

A NEW BLOOD PUMP AND OXYGENATOR SYSTEM
FOR SUPPORT OF INFANTS WITH
NEONATAL RESPIRATORY DISTRESS
PRELIMINARY IN VITRO AND IN VIVO EVALUATION

by

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April, 1979
Blacksburg, VA

DEDICATION



This thesis is dedicated to John Clark Osborne, D.V.M., who died before its completion. Dr. Osborne was responsible for all surgery in this research. He served on my committee and added much to the richness of my education. He was an excellent teacher, a scholar, and friend. His contributions to the quality of the lives of those who knew him are missed.

ACKNOWLEDGEMENTS

This research was supported by the Western Electric Fund. I wish to thank all of the persons responsible for the success of this project. Dr. Leon J. Arp is thanked for his assistance throughout the project. Dr. J.B. Jones is thanked for his efforts to fund my education during this project. Dr. Harrison R. Steeves was of great help in planning my course work. Dr. R.F. Kelly of Food Sciences and Technology, Dr. C.W. Heald of Dairy Science, and Dr. K.E. Webb of Animal Science are thanked for contributions of materials used in the experiments. Without the help of my undergraduate research students; , , , and , not much would have been accomplished. is thanked for the in vitro data collection. is thanked for her assistance in the surgical procedures. A special thanks is extended to my friend, , who shared the task of building the oxygenator and donated his time in helping with the in vivo trials. of Becton-Dickinson Co. is thanked for providing tubing adapters at a time of critical need. Dr. T.L. Bibb is thanked for joining my committee after the death of Dr. John Clark Osborne.

TABLE OF CONTENTS

	<u>PAGE</u>
DEDICATION	ii
ACKNOWLEDGEMENTS	iii
LIST OF FIGURES	vi
LIST OF TABLES	vii
1. INTRODUCTION	1
A. FETAL AND NEONATAL CIRCULATION	1
B. NEONATAL RESPIRATORY DISTRESS	4
C. BLOOD OXYGENATORS	6
D. BLOOD PUMPS	7
E. PUMPS AND OXYGENATORS	9
F. COMPARISON OF OXYGENATOR DAMAGE	10
G. COMPARISON OF PUMP DAMAGE	10
H. SYSTEM TESTED	11
2. REVIEW OF PREVIOUS STUDIES	11
2. PRELIMINARY IN VITRO STUDY	13
A. OBJECTIVES	13
B. MATERIALS AND METHODS	13
C. DISCUSSION AND CONCLUSIONS OF IN VITRO STUDY	19
3. IN VIVO STUDY: SHORT TERM ANIMAL WORK	25
A. PRE-TRIAL CONSIDERATIONS	25
B. MATERIALS AND METHODS	27
C. INDIVIDUAL TRIALS AND DATA	39

	<u>PAGE</u>
D. DISCUSSION OF IN VIVO TESTING	41
E. CONCLUSIONS: PRELIMINARY IN VIVO WORK	47
4. RECOMMENDATIONS	50
5. LITERATURE CITED	52
6. APPENDICES	54
A. CYANOMETHEGLOBIN METHOD FOR PLASMA HEMOGLOBIN DETERMINATION	55
B. TESTS AND MACHINES USED	56
C. BLOOD TYPING	57
D. INJECTION PROCEDURE AND SHAVING	59
E. ACTIVATED PARTIAL THROMBOPLASTIN TIME TEST	61
F. MATERIALS REQUIRED FOR ONE TRIAL	62
7. VITA	65

ABSTRACT

LIST OF FIGURES

<u>FIGURE</u>		<u>PAGE</u>
1	Fetal Circulation	2
2	Neonatal Circulation	3
3	Blood Pumps	8
4	In Vitro Study	14
5	Hemolysis with a Ventricle Type Pump	15
6	Hemolysis with a Ventricle Type Pump and a Membrane Oxygenator	16
7	Oxygen Transfer vs. Blood Flow Rate at Various Oxygen Pressures I	20
8	Oxygen Transfer vs. Blood Flow Rate at Various Oxygen Pressures II	21
9	Oxygen Transfer vs. Blood Flow Rate at 15 p.s.i.g.	22
10	Carbon Dioxide Transfer vs. Blood Flow Rate I	23
11	Carbon Dioxide Transfer vs. Blood Flow Rate II	24
12	Entire In Vivo System in Operation	26
13	Simple Diagram of In Vivo Circuit	29
14	Cannula	33
15	Diagramatic Representation of Extracorporeal Circuit with Transducer Locations	34
16	PCO ₂ vs. Time	48
17	pH vs. Time	49

LIST OF TABLES

<u>TABLE</u>		<u>PAGE</u>
I	OXYGENATION AND HEMOLYSIS IN SEVERAL DIFFERENT OXYGENATORS	12
II	TRIAL #1 DATA	42
III	TRIAL #2 DATA	43
IV	TRIAL #3 DATA	44
V	TRIAL #4 DATA	45
VI	TRIAL #5 DATA	46

1. INTRODUCTION

A. FETAL AND NEONATAL CIRCULATION

A basic understanding of the fetal and neonatal circulation is imperative in treating respiratory deficiency in the neonate. Miscalculation in the treatment of respiratory deficiency can lead to regression from neonatal to the fetal circulatory pattern.

Before birth the circulation to the lungs is almost completely bypassed by the fetal shunting mechanism (1). This foramen ovale and the ductus arteriosus are the pathways in this shunting (see Figure 1). Prior to birth the PaCO_2 is about 40 torr and the PaO_2 is about 20-30 torr. The high PaCO_2 causes a low pH in the blood. The low pH and oxygen tension cause the vasculature of the lungs to constrict, thus causing an increase in resistance to blood flow. The collapsed lungs prior to birth produce mechanical pressure on the pulmonary vasculature, also causing increased resistance to blood flow. The back pressure caused by the increased resistance causes higher pressure in the right side of the heart than the left. This pressure is relieved by the foramen ovale which connects the right atrium with the left atrium. The foramen ovale acts as a check valve which only permits blood flow from the right atrium to the left atrium. The ductus arteriosus also acts to shunt blood away from the lungs. The ductus arteriosus remains patent as long as the PaO_2 remains low (30-40 torr). Only about 15-20% of the total blood volume reaches the lungs when the shunts are open. This is

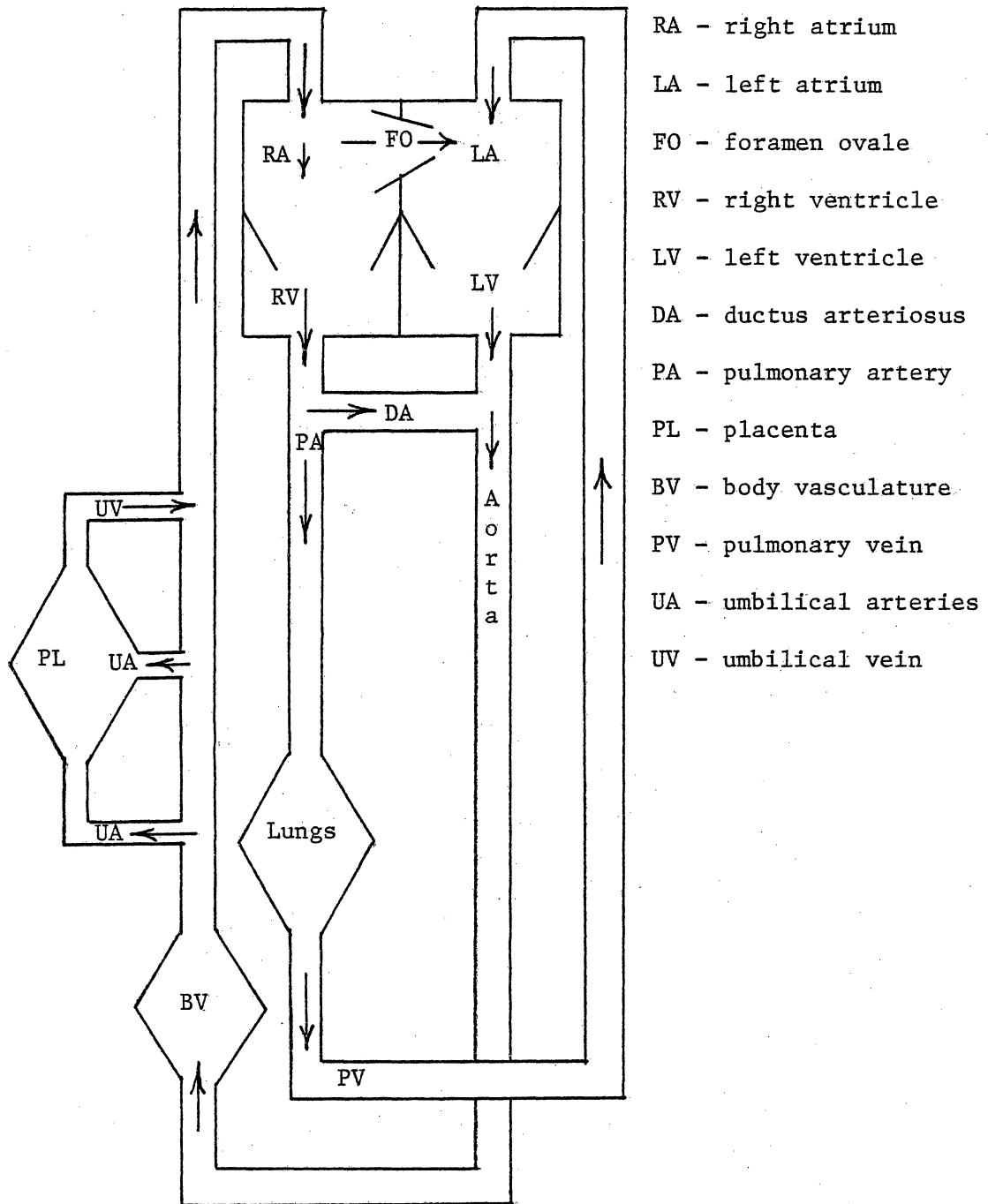


Figure 1. Fetal Circulation (arrows indicate direction of blood flow)

