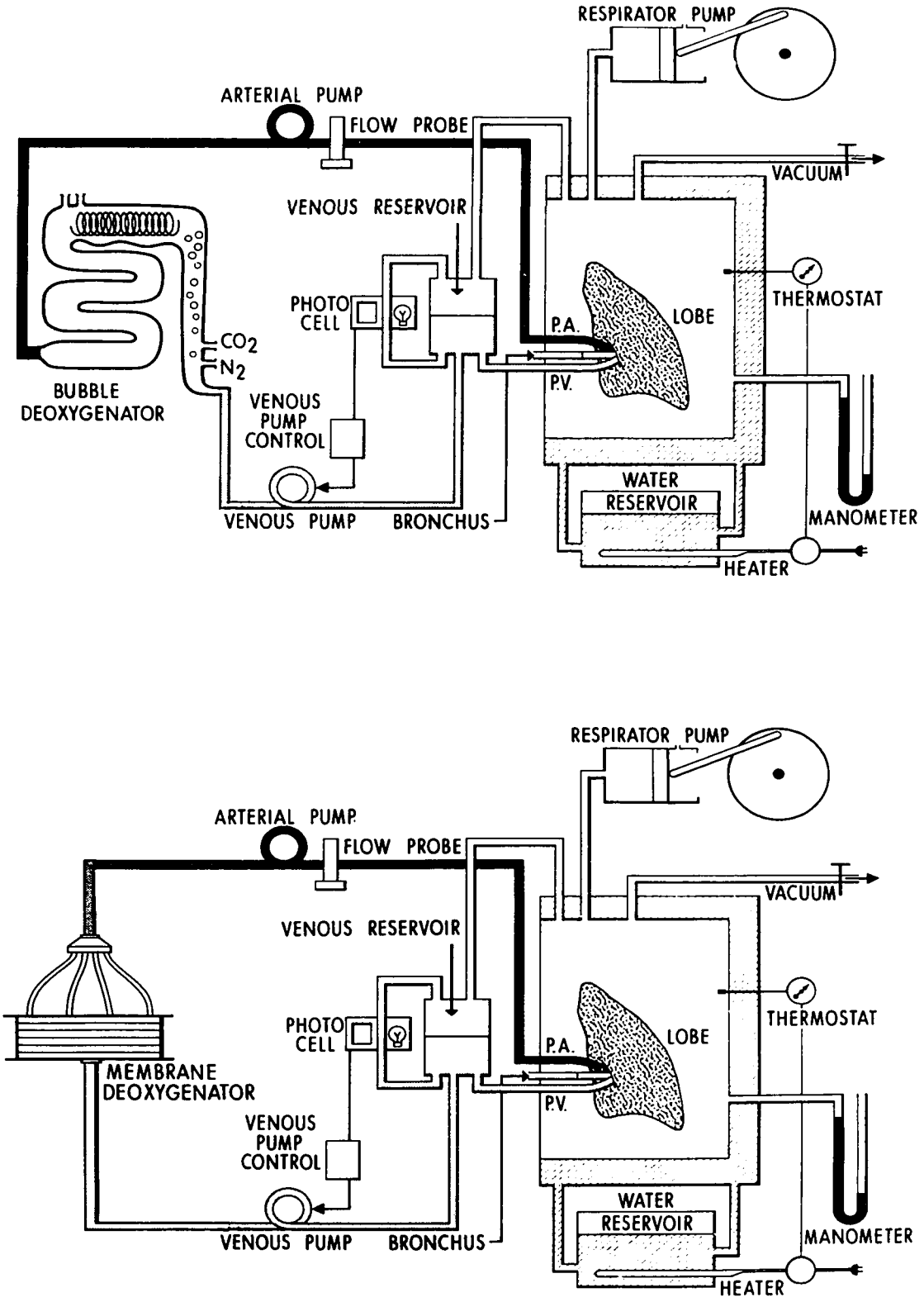

Fig. 1. Perfusion circuits incorporating bubble gas exchanger or membrane gas exchanger in place of support dog.

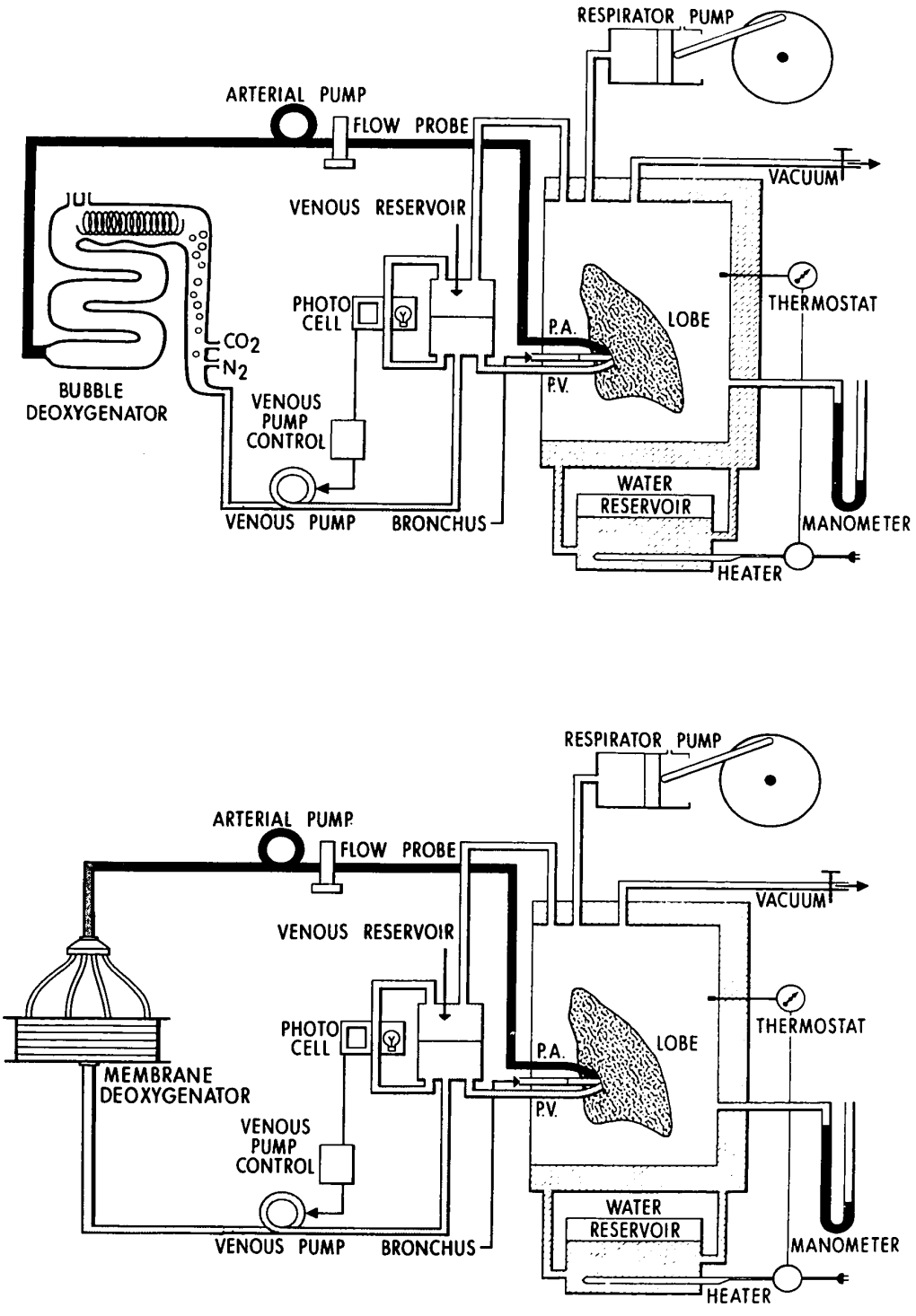

Fig. 1. Perfusion circuits incorporating bubble gas exchanger or membrane gas exchanger in place of support dog.

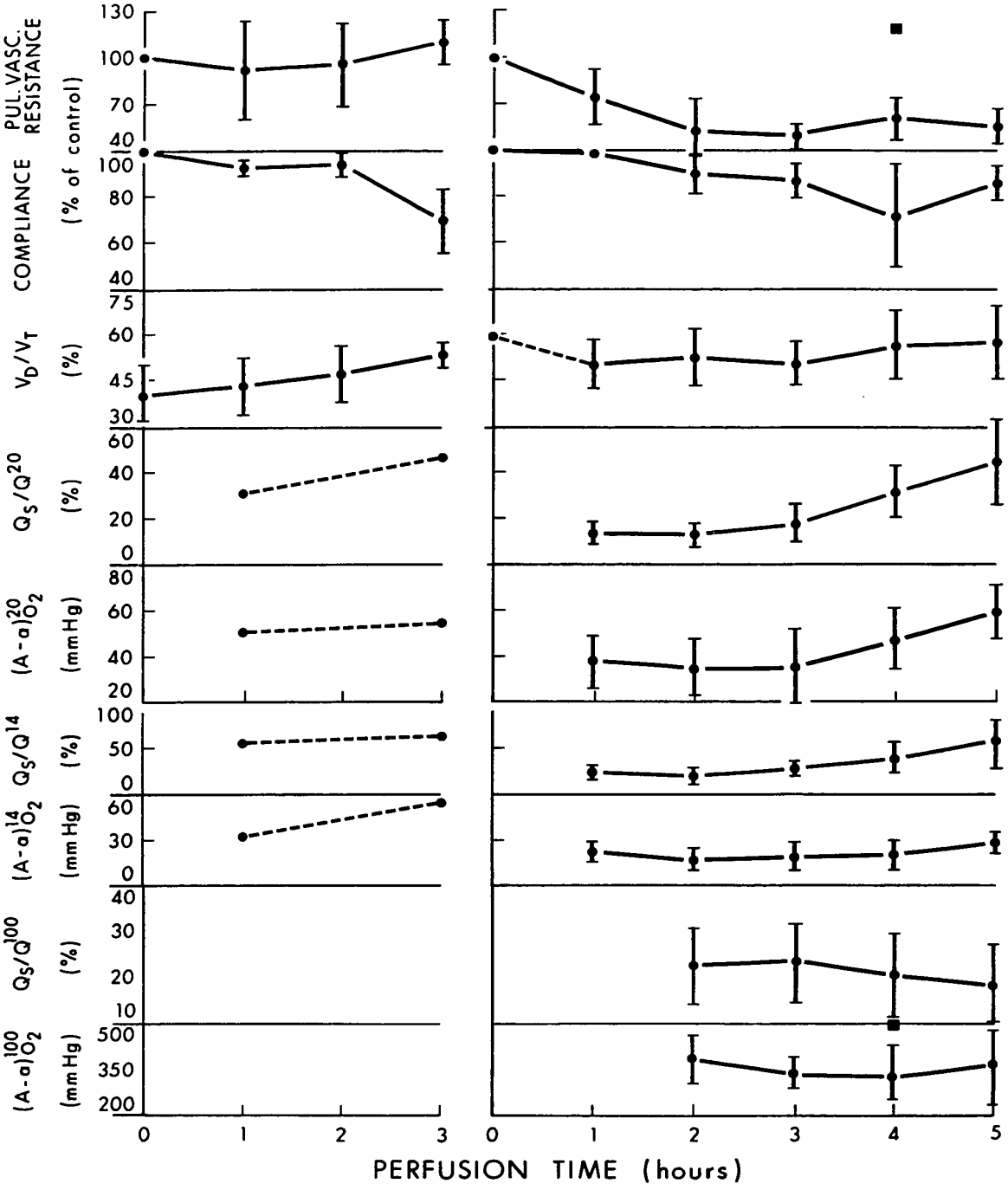

Fig. 2. Function of 4 lungs in bubble gas exchanger circuit (left) and 5 in membrane gas exchanger circuit (right) (■ - 1 lung suddenly destroyed).

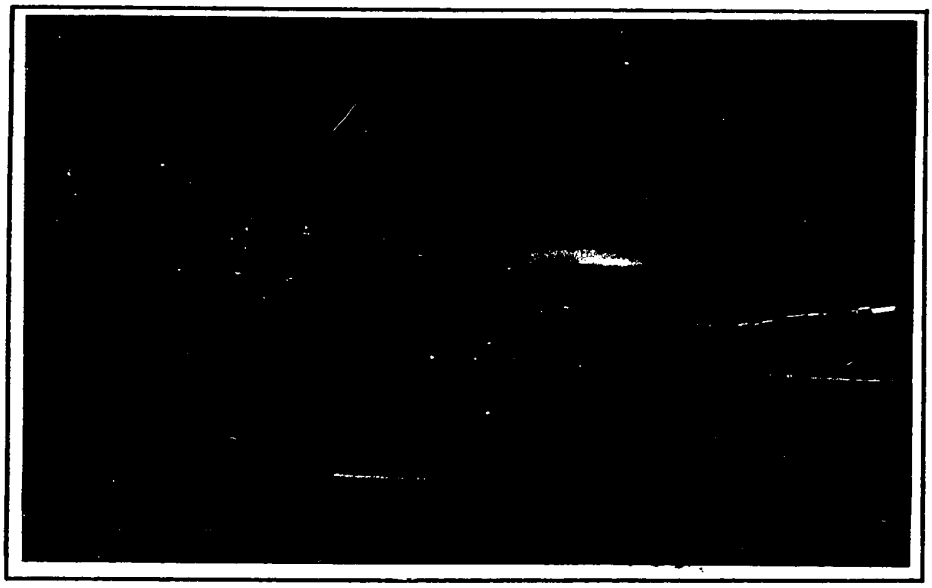

Fig. 3. Lobe after 3 hours in bubble gas exchanger circuit.

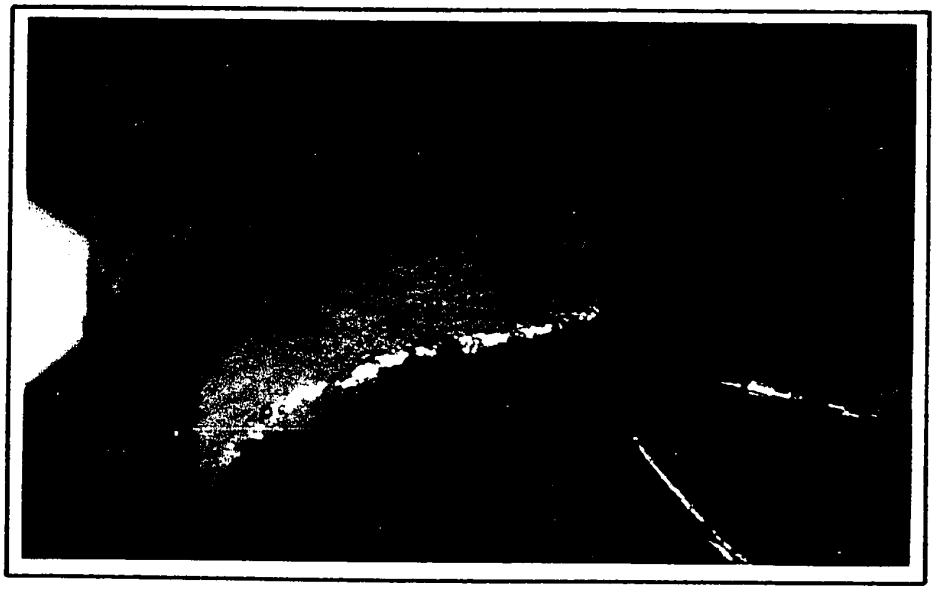

Fig. 4. Lobe after 5 hours in membrane gas exchanger circuit (passively deflated).

Fig. 3. Lobe after 3 hours in bubble gas exchanger circuit.

Fig. 4. Lobe after 5 hours in membrane gas exchanger circuit (passively deflated).

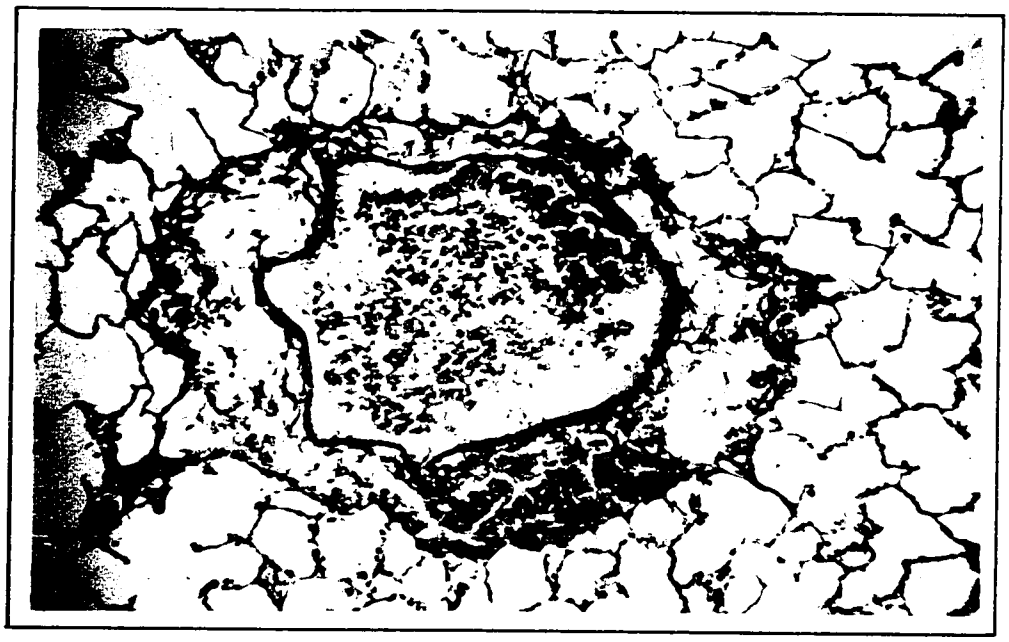

Fig. 5. Lobe in circuit with bubble gas exchanger for 3 hours (VER x 100); perivenular edema, hemorrhage, interstitial edema.

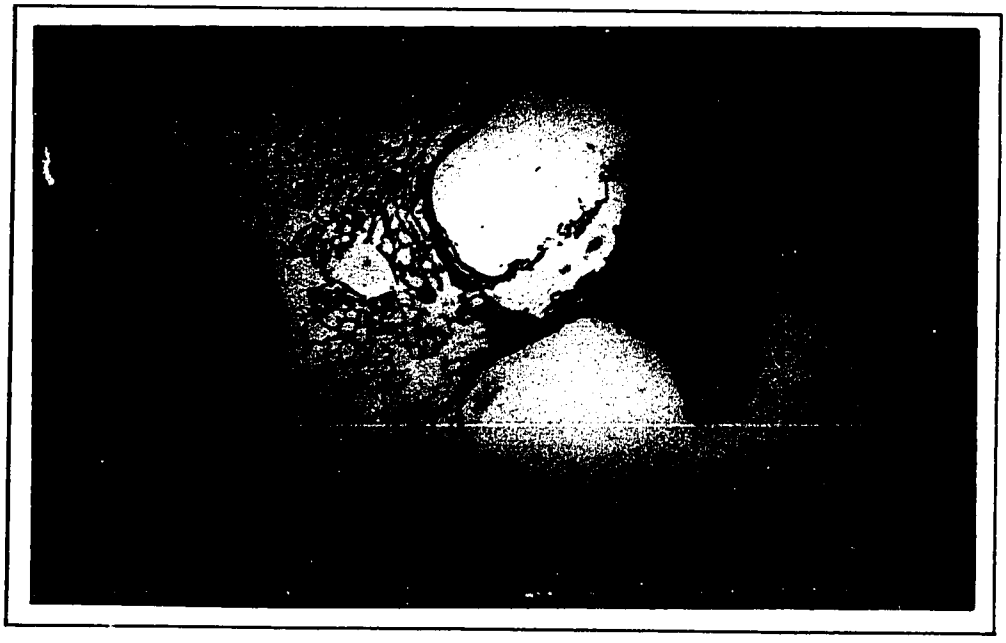

Fig. 6. Lobe in circuit with membrane oxygenator for 5 hours (VER x 25); marked perivascular and interstitial edema, vascular congestion.

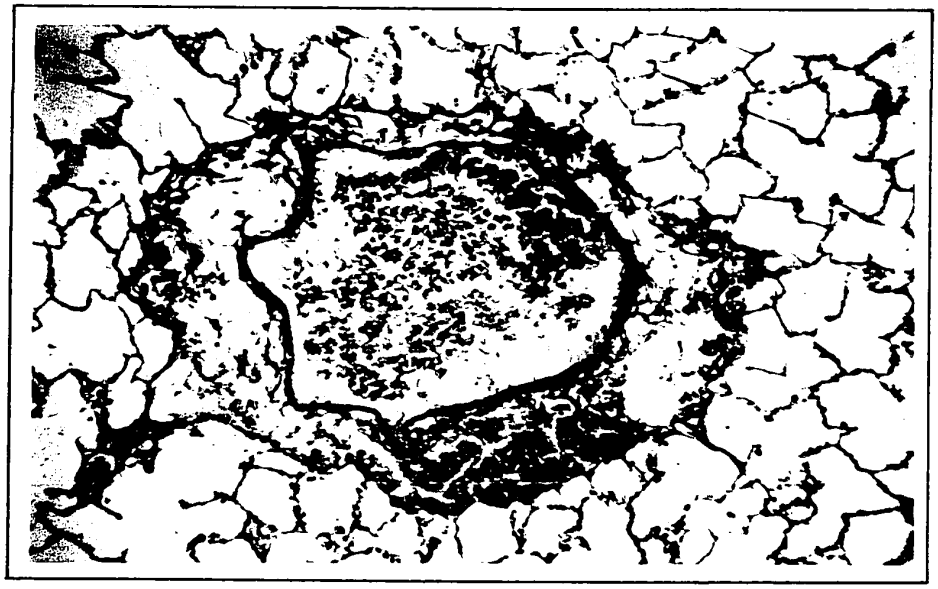

Fig. 5. Lobe in circuit with bubble gas exchanger for 3 hours (VER x 100); perivenular edema, hemorrhage, interstitial edema.

Fig. 6. Lobe in circuit with membrane oxygenator for 5 hours (VER x 25); marked perivascular and interstitial edema, vascular congestion.

CHAPTER IX

PERFUSION OF THE LUNG WITH HOMOLOGOUS BLOOD

Circulatory failure in autologous support dogs has limited the duration of previous perfusions to between five and ten hours. It seemed likely that intact animals would tolerate longer periods of perfusion. This approach would subject the isolated lung to "homologous" blood.

Numerous investigators believe that homologous blood plays a significant role in the pathophysiology of pulmonary dysfunction in cardiopulmonary bypass (Hepps et al, 1963; Neville et al, 1963; Schramel et al, 1963; Sykes et al, 1966; Timmis, 1967) and in isolated perfused lungs (Awad et al, 1966-1; 1966-2; Veith et al, 1967-1; 1967-2).

In the preceding autoperfusions, the entire lung had not been perfused because it is necessary to sacrifice the lung donor in order to obtain a large enough atrial cuff for cannula-control of venous pressure. In order to carry out lung perfusions, a "homologous" support dog would be necessary.

The development of an isolated lung preparation which would allow the objective evaluation of lung storage techniques was one of the author's original objectives. At this stage in our knowledge, the support dog was essential for the optimal perfusion of the isolated lung. Homologous perfusion would be necessary in order to carry out the evaluation of lungs following lengthy periods of storage.

In order to evaluate the effects of homologous blood on the ex vivo lung and in an attempt to prolong the duration of lung perfusion,

the following experiments were carried out.

METHODS

Left lungs from 17 to 25 kilogram mongrel dogs were rapidly excised using methods described in Chapter III. Left lung extirpation required about ten minutes to complete.

25 to 30 kilogram unrelated and unmatched mongrel dogs were anaesthetized and connected to the extracorporeal circuit using transvenous catheters on the pulmonary arterial line. The perfusion circuit was otherwise identical to that which had been used for the previous lobe perfusions. The control of perfusion was also identical to the experiments in the CONTROL group except flow rates were increased to 30 ml per donor kilogram per minute.

RESULTS

General

The four perfusions in this group lasted from four to sixteen hours. In the first and third experiments, venous return from the lungs became mechanically obstructed. The experiments were discontinued when the lungs became irreversibly congested. Air embolism inadvertently occurred at eight hours in the second experiment. The sixteen-hour experiment was terminated when the support dog was rapidly deteriorating.

Figure 1 presents a summary of the data which were obtained in this group. All four experiments provided data during the first five hours of perfusion. The averages allowed comparison to be made with the

CONTROL series of lobes. From the fifth to eighth hours, the averages of the two longer experiments are shown.

Hemodynamics and Mechanics

The pulmonary vascular resistance and compliance remained stable throughout the first five hours of perfusion. The dead space - tidal volume ratio appeared to increase slightly. This change was not significant within the group or in comparison to the preceding isolated lobes.

Gas Exchange

The average percentage of "shunted" blood under conditions of room air breathing was higher than in the CONTROL lobes, but not to a significant degree. This index appeared to improve more noticeably than in the CONTROL lobes during the first five hours.

$[A\text{-}a]O_2^{20}$ paralleled the values in the CONTROL lobes in magnitude and direction of change.

The true shunt appeared greater throughout the first five hours of perfusion in two lungs (15% - 13%) than in the lobes (8% SD 3% to 5% SD 1%). Sufficient data for comparison of the $\dot{Q}s/\dot{Q}^{14}$ and $[A\text{-}a]O_2^{14}$ were not obtained in these four experiments.

The hemodynamic and mechanical properties of the two lungs which were perfused for more than five hours were well preserved from the fifth to eighth hours. The average $\dot{Q}s/\dot{Q}^{20}$ and $[A\text{-}a]O_2^{20}$ for the two longer perfusions deteriorated during this period. These alterations in gas exchange after five hours were much greater in the third lung in the series.

Sixteen Hour Lung

Figure 2 presents the data which were obtained in the sixteen hour lung. Following an initial decrease, the vascular resistance was unchanged during the first eight hours of perfusion. Thereafter, resistance gradually increased. At sixteen hours, vascular resistance was 125% of the initial value. The compliance, on all but one determination, was better than at the beginning of perfusion. At sixteen hours, compliance equalled the initial value. Dead space - tidal volume ratio was 50 percent initially and ranged from 49 to 55 percent. At sixteen hours, $\dot{V}_D/\dot{V}_T$ was 52 percent.

$\dot{Q}_s/\dot{Q}^{20}$ was 52 percent at one hour. Gas exchange gradually improved and $\dot{Q}_s/\dot{Q}^{20}$ was 15 percent at sixteen hours. True shunt also improved from 13 percent at three hours to 7 percent at fifteen hours.

Morphology

Sudden venous obstruction in two lungs and air embolism in one, produced fulminant morphologic deterioration. The sixteen-hour lung weighed 97 grams at the beginning of perfusion and 90 grams following perfusion; a loss in weight of seven percent.

At the termination of the experiments, the first and third lungs were diffusely congested and alveolar edema and hemorrhage had developed. These changes took place rapidly following sudden venous obstruction. The second lung appeared grossly normal after one hour of perfusion. After seven hours, a few focal subpleural hemorrhages and one dependent zone ecchymosis had developed (Figure 3). Subsequent to air

embolism, this lung rapidly developed patchy congestion and pallor and frank tracheobronchial edema and hemorrhage.

Three subpleural hemorrhages were present in the sixteen-hour lung shortly after the beginning of perfusion. These remained at sixteen hours when the lung was otherwise normal in gross appearance (Figure 4). With the exception of air trapping in the apical segment of the upper lobe, the lung collapsed well following the release of negative pressure in the chamber (Figure 5).

In the sixteen-hour experiment, the lungs in the support dog had developed focal subpleural ecchymoses and severe "hepatization" in the dependent zones bilaterally. Peripheral air trapping and surface mottling were also present (Figure 6). The lung in the first three support dogs unfortunately were not examined.

Histologic study of the isolated lungs in the overall group was not rewarding because of the terminal insult in three of the experiments. The sixteen-hour lung demonstrated perivascular and interstitital edema and alveolar irregularity similar to the CONTROL lobes (Figure 7). The lungs in the support dog demonstrated these alterations and in addition, intraparenchymal and alveolar hemorrhage (Figure 8).

DISCUSSION

This group of experiments was small and technical problems resulted in early termination of three experiments. Several useful observations were made, however.

The function of these lungs after five hours of perfusion compared well with the CONTROL lobes. Oxygen gradients and shunts were

higher than in the CONTROL lobes during the initial period of perfusion. These major alterations in gas exchange were likely contributed to by the following factors: difficulty orientating the lungs on the chamber cannulae for optimal distribution of blood flow and ventilation, the greater weight which was borne by the dependent zones of the lungs, failure to minimize hydrostatic pressure differences between upper and lower zones and paren~hymal distortion during the end-expiratory period of respiration (Figure 5). The foregoing problems were encountered in all four experiments.

Mechanical obstruction of the pulmonary veins was the most serious technical problem and resulted in the destruction of two lungs. Venous cannula revision or lobe perfusion would avoid this problem in future.

Subsequent to these experiments, nine lobes were perfused using homologous support dogs and methods which were other wise identical to those used for this series. The lobes functioned as well as autologously perfused lobes (Jirsch, 1970; Jirsch et al, 1970).

These experiments do not rule out the possibility that homologous blood contributes to perfusion damage. Appreciable "mixing" of unmatched blood did not occur in these experiments, because very little donor blood remained in the lung following excision. The effects of homologous blood are likely the result of appreciable volume-mixing in the circulation (Schramel et al, 1963; Sykes et al, 1966; Timmis, 1967). The blood types of the lung donor and support dog in the sixteen-hour experiment may have been "compatible." That experiment would have provided considerable argument against homologous factors had a "mismatch" been documented.

In the experiments of others which have implicated homologous blood, efforts have not been made to demonstrate the blood which was administered was highly compatible. In most clinical situations, only compatible blood is used. The role of homologous blood in alteration of the lung in shock and cardiopulmonary bypass has likely been overestimated.

The sixteen-hour lung functioned well at the termination of perfusion. This experiment provided support for the original assumption that attempts to provide a more "physiologic" environment for the isolated lung enhanced functional preservation. In most respects, the gross and microscopic appearances of this lung were better than the lungs in the support dog. Explanation for this cannot be provided. The isolated lung may elaborate humoral factors which traverse the peripheral microcirculation in the support dog and exert an untoward effect on the in situ lung. This is one of the many possibilities which require further investigation.

SUMMARY

1. Four left canine lungs were perfused for periods of four to sixteen hours using intact dogs to provide metabolic homeostasis in the perfusion circuit.
2. One lung which was not affected by technical problems maintained satisfactory function and morphology for sixteen hours of perfusion.
3. Abnormalities attributable to the use of "homologous" blood did not develop in this group of experiments.

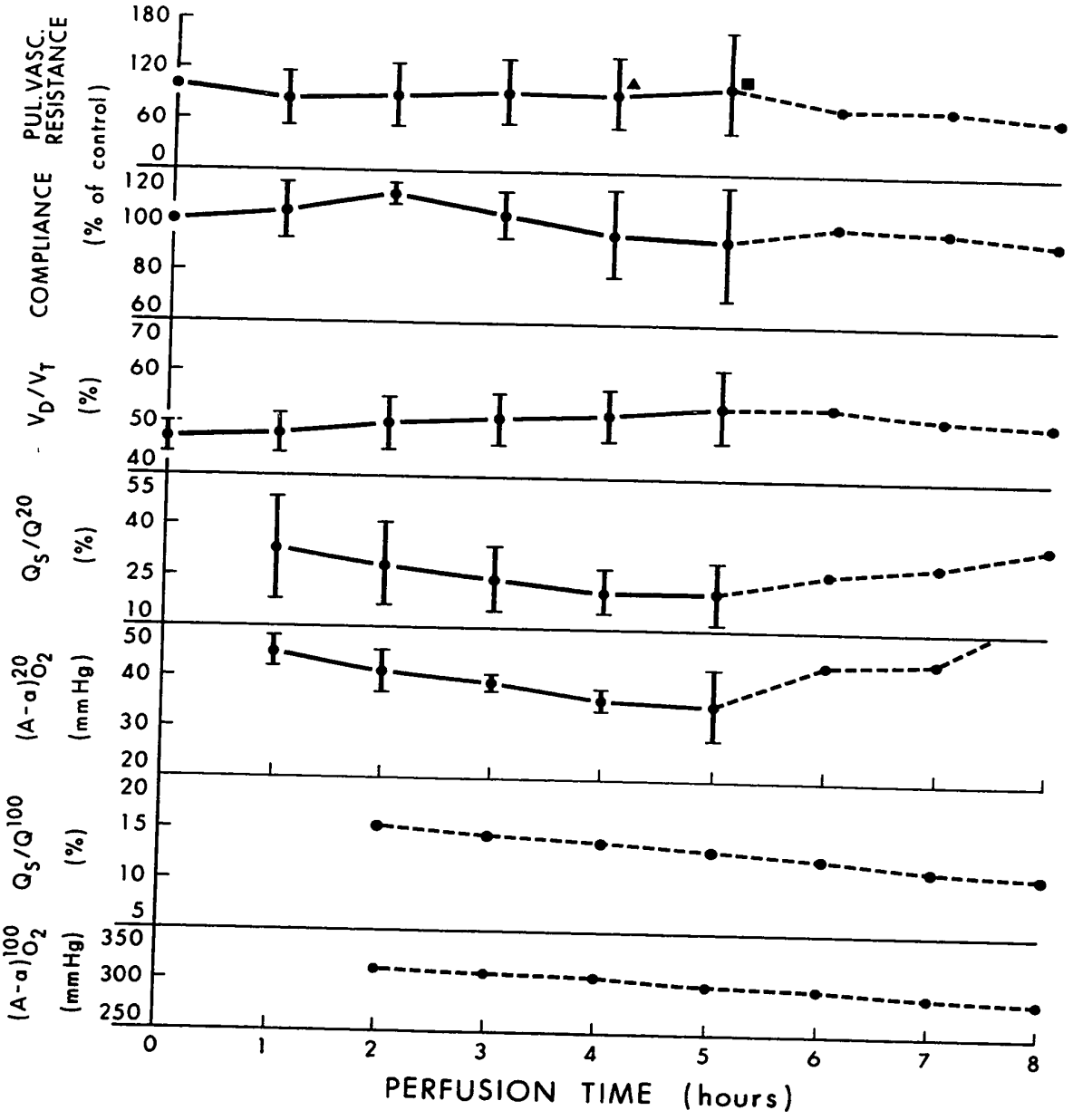

Fig. 1. Function of 4 lungs perfused with homologous blood (▲,■ - venous obstruction, experiments terminated).

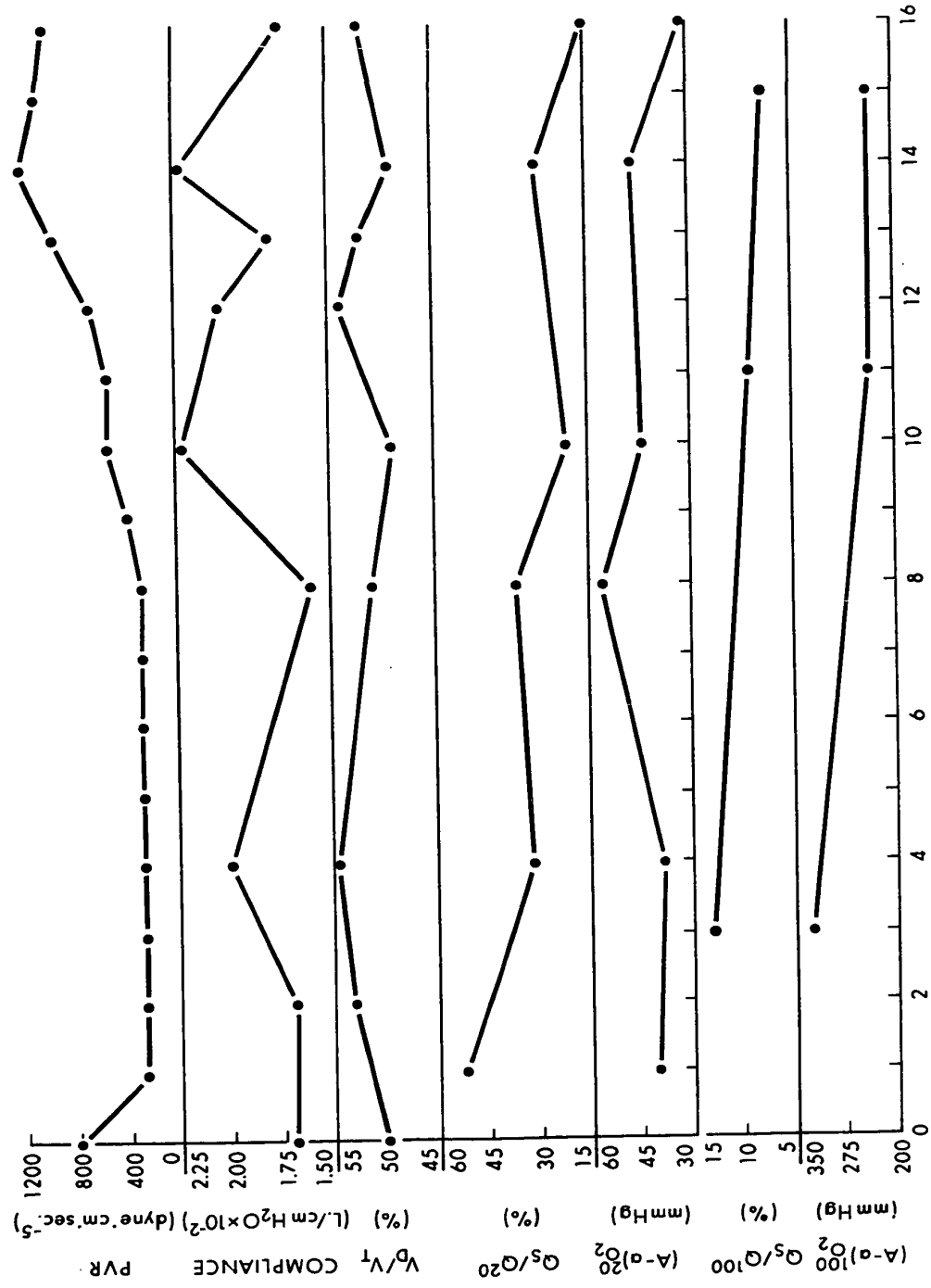

Fig. 2. Function of lung perfused for 16 hours with homologous blood.

Fig. 3. Third lung after 8 hours of homologous perfusion.

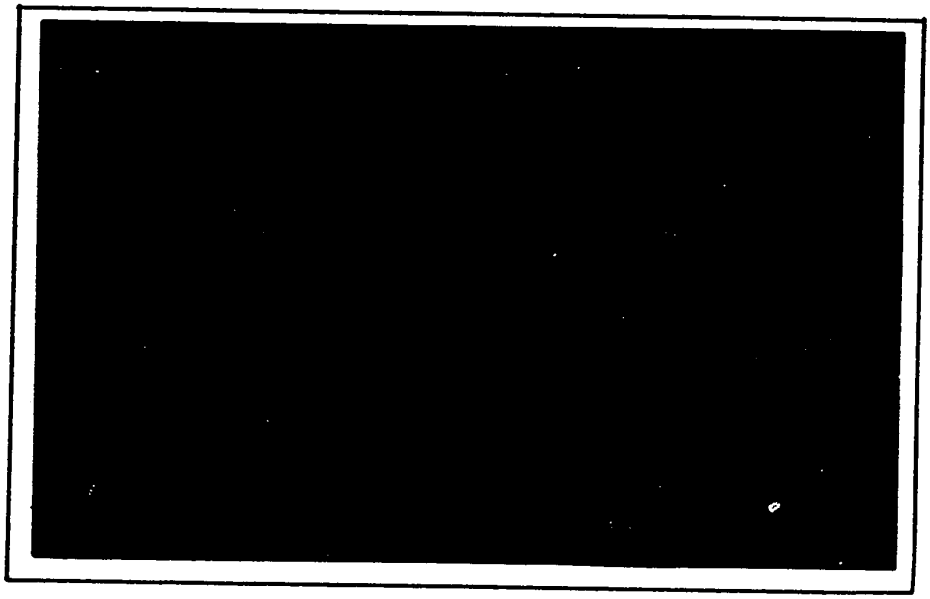

Fig. 4. Fourth lung after 16 hours of homologous perfusion.

Fig. 3. Third lung after 8 hours of homologous perfusion.

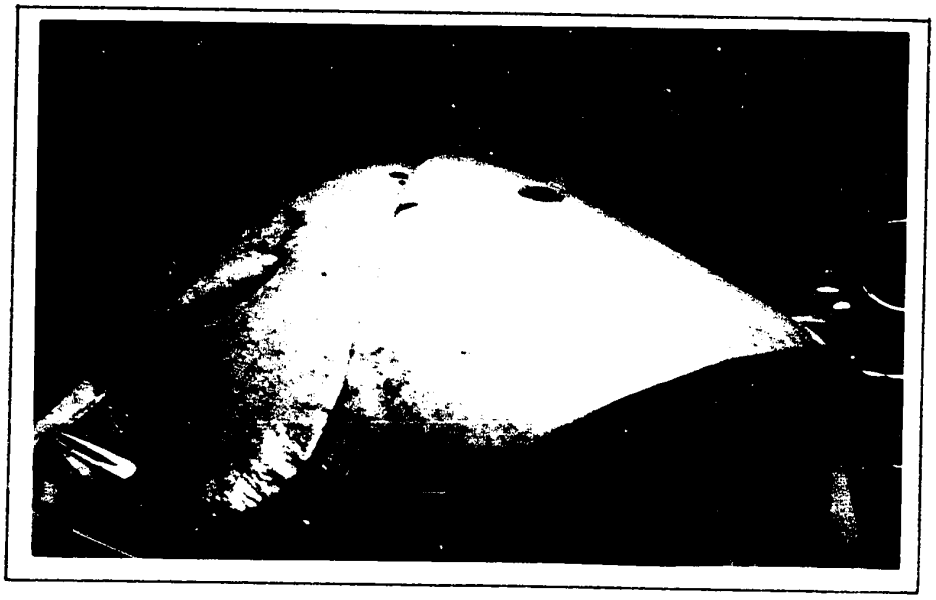

Fig. 4. Fourth lung after 16 hours of homologous perfusion.

Fig. 5. Fourth lung after 16 hours of homologous perfusion (passively deflated).

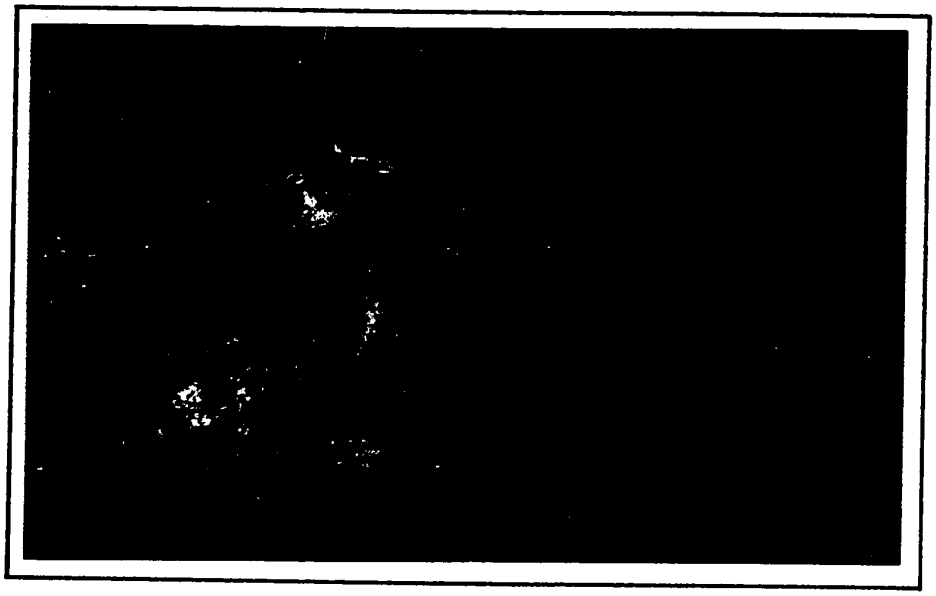

Fig. 6. Right lower lobe from support dog after 16 hours of homologous lung perfusion (passively deflated).

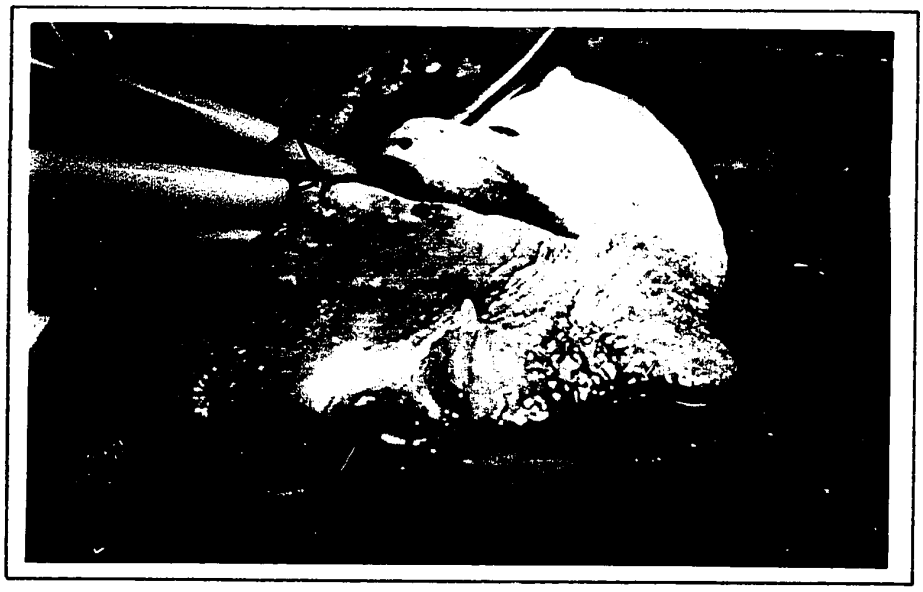

Fig. 5. Fourth lung after 16 hours of homologous perfusion (passively deflated).

Fig. 6. Right lower lobe from support dog after 16 hours of homologous lung perfusion (passively deflated).

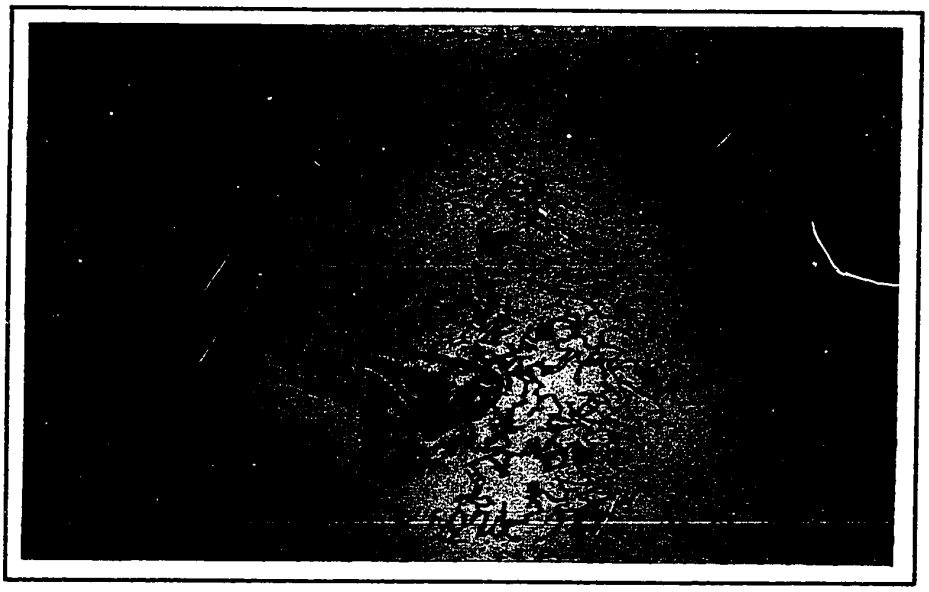

Fig. 7. Lung after 16 hours of homologous perfusion (H & E x 40); mild perivascular and interstitial edema, alveolar irregularity.

Fig. 8. Lung from 16 hour support dog (H & E x 40); marked peribronchial-perivascular edema, intraparenchymal hemorrhage.

Fig. 7. Lung after 16 hours of homologous perfusion (H & E x 40); mild perivascular and interstitial edema, alveolar irregularity.

Fig. 8. Lung from 16 hour support dog (H & E x 40); marked peribronchial-perivascular edema, intraparenchymal hemorrhage.

CHAPTER X

EVALUATION OF LUNG PRESERVATION BY ISOLATION PERFUSION

One of the major problems in lung preservation research, has been the lack of means for objectively assessing the functional adequacy of lungs immediately following preservation attempts. Most investigators have based their assessment of storage adequacy on the survival of unilateral recipients of allografted stored lungs. This approach is complicated by the effects of implantation surgery and by the variable effects of rejection and response to immunosuppression. Unilateral pulmonary function is somewhat difficult to assess. Furthermore, short term survival of recipients which have normal contralateral lungs does not indicate that the stored organ was able to provide almost normal gas exchange immediately following implantation.

Several reports have described "successful" 24-hour preservation of the lung as assessed by the "transplantation" approach (Blumenstock et al, 1962, 1965; Garzon et al, 1966; Hino et al, 1968). None of these studies included a comprehensive evaluation of pulmonary function immediately following transplantation. For this purpose, ex vivo evaluation of functional preservation appeared to be a potentially useful alternative. In order to investigate this possibility and to evaluate lungs which were stored using apparently "satisfactory" methods, the following experiments were carried out.

METHODS

Five left lungs from 16 to 25 kilogram dogs were rapidly excised and flushed with 200 to 300 millilitres of cooled, heparinized, lactated

Ringer's solution (buffered to pH 7.40). Following the placement of a bronchial plug, the lungs were placed in a pressure vessel* where they were immersed in buffered Tyrode's solution (Altman and Dittmer, 1964) under conditions of two atmospheres of oxygen pressure and 4° C.

A large portion of right atrial tissue was incidentally included with the left atrial cuff in the first storage attempt. The heart from the fifth donor was placed with the lung in the storage vessel.

Following 16 hours of storage in the first experiment and 24 hours in the remaining four, the pressure vessel was removed from the ice bath and slowly decompressed over a period of 30 to 45 minutes. The 16-hour lung and two of the 24-hour lungs were then homologously perfused using methods which were described in Chapter X. The upper lobes from two of the 24-hour lungs were removed for histologic study. The lower lobes were homologously perfused. The perfusions were continued until the lungs were destroyed.

RESULTS

While the first lung was being cannulated to the perfusion circuit, the atrial tissue exhibited a spontaneous return of sinus rhythm. Atrial contractile activity remained for the duration of the two-hour perfusion of this lung. The first lung exhibited gas exchange for two hours although all indices of function were considerably deranged initially and all but vascular resistance deteriorated progressively (Table 1).

* Millipore Model YY30, 1 Gallon Pressure Vessel, Millipore Corporation, Bedford, Massachusetts.

Of the four lungs which were stored for 24 hours, one demonstrated very poor and rapidly deteriorating function during 90 minutes of perfusion. The other three were totally destroyed in less than 45 minutes following the careful initiation of perfusion. All of the lungs were easily inflated prior to perfusion. Vascular resistance was elevated when perfusion began in the 24-hour lungs. Shortly after perfusion began, the lungs rapidly lost their compliance. These changes developed despite a reasonably normal gross appearance in two lungs (Figure 1). Three lungs demonstrated patchy areas of severe pallor and congestion (Figure 2).

The heart which was stored with the fifth lung did not demonstrate return of atrial or ventricular contractile activity.

Four of the lungs weighed less following storage than they did prior to storage (average weight loss 5%). They all gained much in excess of 100 percent during the brief periods of perfusion. Edema fluid poured from the bronchial cannulas in all lungs following perfusion. In three, the edema fluid was grossly sanguinous.

The lungs developed severe perivascular and interstitial edema. Moderate perivascular, interstitial and alveolar hemorrhage and vascular congestion accompanied the edema (Figure 3).

Both lobes which were studied following storage but prior to perfusion demonstrated perivascular and interstitial edema grossly and histologically (Figure 4).

The systemic pressure in three of the supporting dogs suddenly decreased when blood flow was begun through the isolated lung. The aortic pressure changes ranged from 20 to 50 percent (average 33 percent) within the first 15 minutes of beginning perfusion. This degree of

change in systemic pressure was not seen in any of the previous experiments. Aortic pressures slowly returned toward the pre-perfusion level during the subsequent 15 minutes despite increasing flow rates through the isolated organ.

DISCUSSION

Normal morphology following storage does not guarantee functional integrity. Metabolic tests of cellular viability have been proposed as means for assessing viability of organs following storage (Hino et al, 1968). Cellular integrity is necessary for the preservation of functional integrity, but the ultimate criteria for successful storage is functional adequacy immediately following storage (Barnes et al, 1968).

The apparent "survival" of lungs which others have stored using hypothermia and hyperbaria requires explanation. In our studies, the stored lobes were unable to tolerate normal flow rates. The lungs which others have implanted were likely not subjected to normal flow rates initially because of reversibly elevated vascular resistance. Healthy unilateral lung recipients retained more than enough normal lung to support life. In that case, the grafts would have been subjected to less than normal flow rates. Clinical lung allografts must tolerate greater than normal unilateral flow immediately following implantation.

The stored lobes in our studies were possibly susceptible to the untoward effects of extracorporeal circulation of blood. This might be considered a criticism of the perfusion approach to storage evaluation. Extracorporeal circulation with a heart-lung machine was required for the intraoperative support of seven of the first twenty-three clinical lung recipients (Wildevuur and Benfield, 1970). Ideally, an adequately

stored lung should tolerate blood which has been circulated extracorporeally. The hematologic alterations which result from total cardiopulmonary bypass with mechanical oxygenation are undoubtedly more severe than those which were produced in our circuit.

The lungs in this study may have been more susceptible to the effects of "homologous" blood than the preceding homologously perfused lungs. In clinical transplantation, allografts must tolerate perfusion with homologous blood.

The persistence of atrial activity throughout perfusion in the first experiment, suggests that the method which was used might be more adequate for preserving the heart than the lung. The absence of contractile activity in the heart which was stored with one of the 24-hour lungs, suggests that the methods were likely inadequate for 24-hour heart preservation. This contrasts with the observations of Largiader and co-workers (1965), who reported that similar degrees of hypothermia and hyperbaria satisfactorialy preserved the canine heart for 24 hours.

Perivascular and interstitial edema were present in the two lungs which were examined prior to perfusion. Tissue osmotic pressure may have been greater than the osmotic pressure of the "preservative" solution. The lobes lost weight, however, during the period of storage. Perivascular and interstitial edema were the predominant lesions following perfusion. The foregoing suggests that perivascular edema was an early change in the sequence of alterations which led to morphologic destruction of the stored lungs.

The magnitude of hypotension in the support dogs immediately following the initiation of perfusion was unexplained. The isolated lungs may have formed or released excessive amounts of vasoactive

materials as a result of storage. In future experiments, attempt should be made to study this possibility. This might provide insight into the nature of the structural damage and requirements for improving lung storage.

In experiments which have preceded this series, no attempts have been made to reimplant lobes or lungs which retained satisfactory morphologic and functional integrity. Normothermic, whole blood perfusion is not practical for clinical organ storage. The assumption that the CONTROL lobes provide an "ideal" standard of comparison for stored lungs can be criticized, however. In this respect, a study of the post-implantation performance of "control" lungs would be a worthwhile endeavour in future.

Addendum:

Subsequent to the foregoing experiments, an additional nine lobes were stored using identical methods and similarly evaluated during homologous perfusion (Jirsch, 1970-1; Jirsch et al, 1970-2). These lobes were compared to nine homologously perfused but non-stored "control" lobes. All nine control lobes retained satisfactory indices of vascular resistance, compliance and gas exchange for four to five hours. The control lobes were almost normal following perfusion.

The stored lobes exhibited one-third of the compliance, one-quarter of the $(a-\bar{v})$ oxygen difference and higher vascular resistance than the control lobes when perfusion began. These indices progressively deteriorated during the first hour of perfusion. All but one of the stored lobes was destroyed following 90 minutes of perfusion.