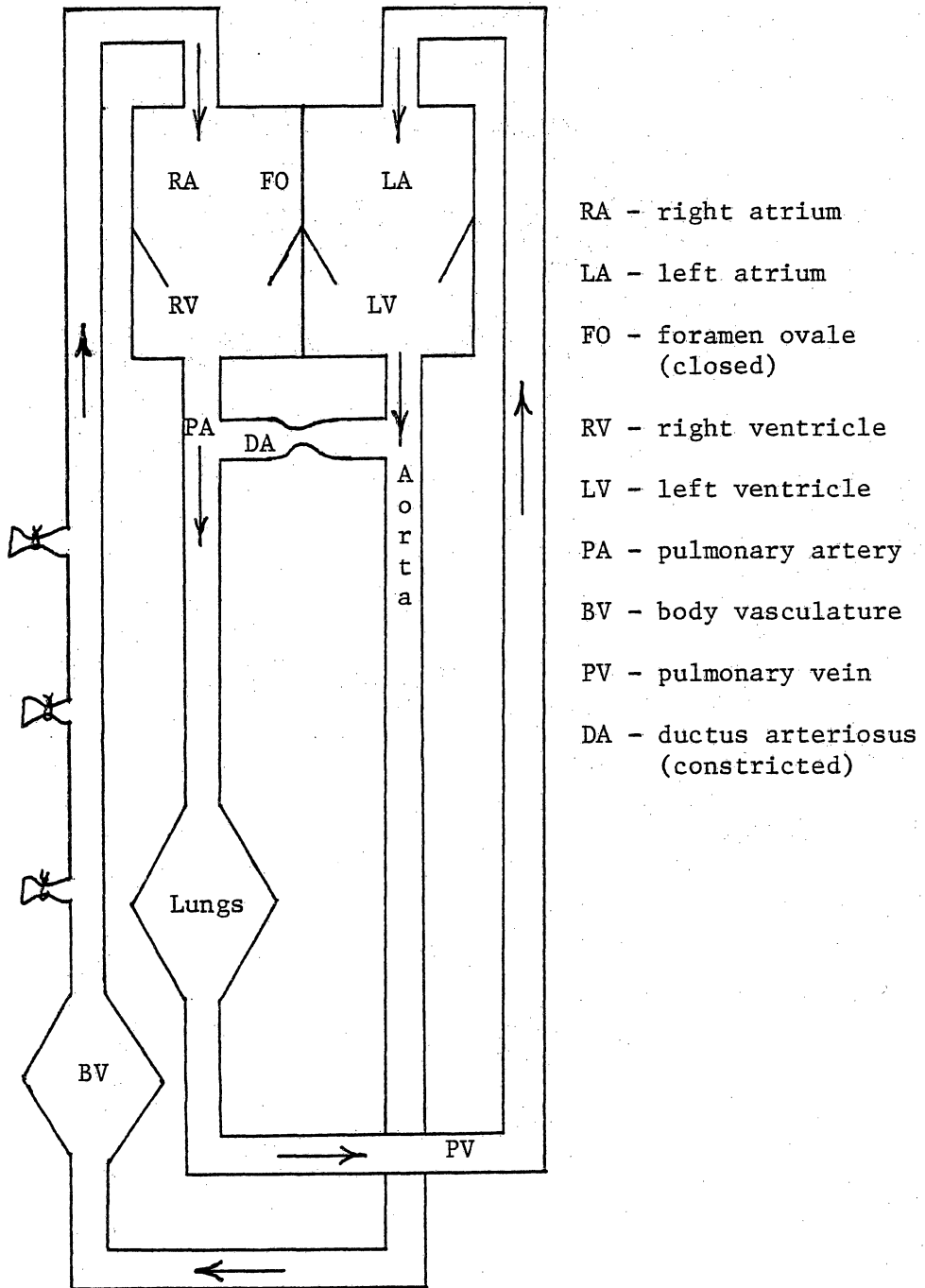


Figure 2. Neonatal Circulation (arrows indicate direction of blood flow)

just enough to support lung tissue metabolism.

At birth the placenta is eliminated from the circulation (see Figure 2). As the neonate breathes, the PaO_2 rises to about 100 torr and the PaCO_2 falls to about 35 torr. The lowering of the PaCO_2 results in a higher pH which causes the lung vasculature to dilate. The increased PaO_2 causes the oxygen sensitive ductus arteriosus to constrict. The reduced resistance to flow of blood in the lungs caused by inflation with the first breaths and dilation of the pulmonary vessels lowers the pressure in the right heart below that of the left heart creating a pressure gradient which holds the foramen ovale closed. The majority of the output of the right ventricle now functionally passes through the lungs.

Any change in the body which causes the PaO_2 to drop and/or the PaCO_2 to rise is dangerous. The fetal circulation may be reinstated without the benefit of the placenta as a site for gas exchange. With only 15-20% of the total blood volume reaching the lungs, 100% oxygen ventilation may not be able to raise the PaO_2 enough to constrict the ductus arteriosus, and the carbon dioxide removal may not be sufficient to raise the pH.

B. NEONATAL RESPIRATORY DISTRESS

Respiratory distress in newborn infants accounts for 30-40% of all newborn deaths each year (2). Of the 60,000 babies born each year with respiratory distress, 25,000 of them die (3). There are many causes of respiratory distress in neonates. Hyaline membrane

disease, known as respiratory distress syndrome, alone kills 12,000 babies annually (4). Other causes of respiratory distress are: Narcosis, prenatal anoxia, perinatal anoxia, intracranial hemorrhage, primary atelectasis, aspiration of amniotic fluid, meconium aspiration, diaphragmatic hernia, lung cysts, pneumothorax, aspiration of food or mucus, pneumonia, lobar emphysema, pulmonary hemorrhage, chilling at birth, tracheal esophageal fistula, and chest wall deformities (5).

Clinical signs of respiratory distress are: Tachypnea, grunting on exhalation, retracting sternum, flaring of external nares, cyanosis on room air, and apneic episodes in severely affected neonates.

The cyanosis associated with respiratory distress is indicative of a low PaO_2 . It is of critical importance that this condition be prevented if possible. Ventilatory support with 100% oxygen is the first step in prevention of immediate regression to the fetal circulation. There is little danger of retrolental fibroplasia if blood PaO_2 is monitored and controlled (2,6). The use of a good ventilator and suitable nursery procedure can raise the survival rate from respiratory distress to 86% (6).

The remaining 14% who do not survive the respiratory therapy are the primary concern of blood oxygenation research. As stated previously, application of 100% oxygen to the lungs after the fetal circulation has been reinstated will not reverse the process because of the very small quantity of blood flowing through the lungs. Use

of an extracorporeal blood oxygenator to substitute for the placenta is a logical solution to this problem. A substitute for the placenta needs a means to provide blood flow. It must also cause minimal damage to the blood.

C. BLOOD OXYGENATORS

Three basic types of blood oxygenators in clinical use are the bubble, disc, and solid membrane. Each type has variations. Some blood oxygenators, known as hybrids, incorporate two principles of blood oxygenation. Each type of blood oxygenator has its advantages and disadvantages.

The bubble oxygenator consists of a chamber containing blood through which oxygen gas is bubbled for oxygenation and carbon dioxide removal. A typical example of a bubble oxygenator in clinical use is the Sarns Miniprime [®] oxygenator.*

The disc type oxygenator exposes a thin film of blood to an oxygen rich atmosphere. Rotating plates or screens are partially submerged in blood. The rotating action carries blood into an oxygen rich atmosphere where it is oxygenated. The Esmond polycarbonate disc oxygenator is representative of this type oxygenator.**

The solid membrane type oxygenator relies upon the properties of oxygen and carbon dioxide which allow them to diffuse across a semi-permeable membrane such as silicon or polypropylene. There is no blood-gas interface as in the bubble and disc type oxygenators. The General Electric-Pierce disposal lung is an example of a solid membrane

oxygenator.***

The Harvey Hybrid Disposable Oxygenator combines two blood oxygenation principles. It acts as both a bubble and a filming oxygenator (as is the disc oxygenator).****

* Miniprime ILF, Travenol Laboratories, Inc., Morton Grove, Ill.

** Fawn Plastics Company, Timokium, Md.

*** Medical Development Operation, General Electric Company,
Schenectady, N.Y.

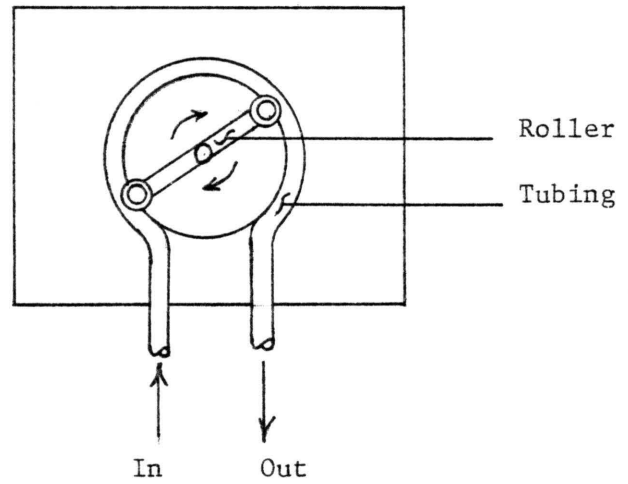
**** William Harvey Corporation, Santa Ana, Ca.

D. BLOOD PUMPS

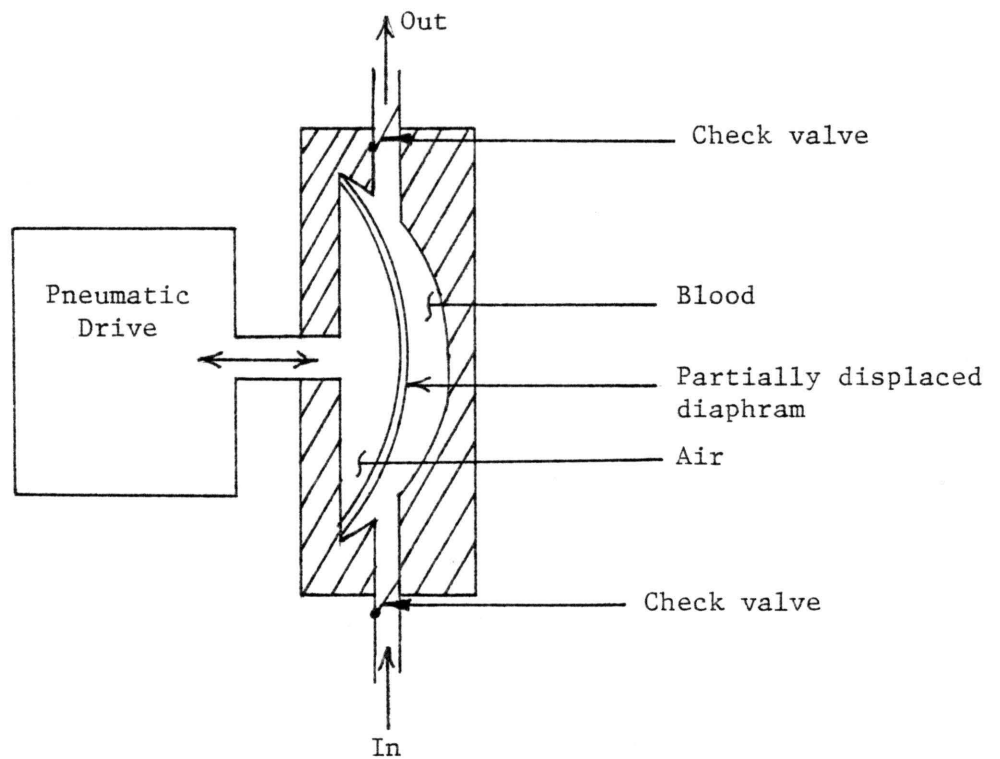
Three basic types of pumps are used for moving blood through an extracorporeal blood oxygenation system. These are the pulsatile, roller, and centrifugal type pumps.