Edema fluid poured from the bronchial cannulas in this group and the lobes demonstrated dependent zone congestion, ecchymotic surface discoloration and petechiae. Interalveolar thickening and edema, alveolar hemorrhage and vascular congestion characterized the stored lobes histologically.

SUMMARY

1. Five lungs were stored for 16 to 24 hours under conditions of hypothermia and hyperbaria. Following storage, the functional adequacy of the lungs was assessed using normothermic homologous perfusion-ventilation. (Identical studies were carried out in nine lobes by the author's successor.)
2. The lungs functioned very poorly immediately following storage and were rapidly destroyed during perfusion.
3. Lungs which have been stored under conditions of hypothermia and hyperbaria do not tolerate isolation-perfusion .
4. Perfusion-evaluation of stored lungs offers several advantages over the transplantation approach to preservation evaluation.
5. These preliminary studies have revealed several important areas for future investigation.

EXPERIMENT NO.	36			39		
DURATION OF STORAGE (HOURS)	16			24		
PERFUSION TIME (MINUTES)	30	60	90	30	60	90
PULMONARY VASCULAR RESISTANCE (dyne · cm · sec.$^{-5}$)	1030	1140	915	5300	5900	9300
COMPLIANCE (Litres/cm x 10^{-2})	2.3	2.2	1.8	1.0		
V_D/V_T (%)	55	60	65	73		
Q_S/Q^{20} (%)	23		32	23		
Q_S/Q^{14} (%)		57				
Q_S/Q^{100} (%)			9.2			
BLOOD VOLUME (ml/kg.dog wt.)		3.4	2.9			

Table 1. Immediate function of lungs following 16 hours storage (#36) and 24 hours (#39).

Fig. 1. Lung stored for 16 hours after 90 minutes of perfusion.

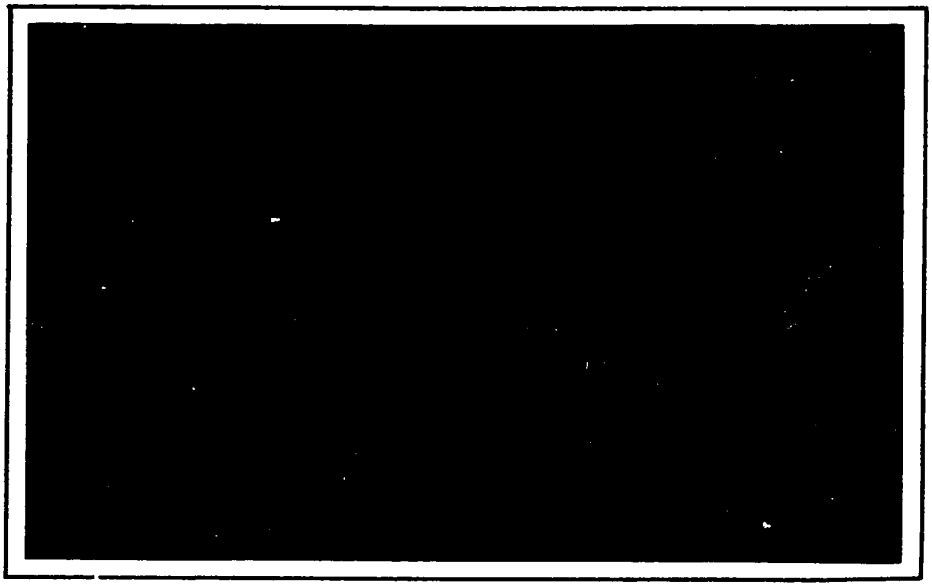

Fig. 2. Lung stored for 24 hours, after 30 minutes of perfusion.

Fig. 1. Lung stored for 16 hours after 90 minutes of perfusion.

Fig. 2. Lung stored for 24 hours, after 30 minutes of perfusion.

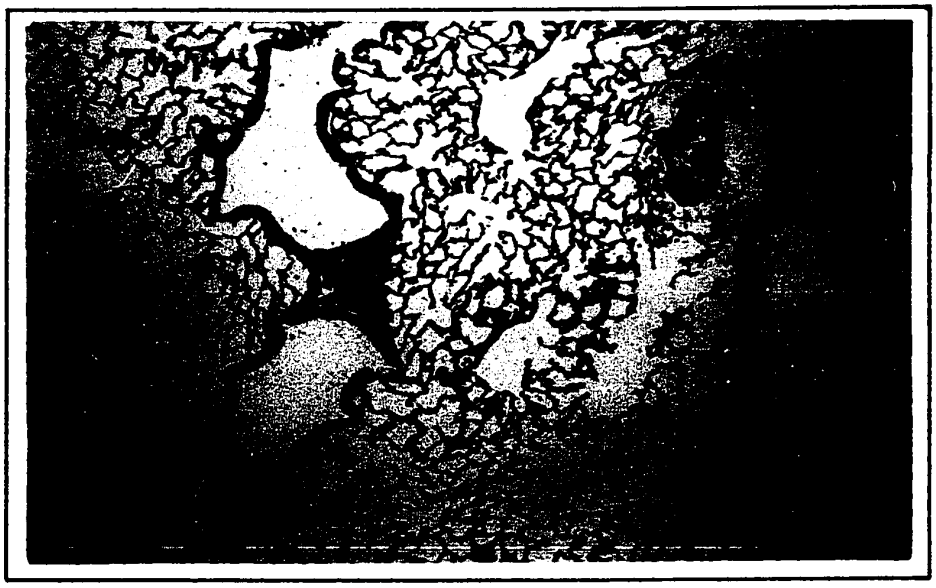

Fig. 3. Lower lobe of lung stored for 24 hours, after 30 minutes of perfusion (H & E x 40); perivascular and interstitial edema, congestion and hemorrhage.

Fig. 4. Upper lobe of lung stored for 24 hours but not perfused (H & E x 40); mild perivascular and interstitial edema.

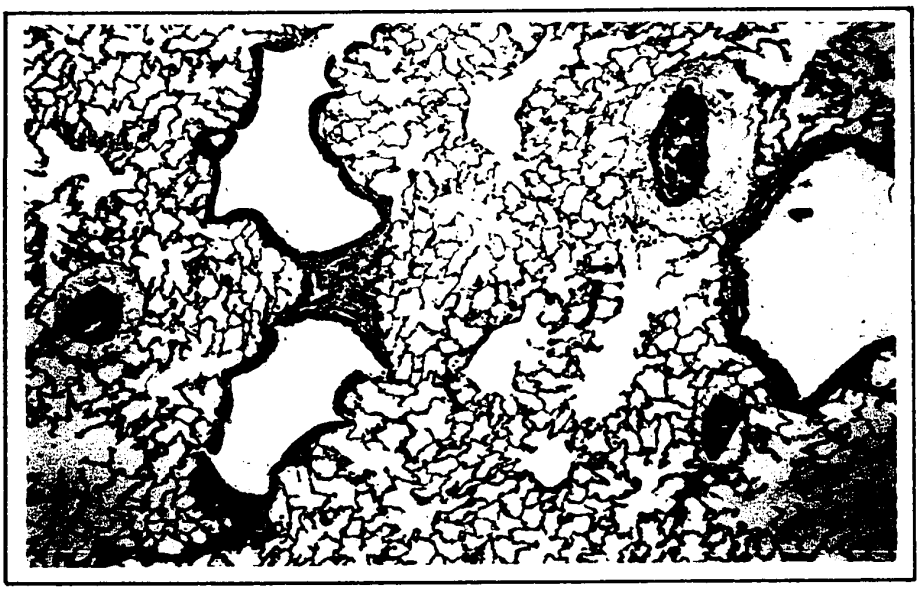

Fig. 3. Lower lobe of lung stored for 24 hours, after 30 minutes of perfusion (H & E x 40); perivascular and interstitial edema, congestion and hemorrhage.

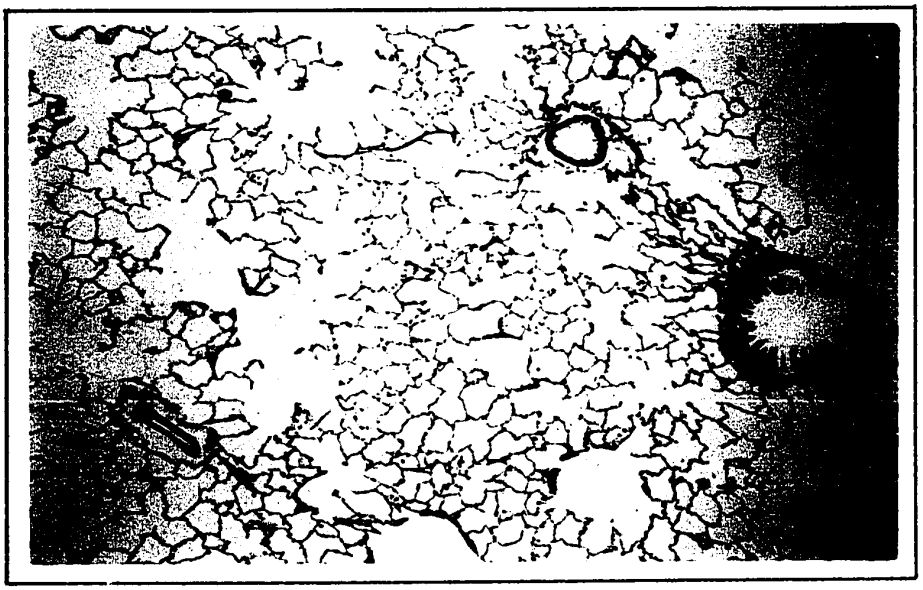

Fig. 4. Upper lobe of lung stored for 24 hours but not perfused (H & E x 40); mild perivascular and interstitial edema.

CHAPTER XI

DISCUSSION

"A perfusion experiment is no better than its weakest point. Perfusion adequate to maintain the organ in its normal physiologic activity is a matter of extreme difficulty and often great confusion is introduced by such methods which are intended to simplify the study of organ function."

Belt, Smith and Whipple, 1920.

The majority of previous isolated lung studies have been carried out using very unphysiologic preparations. Many investigators have attempted to elaborate pathophysiologic mechanisms by searching for factors which modify the severity of alteration in rapidly deteriorating perfused lungs. The author's project was based on the expectation that isolated lung studies would be facilitated if a more physiologic environment was provided for the isolated lung.

An attempt was made to simulate the normal dynamics of perfusion and ventilation and a supporting dog was used to better maintain "metabolic homeostasis." These measures resulted in longer perfusions and less functional and morphologic alteration than has been experienced by other investigators who have not used this approach. Normal function and morphology was not achieved however, and the perfusion durations were limited by deterioration of supporting dogs. Considerable variation in the results of comparable experiments was also encountered. For these reasons, the CONTROL experiments in this study were not ideal.

Surgical manipulation, denervation, temporary ischaemia, lack of collateral circulation, physical abnormalities in the organ environment and metabolic alterations in the perfusate likely contributed to the alterations which developed in all of the author's experiments. These variables are difficult to avoid in isolation-perfusion experiments. The relative roles of these factors in the pathogenesis of alterations which developed in the CONTROL experiments was not clearly apparent.

The aspiration pneumonitis study demonstrated that severe direct pulmonary insult could be studied in the isolated lung. Subsequent experiments were carried out with the object of prolonging, simplifying and hopefully, improving perfusion. Means for eliminating perfusion damage were not found. On the contrary, most methods which differed from those used in the CONTROL experiments resulted in more severe deterioration of isolated lungs. As a result, an ideal isolated lung model for investigating the pathophysiology of other forms of pulmonary derangement, has not yet been developed.

The alterations which occurred in the various groups of experiments did not contrast qualitatively with those in the CONTROL experiments. The role of the different perfusion methods in the quantitative differences in perfusion alterations which resulted cannot at this time be definitely concluded because of the indirect nature of the inferences, the small numbers of experiments in most groups and considerable variation among individual experiments within groups. The experiments which were carried out however provided observations which are useful in the discussion of factors which contribute to perfusion alterations and possibly other forms of acute pulmonary insufficiency.

Most previous investigators have focused their attention on hemorrhage and some have considered this the first sign of perfusion damage (Veith et al, 1967-2, 1968). The studies which have been carried out by the author, provided a wide range in degree of histologic abnormalities. When these are considered, hemorrhage does not appear to be the initial histologic abnormality in the perfused lung.

Perivascular edema was present in all histologic sections of the isolated lungs in these studies. In many sections from functionally better lungs, perivascular edema was the only histologic abnormality which developed. Perivascular hemorrhage never developed in the absence of perivascular edema. When hemorrhage was present, perivascular edema was also present in areas which were not involved by hemorrhage. In lungs which developed intraparenchymal hemorrhage, perivascular edema was more severe. These histopathologic observations indicate that perivascular edema is the first sign of injury to the isolated lung which is detectable by light microscopy.

The development of more severe morphologic alterations can be explained on the basis of progression of perivascular edema and continuing aggravation by the factors which initiated the perivascular fluid accummulation. Perivascular edema proceeds to more diffuse interstitial and eventually alveolar edema. The presence of perivascular edema would also contribute to further alteration in vascular integrity. Eventually formed elements will escape from the vasculature. In the presence of perivascular edema, hemorrhage would extend rapidly. This phenomenon was observed in several experiments (Chapter X,

Addendum 4) and is shown in Figure 1.

Structural characteristics of the lung predispose this organ to perivascular edema. The perivascular tissue includes an invagination of the mediastinal "space" into the lung parenchyma and as such, represents a potential space for fluid accummulation (Tocker and Langston, 1952).

A rich supply of lymphatics courses with the vessels and bronchi from the periphery to the hilum (Courtice, 1953). Fluid which would ordinarily be carried away by lymphatics likely accumulates in isolated perfused lungs. The author did not attempt to exclude lymphatics from arterial and bronchial ligatures in the experiments which have been presented. Lymphatic exclusion by others (West et al, 1965) and subsequently by ourselves, has not prevented edema formation. Some degree of obstruction will likely result regardless of the care which is exercised at the time of lung excision.

Pulmonary lymphatic flow rate is normally very low but pulmonary lymph contains a relatively high concentration of plasma protein (Cameron and Courtice, 1946). Simply ligating the pulmonary lymphatics does not produce edema (Paine et al, 1949; Foldi et al, 1955). Lymphatic obstruction in the presence of other aggravating factors however, contributes to the accummulation of extravascular fluid which has a high osmotic pressure. The role of altered lymphatic flow warrants future study. The clearance of dyes such as T-1824 (Mayerson, 1963) might be useful for such studies if satisfactory means cannot be devised for measuring lymphatic flow directly.

The pulmonary vessels have an inherent tendency to collapse. This results in a critical closing pressure which has been measured at about +7 mm Hg above alveolar pressure (West et al, 1965). Closure of the vessels is opposed by the "tethering" action of the lung interstitium. Surface forces at the alveolar air-liquid interface also exert a negative pressure on the lung interstitium (Bruderman et al, 1964; Pain and West, 1966). The forces on each side of the interstitium of the lung combine to produce an interstitial negative pressure within the lung of about -9 mm Hg (Levine et al, 1967; Meyer et al, 1968) and -7 to -13 mm Hg near the surface of the lung (Mellins et al, 1969).

A monomolecular layer of saturated lecithin normally lines the air-liquid surfaces of the pulmonary alveoli (Clements, 1968). The high degree of surface activity of this so-called surfactant, minimizes surface forces on the unstable alveolar walls. Deficiencies or disturbances in surfactant result in elevated alveolar surface tension and increased negative pressure in the interstitial tissue thereby promoting edema formation. Edema occurs rapidly in the absence of surfactant (Pegg et al, 1962). Several factors will contribute to altering surfactant in isolated perfused lungs.

The half-life of surfactant is normally just a few hours (Clements, 1968). Metabolic alterations will result in diminished replacement of surfactant. Glucose provides energy and precursors for the synthesis of surfactant (Heinemann and Fishman, 1969). Glucose is utilized by the canine lung at a rate of about 10 mg. per

minute per 100 grams of tissue (Lochner and Nasseri, 1956). Blood glucose was not measured in any of the studies which have been presented. Subsequent experiments demonstrated a rapid disappearance of glucose from the perfusate when a support dog was not used (Jirsch, 1970). When glucose was added, functional and morphologic alteration was attenuated in perfused lungs (Modry et al, 1971).

Many of the fatty acid components of the pnospholipids are synthesized by the normal lung from circulating plasma free fatty acids and triglycerides. Alterations in these precursors would affect surfactant production in the isolated perfused lung.

Progressive metabolic acidosis developed in several experiments which did not use a supporting dog in the perfusion circuit. The isolated perfused lung produces lactic acid (Hirsche et al, 1964; Levey and Gast, 1966). The normal lung utilizes lactic acid in the production of surfactant (Heinemann and Fishman, 1969). It is possible that the rate of lactate accummulation in lung perfusion circuits will reflect disturbance of phospholipid synthesis. Future study of this nature is warranted.

The surface activity of lung extracts is altered by some material in blood which has passed through an extracorporeal circuit (Tooley et al, 1961). Whether this is a direct toxic effect on surfactant or a result of alterations in lipid synthesis remains to be determined. Alterations in lipoproteins and plasma proteins result from blood trauma and exposure to abnormal surface forces in a mechanical circuit (Pierce II, 1967). Altered lipoproteins may interfere with lipid hydrolysis at the capillary endothelial membrane (Heinemann and Fishman, 1969).

Morphologic alterations in the Type II surfactant-producing alveolar cells have been associated with interstitial edema in lungs following cardiopulmonary bypass (Sakashita et al, 1968). Edema fluid interferes with the synthesis of surfactant and also results in the binding of existing surfactant with extravasated proteins and fibrin (Clements, 1968). Once the foregoing processes have begun, the stage is set for a viscious circle relationship between surfactant deterioration and edema formation.

The much more rapid edema formation in the lungs which were perfused without a support dog suggested that metabolic alterations and likely surfactant deterioration were important in the pathogenesis of the lesions which developed in those experiments (Chapter VIII). Although methods of assessing surfactant are at best empiric (Clements and Tierney, 1965) attempts should be made in future to correlate changes in surfactant with alterations in metabolic parameters, compliance and edema formation.

Edema will be contributed to by an increase in capillary permeability. Alterations in vascular integrity can result from metabolic and hematologic disturbances which occur during perfusion.

Histamine and serotonin are released from the blood and the lung during lung perfusion (Daly and Hebb, 1966) and accummulate in closed circuits (Jirsch,1967-1). When present in high concentrations, these materials increase capillary permeability (Miles and Wilhelm, 1960) and cause venoconstriction (Greene, 1965).

Electrolyte disturbance, hypoxia and acidosis can all interfere with cellular metabolism and therefore alter membrane stability. Calcium in normal amounts is necessary for endothelial integrity

and platelet adhesiveness (Zwiefack, 1961). When calcium is bound by EDTA, isolated lungs develop fulminant edema (Lunde et al, 1966). Citrate binding of calcium, may in part explain why stored blood has produced more severe pulmonary alterations when used as a perfusate by others (Awad et al, 1965-2; Veith et al, 1967-1). The minor "incompatibility" of stored, matched blood may be less important than electrolyte alterations in development of the so-called "homologous blood syndrome". Homologous perfusion per se has not been associated with more severe damage to the isolated lung in our experience (Chapter IX).

Heparin may contribute to increased capillary permeability. Heparin interferes with the normal adherence of fibrinogen, platelets and leukocytes to the vascular wall. Plasma protein leakage is increased by heparin (Zwiefack, 1961). As a result of interference with the coagulation mechanism, hemorrhage can occur more precipitously through a damaged vascular wall. This helps to explain the rapid development of hemorrhage in already edematous isolated lungs (Chapter X).

Circulating platelet numbers are diminished by mechanical blood trauma, hemolysis, accummulation of metabolites and by diffuse vascular damage. The lung appears to be an important site of platelet binding and release (Hague et al, 1966). Platelet aggregation is dependent on the presence of A.D.P. (Marcus, 1969). The relationship of platelets to energy transfer and maintenance of integrity of the pulmonary endothelium remains to be investigated further. Platelets were undoubtedly depleted from the perfusion circuits in the experiments which have been carried out and particularly in those which did not

utilize a support dog. Platelet studies unfortunately were not carried out in the author's experiments. Much useful information will derive from further studies of this nature.

Many other biochemical and hematologic factors influence vascular integrity. Glucocorticoids exert a stabilizing influence on cell membranes and ultrastructural organelles. Yamada and co-workers (1965) have shown that the administration of dexamethasone decreases the severity of morphologic and biochemical alterations in the perfused lung. They also achieved significant prolongation of their preparation by periodically exchanging their blood perfusate.

Several groups have observed that vasoactive responses of the lung to pharmacologic and hypoxic stimuli require the presence of plasma in the perfusate (Yamada et al, 1965; Waaler et al, 1966; Gorski and Lloyd, 1967). The specific nature and action of the plasma factors responsible for vasoactive integrity have not yet been clearly defined. These factors may also prove to be important in maintaining the normal capillary permeability. It may be useful to study changes in capillary filtration coefficient as described by Gaar and co-workers (1967-1).

Decreased osmotic pressure in a perfusate can contribute to edema formation. Hypoproteinemia decreases the critical capillary pressure for the development of pulmonary edema in proportion to the degree of protein deficiency (Guyton and Lindsay, 1959). Moderate hypoproteinemia does not alone result in extravasation of fluid from the pulmonary vasculature, but may contribute significantly in the presence of other predisposing abnormalities.

The osmotic pressure effect of erythrocytes has been largely overlooked. Perfusion of the lung with erythrocyte-free perfusate, which is otherwise "iso-osmolar" has been associated with fulminant edema formation (Lunde et al, 1966; Brownlee et al, 1968). Severe edema occurred rapidly in lungs which the author perfused with plasma (Addendum 4).

Tybjaerg-Hanson (1961) theorized that erythrocytes contribute to intravascular osmotic pressure at the microcirculatory level. This effect might be particularly important in the lung where interstitial pressure is negative. The lungs become edematous in clinical conditions associated with red cell damage (cardio-pulmonary bypass) and hemodilution associated with massive fluid infusion (hypovolemic shock). It is possible that a decrease in effective osmolarity of the erythrocytes contributes to pulmonary edema in these conditions. The relationship between alterations in hematocrit and the development of edema in the isolated lung would be a worthwhile future study.

Altered hemodynamics which produce elevated intravascular pressure in the lung is a primary cause of pulmonary edema (Greene, 1965). The otherwise normal canine lung can tolerate rather prolonged periods of left atrial pressures up to 25 mm Hg without the development of severe edema (Guyton and Lindsay, 1959; Rabin and Meyer, 1960; Gaar et al, 1967-2). The isolated perfused lung is likely much less tolerant of elevated venous pressures (Addendum 1).

Syphonage has been applied to the veins of isolated lungs but edema has worsened (Bryant et al, 1968). Excessive negative pressure

in the venous catheter may produce elevated venous pressures proximal to the point of forced venous collapse at the end of the catheter. Lowering venous pressure has not minimized edema (Addendum 1). Our experiences and those of others (West et al, 1965) indicate that the venous pressure should be positive to the point of producing optimal flow distribution with minimal arterial pressures.

Positive pressure ventilation has little effect on pulmonary vascular resistance in the closed chest. Increases in trans-pulmonary pressure increase venous and arterial pressures concomitantly. This method of respiratory assistance likely exerts its beneficial effect by diminishing venous return to the failing heart, increasing functional residual capacity and relieving the work of breathing. It has not been clearly shown that positive alveolar pressure opposes the hydrostatic forces which promote edema formation (Greene, 1965). Recent work has shown that positive pressure ventilation promotes fluid retention and pulmonary edema in the absence of cardiac failure (Sladen et al, 1968).

Positive pressure ventilation of the isolated lung has been associated with progressively increasing vascular resistance and fulminant edema and hemorrhage. In the exposed lung, positive airway pressures will elevate vascular resistance unless venous pressure is increased by the same magnitude as alveolar pressure. (Addendum 2). Elevated resistance and the diversion of flow to the dependent regions of the lung will contribute to excessive pre-capillary and dependent zone intravascular pressures and thereby edema and hemorrhage in those regions.

The theory of primary arteriolar spasm has arisen from studies which have employed very unphysiologic hemodynamics in isolated lungs (Veith et al, 1967-2). The author found no evidence of arteriolar spasm when the negative pressure-ventilated isolated lung was subjected to blood which circulated through an extracorporeal oxygenator (Chapter VIII). Furthermore, most lungs in the author's studies were repeatedly subjected to periods of vasospasm associated with alveolar hypoxia. One lung was subjected to intense vasospasm in response to a ventilation with nitrogen (Addendum 3). Blood volumes did not decrease significantly during periods of elevated resistance suggesting that much of the increase in resistance occurred at the pre-capillary level. Vascular resistance almost always returned to the pre-hypoxic level following resumption of room-air breathing. No significant change in the gross appearance of the lobes was noted during or immediately following the period of elevated resistance. The foregoing contradicts the theory that arteriolar constriction initiates damage to the isolated perfused lung.

Previous reports which describe the lesions in isolated perfused lungs emphasize that edema and hemorrhage are focused in the peribronchial and subpleural areas. Such was also the case in the author's study (Figure 2).

Bronchial and peribronchial structures (including pulmonary arteries and veins) and the pleura are nourished by the bronchial arteries (Daly and Hebb, 1966, pp. 48-50). Bronchopulmonary hemodynamics are grossly disturbed in isolated perfused lungs. The bronchial arteries are not perfused and bronchopulmonary communications are known to exist. All forms of inter-bronchial, inter-pulmonary

and broncho-pulmonary arterio-arterial, arterio-venous and veno-venous anastomoses are present in the human lung (Pump, 1963). In the canine lung, all but the broncho-pulmonary arterio-arterial anastomoses have been demonstrated (Daly and Hebb, 1966, pp. 52-88). As a result of the free communication between the two vascular systems, either can be injected from the other depending on their relative pressures.

Peri-hilar bleeding did not occur in our experiments. Intravascular pressure in the pulmonary circulation was likely transmitted to the bronchial system however, through normal channels of communication. The bronchial vessels are often congested in lungs which are perfused with blood (Yamada et al, 1965; Awad et al, 1967). In several of our experiments (Addendum IV) erythrocytes remained in the bronchial vessels following several hours of plasma perfusion (Figure 3). During isolation-perfusion, structures which are normally supplied by the bronchial vessels are likely relatively ischemic. Ischemia and pressure-stasis in the bronchial channels would further contribute to perivascular edema and hemorrhage in the isolated lung.

Perivascular edema and hemorrhage are also focused in the peri-bronchial and subpleural regions in the post-perfusion and shock lung (Veith et al, 1967-2; 1968-1). Respirator therapy, which is often introduced in these conditions will reduce bronchial flow further (Finley et al, 1960; Daly and Hebb, 1966, p. 76). Bronchopulmonary flow disturbance may contribute to the lesions which develop in these states. Investigators have recently emphasized the importance of bronchial flow to the integrity of transplanted lungs (Haglin, 1970). Bronchial perfusion may be important for preservation of the lung.

Indirect methods for the measurement of bronchial flow in situ have been developed (Deal et al, 1968; Awad et al, 1969). Methods for controlled perfusion of the bronchial circulation have been described (Berry et al, 1931; Daly and Hebb, 1966). In view of the possible role of bronchopulmonary flow disturbance in the pathogenesis of perfusion lesions and various forms of clinical pulmonary failure, studies of the bronchial circulation deserve increased attention.

Several physical and technical factors associated with the author's methods may have contributed to alterations which occurred and difficulties which were experienced in assessing functional and morphologic changes.

The dependent surfaces of the lungs were weight bearing. This interfered with ventilation of the dependent zones and may have produced mechanical trauma. Suspension of the lungs would minimize these problems. The lungs were not periodically rotated. During perfusion, the dependent regions were subjected to relatively greater intravascular pressure. Periodic rotation of the perfused lung would redistribute intravascular pressures and may help to decrease the degree of edema.

Periodic hyperinflation was carried out in attempt to restore normal surface forces (Clements, 1968), and expand temporarily atelectatic areas of lung. On occasion, the degree of alveolar expansion may have been excessive enough to increase capillary permeability (Greene, 1965) or increase negative interstitial pressure in regions which were poorly compliant (Greene, 1952). The negative pressure was evidently relatively excessive on the several occasions when fulminant edema formation suddenly accompanied a routine hyperinflation

(Chapter VIII; Addendum IV).

Attempt was made to maintain all components of the perfusion system at normothermia. Differences of several degrees in temperature between the alveoli, parenchyma and arterial and venous blood undoubtedly occurred. Temperature gradients across the membranes of the lung may have altered capillary integrity. These temperature differences also introduce problems in physiologic measurement. Application of more efficient heat exchange devices should be used to better control the temperature of inspiratory gases and the temperature of blood in the extracorporeal circuit.

Humidification of the chamber was maintained with water vapor. Drying of the surfaces of the lungs was prevented but the pleural surface may have absorbed water from the chamber environment.

In none of the lung perfusions were steps taken to assure a sterile circuit. In several longer experiments using similar circuitry (Fisk et al, 1968), cultures of the perfusate revealed contamination with coliform organisms (unpublished observations). Endotoxin may have been circulating in many of the lung perfusion experiments. This could have contributed to both venospasm (Greene, 1965) and increased capillary permeability (Zwiefach, 1961). Endotoxemia may also have contributed to hypotension in the supporting dogs. In subsequent studies, attempts should be made to assure sterility of the circulating perfusate.

Embolism of platelet, leukocyte, lipid and fibrin aggregates have been implicated in many forms of pulmonary dysfunction, particularly those associated with endotoxemia and sepsis (Collins, 1969) and in the damage resulting from extracorporeal circulation (Belzer

et al, 1968-1). The pathophysiologic effects of microembolism are not well understood. In the author's studies, embolic material was not observed. This phenomena may however, be missed on light microscopy (McKay et al, 1967). Blood filters should be employed if macro-aggregate embolism can be demonstrated by more careful study for this problem.

In future experiments morphologic studies must be improved. Histologic specimens must be obtained from identical representative areas of the lungs in different experiments. Histological changes must be more accurately quantitated. Staub and Storey (1962) described a method for freeze preparation of lungs for better correlation of morphologic and physiological events. This has been applied to the study of hemodynamic factors which contribute to perivascular edema (Hughes et al, 1968). Means for semi-quantitation of perivascular edema based on edema cuff to vessel lumen size have been described (Glazier et al, 1969). Direct visualization of the microcirculation should be considered (Wagner, 1969). Electron microscopy will be necessary to study the alterations which take place at the sub-cellular and membrane level of the pulmonary microcirculation.

In the author's experiments where support dogs were used, attention was focused on the isolated lungs. Alterations were also found in the in situ lungs when these were examined (Chapters IV, VI, IX). The cause for these alterations was not clear in the experiments which have been carried out to date. Abnormal pulmonary hemodynamics, systemic hypotension and humoral factors may all have played a role in damaging the in situ lungs. The sudden transient hypotension which

developed in the support dogs when blood was returned to them from damaged isolated lungs which had been stored (Chapter X), suggested that potent humoral agents were liberated from the isolated lungs. These materials may have played a role in damaging the in situ and ex vivo lungs. In future experiments greater attention to events in the support dog lungs may yield valuable information.

Closed circuit studies which eliminate the supporting dog are not as study models analogous to any of the clinical forms of acute pulmonary insufficiency. They do however, facilitate control and measurement of metabolic, hematologic and humoral factors which effect the isolated lung. Studies of this nature should continue in order to elaborate pathophysiologic mechanisms which may participate in damaging the lung in clinical disease.

The isolated lung was more tolerant of blood which circulated through a membrane oxygenator than blood in a bubble gas exchanger circuit. The totally mechanical circuit may be a useful empiric approach for assessing the hematologic and pulmonary effects of newer gas exchange devices as these become available. The foregoing should also apply to other aspects of extracorporeal circulation technology and the development of "biologic" materials for blood handling.

Means for satisfactorily preserving the lung for transplantation are not available at the present time. Functional integrity of the perfused lung is best preserved by attempting to simulate physiologic conditions in a perfusion circuit. Even then, the lung is not preserved for periods substantially longer than if the lung is not perfused at all (Stevens et al, 1968). Physiologic perfusion is technically difficult

and impractical for clinical organ storage. To date the lung has demonstrated increasing degrees of perfusion damage as the departures from physiologic conditions increase. At the moment, perfusion of the lung does not appear to hold much promise for clinical lung preservation. Alternative approaches to the problem such as whole organ freezing and the use of metabolic inhibitors should be considered in greater depth.

The isolated perfused lung should be useful for future studies related to preservation and transplantation. Ex vivo evaluation of the preserved lung should expedite the assessment of newer storage techniques. Factors which contribute to altered integrity of isolated perfused lungs may be responsible for limited initial function in the transplanted lung. Surgical manipulation, denervation, interruption of bronchial and lymphatic circulation and exposure to blood from an extracorporeal circuit are imposed on both the isolated lung and the lung graft. Further assessment of the role of these factors in the isolated lung may provide important information relating to altered function in the transplanted lung.

The experiments on aspiration pneumonitis in the isolated lung suggested that several aspects of the pathophysiology of other forms of severe direct pulmonary insult should be amenable to study in the isolated lung. The applicability of the isolated lung to the study of pulmonary air, lipid and thromboembolism remain to be explored.

Future attempts to prolong function and minimize alteration in the isolated lung should continue. This approach will improve on the lung study model and should also provide insight into the pathogenesis of pulmonary derangement in conditions which develop alterations

analogous to perfused lung lesions.

Since Le Gallois speculated in 1812 that life might be preserved in blood-perfused organs separated from the body, the imagination of scores of investigators has been stimulated by this surgical and technical challenge. Used initially as a model for fundamental investigation, the isolated lung has contributed to much of our current knowledge of normal pulmonary function.

The lung is characterized by a limited variety of reactions to injury. Alterations in isolated perfused lungs closely resemble the derangements which occur in a wide variety of clinical conditions. Attempts to eliminate perfusion lesions will provide an improved model for fundamental and pathologic investigation. Carefully selected and cautiously interpreted isolated lung studies should contribute considerably to the eventual solution of a wide range of pathophysiologic problems ranging from acute pulmonary insufficiency to lung preservation and transplantation.

Fig. 1. Extension of perivascular edema and hemorrhage along perivascular planes. (VER x 25).

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Fig. 2. Typical peribronchial focus of advanced "perfusion lesions" (H & E x 25).

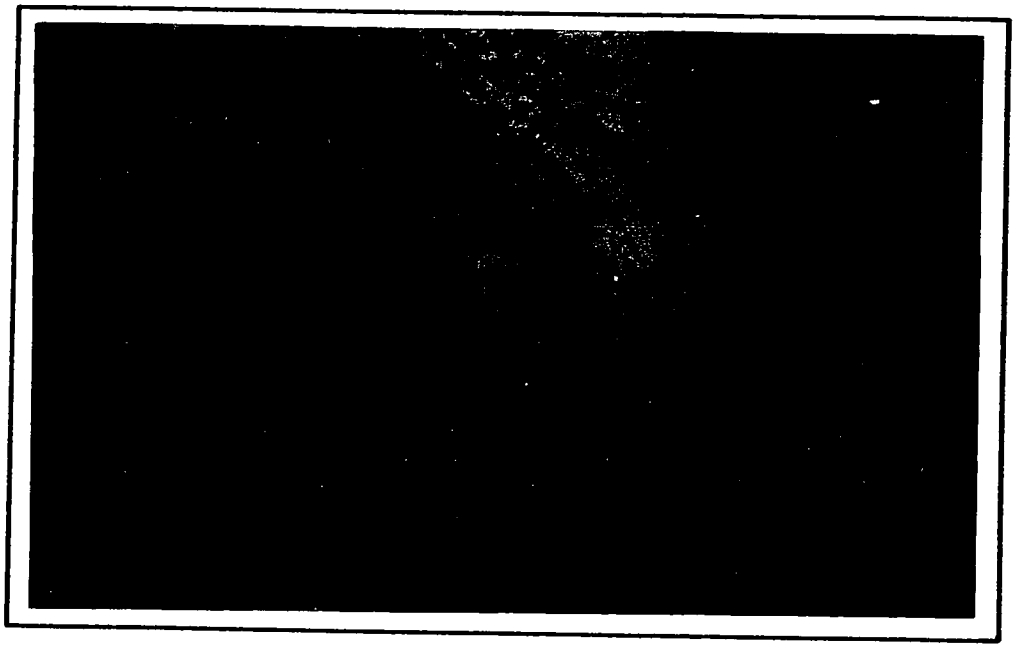
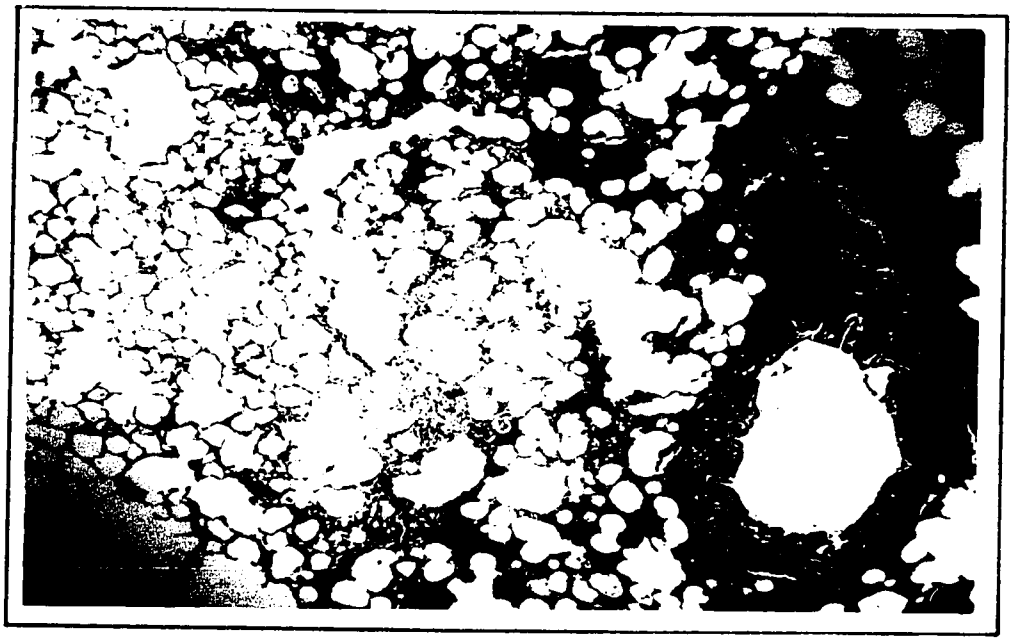


Fig. 3. Peribronchial interstitial edema in plasma-perfused lung; erythrocytes remain in bronchial vessel (VER x 100).



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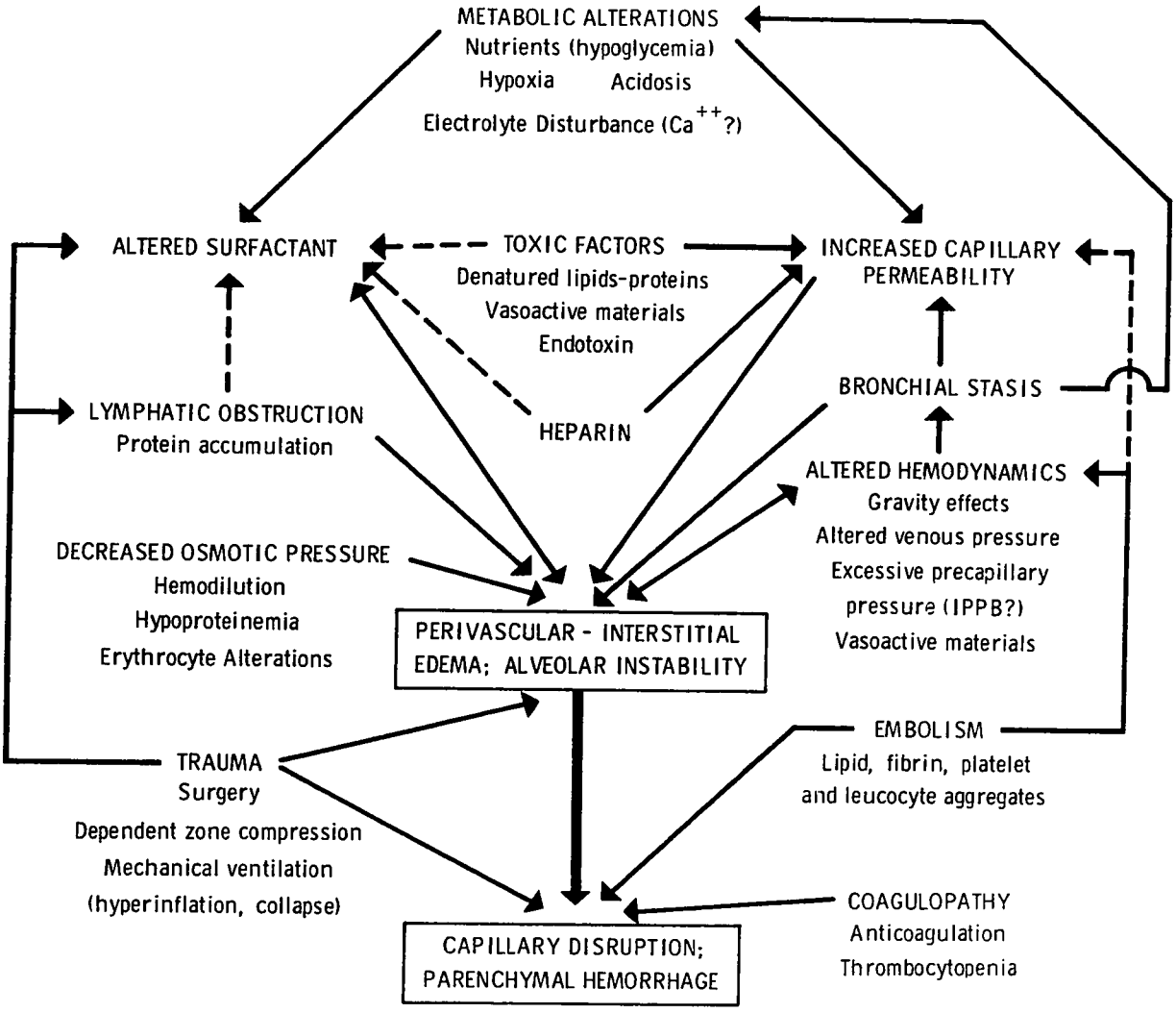

Fig. 4. Factors contributing to perfusion damage of isolated lungs.

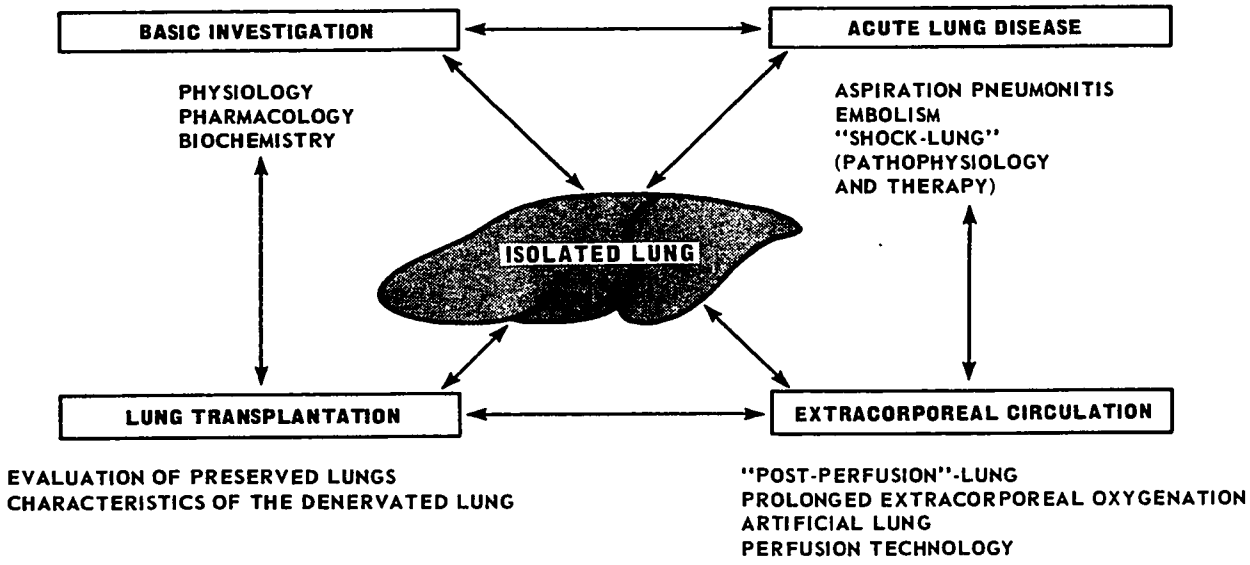

Fig. 5. The diverse applications of the isolated perfused lung.

CHAPTER XII

CONCLUSIONS

In an attempt to prolong the functional and morphologic integrity of isolated functioning lungs an effort was made to simulate physiologic hemodynamics, respiration and physical environment for the perfused organ. To accomplish this a special perfusion apparatus was devised and a support dog was used in order to regulate metabolic conditions in the blood perfusate. These measures resulted in continuous function of autoperfused left lower lobes for five to ten hours (Chapter IV and VI) and homologously perfused left lungs for up to sixteen hours (Chapter IX).

The perfusion apparatus was designed with emphasis on the automatic control of blood flow, arterial and venous pressures in the perfused organ and temperature and pressure in the organ environment. The apparatus facilitated the study of the perfused lung under control and various altered conditions.

Acute aspiration pneumonitis can be produced in the isolated functioning lung (Chapter V). The affected lung develops a marked increase in vascular resistance and concomittant decrease in compliance and gas exchange. The foregoing functional abnormalities undoubtedly contribute to systemic failure in this condition. In addition, the isolated lung studies demonstrated that absorption of acid and possibly other metabolic factors play a role in the pathogenesis of systemic

deterioration in severe aspiration pneumonitis. The model demonstrated potential for the future study of pathophysiology and treatment methods in this condition and perhaps other forms of acute direct pulmonary insult.

The autoperfusion of ten left lower lobes under conditions of negative pressure ventilation and positive venous pressure resulted in relatively stable hemodynamics, mechanics and gas exchange characteristics for an average of 6.25 hours (Chapter V). Absolute gas exchange values were not normal. These alterations appeared largely attributable to mechanical abnormalities which prevented physiologic ventilation-perfusion matching and to perivascular edema which was the most frequently observed histologic abnormality. The duration of perfusions was usually limited by progressive cardiorespiratory deterioration of the supporting dogs. In many of these, the in situ lungs developed more severe morphologic alteration than the ex vivo lung.

In four experiments, the supporting dog was intentionally subjected to hypovolemic hypotension (Chapter VII). Although few in number, these experiments did not incriminate the cardiorespiratory status of the supporting animal as a predominant factor in the pathogenesis of abnormalities which developed in experiments which used support dogs.

The substitution of a mechanical gas exchanger for the supporting dog results in early destruction of isolated perfused lungs (Chapter VIII). The rate of deterioration is less marked when a membrane gas exchanger is used rather than a bubble device. Severe damage developed despite the absence of a significant increase in vascular resistance in

the bubble device group and in the presence of a substantial decrease in vascular resistance when a membrane device was used.

At the present stage of our knowledge, reasonable preservation of the isolated perfused lung appears more related to the presence or not of a support dog in the perfusion circuit than to the general condition of the support dog. The support dog likely provides essential metabolic requirements and clears the blood of many undesirable materials which are liberated in an extracorporeal circuit. Substitution of mechanical gas exchangers removes the foregoing means of metabolic control and in addition, exaggerates the untoward effects of extracorporeal circulation of blood.

Within the time limits of support dog survival the isolated lung does not demonstrate abnormalities attributable to perfusion with homologous rather than autologous blood (Chapter IX).

Lungs which are stored for sixteen to twenty-four hours under conditions of hypothermia (4° to 8° C) and hyperbaric oxygen (two atmospheres) are very rapidly destroyed by ex vivo normothermic perfusion-ventilation (Chapter X). Recipients of unilateral allografts of lungs stored in an identical manner reportedly survive for up to one week. Adequately stored lungs should tolerate isolation-perfusion nearly as well as freshly excised lungs. Hypothermic-hyperbaric storage of the lung does not provide satisfactory functional preservation. Mere survival of recipients of unilateral stored lung grafts does not adequately reflect the efficacy of a preservation method. Ex vivo perfusion-evaluation should facilitate the assessment of future lung preservation methods.

In none of the author's experiments did primary arteriolar constriction appear to be responsible for the perfusion damage. Many lungs were repeatedly subjected to marked vasospasm in response to alveolar hypoxia and the elevated resistance appeared to be mainly arteriolar. The organs which demonstrated the greatest vasoconstrictor response were among the best preserved morphologically. The arteriolar constriction theory has arisen from isolated lung studies which have used positive pressure ventilation in association with low venous pressure and bubble gas exchangers in the perfusion circuit. The former hemodynamic determinants appear to result in progressive elevation in vascular resistance (Addendum II). The mechanical deoxygenator circuit produces severe perivascular edema and hemorrhage. The combination of grossly unphysiologic conditions in a perfusion method can lead to difficulty and error in interpretation of pathophysiologic mechanisms.

The provision of a physiologic environment for the perfused lung is essential for preservation of normal function and morphology. Although the author's attempts in this regard resulted in relatively prolonged periods of reasonable function, none of the lungs were normal morphologically. The lung is intolerant of perfusion to the degree that conditions of perfusion differ from physiologic conditions for the in situ lung. Even when all requirements of the lung are well understood, "physiologic" perfusion of the lung will undoubtedly remain technically complex and therefore impractical as a means for clinical lung preservation. Nevertheless, further attempts to "physiologically" perfuse the lung should continue in order to improve the isolated lung as a study model and advance knowledge regarding factors respon-

sible for the lung's reaction to injury.

The morphologic alterations which develop in isolated perfused lungs are characterized by the early development of peri-vascular edema which progresses to vascular disruption and rapidly extending intraparenchymal hemorrhage. Perfusion lesions focus in the peri-bronchial tissues. The etiology and pathogenesis of perfusion lesions are not yet well understood but clearly appear to be multi-factorial. Morphologic characteristics of the lung predispose this organ to the development of perivascular edema. The role of bronchial and lymphatic vessel disturbance requires specific study. Altered metabolism with emphasis on surfactant properties should receive special attention. Hematologic, pharmacologic and toxic alterations must be further investigated.

Perfusion lesions resemble the morphologic abnormalities which develop in many forms of acute pulmonary dysfunction. The conditions which develop similar morphologic alterations may share common etiologic factors. Future isolated lung studies will likely yield information of greater clinical value if investigation is directed toward the definition of common denominator factors in the pathogenesis of perfused lung lesions.

A D D E N D U M

ADDENDUM I

THE EFFECTS OF ALTERED VENOUS PRESSURE ON THE ISOLATED PERFUSED LUNG: PILOT STUDIES

When the lung perfusion method was first implemented, the maintenance of positive venous pressure seemed important for several reasons. The majority of previous lung experiments had been carried out using gravity venous drainage. These experiments were limited by the development of severe edema. Edema was worsened by the application of syphonage to the venous outflow (Wesolowski et al, 1952; Bryant et al, 1968). Positive venous pressures have improved the perfusion of kidneys (De Falco et al, 1965; Humphries, 1967) and the liver (Van Wyk et al, 1966). Positive venous pressure decreases the degree of "vascular collapse" in the lung (Permutt et al, 1962; West et al, 1964) and improves blood flow distribution (Lawson et al, 1964).

In early experiments, venous pressure was maintained at a mean of +7 mm Hg. The lungs appeared congested but did not develop frank edema. The CONTROL series was carried out using a mean venous pressure of +3 mm Hg. The lungs appeared normal in color and maintained satisfactory function for over five hours. In order to assess the effect on hemodynamics and function of variations in venous pressure, the following pilot experiments were carried out.

METHODS

Five left lower lobes from 19 to 24 kilogram dogs were autologously perfused under conditions which were identical to those in the

CONTROL group except for venous pressure variations. In two experiments, venous pressure was increased after the second hour from +3 mm Hg to +7 mm Hg at which it was maintained for three hours. Venous pressure was then returned to +3 mm Hg for the final one hour of perfusion. In two experiments, the venous pressure was reduced during the third to fifth hour from +3 mm Hg to -7 mm Hg.

In the fifth experiment, venous pressure was maintained at 0 mm Hg for the first two hours following which it was increased to +6 mm Hg for one hour. The pressure was then decreased to -6 mm Hg for one hour. This experiment was completed by a final hour of perfusion with a venous pressure of 0 mm Hg.

RESULTS

General

The first four experiments were terminated after six hours of study. The data from the first two experiments (elevated venous pressure) were averaged and compared with the average data for the second two experiments (low venous pressure).

Many of the indices of function for both pairs of lobes remained quantitatively within the range of values which were observed in the CONTROL lobes wherein venous pressure had been maintained at +3 mm Hg throughout. The direction of change in many of these values during perfusion was different.

Hemodynamics (Figure 1)

Vascular resistance remained near the control value when the venous pressure was elevated from +3 to + 7 mm Hg. In contrast, the

vascular resistance increased progressively to 250 percent of the initial value under conditions of low venous pressure.

Blood volume remained between 95 and 105 percent of the initial value when the venous pressure was +3 mm Hg or higher. When venous pressure was lowered, blood volume steadily decreased. The decrease in blood volume was more exaggerated when the venous pressure was decreased from +6 mm Hg to -6 mm Hg in the fifth experiment. When venous pressure was increased, blood volume and vascular resistance were quite stable. When venous pressure was lowered to produce syphonage, blood volume decreased and vascular resistance increased.

Mechanics (Figure 1)

Compliance tended to decrease in all experiments, but the rate of change was more marked when venous pressure was lowered. Dead space - tidal volume ratio gradually increased in all experiments and there was no appreciable difference between the two groups.

Gas Exchange (Figure 2)

The gas exchange indices in the first pair of lobes were comparable to the CONTROL lobes with the exception of the values under conditions of low oxygen breathing. Elevation of venous pressure resulted in an increase in the $\dot{Q}s/\dot{Q}^{14}$ and $[A\text{-}a]O_2{}^{14}$. When abnormally low venous pressures were maintained, all gas exchange indices deteriorated. The $[A\text{-}a]O_2{}^{14}$ appeared to be more severely affected under conditions of low venous pressure than high venous pressure. Unfortunately, gas exchange data were not obtained for the second pair of

experiments after venous pressure was altered from -7 mm Hg to + 3 mm Hg.

Morphology

Weight gain of the lobes in this series averaged 26 percent and ranged from 23 percent to 29 percent.

The first two lobes appeared grossly congested during the period of elevated venous pressure. Little congestion remained when venous pressure was returned to +3 mm Hg. The second two lobes demonstrated patchy pallor during the second period of low venous pressure. The areas of pallor improved, but did not resolve completely when venous pressure was raised for the final hour (Figure 3). Areas of plethora and then patchy pallor were observed in the fifth lobe when venous pressure was changed from positive to negative levels respectively.

All of the lobes in this study demonstrated more histologic alteration than was seen in the CONTROL experiments. Perivascular and interstitial edema were present in all lobes. Interstitial edema was more noticeable in the pale (low venous pressure) lobes.

In the fifth lobe, sanguinous edema poured from the airway at the end of the experiment. More severe perivascular and interstitial edema and hemorrhage developed than in any of the preceding four lobes.

DISCUSSION

Mocerate elevation in venous pressure appeared to result in less disturbance to function than abnormally low venous pressure.

Under both conditions, the lobes did not function as well during the period of hemodynamic alteration as the CONTROL group. The hemodynamic and mechanical changes which were associated with changes in venous pressure began to reverse after the restoration of venous pressure to +3 mm Hg.

The magnitude of venous pressure change when it was elevated above 3 mm Hg (+4 mm Hg) was less than when pressure was reduced (-10 mm Hg). At +7 mm Hg, the venous pressure was hydrostatically about 2 - 3 cm H_2O above the level of the highest part of the lung from the venous and arterial cannulae. -7 mm Hg was 2 - 3 cm H_2O below the most dependent area. For purposes of comparing function before and after pressure changes, an initial level of 0 mm Hg might have been more desirable. Because the CONTROL group of experiments were perfused at venous pressures of +3 mm Hg, this level was chosen as the base line in order to compare these lobes with that group.