The roller pump functions by the action of rollers on the ends of rotating arms which pinch a section of tubing as they move, thus pushing the blood ahead as they move (see Figure 3.a). The roller pump is in wide clinical use.

The pulsatile pump acts like the ventricles of the heart. It requires check valves to achieve unidirectional flow. A length of pliable tubing or bladder passes through an airtight chamber (see Figure 3.b). By applying alternating positive and negative pressure to the chamber the tube or bladder can be squeezed for systole and expanded for diastole. There are many variations of this principle possible today. The pulsatile pump causes much less damage to the



(a) Roller Pump



(b) Pulsatile Pump (type used in this project)

Figure 3. Blood Pumps

blood than the roller pump (7).

E. PUMPS AND OXYGENATORS

Various combinations of pumps and oxygenators are in the use today. In some, such as the Longmore Pump-oxygenator, the pumping and oxygenation are achieved in a single unit (8).

F. COMPARISON OF OXYGENATOR DAMAGE

Various types of oxygenators have been compared (Table 1.) (9). Hemolysis, indicated by the amount of hemoglobin released into the plasma by lysing of red blood cells, is related to the type of oxygenator used. The least harmful of the blood oxygenators is the solid membrane type, followed by the disc, and then the bubble type oxygenator.

G. COMPARISON OF PUMP DAMAGE

The rate of hemolysis is much lower with the pulsatile pump than the rotary roller pump (10). The hemolysis index (mg of hemoglobin produced in plasma for every 100 ml pumped) is used to compare damage caused by blood pumps and is calculated as follows:

$$\frac{[\text{Plasma Hemoglobin (mg \% above control value)}] [\text{Volume of system (ml)} \div 100]}{100} = \text{absolute hemoglobin in mg}$$

$$[\text{Pump Rate (ml/min)}][\text{Total Pumping Time (min)}] = \text{Total Volume Pumped}$$

$$\frac{[\text{Absolute Hemoglobin}]}{[\text{Total Volume Pumped}]} = \text{mg hemoglobin per ml pumped .}$$

$$[\text{mg hemoglobin per ml pumped}] \left[\frac{100}{100} \right] = \text{mg hemoglobin per 100 ml pumped .}$$

The hemolytic index of the pulsatile pump used was 0.028 mg hemoglobin per 100 ml pumped after 120 hours of pumping a 500 ml volume at 150 ml per min. The hemolytic indices for roller pumps are from 0.04

to 0.23 mg hemoglobin per 100 ml pumped after just a few hours of pumping a 500 ml volume at 4000 ml per min (10).

H. SYSTEM TESTED

The system tested combined the best of the two elements required for a successful extracorporeal oxygenator. A solid membrane blood oxygenator and a pulsatile pump were used. The blood oxygenator is one in which blood flows around silicon capillary tubes through which oxygen flows. The pump is of pulsatile design and has a pneumatic drive system.

I. REVIEW OF PREVIOUS STUDIES

This thesis is a direct continuation of the work done by Randall Lester. There is a thorough review of related literature and an extensive bibliography in Mr. Lester's thesis (9). Reproduction of these would be redundant.

TABLE I. OXYGENATION AND HEMOLYSIS IN SEVERAL DIFFERENT OXYGENATORS (9)

WORKER AND DATE	TYPE OF OXYGENATOR	BLOOD FLOW (ml/min)	OXYGEN: TRANSFERRED OR SATURATION %	HEMOLYSIS (mg%)
Pierce (1969)	DISC MEMBRANE	-----	----- 35 ccO ₂ /min/m ²	115 after 6 hours 50 after 6 hours
Clark (1950)	BUBBLE	-----	95%	300-500 after 1 hour
Zingg (1969)	MEMBRANE TUBES	-----	-----	58 after 1 hour
Katsuhara (1964)	MEMBRANE STACKS	300-400	90%	200-300 after 5 hours (in vivo)
Lande (1968)	MEMBRANE STACKS	500 1500	40 ccO ₂ /min/m ² 75 ccO ₂ /min/m ²	----- -----
Dantowitz (1970)	MEMBRANE LINED CHANNELS	230 920	32 ccO ₂ /min/m ² 92 ccO ₂ /min/m ²	----- -----
Cresenzi (1960)	MEMBRANE STACKS	45	4.0 ccO ₂ /min/m ²	139 after 1½ hours
Rush (1969)	MEMBRANE TUBES	-----	65 ccO ₂ /min/m ²	35 after ¾ hour
Kolobow (1970)	MEMBRANE SPIRAL COIL	-----	40 ccO ₂ /min/m ²	-----
Dutton (1971)	MEMBRANE TUBES	-----	60 ccO ₂ /min/m ²	40 after 24 hours

2. PRELIMINARY IN VITRO STUDY

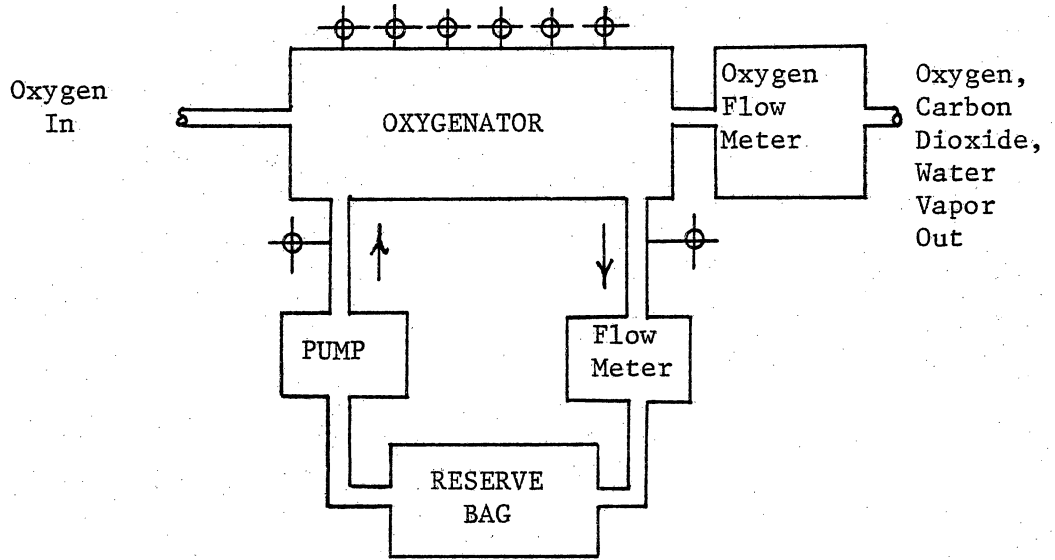
A. OBJECTIVES

The preliminary in vitro study involved the testing of the ability of the oxygenator to oxygenate the blood and to remove carbon dioxide. Hemolysis by the oxygenator was tested during a five day trial. The pump hemolysis was previously determined (9). Based on the results of these tests, the decision to continue with preliminary in vivo work was made.

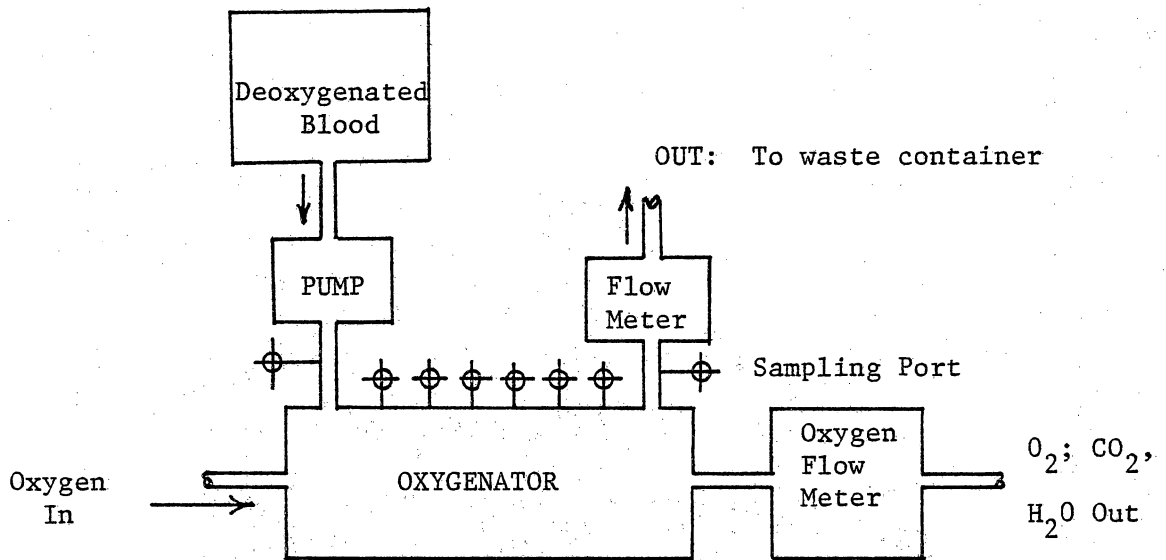
B. MATERIALS AND METHODS

HEMOLYSIS

For the five day hemolysis trial, great care was taken to insure minimal blood damage during collection and handling. Bovine blood for this procedure was obtained by venipuncture of the jugular vein. The blood was collected in standard plastic blood banking bags which contained 67.5 ml of acid citrate dextrose anticoagulant (2.2 gm sodium citrate, 0.73 gm citric acid monohydrate, 2.45 gm dextrose per 100 ml of distilled water) per 450 ml of blood. The circuit for the hemolysis test is shown (Figure 4.a). It was primed by allowing the blood to flow from the bags by gravity. The entire circuit was then placed in the refrigerator at 5°C. A control bag containing 450 ml of blood was also placed in the refrigerator. Plasma hemoglobin was determined over the five day period. These data are presented (Figures 5 and 6).