Elevated venous pressure increases the degree of perivascular edema in the dependent zone of the lung (West et al, 1965). This could contribute to an increase in vascular resistance in these areas. Elevated venous pressure and perivascular edema in the dependent zone will distribute flow to the higher levels of the vasculature and increase the cross-sectional area of the pulmonary vascular bed (Lawson et al, 1964). The increase in blood volume reflects passive distention of the vasculature and an increase in cross-sectional area. The net effect was little increase in the resistance. The subsequent lowering of venous pressure could reverse these effects. The perivascular edema which formed during elevated venous pressure may have resolved. This is known to occur when

venous pressure is subsequently lowered (West et al, 1965). The lobes tolerated venous pressure elevation quite well.

The mechanism of the more marked increase in vascular resistance under conditions of excessively low venous pressure is likely related to the effect of venous pressure on flow distribution. When venous pressure is very low, proportionately greater flow passes through more dependent zones of the lung concomittant with collapse of the vessels in the higher areas. Perivascular edema in the dependent regions and a decrease in vascular cross-sectional area then both contribute to an increase in vascular resistance.

Several observations suggest that the distribution of vascular resistance is shifted in a downstream direction under conditions of excessively low venous pressure. Compliance was less affected in the lobes which were subjected to elevated venous pressure than in those which were subjected to low venous pressure. Interstitial edema was more severe in the latter. Perivascular edema was no more severe in this group than in the first lobes, but resistance progressively increased. The restoration of initial venous pressures did not bring about improvement in compliance, nor appreciable improvement in resistance, despite a reasonable improvement in blood volume.

In the experience of others, alveolar edema has worsened when venous syphonage has been increased (Bryant et al, 1968).

The morphologic abnormalities which developed in the fifth lobe indicated that the isolated lung is intolerant to even brief periods of major alteration in venous pressure. The other data cannot be directly compared to the first four lobes because the venous pressure in this lobe was 0 mm Hg before and after the venous pressure alterations.

The "ideal" venous pressure for the isolated lobe was not defined by these experiments. Previous experiments suggested, but did not confirm, that moderately positive venous pressure contributed to an improvement in hemodynamics and function. The pulmonary vasculature is abnormally suffused when pressures of +7 mm Hg are used. Pulmonary function and morphology irreversibly deteriorates and the lung is abnormally pale and unevenly perfused under conditions of abnormally low venous pressure. +3 mm Hg appears to be a practical and reasonably physiologic level at which to maintain venous pressure in this isolated lung preparation.

SUMMARY

1. Five left lower canine lobes were autologously perfused for periods of five to six hours during which the effects of varying the mean venous pressure were studied.
2. The isolated perfused lung is sensitive to venous pressure alterations.
3. In these few experiments, undesirable alterations in function and morphology were produced by raising venous pressure from +3 mm Hg to +7 mm Hg. More severe alteration resulted from the lowering of venous pressure from +3 mm Hg to -7 mm Hg.
4. Attempts to simulate physiologic venous pressures appears to be important to the maintenance of function and morphologic integrity in the isolated perfused lung.
5. The use of grossly unphysiologic venous pressure may contribute to perfusion damage which others have encountered. Additional studies would be necessary, however, to confirm this impression.

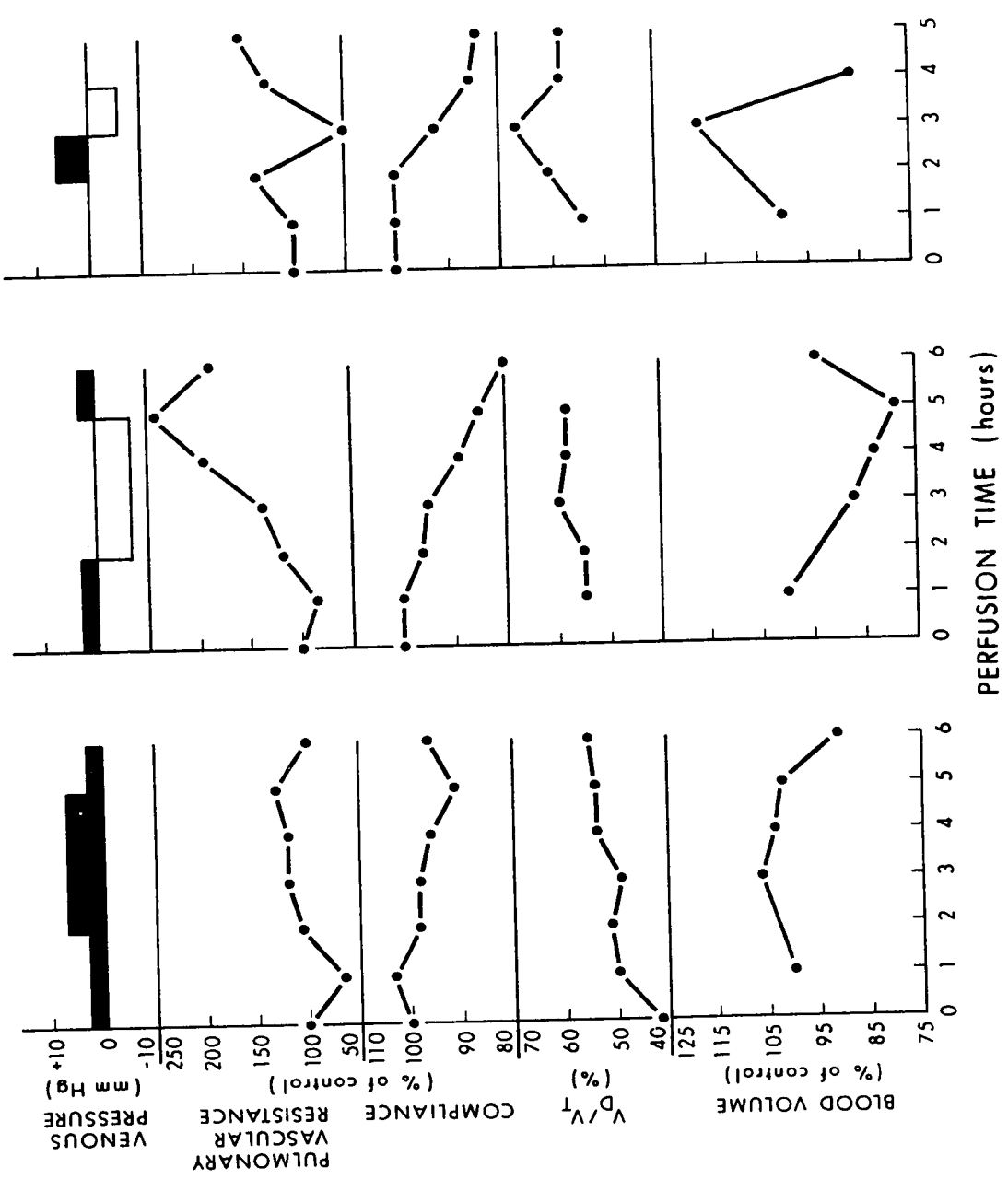

Fig. 1. Effects on hemodynamics and mechanics of altered mean venous pressure in 5 lobes.

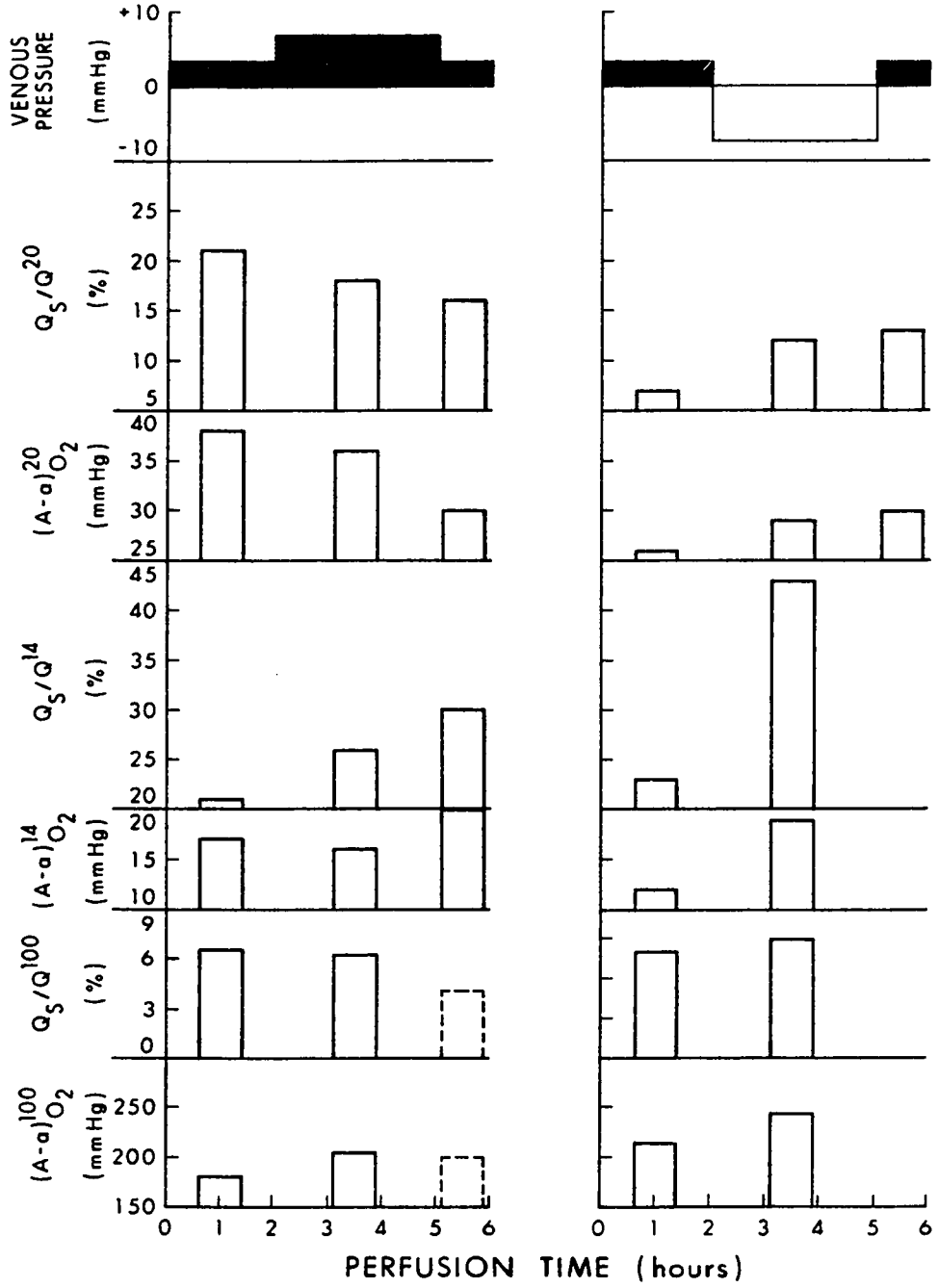

Fig. 2. Effects on gas exchange of altered mean venous pressure in 4 lobes.

Fig. 3. Lobe when venous pressure - 7 mm Hg.

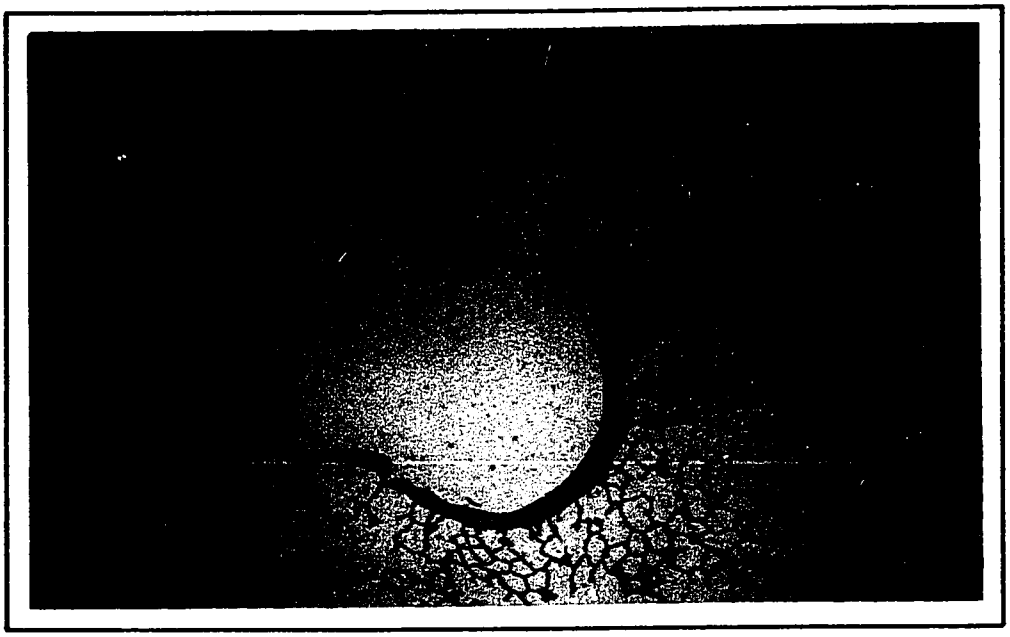

Fig. 4. Lobe subjected to elevated and lowered venous pressure (H & E x 40); peribronchial hemorrhage, interalveolar thickening.

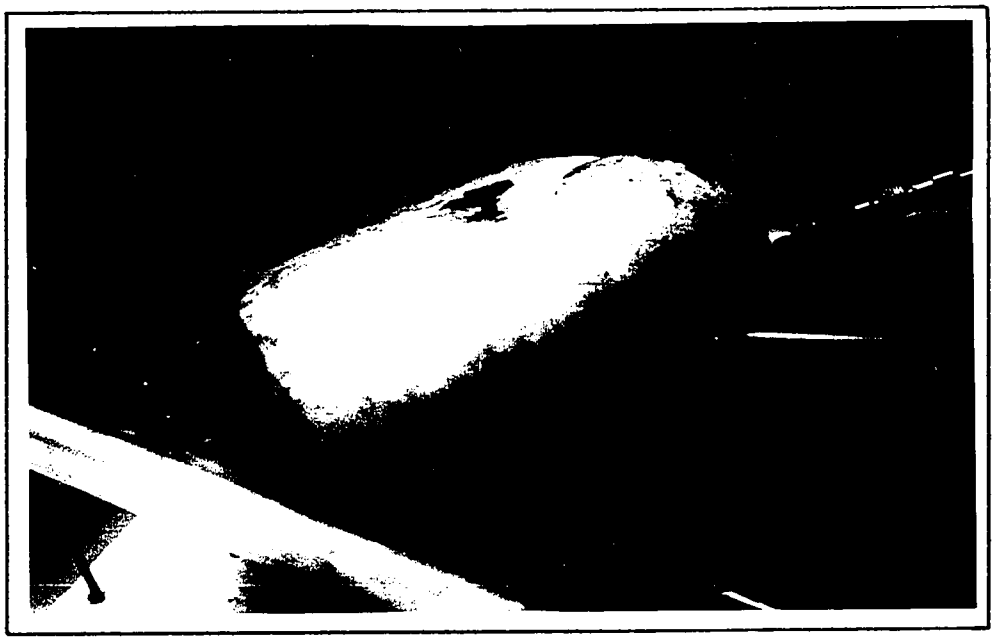

Fig. 3. Lobe when venous pressure - 7 mm Hg.

Fig. 4. Lobe subjected to elevated and lowered venous pressure (H & E x 40); peribronchial hemorrhage, interalveolar thickening.

ADDENDUM II

THE EFFECTS OF POSITIVE PRESSURE VENTILATION ON THE ISOLATED PERFUSED LUNG: PILOT STUDIES

In the majority of experiments which have been carried out by other investigators, positive pressure has been used to ventilate the isolated lung. Several investigators have considered that positive pressure ventilation is essential to minimize edema in the isolated lungs (Nisell, 1949; Bryant et al, 1968, Clarke, 1969). The advantage of ventilating isolated lungs with positive pressure has never been specifically investigated.

When the perfusion method was implemented, negative pressure ventilation was considered to be essential for the simulation of normal pulmonary hemodynamics and function. In order to investigate the effect of positive pressure ventilation on the isolated lung, the following experiments were undertaken.

METHODS

Left lower lobes from three 19 to 25 kilogram dogs were autologously perfused for five to six hours. A three-hour period of positive pressure ventilation was introduced during perfusion.

Following a two-hour control period, the respirator pump was connected to the bronchial cannula in order to produce tidal volumes comparable to those in the preceding negative pressure ventilation period. The lobes were allowed to collapse in expiration. Respirator stroke volume and rate were maintained unchanged for the three-hour

period of positive pressure ventilation. Endobronchial pressures were observed on a water manometer which was connected to the bronchial cannula. The venous reservoir was lowered to produce a pressure of 0 mm Hg in the lobe veins. Following three hours of positive pressure ventilation, negative pressure ventilation was restored for a final one hour of perfusion.

RESULTS

Figure 1 presents a summary of the observations which were made in this group of experiments.

During the first two hours of perfusion, all indices of function were comparable to the CONTROL group of experiments. When positive pressure ventilation was imposed on the lobes, the vascular resistance increased 45 percent during the first hour and reached 160 percent of the initial value by five hours.

Compliance decreased by 40 percent and remained at that level throughout the period of positive pressure ventilation. In order to produce comparable degrees of lobe inflation under conditions of positive pressure ventilation, endobronchial pressures of +16 to +20 cm H_2O were required. All three lobes appeared to deflate much more rapidly and inflation became quite irregular by the end of the positive pressure ventilation period.

Dead space - tidal volume ratio decreased very slightly (58 to 56%) and was quite stable. The blood volume decreased 24 to 34 percent. Most indices returned toward the control values following positive pressure ventilation. Compliance remained less and $\dot{V}_D/\dot{V}_T$ slightly higher

than during the initial period.

During the period of positive pressure ventilation, the gas exchange indices demonstrated a mild but insignificant improvement. When negative pressure ventilation was restored, these values were comparable to those which were obtained during the early period of negative pressure ventilation.

The lobes in this group gained from 19 to 25 percent, averaging 23 percent. None of the lobes demonstrated frank endobronchial edema or hemorrhage. All developed several areas of sub-pleural ecchymosis during the period of positive pressure ventilation. During this period, patchy areas of pallor were evident on the pleural surface. These changes were similar to those in previous lobes which had been subjected to negative venous pressures. These areas did not resolve following the resumption of "physiologic" ventilation pressures (Figure 2).

Histologically, all lobes demonstrated alveolar irregularity and mild to moderate degrees of perivascular edema and interstitial edema. Two lobes developed perivascular hemorrhage. Mild vascular congestion was seen in one lobe.

DISCUSSION

These pilot experiments were few in number. The introduction of constant volume positive pressure ventilation, alteration in venous pressure and allowing the lobes to collapse at end-expiration all represent conditions which were different from those in the CONTROL series. A direct comparison with previous experiments therefore cannot be made. Several observations seem worthy of comment.

The altered conditions in these experiments produced an immediate and progressive increase in vascular resistance. This change was also observed in a subsequent series of well controlled experiments which were carried out by Jirsch (1970-1). Under the conditions of these experiments, the resistance did not increase so rapidly as others have observed (Veith, 1967-1; 1968-2; Thelmo, 1970), but resistance increased considerably more than in previous negative pressure ventilated lobes. The initial increase in resistance appeared to result from pressure effects on the vascular cross-sectional area as reflected by the immediate decrease in blood volume.

The blood volume did not continue to decrease during the entire period of positive pressure ventilation commensurate with continuing increase in the vascular resistance. This suggested that vascular resistance was progressively increasing in upstream vessels. Positive alveolar pressure should exert most of its effect on the alveolar capillaries. The pallor which was observed during the period of positive pressure ventilation supports this assumption, as do the hemodynamic studies which have been carried out by others (Macdonald and Butler, 1967; Lopez-Muniz et al, 1968).

Positive alveolar pressure promotes alveolar capillary "collapse" unless venous pressure is increased to the same degree. When constant flow rate is imposed, arterial pressure increases. Elevated upstream pressure would promote periarteriolar and interstitial edema. These abnormalities developed to a greater degree than in the average CONTROL lobe. Jirsch (1970-1) observed progressively increasing vascular resistance during positive pressure ventilation when end-expiratory volume was preserved. The foregoing is forwarded as partial explanation for

the prevalence of arteriolar disruption which has often been associated with positive pressure ventilation of the isolated lung (Veith, 1966, 1967-2).

Compliance of the lobes was less under conditions of positive pressure ventilation. Part of this was likely related to a greater pressures required to overcome surface forces which developed as a result of end-expiratory collapse. Irregular inflation was grossly apparent during the final period of positive pressure ventilation. Compliance appeared fairly stable during the period of positive pressure ventilation, but was less at the end of these experiments than it was in the CONTROL lobes.

The satisfactory gas exchange indices during the period of positive pressure ventilation likely resulted from the use of fixed-volume ventilation and the relatively small effect that areas of high $\dot{V}_A/\dot{Q}$ have on venous shunting (West and Jones, 1965). Positive pressure ventilation exaggerates the effects of hydrostatic pressure differences on blood flow distribution (Bergman, 1963). This effect was likely minimized in these experiments by the horizontal posture of the lobes during perfusion.

Positive pressure ventilation increases the dead space (Nash et al, 1967). A significant proportion of the tidal volume was used to restore the functional residual capacity at the beginning of each inspiration. This may explain why $\dot{V}_D/\dot{V}_T$ did not increase during the period of positive pressure ventilation in these experiments.

The elevated vascular resistance, diminished compliance, and the more severe morphologic alterations which were observed in these

experiments, were undesirable. On the basis of these pilot experiments, it appeared important to ventilate the isolated lung with negative pressure, maintain a positive venous pressure and preserve end-expiratory volume. The relative importance of these individual conditions requires additional study.

SUMMARY

1. Three isolated lobes were autologously perfused and studied under conditions of positive pressure ventilation, 0 mm Hg venous pressure and end-expiratory collapse.
2. Negative pressure ventilation is not only more "physiologic" but has indirectly appeared to contribute to the preservation of functional and morphologic integrity in isolated lobes which have been maintained under these conditions.
3. The possible contribution of positive pressure ventilation to precapillary disruption which many investigators have observed, requires further study.

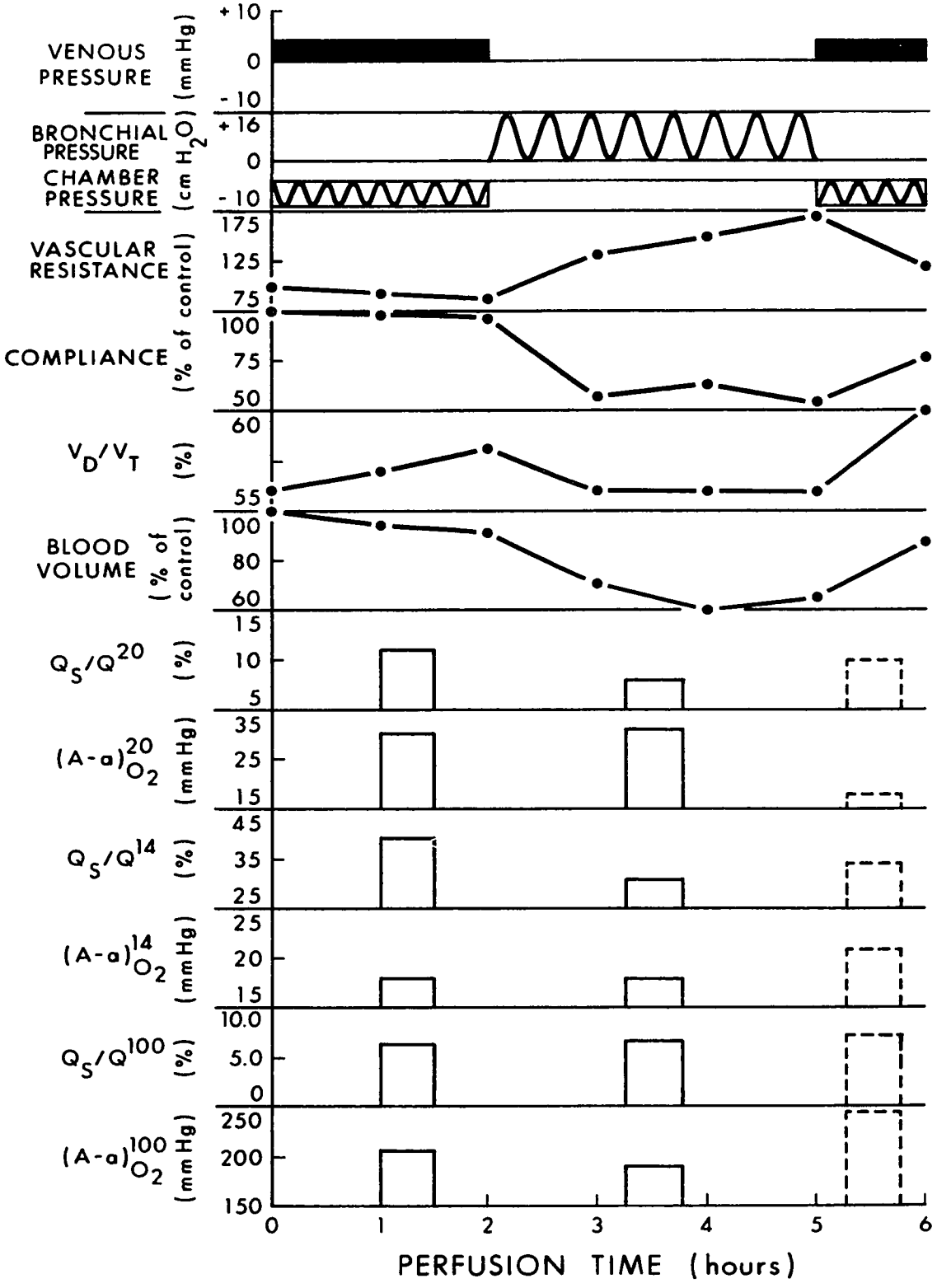

Fig. 1. Effects on hemodynamics, mechanics and gas exchange of 3 hours of positive pressure ventilation in 3 lobes.

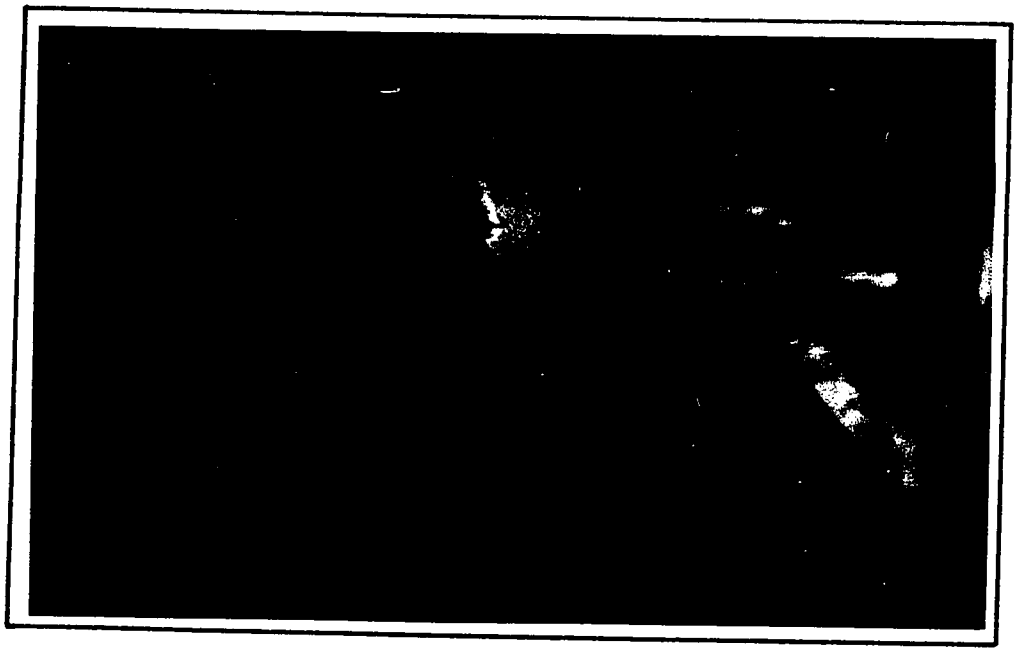

Fig. 2. Lobe after 3 hours of positive pressure ventilation.

Fig. 3. Lobe after 3 hours of positive pressure ventilation (H & E x 40); peribronchial-perivascular edema and hemorrhage, alveolar irregularity.

Fig. 2. Lobe after 3 hours of positive pressure ventilation.

Fig. 3. Lobe after 3 hours of positive pressure ventilation (H & E x 40); peribronchial-perivascular edema and hemorrhage, alveolar irregularity.

ADDENDUM III

LUNG PRESERVATION PILOT STUDIES

Belzer (1967) and Humphries (1968) and their co-workers have developed methods which adequately preserve kidneys for 72 hours. These investigators have used hypothermia to 4 to 8° C (to reduce metabolic requirements of the organ), pulsatile arterial flow and membrane oxygenation of acellular perfusate in order to minimize progressive perfusate deterioration.

Humphries used pooled plasma or diluted blood (1968). Belzer (1967) discovered that ultrafiltration of cryoprecipitated plasma removes plasma proteins which damage the vasculature of the perfused kidney. Plasma which has been treated in this way apparently retains essential nutrients and adequate plasma osmotic pressure. Belzer's method has been the more satisfactory of the two.

It appeared essential to evaluate the applicability of Belzer's techniques to lung preservation. Pilot experiments were first carried out in order to assess modifications of the lung perfusion methods which had been used to this point.

Belzer has emphasized the importance of pulsatile arterial flow to the maintenance of vascular integrity in the perfused kidney. The importance of pulsatile perfusion of the isolated lung has been disputed. Clarke (1969) reported that the isolated lung functioned no better when pulsatile flow was used rather than isogravimetric flow. Maloney and co-workers (1968) showed however, that better distribution of flow resulted when pulsatile perfusion was used.

One isolated lobe was perfused using totally depulsated ("isogravimetric") arterial flow. The results of this experiment were compared with those of another lobe perfusion in which an artificial ventricle produced pulsatile flow.

If the lung served as its own oxygenator, perfusion would be simplified and perfusate deterioration would be minimized. In order to investigate this possibility, one experiment was carried out to determine the effect of bypassing the support dog by "recirculating" blood through the isolated lung.

If the lung was going to be ventilated and perfused at low temperatures, information was needed regarding compliance and vascular resistance under these conditions. The fourth experiment in this group was carried out to investigate these factors.

In the fifth experiment, an attempt was made to determine the composition of inspired gas which would provide optimal hemodynamics and compliance of the lung in a "recirculation" circuit.

METHODS

Isogravimetric Versus Pulsatile Arterial Perfusion

In two experiments, left lower lobes were autologously perfused as they had been in the CONTROL series. After the first hour of perfusion, arterial pressure characteristics were altered. In the first experiment, blood was pumped through a reservoir which was located above the arterial cannula. The height of the bag was adjusted to provide a constant arterial flow rate of 20 millilitres per donor kilogram per minute. After the first hour in the second experiment, arterial flow was

controlled by an air driven, sac-type ventricular pump.* The ventricular contraction rate was 90 per minute and systolic and diastolic durations were set at 0.2 and 0.46 seconds respectively.

After four hours of altered arterial pressures in both experiments, roller pump perfusion was resumed for the sixth hour of perfusion.

RESULTS

(Figures 1 and 2)

During isogravimetric perfusion, vascular resistance did not increase. Resistance increased slightly in the lobe which was subjected to pulsatile perfusion. Blood volume decreased approximately 20 percent in both lobes, but the decrease in volume occurred earlier in the first lobe.

Compliance decreased to 68 percent of control during isogravimetric perfusion and to 78 percent during pulsatile perfusion. At the end of both experiments, the compliance was nearly normal. Dead space - tidal volume ratio did not change appreciably in either experiment.

The venous shunt and oxygen gradients were quite stable during the six hours of perfusion. $\dot{Q}s/\dot{Q}^{20}$ increased slightly following the initiation of pulsatile perfusion in the second experiment.

The first lobe appeared grossly normal and gained 11 percent in weight. Histologically, the lobe demonstrated mild perivascular and interstitial edema. The second lobe demonstrated moderate vascular congestion and several subpleural ecchymoses (Figure 3). The lobe gained

*Knight experimental pneumatic-driven sac-type ventricular pump with infant ventricle (Courtesy Mr. D. Wilson). Cardiovascular Specialties Ltd., Scarborough, Ontario.

52 percent in weight. The cut surface of the lobe demonstrated cuffs of hemorrhage around most of the grossly visible vessels. Marked perivascular edema and hemorrhage and interstitial edema were seen histologically (Figure 4).

Whole Blood Recirculation

Two lobes were autologously perfused for a one-hour control period. For three hours thereafter, the support dog was bypassed using a perfusion circuit which is shown in Figure 5.

In the first experiment, the chamber temperature was maintained between 38 and 40° C. During the period of recirculation in the second experiment, the temperature of the chamber was gradually reduced from 39° C to 24° C. After the second hour of hypothermia, the chamber was re-warmed to 38° C.

During the period of hypothermia, the blood flow rate was gradually reduced to maintain arterial pressure at the initial value. Both lobes were breathing 5 percent CO_2 in 95 percent room air during the recirculation period.

At the end of the fourth hour of perfusion, the "autologous" circuit was restored for a final hour. Gas exchange data was not obtained in the normothermic experiment.

RESULTS

(Figure 6)

During normothermic recirculation, vascular resistance increased progressively but not significantly more than in the initial CONTROL

lobes. Blood volume and compliance were well preserved. The lobe appeared grossly normal after five hours, but had gained 20 percent in weight. Perivascular and interstitial edema and hemorrhage were present on the histologic sections.

In the second lobe, hypothermia resulted in a considerable increase in vascular resistance. At 24° C the flow was reduced 40 percent in order to maintain arterial pressure at the pre-hypothermia level. Blood volume was 84 percent of the control level at 24° C and decreased to 72 percent during the period of re-warming. Compliance was 66 percent of the initial value at 24° C, but increased to 88 percent after re-warming.

Dead space - tidal volume ratio increased slightly but pre and post-hypothermia venous shunts on room air were comparable. $\dot{Q}s/\dot{Q}^{14}$ was high initially and rose to 40 percent following hypothermia. $\dot{Q}s/\dot{Q}^{100}$ was essentially unchanged.

The lobe gained 17 percent in weight and its gross appearance was well preserved. Mild perivascular and interstitial edema were noted histologically.

Effects of Inspired Gas Composition

One left lower lobe was autologously perfused for three hours, following which recirculation was carried out for two hours as in the preceding two experiments. During the recirculation period, oxygen, nitrogen and carbon dioxide were altered in the inspired gas mixture. Fifteen minutes was allowed for alveolar gas to equilibrate with each inspired gas mixture. pO_2, pCO_2, and pH were not measured in the perfusate.

RESULTS

(Figure 7)

When recirculation began, CO_2 was eliminated from the perfusate. This was accompanied by a decrease in both resistance and compliance. When 5 percent CO_2 was added to the inspired air, these indices returned toward the values which were measured prior to bypassing the donor. One hundred percent N_2 resulted in a profound increase in resistance and fall in compliance. The subsequent addition of 5 percent CO_2 only partially alleviated the response to alveolar hypoxia. When 9 percent O_2 and 5 percent CO_2 in nitrogen were inspired, vascular resistance improved. A subsequent increase in oxygen from 9 to 19 percent did not alter resistance, but appeared to improve compliance slightly. When the "autologous" circuit was restored, resistance decreased further. Elimination of CO_2 from the inspired gas resulted in an increase in resistance.

Despite the marked alterations in resistance which took place in this lobe, it appeared grossly normal at the termination of the experiment. The lobe gained 9 percent in weight. Mild perivascular and interstitial edema and slight congestion and alveolar irregularity were seen on histologic section. No perivascular hemorrhages developed.

COMMENT

Arterial depulsation has been used by many investigators in an attempt to minimize perfusion damage to the isolated lung. Specific studies have not been carried out previously to prove that pulse-damped arterial flow is better for the isolated lung. The author empirically

used a roller pump for all previous experiments.

The occlusive roller pump produced a pressure pulse which was three-quarters systolic phase and one-quarter diastolic phase in each pulse cycle (Figure 1). The ventricular pump produced one-quarter cycle systole and three-quarters diastole. As a result, the pulse-pressure which was produced by the roller pump was only one-quarter to one-third as great as that generated by the ventricular pump. The pressure dynamics which resulted from the roller pump, more closely resembled the "depulsated" circuit than those which were produced by the ventricular pump.

The lobe pulsated with each systolic contraction of the ventricular pump. The blood volume undoubtedly increased abnormally during systole because the lobe was not supported on all pleural surfaces. Much of the systolic kinetic energy was likely dissipated in distending the vasculature of the lobe during systole. The greater pulse pressure may have contributed to the severe perivascular hemorrhage which developed in the second experiment. These two experiments did not allow any conclusions to be drawn, but the value to the isolated lung of pulsatile perfusion appeared questionable.

Normothermic recirculation of blood through a lobe breathing 5 percent CO_2 and 95 percent air appeared to be reasonably well tolerated. Histologic alterations were slightly more pronounced than in the CONTROL lobes.

The progressive rise in resistance during recirculation, suggested that humoral factors may have been accumulating or the circuit may have been progressively depleted of nutrients. This remains a subject for future investigation. The simple low-volume recirculation

circuit appears to be a potentially useful preparation for investigation of lung metabolism.

If the depletion of nutrients results in the vascular alterations, the use of hypothermia should decrease or delay these effects. The combination of moderate hypothermia and recirculation appeared to decrease the degree of vascular alteration in the second experiment. Compliance and vascular resistance alterations which occurred during hypothermia reversed when normothermic conditions were restored. The blood volume did not increase during re-warming, however. This, in addition to the increase in the low oxygen gradients suggests that diffuse membrane damage had occurred.

Continuation of normal respiratory rates may have been harmful to the hypothermic lung. Respiratory excursions were sluggish during the period of hypothermia. In previous experiments, appreciable loss of compliance was invariably associated with edema formation and grossly evident uneven ventilation. The lobe in this experiment did not show these changes. The venous shunt and true shunt data would suggest that irreversible alterations had not taken place. Perhaps the respiratory rate should be decreased in order to maintain normal tidal volumes in a less compliant, cooled organ. Although this was just a single experiment, the observations support the proposal of others that moderate hypothermia protects the lung from the deleterious effects of perfusion (Veith et al, 1967-1).

Information obtained from the fifth experiment indicated that 5 percent carbon dioxide in air is a satisfactory inspired gas mixture. Unfortunately, the effect of inspiring oxygen-enriched mixtures was not investigated in this experiment. High levels of inspired oxygen are

likely not desirable in this form of preservation attempt. High inspired oxygen levels over prolonged periods are toxic to the lung Pratt, 1958; Soloway et al, 1968). In the recirculation circuit, the perfusate will contain unphysiologically elevated pO_2's. In the last lobe, when the arterial pO_2 was reduced from high levels to that in mixed venous blood, the resistance of the lobe decreased. The partial pressure of oxygen in the milieu should be adequately maintained without using high inspired oxygen levels, providing ventilation and perfusion are well distributed.

One hundred percent and 95 percent pIN_2 markedly elevated vascular resistance for a period of nearly 30 minutes in the last lobe. Despite this insult, the lobe retained excellent vasomotor activity and grossly and histologically appeared comparable to the CONTROL lobes. This again was only a single experiment, but these observations raise some question regarding the theory that vasospasm initiates damage to the isolated lung (Veith et al, 1968-1; 1968-2). This question should be investigated in greater depth in future.

SUMMARY

1. Isolated lobes were perfused with autologous blood in five pilot experiments. Arterial flow characteristics or temperature or inspired gas composition were altered in order to obtain preliminary information regarding the effects of these conditions on the isolated lung.
2. The isolated lung may be subject to more severe vascular damage when pulsatile perfusion is used.
3. The isolated lung tolerates brief periods of normothermic or moderately hypothermic recirculation-perfusion.

4. Five percent carbon dioxide in air appears to be a satisfactory inspired gas composition for the isolated lung in the recirculation circuit.
5. Brief periods of severe vasospasm and hypoxia do not appear to initiate damage to the isolated perfused lung.

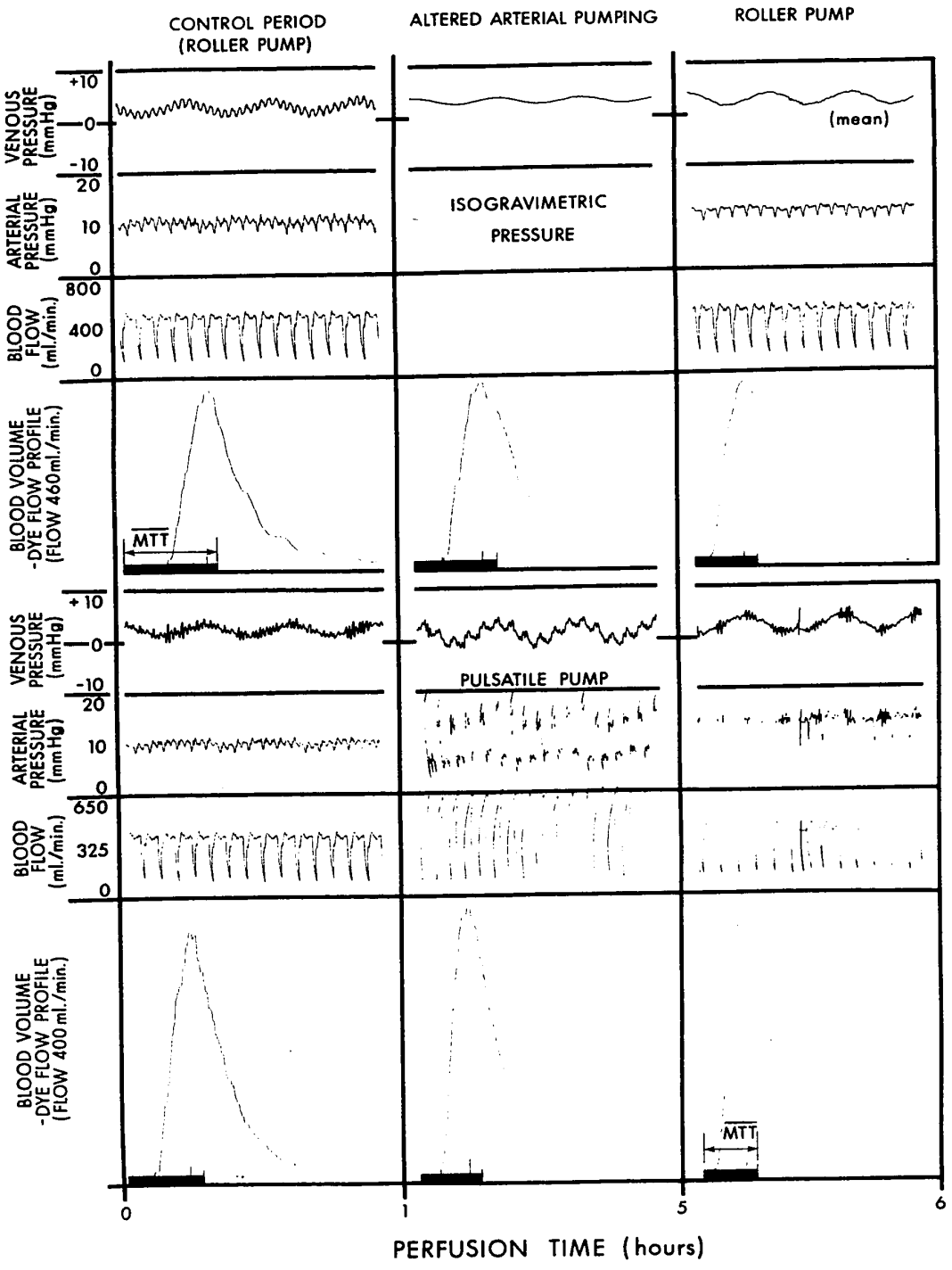

Fig. 1. Hemodynamics in lobe with "depulsated" arterial flow vs. lobe with pulsatile flow.

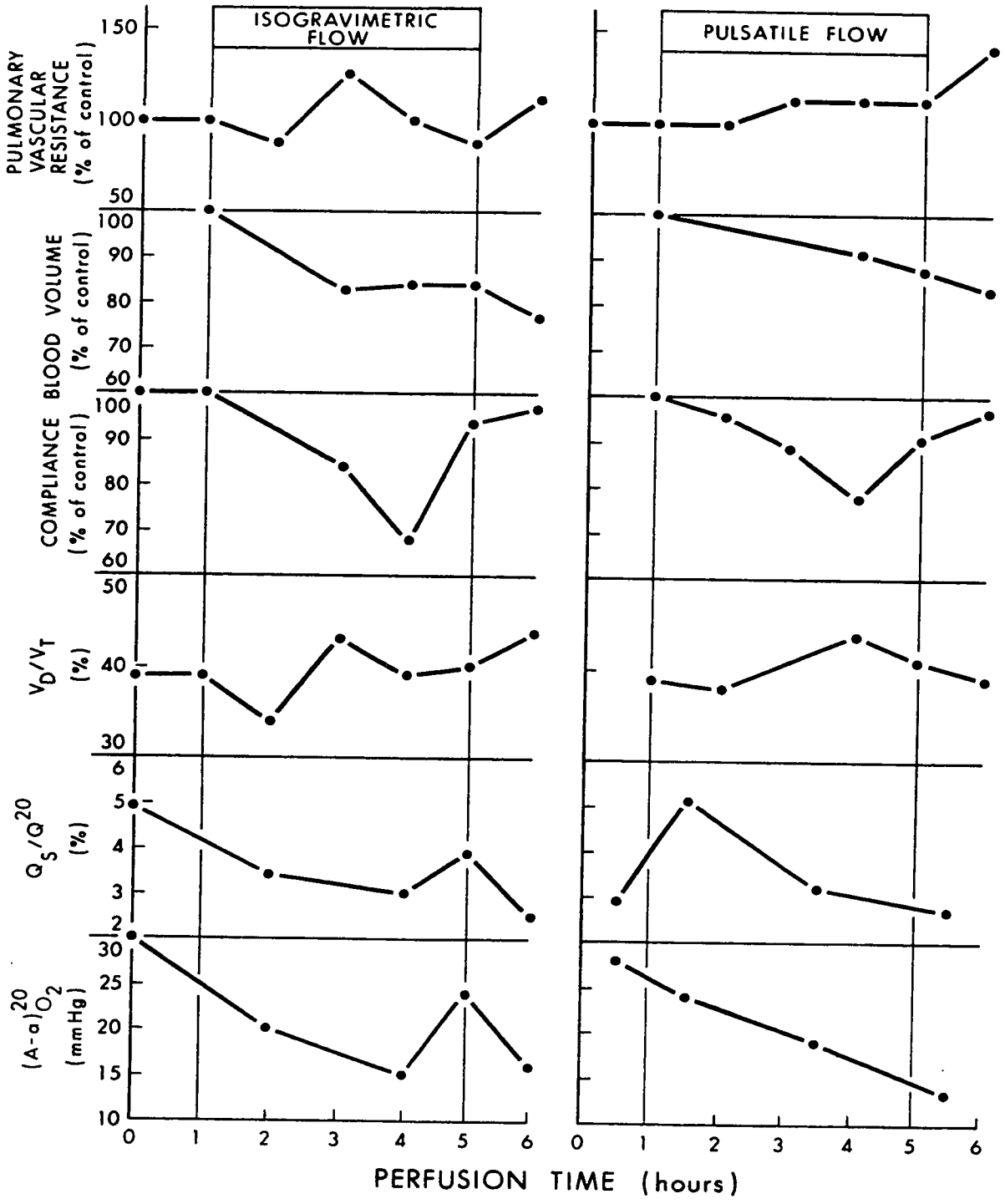

Fig. 2. Function of lobe perfused with "depulsated" flow vs. lobe with pulsatile flow.

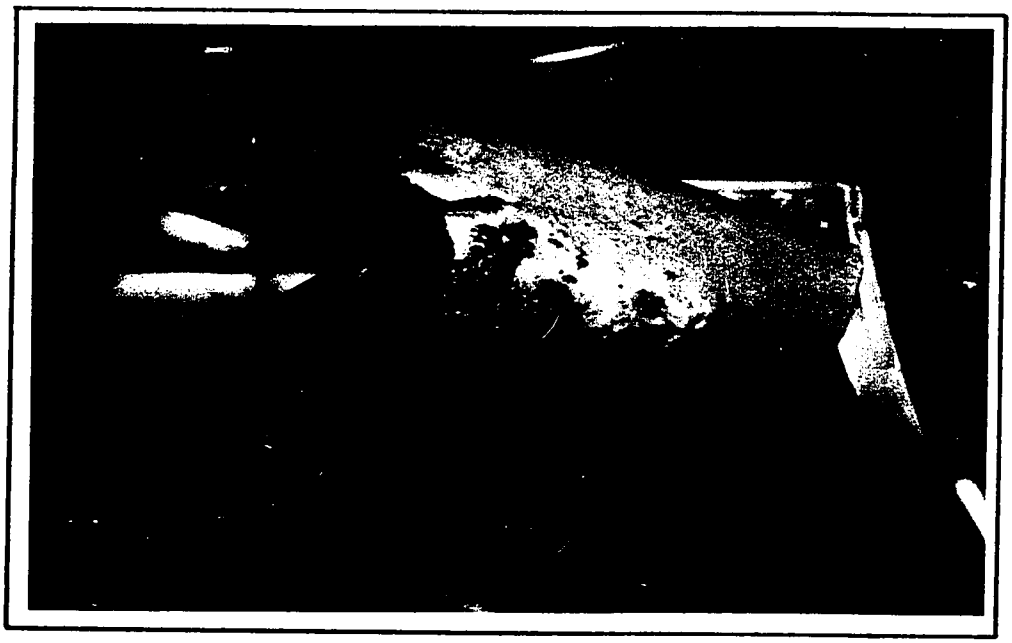

Fig. 3. Lobe after 4 hours of pulsatile perfusion.

Fig. 4. Lobe after 4 hours of pulsatile perfusion (H & E x 40); marked perivascular hemorrhage, interstitial edema.

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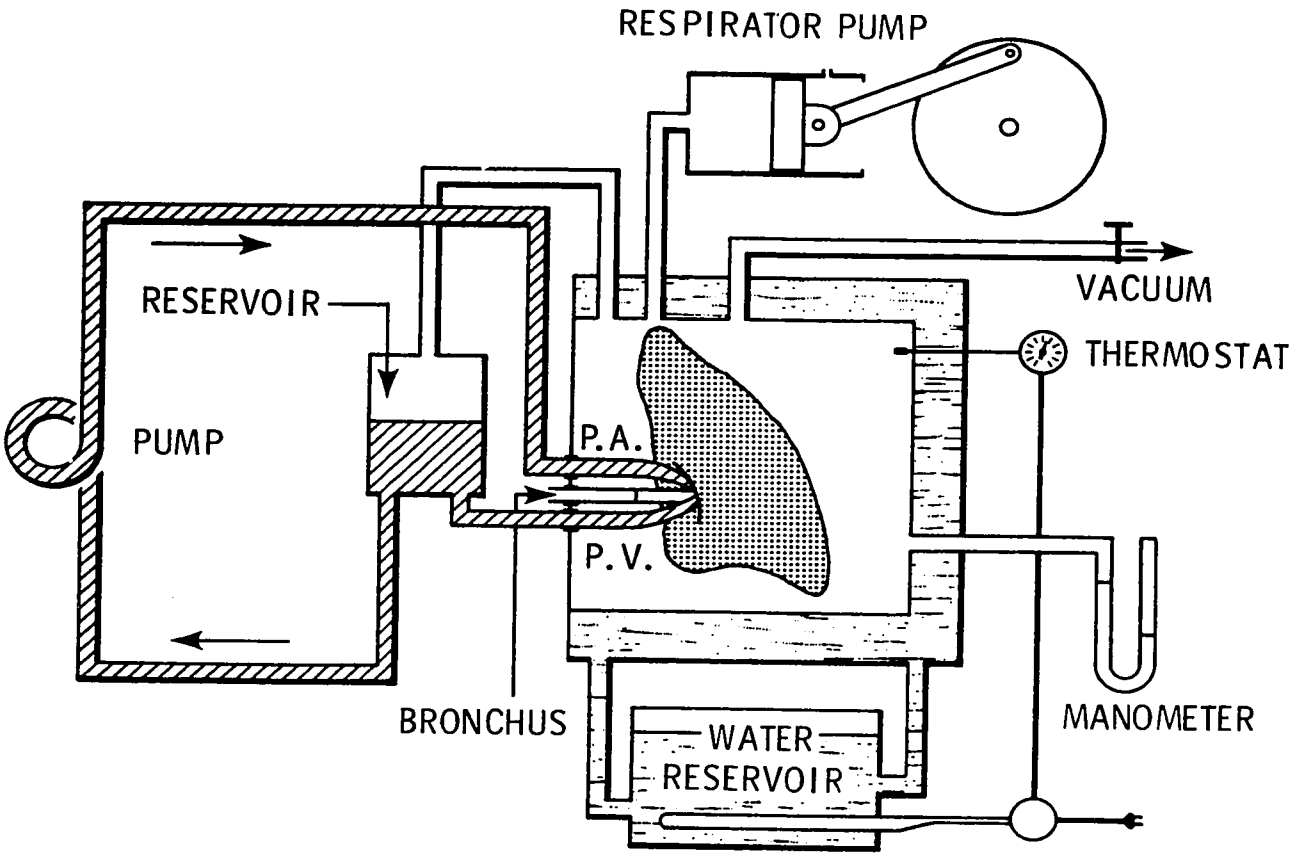

Fig. 5. Perfusate "recirculation" circuit.

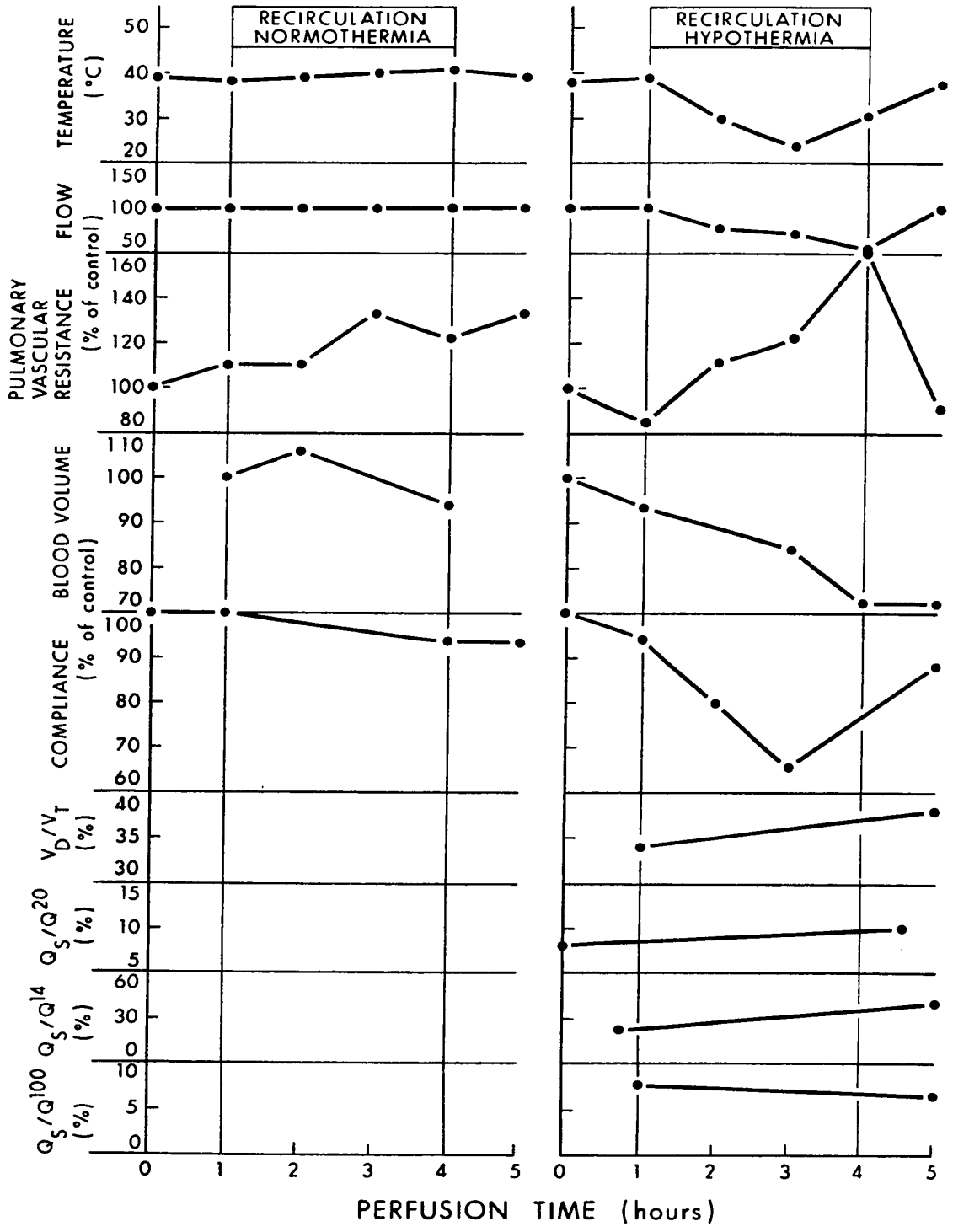

Fig. 6. Function of 1 normothermic vs. 1 hypothermic lobe in recirculation circuit.