(a) Hemolysis Study



(b) Oxygenation and Carbon Dioxide Removal Study

Figure 4. In Vitro Study

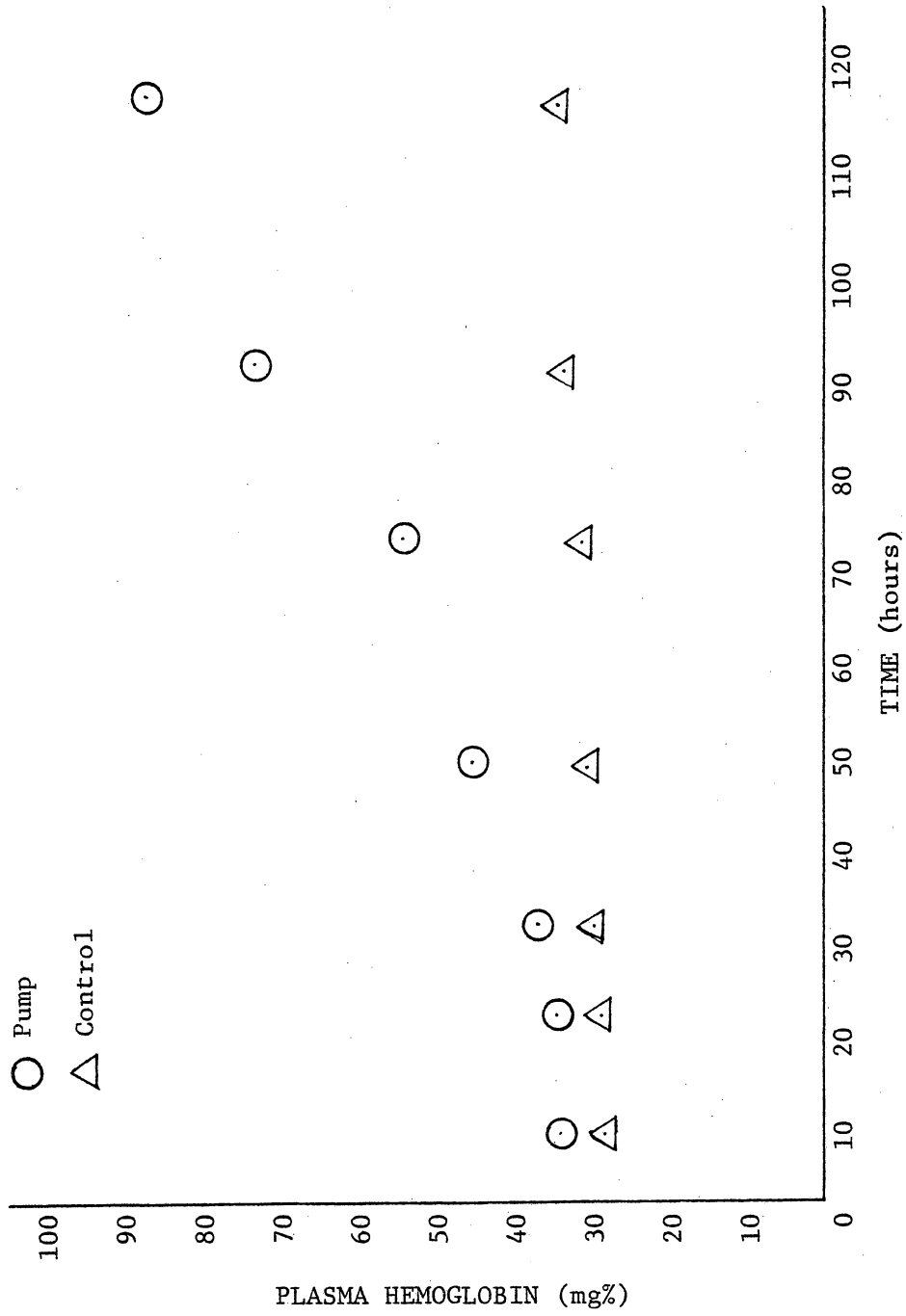


Figure 5. Hemolysis with a Ventricle Type Pump (9)

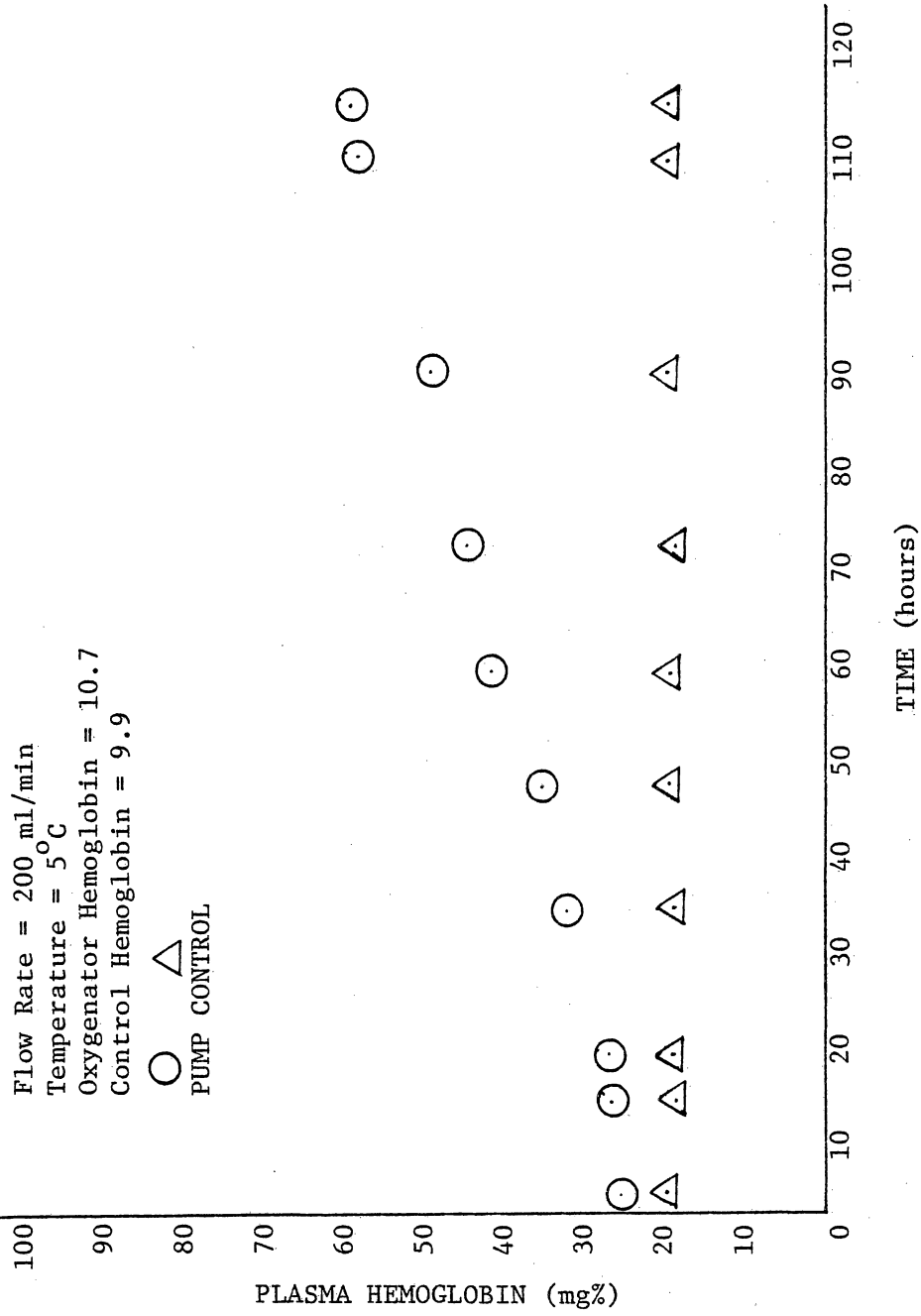


Figure 6. Hemolysis with a Ventricle Type Pump and Membrane Oxygenator

OXYGEN AND CARBON DIOXIDE TRANSFER

For these studies fresh bovine blood was collected with acid citrate dextrose as the anticoagulant. 67.5 ml of acid citrate dextrose was used per 450 ml of blood. As large quantities of blood were used in this study, the bovine blood was collected in an 11.32 liter plastic jug which had been thoroughly rinsed with normal saline and contained the acid citrate dextrose at the time of collection. The blood was collected from a severed jugular vein of a sacrificed cow. The blood was deoxygenated by bubbling nitrogen through it. As shown in Figure 4.b, the deoxygenated blood was pumped through the oxygenator and collected in a waste container. Samples of blood could be taken at eight different sample ports in the system. Six of these sampling ports were located on the oxygenator and the other two were located at the input and output lines of the oxygenator. A variety of tests were performed to determine oxygen and carbon dioxide transfer rates. Test data is presented in Figures 7, 8, 9, 10, and 11. PO_2 , PCO_2 , pH, hemoglobin saturated with oxygen, hemoglobin saturated with carbon monoxide, hemoglobin, oxygen flow rate, oxygen pressure, and blood flow rate were also ascertained.

OXYGEN TRANSFER RATE

The oxygen transfer rate is dependent upon several factors:

- 1) Oxygen binding capacity of hemoglobin.
- 2) Surface area of the membrane.
- 3) Rate of hemoglobin passing through the oxygenator.
- 4) Starting percentage of hemoglobin saturated with oxygen.

5) Final percentage of hemoglobin saturated with oxygen.

The hemoglobin value for each test was determined by each of two methods. The IL Co-Oximeter and the Coleman Spectrophotometer were used. The oxygen binding capacity of bovine hemoglobin is 1.36 cc of O_2 /gm. The surface area of the membrane was 0.18 m^2 . The starting and final percentage of hemoglobin saturated with oxygen were determined using the IL Co-Oximeter. A sample calculation to determine the oxygen transfer is as follows:

$$[\text{Hb}(\text{gm}/100 \text{ blood})] [\text{Flow Rate}(\text{ml}/\text{min})] = [\text{gm Hb through oxygenator}/\text{min}]$$

$$[\text{Percentage of Hb Sat with } O_2, \text{Final}] - [\text{Percentage of Hb Sat with } O_2, \text{In}] =$$

Net change in Hb saturated with oxygen

$$[\text{gm of Hb through oxygenator}/\text{min}] [\text{Net change in Hb sat with } O_2] = \text{gm}$$

of hemoglobin oxygenated per minute

$$[\text{gm of Hb oxygenated}/\text{min}] [1.36 \text{ cc } O_2/\text{gm Hb}] = \text{cc of } O_2 \text{ across membrane}$$

per minute

If the membrane is 0.18 m^2 , then the amount of oxygen transferred per minute per square meter is equal to: cc O_2 across membrane per minute divided by the surface area of the membrane.

CARBON DIOXIDE TRANSFER RATE

The carbon dioxide transfer across the membrane is determined by measuring input and output PCO_2 and converting PCO_2 to carbon dioxide

content by the following equation:

$$\text{Carbon Dioxide Content} = 0.041611(\text{PCO}_2)^{0.6156} \text{ liters/liter}$$

This difference in carbon dioxide content is multiplied by the blood flow rate to find carbon dioxide transfer across the membrane.

Dividing cc CO₂ by 0.18 m² gives the cc CO₂ transferred per minute per square meter.

C. DISCUSSION AND CONSLUSIONS OF IN VITRO STUDY

The data obtained in this in vitro study provided enough evidence to warrant an in vivo evaluation of the system. The hemolysis rate was extremely low (see Figure 5). Oxygenation of the blood seemed to have a membrane stabilizing effect on the red blood cells. Oxygenation actually decreased hemolysis as can be seen by comparing Figures 5 and 6.

Oxygen transfer in the new system was excellent. As can be seen in Figure 9, up to 189 cc of oxygen per square meter of membrane can be transferred at the blood flow rate of 250 ml/min. This is twice the oxygen transfer of the Dantowitz membrane oxygenator (see Table 1).

The carbon dioxide transfer was good enough to suggest that in vivo evaluation should be attempted. Prior to the in vivo evaluation of the blood pump and oxygenator system, several alterations to enhance carbon dioxide transfer were made to the outer casing of the oxygenator. These alterations could have no effect on the rate of hemolysis.

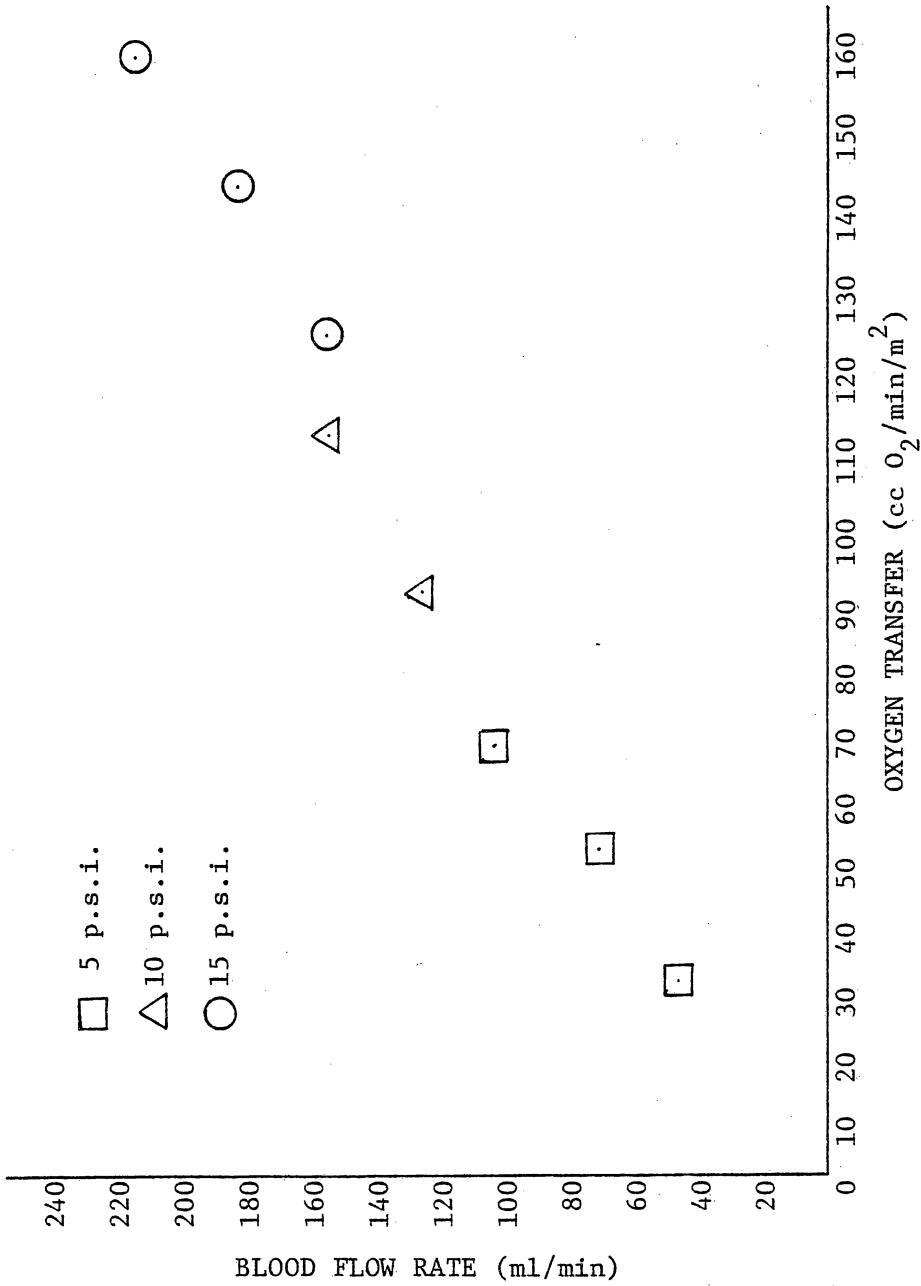


Figure 7. Oxygen Transfer vs. Blood Flow Rate at Various Oxygen Pressures I

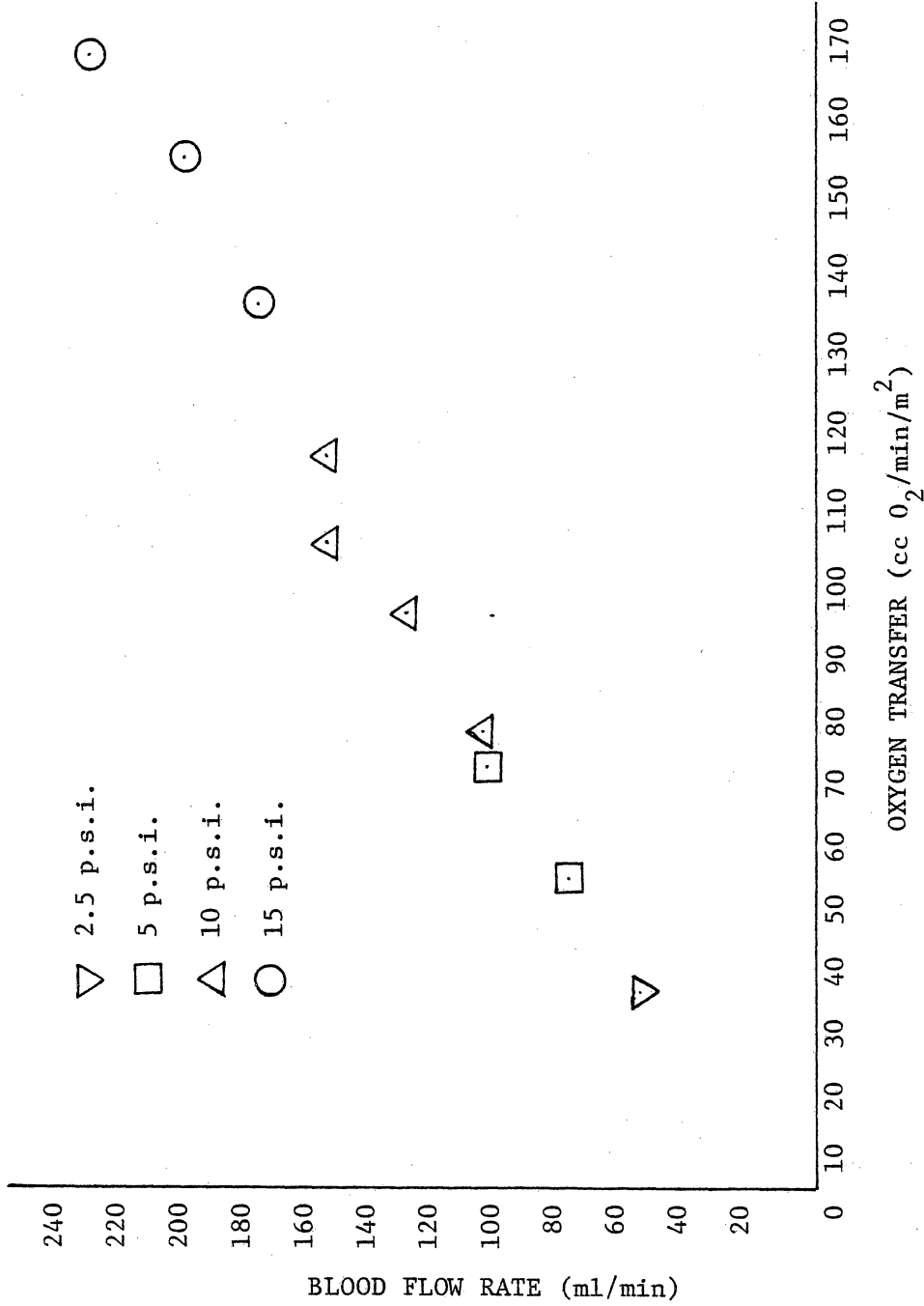


Figure 8. Oxygen Transfer vs. Blood Flow Rate at Various Oxygen Pressures II

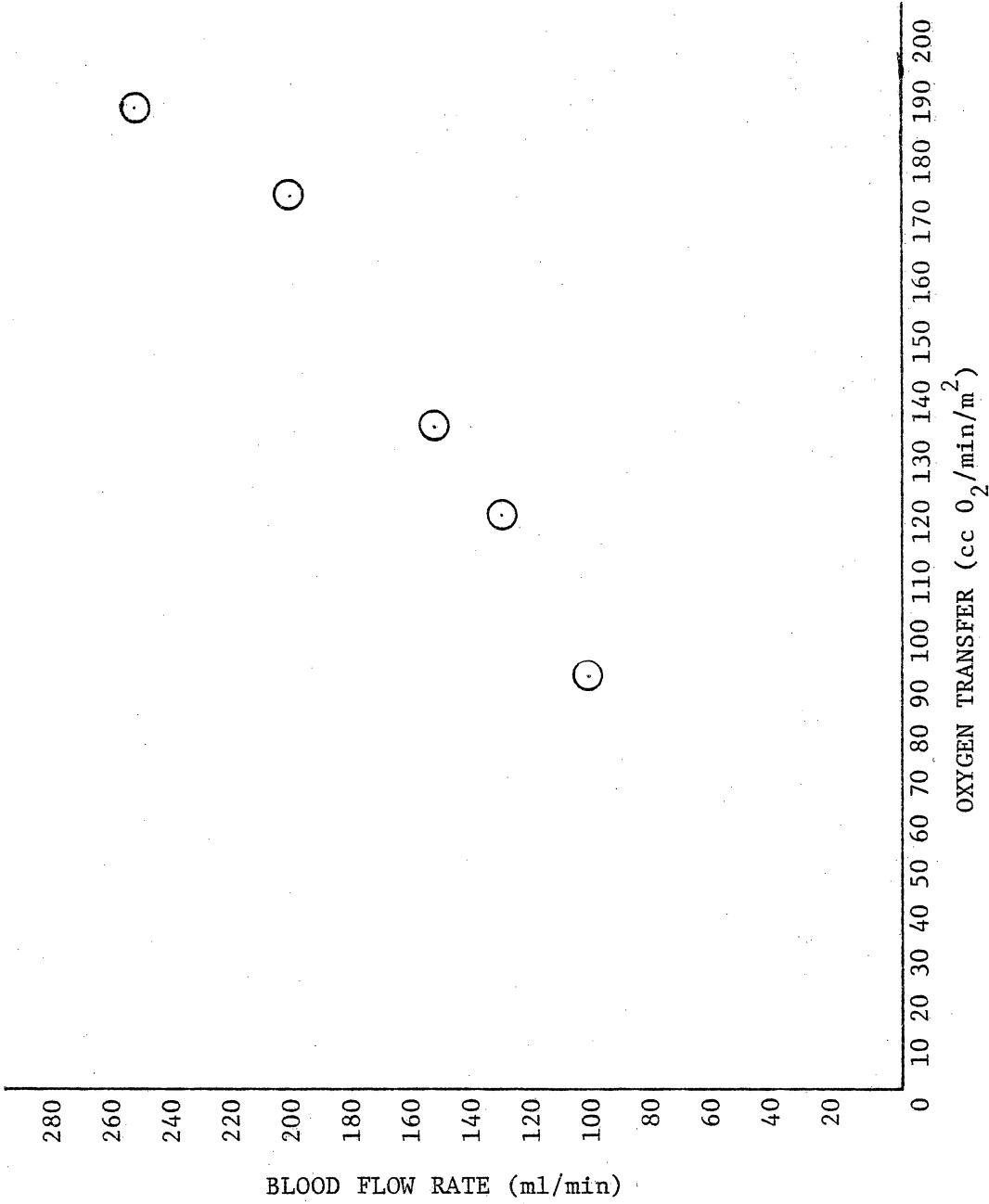