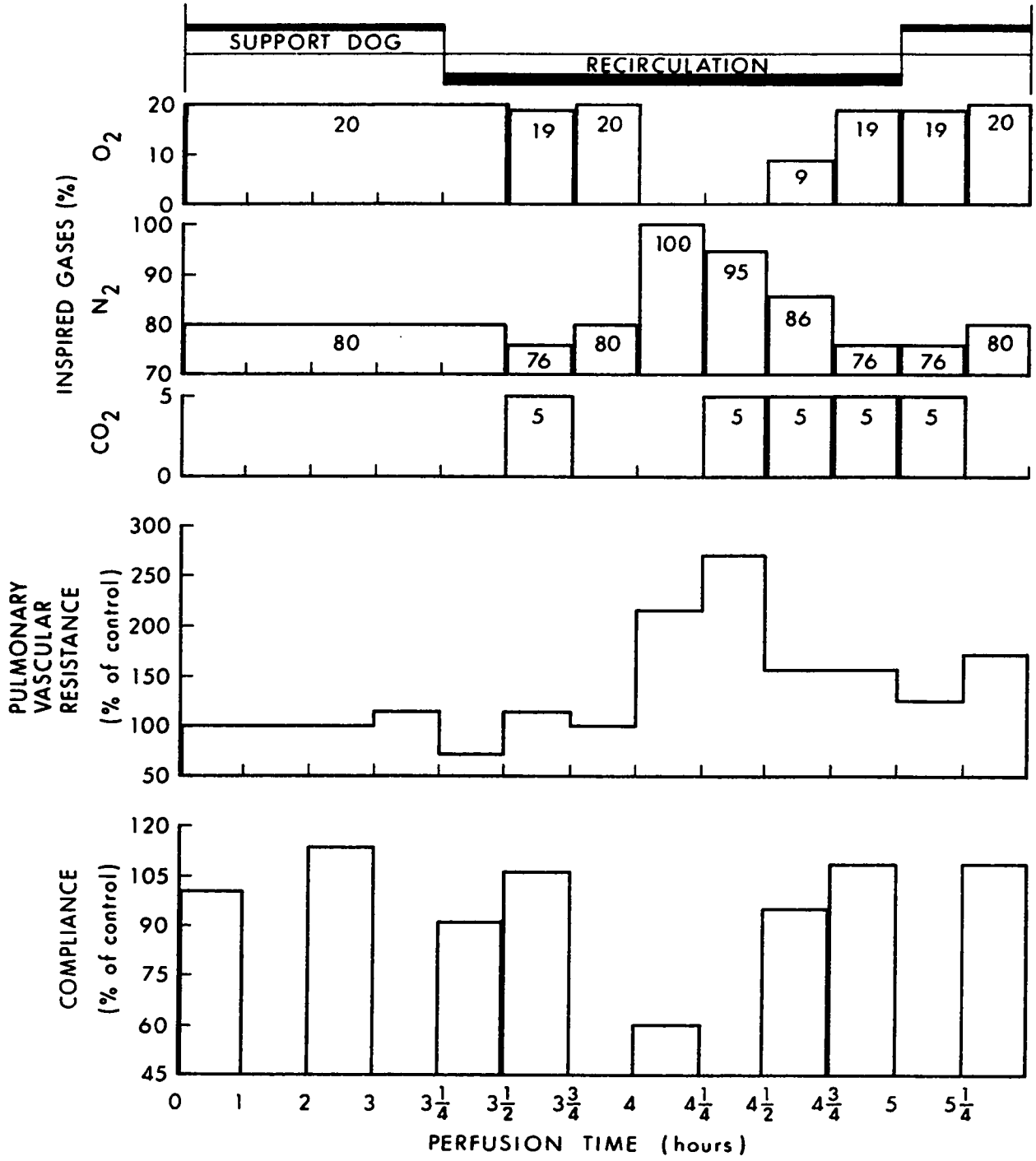

Fig. 7. Vascular resistance and compliance with altered inspiratory gas during blood recirculation.

ADDENDUM IV

HYPOTHERMIC PLASMA PERFUSION OF THE LUNG

Room temperature plasma recirculation would be a very convenient method of lung preservation. In the fourth of the preceding experiments, the lung was adequately preserved following a brief period of room temperature perfusion. Compliance decreased 50 percent and vascular resistance increased under these conditions.

It was thought that plasma perfusion might alleviate the resistance changes and that reduction in respiratory rate might minimize the effects of altered compliance. In order to investigate this possibility, the first experiment in this series was carried out. It became apparent that the problem of optimal hemodynamics and perfusate oxygenation could not be easily solved. Subsequent experiments were carried out under various conditions of temperature, flow and ventilation in attempt to assess the potential or limitations of hypothermic plasma perfusion for lung preservation.

METHODS

General

A perfusate of ultra-filtrated plasma was prepared according to the method of Belzer and co-workers (1967; 1968-2). Heparinized blood from healthy mongrel dogs was centrifuged and the supernatant plasma was decanted and freeze-stored at -10 to -20° C. Immediately prior to use, the plasma was rapidly thawed by immersion of the plasma containers in water at 60 to 70° C. The cryoprecipitate was removed by

seven millilitres per donor kilogram per minute after 90 minutes of perfusion. A respiratory rate of eight per minute was maintained throughout.

A respiratory rate of five per minute was maintained throughout the second experiment. A flow rate of 25 millilitres per donor kilogram per minute was initiated at room temperature. When the temperature reached 10° C at three hours, the flow was reduced to 2.5 millilitres per kilogram per minute.

Throughout the room-temperature perfusion in the third experiment, the respiratory rate was five per minute and the flow was six millilitres per donor kilogram per minute.

All of the first three lungs were breathing five percent CO_2 in air.

In the fourth experiment, the lung inspired five percent CO_2 in oxygen at a respiratory rate of five per minute for two minutes each hour. The lung was perfused at a rate of 100 millilitres per minute (five millilitres per donor kilogram per minute) during the periods of ventilation. The venous pressure was maintained at 0 mm Hg during this experiment. Functional residual capacity was maintained by a chamber pressure of -6 cm H_2O between the periods of perfusion-ventilation. The temperature was maintained throughout at 8° C and 100 percent O_2 continuously flowed through the chamber interior. At the end of the storage attempt, the circuit was primed with blood from an appropriately cannulated homologous dog and perfusion was begun after warming the chamber to 23° C.

RESULTS

(Figure 1)

In the first experiment, the lung was becoming edematous following one hour of perfusion. When resistance increased at 90 minutes, the flow rate was reduced. Vascular resistance and edema formation progressively increased. The lung was destroyed following 210 minutes of perfusion, at which time vascular resistance was seven times the initial value. Throughout the period of perfusion, the perfusate pO_2 and pCO_2 were constant at 75 and 30 mm Hg respectively (150 and 40 mm Hg corrected for temperature - Bradley et al, 1956).

The gross appearance of the lung remained unchanged during the period of perfusion (Figure 2), but it gained in excess of 100 percent in weight and edema fluid poured from the bronchus. The cut surface of the lung displayed cuffs of edema fluid around the vessels. Marked perivascular and interstitial edema with mild alveolar irregularity were seen microscopically (Figure 3).

Despite a reduction of flow rate and arteriovenous pressure difference at lower temperatures, the second lung was becoming edematous after three hours. At two hours, the venous pressure was raised from +1 to +3 mm Hg for a period of one minute. Within two minutes, the arterial pressure had increased from 6 to 12 mm Hg. Thirty minutes elapsed before the arterial pressure returned to 8 mm Hg. Compliance at 10° C after four hours of perfusion was 70 percent of the compliance at 23° C. The arteriovenous pressure difference began to increase after four hours of perfusion. Perfusion was discontinued at four and one-

half hours when severe edema developed. Throughout the experiment, the perfusate pO_2 and pCO_2 were comparable to the first experiment, the pH, however, dropped from 7.30 after one hour to 7.18 by four hours. The lung gained 47 percent in weight and grossly and histologically demonstrated changes comparable to the first lung.

Vascular resistance progressively decreased during the first hour in the third experiment. Thereafter, resistance was stable for five hours. Perfusate pO_2 ranged from 65 to 75 mm Hg and pCO_2 from 21 to 30 mm Hg (temperature corrected). The pH was 7.36 when perfusion began and 7.18 after six hours. Circulating fluid volume decreased after the fourth hour. At six hours, the compliance was 15 percent less than at one hour. Vascular resistance did not increase until six and one-half hours, at which time edema fluid was present in the airway. The lung gained 60 percent in weight and appeared grossly and histologically similar to the preceding lungs. The histologic alterations were more severe than in the second lung.

At a temperature of 8° C, the fourth lung displayed a high arteriovenous pressure difference initially. This had doubled at ten hours despite brief periods of intermittent perfusion. The perfusate slowly returned to the reservoir during the thirty minute period which followed each hourly 200 millilitre perfusion. The perfusate pO_2 "measured" 550 to 870 mm Hg and the pCO_2, 40 to 50 mm Hg. The pH remained between 7.27 and 7.31.

After ten hours, the lung had retained approximately 100 millilitres of perfusate. Edema was localized to the dependent areas. At ten hours, the lobe appeared otherwise normal. An attempt at perfusion-

evaluation was made following the tenth hour of "storage."

Homologous perfusion was initiated using a flow rate of 80 millilitres per minute (3.5 millilitres per donor kilogram per minute) and a venous pressure of +2 mm Hg. The lung inspired room air at eight breaths per minute. Perfusion was continued for only four minutes. During that time, no blood returned to the venous reservoir. The lung became totally unresponsive to chamber pressure fluctuations and developed large patches of congestion and ischaemia (Figure 4). Watery edema fluid poured from the bronchus. Microscopically, the lung resembled those in the preceding three experiments, except vascular congestion and perivascular hemorrhage were present (Figure 5).

COMMENT

These experiments must be considered pilot studies only. In the first three experiments, the lungs were becoming edematous before vascular resistance increased. The characteristics of the edema formation were identical to all previous experiments. These alterations developed despite adequate oxygen levels in the perfusate.

The resistance did not increase in the first three experiments until the edema was already developing. Edema developed despite very low flows and perfusion pressures. The osmotic pressure of the plasma perfusate may have been reduced as a result of the removal of lipoproteins. Plasma osmotic pressure must be measured in future studies. The reduction of flow rates and lowering of temperature appeared to delay the onset of edema formation.

The fourth lung temporarily retained perfusate concomittant with an increase in resistance during the periods of intermittent perfusion. In order for this to occur, much of the resistance must have been located downstream from the "capacitance" vessels. The exaggeration in arterial pressure in response to a small increase in venous pressure which was observed in the third experiment, may have resulted from the sudden development of partially reversible perivascular edema.

As the departure from physiologic conditions became greater through this series, vascular derangements developed following smaller total volumes of perfusate pumped to the lobes. To state this another way, perfusion damage was greater as the departure from physiologic conditions increased.

The initiation of homologous perfusion at 24° C in the fourth lung may have contributed to the damage which resulted. The lung in the preceding group which was perfused with blood at 24° C, however, withstood rewarming to 37° C well. The temperature of lungs which are transplanted will likely approximate room temperature when in situ flow is initiated.

The fourth lung displayed a very high fixed vascular resistance. This likely resulted more from anatomic derangement than vascular spasm. The lung "trapped" a large volume of blood (320 ml) before congestion and hemorrhage fulminantly developed. Severe perivascular and interstitial edema were likely present before blood perfusion began. The development of perivascular hemorrhage indicates that this can take place rapidly when perivascular edema pre-exists.

The acidosis which developed in the second and third experi-

ments indicates that anaerobic metabolism was taking place. How much this was contributed to by derangements in ventilation and perfusion and deficiency in the perfusate remains a subject for future study.

SUMMARY

1. Four experiments were carried out using various flows, temperatures and respiratory rates to investigate the potential of hypothermic plasma perfusion for lung preservation.
2. The lung remains very intolerant to perfusion-preservation as a result of its propensity to develop severe perivascular and interstitial edema under non-physiologic conditions.
3. These studies suggested, but have not concluded that the Belzer kidney preservation technique will not be suitable for lung preservation.

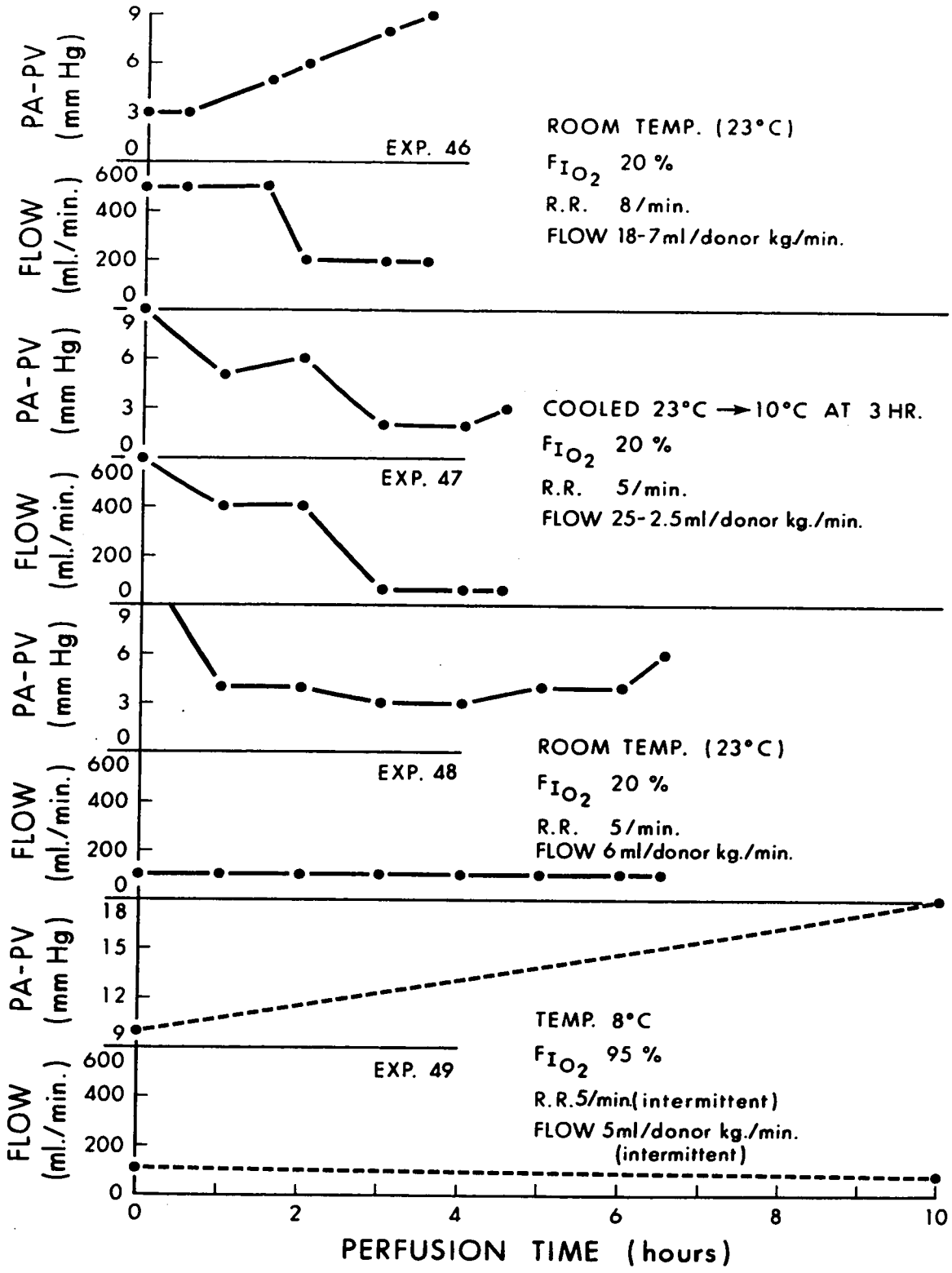

Fig. 1. Pressures and flows among 4 plasma-perfused lungs at various temperatures, and respiratory rates.

Fig. 2. Lung after 3.5 hours of hypothermic plasma perfusion.

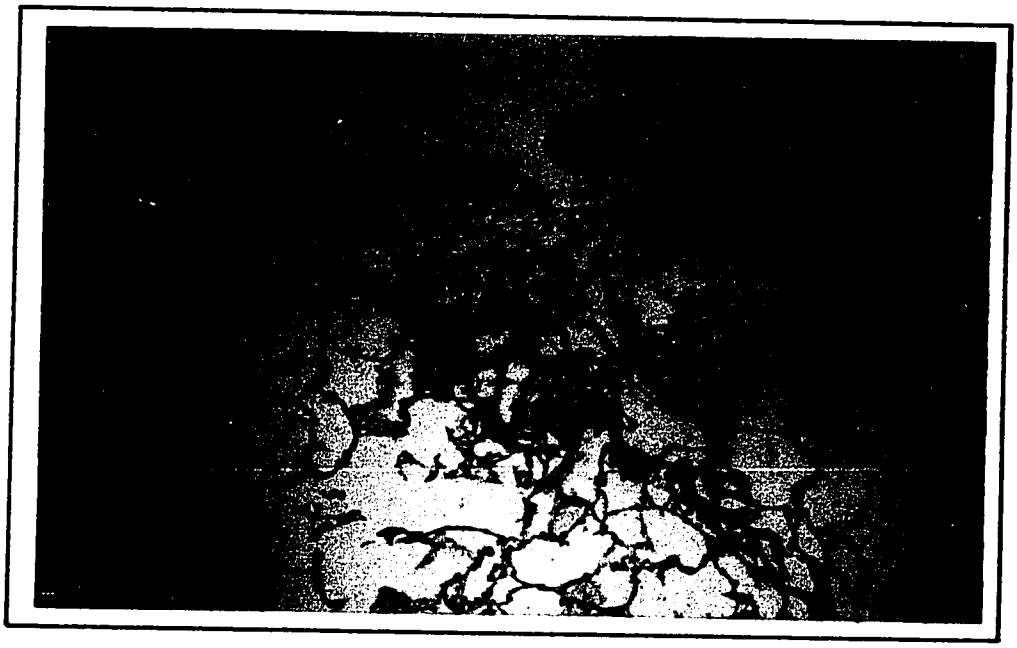

Fig. 3. Hypothermic plasma-perfused lung (VER x 100); marked perivascular edema, interstitial edema, distended lymphatic; disruption of elastic fibres.

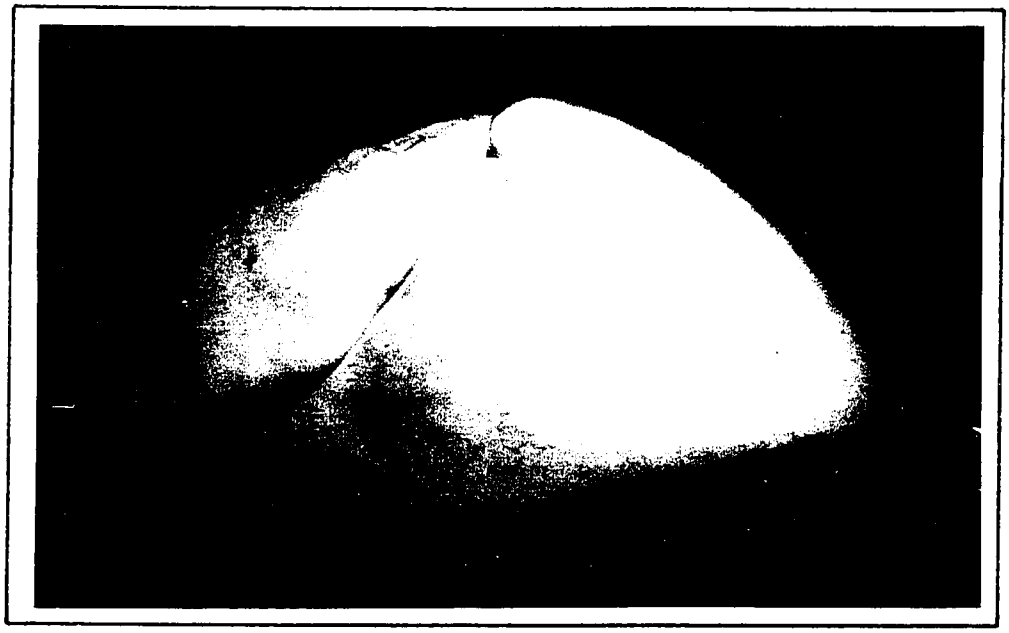

Fig. 2. Lung after 3.5 hours of hypothermic plasma perfusion.

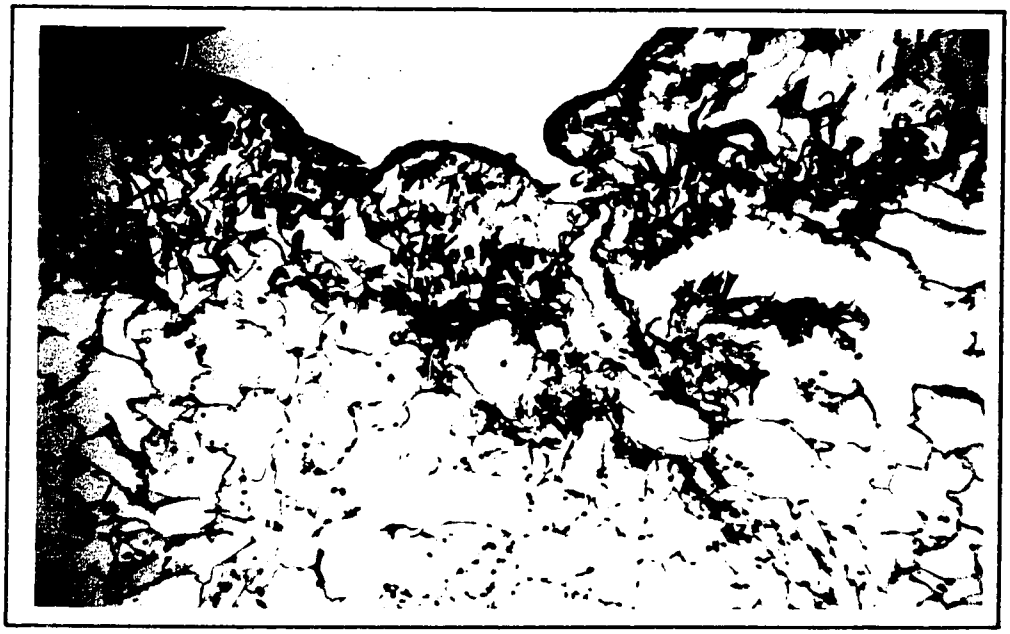

Fig. 3. Hypothermic plasma-perfused lung (VER x 100); marked perivascular edema, interstitial edema, distended lymphatic; disruption of elastic fibres.

Fig. 4. Lung perfused with blood after 10 hours of hypothermic plasma perfusion.

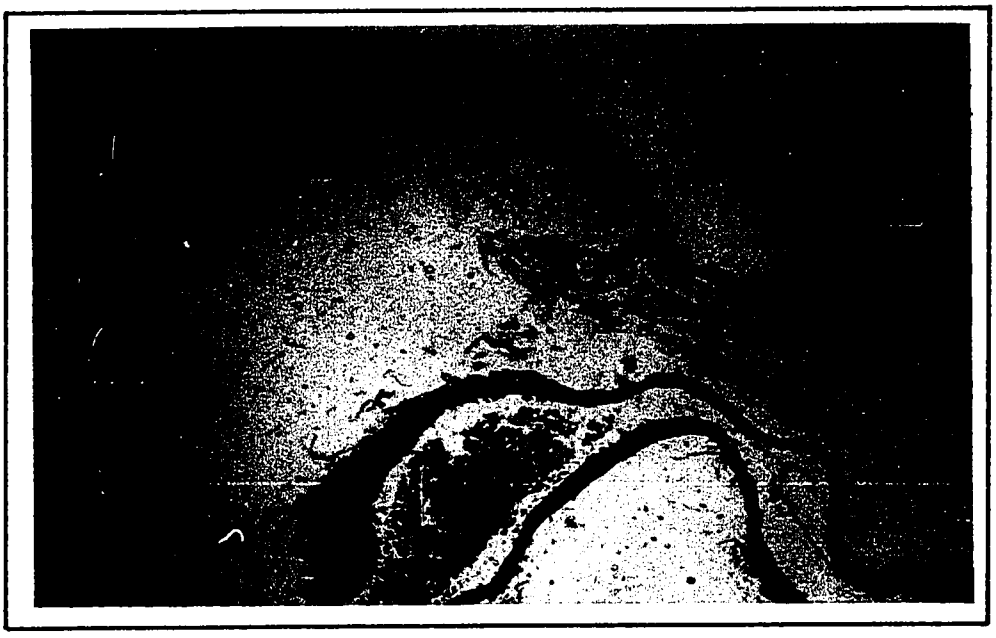

Fig. 5. Lung after 10 hours of hypothermic plasma perfusion and 4 minutes of blood perfusion (H & E x 40); perivascular and interstitial edema.

Fig. 4. Lung perfused with blood after 10 hours of hypothermic plasma perfusion.

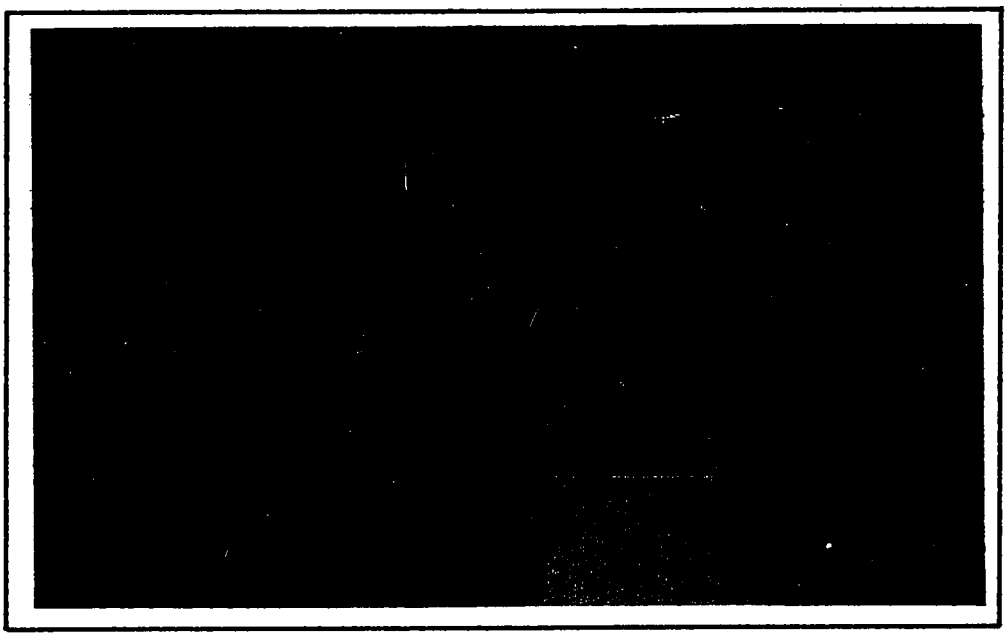

Fig. 5. Lung after 10 hours of hypothermic plasma perfusion and 4 minutes of blood perfusion (H & E x 40); perivascular and interstitial edema.