Figure 9. Oxygen Transfer vs. Blood Flow Rate at 15 p.s.i.g.

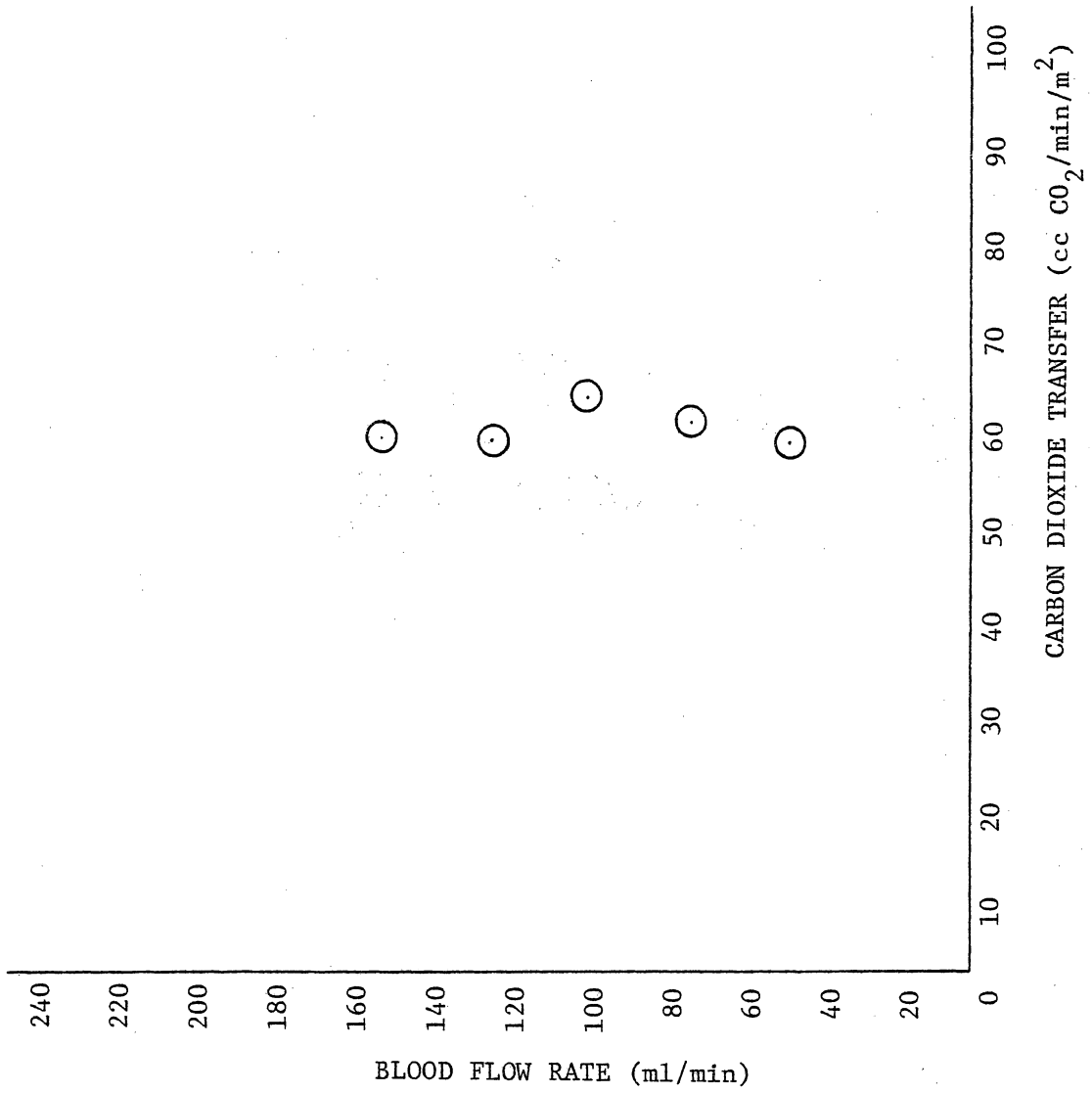


Figure 10. Carbon Dioxide Transfer vs. Blood Flow Rate I

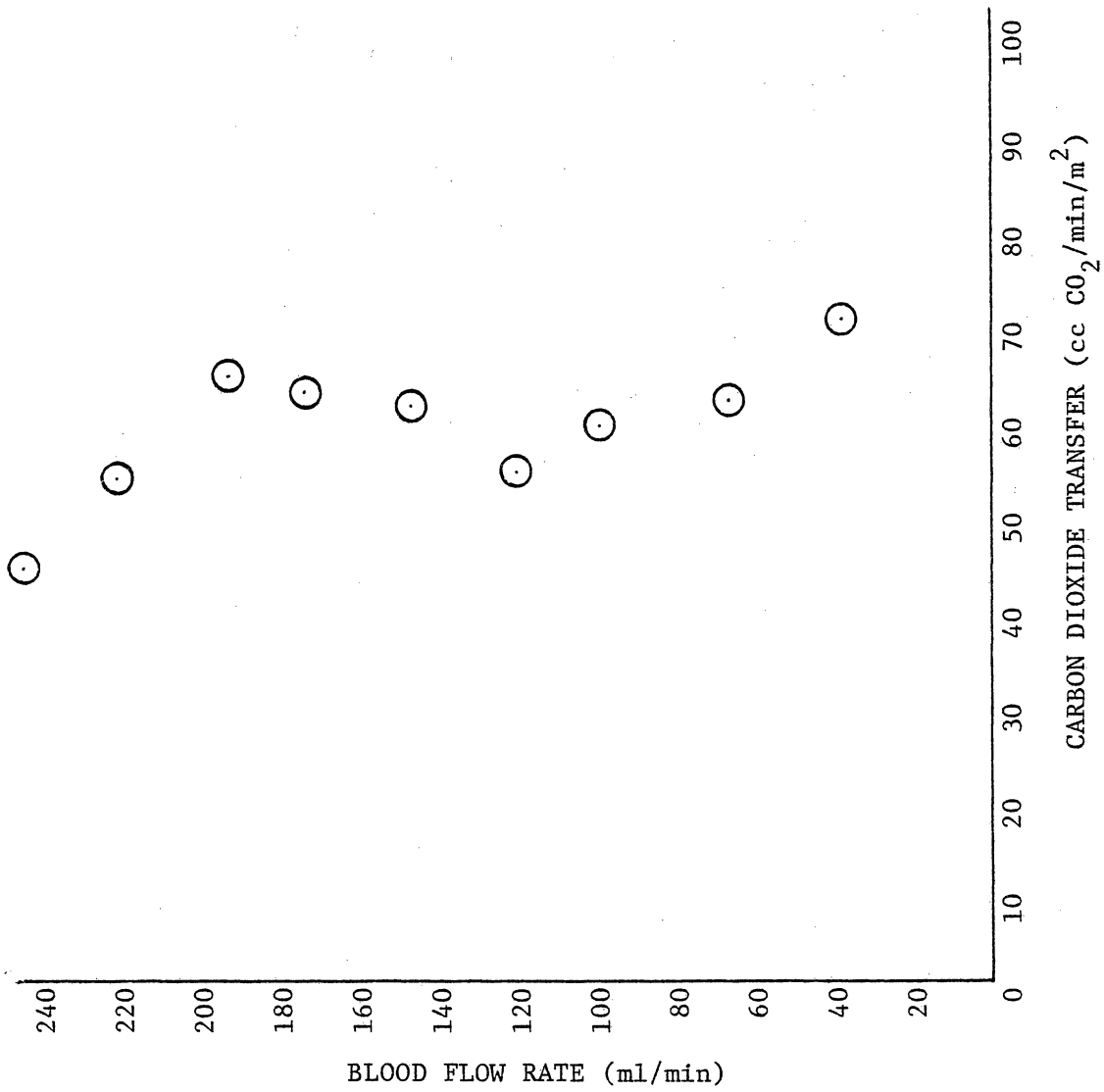


Figure 11. Carbon Dioxide Transfer vs. Blood Flow Rate II

3. IN VIVO STUDY: SHORT TERM ANIMAL WORK

A. PRE-TRIAL CONSIDERATIONS

The transition from in vitro to in vivo work brought it many factors to be considered. As can be seen in Figure 12, the in vivo system was very complicated.

The first consideration was the choice of animal to be used in the experiments. Rabbits were chosen for a variety of reasons. Size of the rabbit is close to that of the newborn infant. Rabbits are easy to care for. Because the blood pump and oxygenator system required priming with blood compatible with the animal in the experiment, an animal which could be easily typed for blood compatibility was needed. 97% of all rabbits have the same blood group (11), and rabbits are inexpensive relative to other laboratory animals of the size range required.

Similar studies with oxygenators on rabbits have been conducted (11). Cannulas were placed in the carotid arteries and jugular veins. After an anatomical study of the veins and arteries of the rabbit, we decided that the use of the carotids and jugulars were the best routes for connecting the new system to the rabbit.

The anticoagulant used in the in vitro study was acid citrate dextrose. It was not possible to use acid citrate dextrose for the in vivo work. Rapid infusion of this anticoagulant can greatly decrease blood calcium resulting in tetany and convulsions (12). Heparin was chosen as the anticoagulant. Heparin therapy is easy

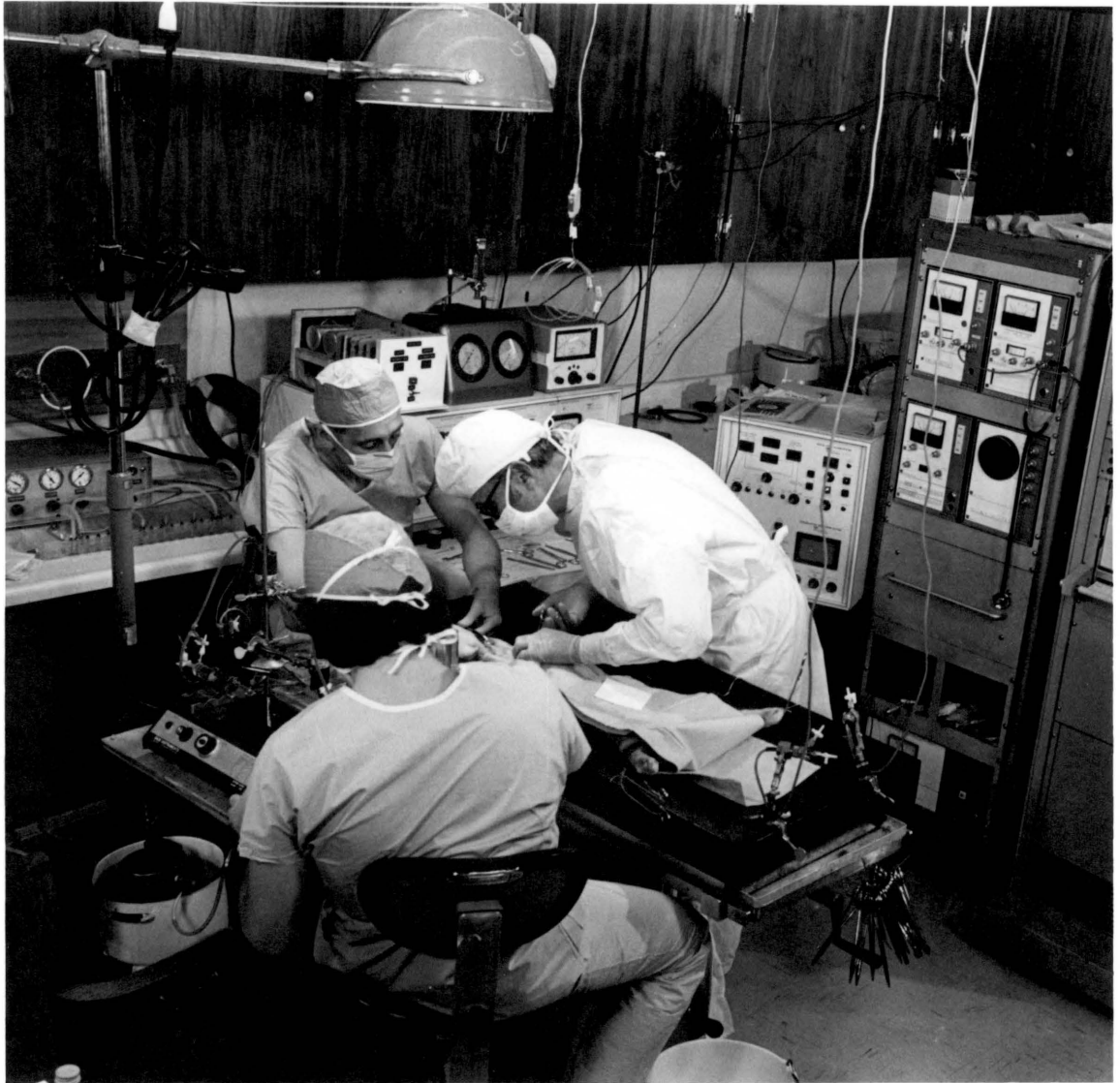


Figure 12. Entire In Vivo System During Surgery

to monitor (13), and over heparinization can be controlled by the administration of a heparin neutralizer, protamine sulfate (14).

To evaluate the blood pump and oxygenator we needed a way to stop respiration in the lungs. After trying several methods during our first attempts at hooking the rabbits up to the circuit we found that the best way was to administer carbon dioxide and nitrogen to the lungs via a face mask secured to the rabbit. The PCO_2 of the exhaled gasses of the rabbit at rest was determined to be 27 torr. By mixing nitrogen and carbon dioxide we were able to match this exhaled PCO_2 , thus there was no net loss of carbon dioxide across the lung. All exchange of gases took place across the membrane of the oxygenator.

The anesthetic used was sodium pentobarbital given intravenously (15). This anesthetic was chosen because it presented us with no complications and it was easy to administer.

B. MATERIALS AND METHODS

Each animal trial on the pump and oxygenator system involved basically four steps:

- 1) Presurgical
- 2) Surgical
- 3) Hook-up of the system
- 4) Data collection and maintenance

(See Appendix for a list of materials needed for one trial)

The main objective of surgery was to place cannulas in each of two carotid arteries and one jugular vein. The cannulas were connected to the system containing the blood pump and oxygenator. Blood flowing out the carotid arteries was pumped through the oxygenator, oxygenated, and returned to the rabbit via the jugular vein cannula. A simple diagram showing the experimental set-up is shown (Figure 13).

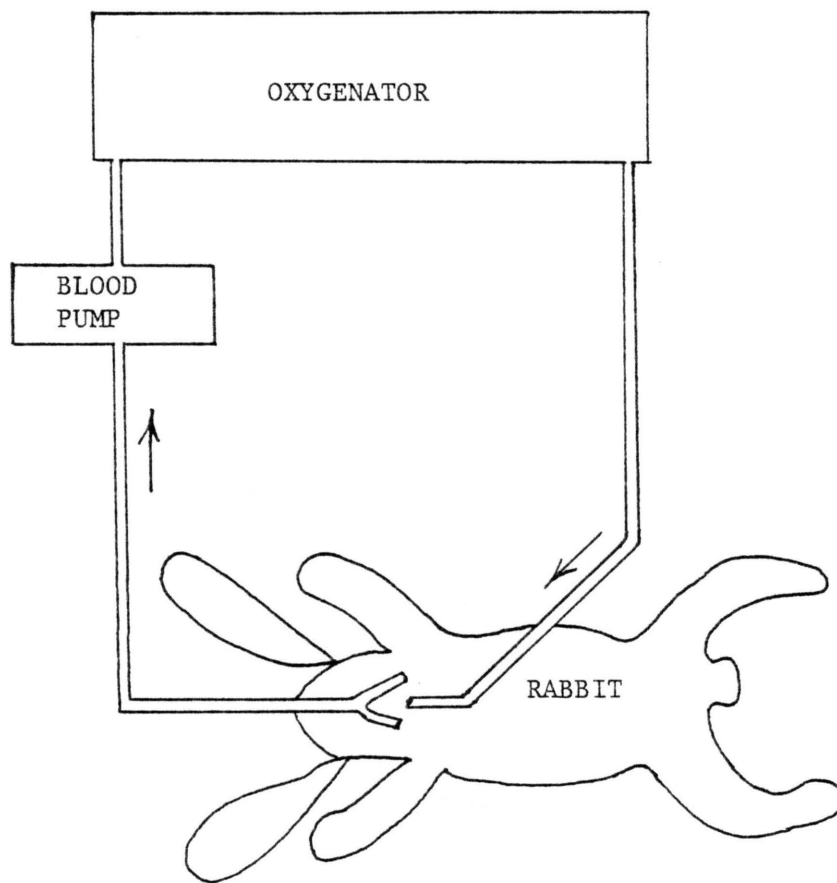


Figure 13. Simple Diagram of In Vivo Circuit

PRESURGICAL PROCEDURE

PROCUREMENT AND BLOOD TYPING

Rabbits weighing 2 to 4 kilograms were obtained and kept in separate cages. The test performed on each rabbit was the determination of ABO group and Rh factor. Each of the thirty-one rabbits used had blood type B with a negative Rh factor. (See Appendix for blood typing procedure.)

PRIMING THE SYSTEM

To prime the system several rabbits were sacrificed and blood was collected. The rabbits were shaved and surgical anesthesia was applied by administration of 20 mg of sodium pentobarbital per kilogram body weight. (See Appendix for shaving and injection procedure) 225 units of heparin were given to the rabbits with the surgical anesthesia. To prevent clotting the blood was collected in flasks which contained three cc of heparin-normal saline (75 units/cc). Additional anesthesia was administered if required. The throat region was rinsed with sterile normal saline to minimize hair collected in the blood. A suprasternal midline incision through all tissue planes to the trachea was made. The carotid arteries were identified and severed with the rabbit in the inverted position over the collection funnel. Each animal provided 50-75 cc of blood.

Prior to priming the circuit, the blood pump and oxygenator system was filled with sterile normal saline and heparin (40 units/cc) for

twenty-four hours.

The blood pump and blood oxygenator system was filled with the donor blood which had been filtered through a fine nylon mesh filter to remove any clots or hair. The total priming volume was approximately 175 cc. To prime the system, the same configuration as shown in Figure 4.a for the in vitro tests was used. Blood pumped through the system forced the heparin-saline solution out ahead of it. The buffer bag was then filled and the system was closed (see Figure 4.b).

SURGERY

The rabbit to be used in the system was shaved and anesthetized as previously described with the exception that no heparin was given with the sodium pentobarbital. It was then secured to the operating table on its back. A skin incision directly over the trachea was made starting at the upper third of the throat and extending caudally for about five centimeters. The fascia was cut and the carotid arteries and jugular vein were exposed using blunt dissection. Each of the three vessels was cannulated following this procedure: Two loose ligatures were passed under the vessel. The ligature at the cephalic end of the vessel was tied. The ligature at the caudal end of the vessel was gently raised to expose the vessel for cannulation. A Teflon [®] cannula was then placed in the vessel via a small incision cut at a slight angle to form a "V" in the vessel wall. The cannula most commonly used was 0.071" O.D. and 0.047" I.D. After the cannula was inside the vessel in the caudal direction, the caudal ligature was

tied around and it was secured by tying the cephalad ligature around it also. Each cannula was filled with a mixture of heparinized normal saline (500 units/cc) and normal saline. The amount of heparin was dependent upon how much heparin was required to initially begin anticoagulant therapy in the rabbit. This was determined to be 220 units of heparin per kilogram body weight. This number was divided equally among the three cannulas placed. A series of connectors (see Figure 14) adapted the extracorporeal ends of the cannulas to a standard luer-lock stopcock. The end of the cannula placed could be cleared by injecting normal saline through the three way stopcock.