Fig. 4. Lung perfused with blood after 10 hours of hypothermic plasma perfusion.

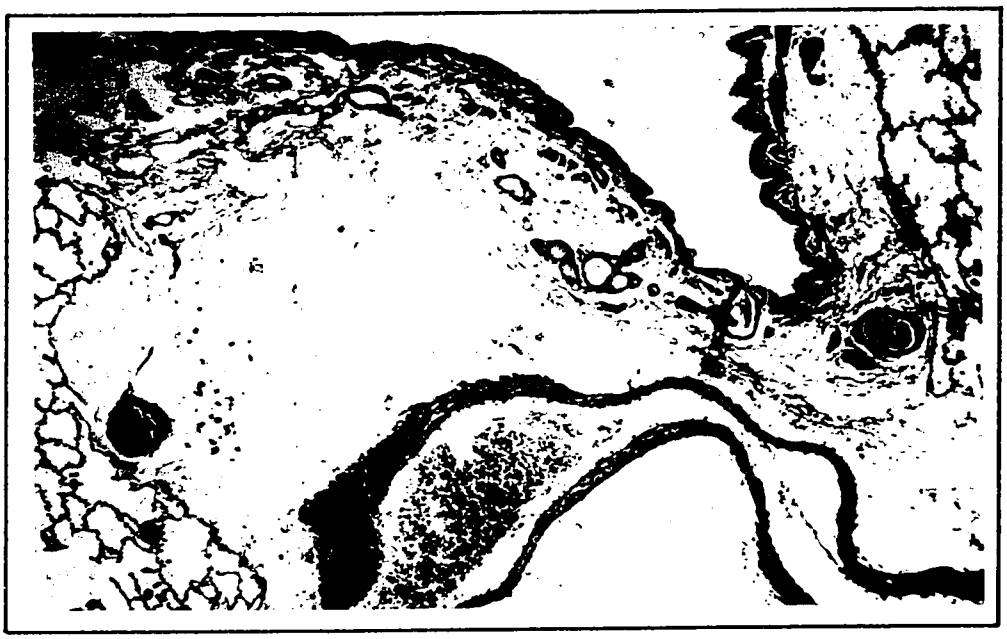

Fig. 5. Lung after 10 hours of hypothermic plasma perfusion and 4 minutes of blood perfusion (H & E x 40); perivascular and interstitial edema.

APPENDIX

TABLES

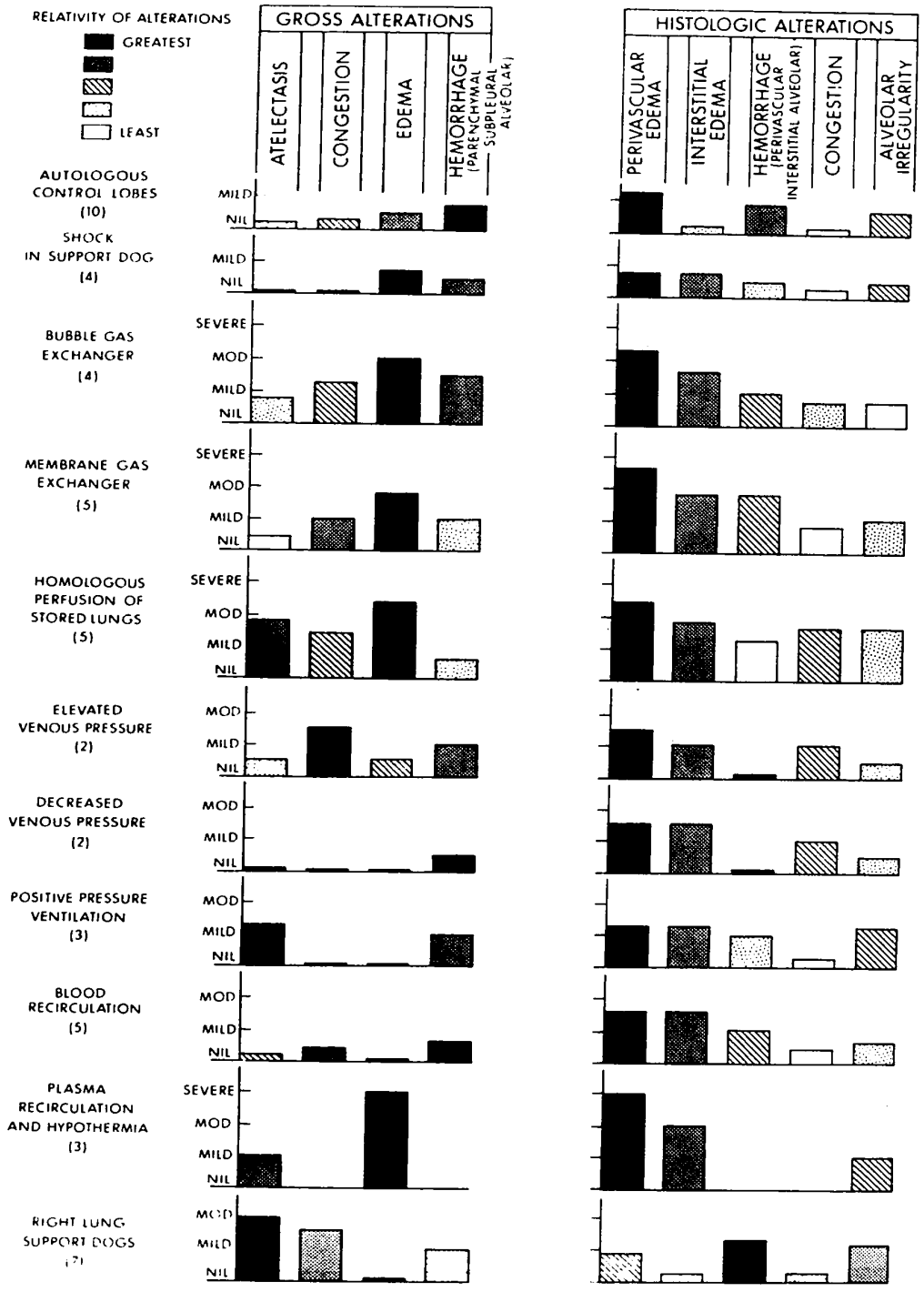

Fig. 1. Severity and pattern of morphologic alterations in isolated perfused lungs under various conditions ( ) - number of lungs.

TABLE 2.

FUNCTION OF LOBES AND CARDIORESPIRATORY INDICES
OF SUPPORTING DOGS IN 8 AUTOLOGOUS LOBE PERFUSIONS
(Chap. IV, Figs. 2 & 5)

ISOLATED LOBE

Hour of Perfusion	0		1		2		3		4		5	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
Arterial Pressure (mm.Hg.)	15.8	2.5	14.6	3.3	15.4	3.0	15.4	3.6	16.8	4.8	17	5.4
Vascular Resistance (% of control)	100		89	11	102	12	102	17	122	26	127	37
Compliance (% of control)	100		97	9	97	11	94	13	91	14	85	19
$(a - \bar{v})\ pO_2$ (mm. Hg.)	59	12	60	11	63	10	61	8	60	13	64	11
$\dot{V}\ O_2$ (ml/donor kg./min.)	0.78	.20	0.89	.33	1.13	.30	1.08	.26	1.13	.30	1.10	.33
SUPPORT DOG												
Arterial Pressure (mm.Hg.)	114	13	102	15	92	22	93	19	82	13	70	19
$p\bar{v}O_2$ (mm. Hg.)	46	8	43	7	40	6	37	5	36	6	33	7
$p\bar{v}H$	7.27	.08	7.24	.07	7.24	.09	7.21	.08	7.19	.10	7.11	.14

TABLE 3. FUNCTION OF 8 CONTROL (GROUP 2), 10 PNEUMONITIS (GROUP 3) AND 5 "TREATED" (GROUP 4) PNEUMONITIS LOBES (CHAPTER V, FIG. 3)

VASCULAR RESISTANCE PERFUSION TIME(HOURS)

TIME (HRS.)	0	1		2		3		4	
		%	SD	%	SD	%	SD	%	SD
GROUP 2	100	89	11	102	12	102	17	122	26
GROUP 3	100	148	24	188	34	215	44	307	79
GROUP 4	100	169	42	200	73	225	64	252	67

COMPLIANCE

GROUP 2		97	9	97	11	94	13	91	14
GROUP 3	100	73	7	55	16	45	17	34	12
GROUP 4	100	84	6	88	7	88	12	80	16

$(a - \bar{v})\ pO_2$

GROUP 2	100	102	15	108	15	107	24	105	30
GROUP 3	100	48	16	41	16	32	18	28	17
GROUP 4	100	76		80		72		68	25

AORTIC PRESSURE

GROUP 2	100	91	14	81	21	82	18	72	12
GROUP 3	100	77	15	67	22	54	21	44	22
GROUP 4	100	88	13	78	21	78	17	76	7

TABLE 4. PULMONARY VENOUS MINUS PULMONARY ARTERIAL BASE EXCESS (DEFICIT) IN CONTROL AND PNEUMONITIS LOBES (CHAPTER V, FIG. 6)

	PERFUSION TIME (HRS.)	0	1	2	3	4
		$(a-\bar{v})$	$(a-\bar{v})$	$(a-\bar{v})$	$(a-\bar{v})$	$(a-\bar{v})$
PNEUMONITIS GROUP 3		-1	-1	-1	0	0
		+1	0	-1	-1	-2
		+1	-0.5	-0.5	-1.5	-2.5
		-1	-1	-1	-1	-2
		-1	-1.5	-2	-1.5	-2
		-1	-3	-1.5	-3	-2.5
		-0.5	-3	-1.5	-2.5	-1.5
		-1	-2.5	-1	0	0
	AV & SD	-0.45(.7)	-1.56(1.1)	-1.2(0.4)	-1.32(1)	-1.56(1)
CONTROL GROUP 2		+1	+2	+1.5	+1.5	+2
		-0.5	+.5	-1.0	+1	+1
		+1	+1	+1.5	-1.5	+1
		0	-.5	0	0	-.5
		+1	-1	0	+.5	0
		-0.5	-.5	+.5	0	+1
		+0.5	0	+1	+1	-1
		0	-.5	+1.5	+1.5	+.5
	AV & SD	+.3(.4)	+0.1(1)	+.6(.9)	+.6(.9)	+.5(.9)

TABLE 5.

INDICES REFLECTING CONDITIONS IN SUPPORTING DOGS AND PERFUSION SYSTEM TO AUTOLOGOUSLY PERFUSED LOBES (CHAPTER VI, FIG. 1)

PERFUSION TIME (HRS.)	0		1		2		3		4		5	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
DONOR ARTERIAL PRESSURE (mm.Hg.)	123	21	116	27	112	21	108	24	105	17	97	22
DONOR ARTERIAL pO_2 (mm.Hg.)	79	12	81	7	86	15	89	16	87	15	81	16
DONOR ARTERIAL pH	7.43	.05	7.42	.04	7.40	.05	7.37	.07	7.31	.11	7.28	.10
HEMOGLOBIN (gm./100 mc.)	14.7	3.2	14.3	2.0	14.5	2.0	14.0	2.0	13.5	2.0	13.5	2.0
DONOR RECTAL TEMP. (^{o}C)	36.5	1	36	1	36	2	36	1	35.5	1	35.5	1
CHAMBER TEMP. (^{o}C)	37	1	39	1	38.5	1	37	1	38	1	37.5	1

TABLE 6.

HEMODYNAMICS, MECHANICS AND GAS EXCHANGE
OF 10 AUTOLOGOUSLY PERFUSED LOBES
(CHAPTER VI, FIG. 2 & 3)

PERFUSION TIME (HRS.)	0		1		2		3		4		5	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
VASC. RES. (% of Control)	100		92	14	112	29	112	32	131	35	119	31
COMPLIANCE (% of Control)	100		99	4	93	4	95	6	95	5	95	6
$V_D/\dot{V}_T$ (%)	48	8	49	8	50	8	49	8	48	9	48	11
$(a-\bar{v})pO_2$ (mm.Hg.)	37	12	42	10	42	6	45	10	39	4	46	7
$\dot{V}O_2$ (ml/min)	34	16	32	12	32	11	38	13	38	8	51	15
$\bar{v}_sO_2$ (%)	62	6	60	8	55	8	51	10	43	14	38	13
$\dot{Q}_S/\dot{Q}^{20}$ (%)	19	11	15	8	15	6	15	7	15	5	11	5
$[A-a]O_2^{20}$(mm.Hg.)	44	6	40	7	38	7	35	10	33	10	30	7
$\dot{Q}_S/\dot{Q}^{14}$ (%)			31	6	30	8	32	9	32	11	27	7
$[A-a]O_2^{14}$ (mm.Hg.)			27	3	24	5	23	4	20	1	18	3
$\dot{Q}_S/\dot{Q}^{100}$ (%)			8	3	9	3	7	2	7	2	5	1
$[A-a]O_2^{100}$(mm.Hg.)			270	65	290	70	250	50	245	40	220	30

TABLE 7. 10 HOUR AUTOLOGOUSLY PERFUSED LOBE
(CHAP. VI, FIG. 4)

Donor Weight 28Kg.
Lobe Weight Pre-Perfusion 74 grams.
Post-Perfusion 71 grams.
Flow Rate 600 ml/min.

Mean Venous Pressure +2 mm. Hg.
Chamber Pressure -10/-5 cm. H_2O

Respiratory Rate 17/min.

PERFUSION TIME (HRS.)	0	1	3	5	10
DONOR ARTERIAL PRESSURE (mm. Hg.)	140	130	120	100	60
DONOR ARTERIAL pO_2 (mm. Hg.)	73	76	104	76	72
DONOR ARTERIAL pH	7.36	7.32	7.30	7.15	7.16
HEMOGLOBIN (gm.%)	19.72	17.00	15.60	13.60	9.20
DONOR TEMP. (^oC)	35.5	35	35.5	35	35.5
CHAMBER TEMP.(^oC)	36.5	37	38.5	37	38
VASC. RES. dyne.sec.cm^{-5}	930	532	400	665	930
COMPLIANCE (L/cm. H_2O/Kgm.10^{-4})	7.6	7.5	7.45	7.15	6.4
$\dot{V}_D/\dot{V}_T$ (%)	52	49	46	48	58
$(a-\bar{v})pO_2$ (mm. Hg.)	19	45	39	36	48
$[A-a]^{20}_{O_2}$ (mm. Hg.)	44	26	24	28	28
$\dot{Q}_S/\dot{Q}^{20}$ (%)	37	10	13	20	11
$[A-a]^{14}_{O_2}$ (mm. Hg.)		21	19	15	17
$\dot{Q}_S/\dot{Q}^{14}$ (%)		30	43	19	34
$[A-a]^{100}_{O_2}$ (mm. Hg.)		219	352	244	218
$\dot{Q}_S/\dot{Q}^{100}$ (%)		5.4	8.4	5.4	6.2

TABLE 8. HEMODYNAMICS, MECHANICS AND GAS EXCHANGE OF ISOLATED LOBES DURING HYPOTENSION IN SUPPORTING DOGS (CHAPTER VII, FIG. 1 & 2)

PERFUSION TIME (HRS.)	0		1		3		4		5		6	7	8	9	10
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV				
DONOR ARTERIAL PRESSURE (mm.Hg.)	126	8	120	7	53	8	45	11	45	6	45	45	45	40	40
MIXED VENOUS pH	7.38	.06			7.25	.11	7.17	.13	7.19	.07	7.19	7.18	7.18	7.18	7.18
VASC. RES. (% of Control)	100		89	17	126	33	127	42	100		100	113	113	113	113
COMPLIANCE (% of Control)	100		100		91.6	9	89	5	85	5	80	72	73	74	75
$\dot{V}_D/\dot{V}_T$ (%)	47		49	4	57	3	58	1	58	2	58	55	54	53	51
BLOOD VOLUME (% of Control)	100		100		95		89		84		78	73	69		
$\dot{V}_{O_2}$ (ml./min.)	27	4			40	7	42	2	42	4	42	48			
$\dot{Q}_S/\dot{Q}^{20}$ (%)			9	3	9	6	11	5	14	9	11	8	15	23	
$[A\text{-}a]^{20}_{O_2}$ (mm. Hg.)			28	4	21	8	19	3	25	8	25	31	39	47	55
$\dot{Q}_S/\dot{Q}^{14}$ (%)			28	20	35	16	37	8	28		29	33	37		
$[A\text{-}a]^{14}_{O_2}$ (mm. Hg.)			16	12	15	4	13	5	11		15	17	19		
$\dot{Q}_S/\dot{Q}^{100}$ (%)			6	1	5	.05	5	1	6					5.9	
$[A\text{-}a]^{100}_{O_2}$ (mm. Hg.)			201	19	224	31	221	30	244					270	

TABLE 9.

FUNCTION OF 4 LOBES IN BUBBLE OXYGENATOR CIRCUIT
(CHAPTER VIII, FIG. 2)

PERFUSION TIME (HRS.)	0		1		2		3	
	AV	SD	AV	SD	AV	SD	AV	SD
VASC. RES. (% of Control)	100		92	32	95	27	111	14
COMPLIANCE (% of Control)	100		92	3	93	5	69	14
$\dot{V}_D/\dot{V}_T$ (%)	41	9	43	9	47	9	53	4
$\dot{Q}_S/\dot{Q}^{20}$ (%)			31				46	
$[A\text{-}a]^{20}_{O_2}$ (mm. Hg.)			51				54	
$\dot{Q}_S/\dot{Q}^{14}$ (%)			52				58	
$[A\text{-}a]^{14}_{O_2}$ (mm. Hg.)			33				54	

TABLE 10.

FUNCTION OF 5 LOBES IN MEMBRANE OXYGENATOR CIRCUIT
(CHAPTER VIII, FIG. 2)

PERFUSION TIME (HRS.)	0		1		2		3		4		5	
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
VASC. RES. (% of Control)	100		74	18	52	15	48	8	59	13	56	11
COMPLIANCE (% of Control)	100		99		89	8	86	7	71	22	85	7
$\dot{V}_D/\dot{V}_T$ (%)	59		50	8	52	9	50	7	56	11	57	12
$\dot{Q}_S/\dot{Q}^{20}$ (%)			13	4	14	4	17	8	31	11	44	19
$[A\text{-}a]_{O_2}^{20}$ (mm. Hg.)			37	11	34	13	35	16	47	13	59	11
$\dot{Q}_S/\dot{Q}^{14}$ (%)			21	4	19	9	27	6	38	17	56	22
$[A\text{-}a]_{O_2}^{14}$ (mm. Hg.)			22	6	16	8	18	9	19	10	27	7
$\dot{Q}_S/\dot{Q}^{100}$ (%)					23	8	24	8	21	9	19	9
$[A\text{-}a]_{O_2}^{100}$ (mm. Hg.)					385	76	345	49	341	91	360	10

TABLE 11. FUNCTION OF 4 LUNGS PERFUSED WITH HOMOLOGOUS BLOOD (CHAPTER IX, FIG. 1)

PERFUSION TIME (HRS.)	0		1		2		3		4		5		6	7	8
	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD			
VASC. RESIS. (% of Control)	100		84	32	86	37	92	40	94	42	106	66	76	76	71
COMPLIANCE (% of Control)	100		104	11	111	4	103	9	96	17	93	24	100	99	94
$\dot{V}_D/\dot{V}_T$ (%)	47	3	48	4	50	5	51	5	52	5	54	7	54	52	51
$\dot{Q}_S/\dot{Q}^{20}$ (%)			33	15	28	13	24	10	21	7	21	8	27	29	36
$[A\text{-}a]_{O_2}^{20}$(mm.Hg.)			45	3	41	4	39	1	36	2	35	7	43	44	
$\dot{Q}_S/\dot{Q}^{14}$ (%)					26				60				44		
$[A\text{-}a]_{O_2}^{14}$(mm.Hg.)					11				29				17		
$\dot{Q}_S/\dot{Q}^{100}$ (%)					15.5		14.8		14.0		13.3		12.5	11.8	11.1
$[A\text{-}a]_{O_2}^{100}$(mm.Hg.)					310		306		302		297		293	288	284

TABLE 12. LUNG PERFUSED WITH HOMOLOGOUS BLOOD FOR 16 HOURS (CHAPTER IX, FIG.2)

Lung Donor Weight - 18.5 Kg.
Lung Weight Pre-Perfusion 97 gm.
Post-Perfusion 90 gm.

Flow Rate 600 ml/min.
Chamber Pressure -9/4 cm. H_2O
Venous Pressure +3 mm. Hg.
Respiratory Rate 16/min.

PERFUSION TIME (HRS.)	0	1	2	3	4	8	10	11	14	15	16
VASC. RES. (dynes·cm.·sec^{-5})	800	266	266	266	266	266	533	533	1200	1066	1000
BLOOD VOL. (ml.)		59	64	66			74	68			
COMPLIANCE (L/cm. $H_2O(10^{-2})$)	1.7		1.7		2.1	1.6	2.24		2.24		1.74
$\dot{V}_D/\dot{V}_T$ (%)	50		53		55	51	49		49		53
$\dot{Q}_s/\dot{Q}^{20}$ (%)		52			31	36	21		30		15
$[A\text{-}a]^{20}_{O_2}$ (mm. Hg.)		40			39	56	44		46		32
$\dot{Q}_s/\dot{Q}^{100}$ (%)				12.9				9.0		7.4	
$[A\text{-}a]^{100}_{O_2}$ (mm. Hg.)				324				236		234	
ARTERIAL PRESSURE SUPPORT DOG (mm. Hg.)	160	180	180	170	170	160	140	130	110	90	80

TABLE 13. EFFECTS ON HEMODYNAMICS, MECHANICS AND GAS EXCHANGE OF VENOUS PRESSURE CHANGES (ADDENDUM I, FIG. 1 & 2)

2 LOBES

PERFUSION TIME (HRS.)	0	1	2	3	4	5	6
VENOUS PRESSURE (mm. Hg.)	+3	+3	+7	+7	+7	+7	+3
VASC. RES. (% of Control)	100	63	102	120	120	130	100
COMPLIANCE (% of Control)	100	103	98	98	96	91	96
$\dot{V}_D/\dot{V}_T$ (%)	40	50	51	49	54	54	55
BLOOD VOLUME (% of Control)		100		106	103	102	91
$\dot{Q}_S/\dot{Q}^{20}$ (%)		21			18		16
$[A\text{-}a]_{O_2}^{20}$ (mm. Hg.)		38			36		30
$\dot{Q}_S/\dot{Q}^{14}$ (%)		21			26		30
$[A\text{-}a]_{O_2}^{14}$ (mm. Hg.)		17			16		20
$\dot{Q}_S/\dot{Q}^{100}$ (%)		6.5			6.2		3.9
$[A\text{-}a]_{O_2}^{100}$ (mm. Hg.)		180			205		202

TABLE 14. EFFECTS ON HEMODYNAMICS, MECHANICS AND GAS EXCHANGE OF VENOUS PRESSURE CHANGES (ADDENDUM I, FIG. 1 & 2)

PERFUSION TIME (HRS.)	0	1	2	3	4	5	6
2 LOBES							
VENOUS PRESSURE (mm. Hg.)	+3	+3	-7	-7	-7	-7	-7
VASC. RES. (% of Control)	100	83	117	133	191	240	185
COMPLIANCE (% of Control)	100	100	96	95	89	85	80
$\dot{V}_D/\dot{V}_T$ (%)		55	55	60	58	58	
BLOOD VOLUME (% of Control)		100		87	83	79	94
$\dot{Q}_S/\dot{Q}^{20}$ (%)		7			12		13
$[A\text{-}a]_{O_2}^{20}$ (mm. Hg.)		26			29		30
$\dot{Q}_S/\dot{Q}^{14}$ (%)		23			43		
$[A\text{-}a]_{O_2}^{14}$ (mm. Hg.)		12			19		
$\dot{Q}_S/\dot{Q}^{100}$ (%)		7.0			7.1		
$[A\text{-}a]_{O_2}^{100}$ (mm. Hg.)		213			243		
1 LOBE							
VENOUS PRESSURE (mm. Hg.)	0	0	0	+6	-6	0	
VASC. RES. (% of Control)	100	100	138	50	125	150	
COMPLIANCE (% of Control)	100	100	100	92	85	84	
$\dot{V}_D/\dot{V}_T$ (%)		54		67	58	58	
BLOOD VOLUME (% of Control)		100		116	86		

TABLE 15. EFFECTS ON HEMODYNAMICS, MECHANICS AND GAS EXCHANGE OF POSITIVE PRESSURE VENTILATION IN 3 LOBES (ADDENDUM II, FIG. 1)

PERFUSION TIME (HRS.)	0	1	2	3	4	5	6
VENOUS PRESSURE (mm. Hg.)	3	3	3	0	0	0	3
BRONCHIAL PRESSURE (cm. H_2O)	0	0	0	10	10	10	0
CHAMBER PRESSURE (cm. H_2O)	-10/5	-10/5	-10/5	0	0	0	-10/5
VASC. RES. (% of Control)	100	94	91	131	150	168	118
COMPLIANCE (% of Control)	100	99	95	57	63	54	75
$\dot{V}_D/\dot{V}_T$ (%)	56	57	58	56	56	56	60
BLOOD VOLUME (% of Control)	100	95	91	69	60	65	86
$\dot{Q}_S/\dot{Q}^{20}$ (%)		11			8		10
$[A\text{-}a]^{20}_{O2}$ (mm. Hg.)		30			31		18
$\dot{Q}_S/\dot{Q}^{14}$ (%)		39			31		34
$[A\text{-}a]^{14}_{O2}$ (mm. Hg.)		18			18		21
$\dot{Q}_S/\dot{Q}^{100}$ (%)		6.5			6.8		7.4
$[A\text{-}a]^{100}_{O2}$ (mm. Hg.)		205			192		246

TABLE 16.

FUNCTION OF LOBE PERFUSED WITH "DEPULSATED" FLOW
VS. LOBE WITH PULSATILE FLOW
(ADDENDUM III, FIG. 2)

	ISOGRAVIMETRIC FLOW							PULSATILE FLOW						
PERFUSION TIME (HRS.)	0	1	2	3	4	5	6	0	1	2	3	4	5	6
VASC. RES. (% of Control)	100	100	88	125	100	85	112	100	100	100	114	114	114	142
COMPLIANCE (% of Control)	100	100		83	84	84	77		100			92	88	84
$\dot{V}_D/\dot{V}_T$ (%)	39	39	34	43	39	40	44		39	38		44	41	39
$\dot{Q}_S/\dot{Q}^{20}$ (%)	4.9		3.4		3.0	3.9	2.5	2.9		5.1		3.2		2.7
$[A\text{-}a]O_2$ (mm. Hg.)	30		20		15	24	16	28		24		19		13

TABLE 17. FUNCTION OF 1 NORMOTHERMIC VS. 1 HYPOTHERMIC LOBE IN RECIRCULATION CIRCUIT (ADDENDUM III, FIG. 6)

	RECIRCULATION NORMOTHERMIA						RECIRCULATION HYPOTHERMIA					
PERFUSION TIME (HRS.)	0	1	2	3	4	5	0	1	2	3	4	5
TEMPERATURE (°C)	39	38	39	40	40.5	39	38	39	30	24	31	38
VASC. RES. (% of Control)	100	111	111	133	122	133	100	85	112	122	160	91
FLOW (% of Control)	100	100	100	100	100	100	100	100	81	74	58	100
BLOOD VOLUME (% of Control)		100	106		94		100	93		84	72	72
COMPLIANCE (% of Control)	100	100			94	94	100	94	80	66		88
$\dot{V}_D/\dot{V}_T$ (%)								34				38
$\dot{Q}_S/\dot{Q}^{20}$ (%)							8					9
$\dot{Q}_S/\dot{Q}^{14}$ (%)								22				40
$\dot{Q}_S/\dot{Q}^{100}$ (%)								9				7

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