HOOK-UP

The next step was to attach the vessel cannulas to the rest of the system. To minimize blood loss the silicone tubing between the adapters was clamped, the stopcock removed, and the luer fitting was attached to the corresponding fitting in the circuit. After each of the three cannulas was attached to the circuit, the clamps were removed and pumping commenced.

TESTS AND MAINTENANCE

The total system had many inputs and outputs (see Figure 15). When possible, in line data was collected. A detailed account of the course of the blood in the system is as follows: The blood flowed through the arterial cannulas (A) from the rabbit. It passed into the buffer bag (D) which was in the system to prevent back pressure from

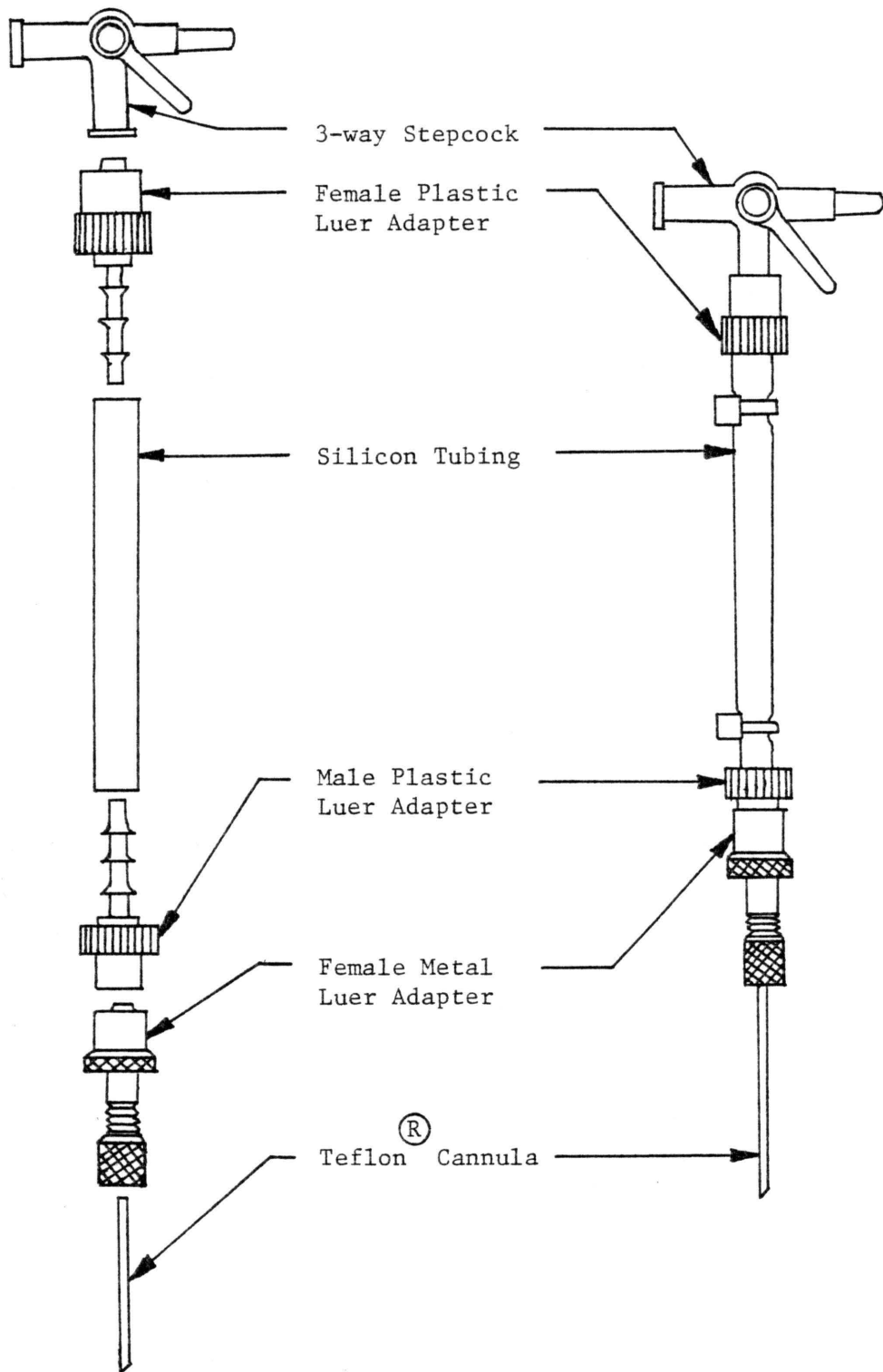


Figure 14. Cannula

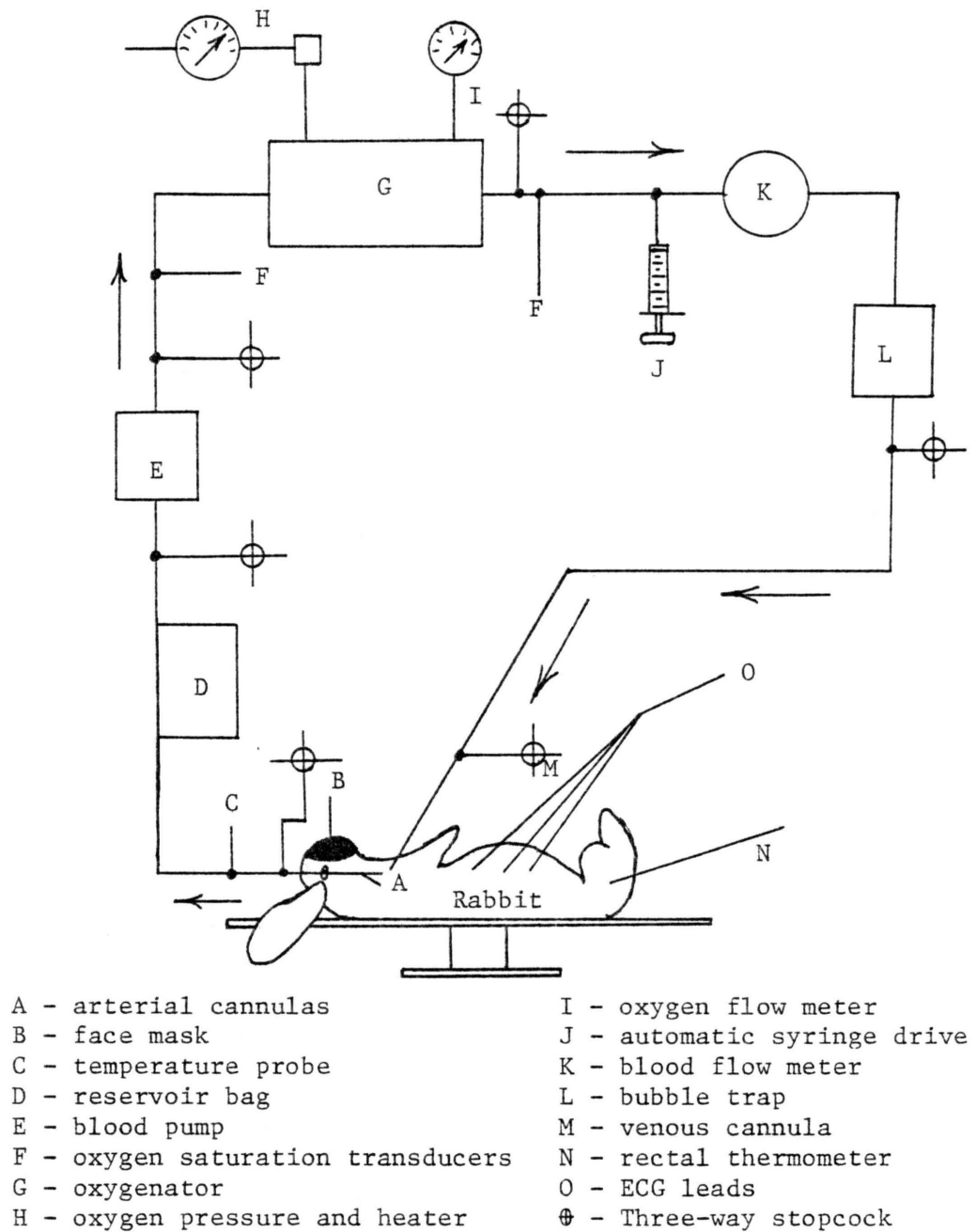


Figure 15. Diagrammatic Representation of Extracorporeal Circuit with Transducer Locations

the pump and to respond to changes in intracorporeal volume changes of the rabbit. The blood flowed into the pump (E) via a check valve and was pumped out of the pump via a check valve. It passed into the oxygenator (G) where it lost carbon dioxide and was oxygenated. As the blood passed through the oxygenator it was heated to maintain normal body temperature. As the oxygen rich blood passed out of the oxygenator, heparin and dextrose were added via the automatic syringe drive (J). The blood passed through a bubble trap (L) to remove any air bubbles. The oxygenated blood then flowed back into the venous cannula (M) and back into the rabbit to supply the body tissues with oxygen.

Regulation and monitoring of homeostasis after the rabbit was attached to the system were accomplished in many ways. When possible, in line measurements were made and continuously recorded on a Sanborn 8 channel chart recorder. Blood flow rate (ml/min), electrocardiogram, blood temperature, percentage of hemoglobin saturated with oxygen in and out of the rabbit, and arterial and venous pressure could be recorded in this way. Each of these parameters on the recorder was monitored and adjustments were made to maintain homeostasis.

BLOOD GAS AND pH

At regular intervals 2.5 cc samples of blood were drawn from the arterial and venous sides of the oxygenator. These samples were tested for PO_2 , PCO_2 , pH, and percentage of hemoglobin saturated with oxygen. The PO_2 , PCO_2 , and pH were ascertained by using the

Instrumentation Laboratory Ultra-Micro pH and Blood Gas Analyzing System Model 113-S1. The percentage of hemoglobin saturated with oxygen was determined with the Instrumentation Laboratory Co-Oximeter (R) Model 182 (hemoglobin was also determined from this sample). In line percentages of hemoglobin saturated with oxygen were also determined by the use of an experimental oximeter (16). Changes in any of these four parameters could be regulated within certain limits.

PO_2 could be controlled by changing the oxygen flow rate or oxygen pressure. Percentage of hemoglobin saturated with oxygen could be controlled in the same manner.

PCO_2 was mainly affected by blood flow rate through the oxygenator. Flow over 225 ml/min were desirable to maintain normal PCO_2 . Increased oxygen flow through the oxygenator also aided in removal of carbon dioxide from the blood.

The pH dropped as the PCO_2 went up as would be expected. Control of the PCO_2 was the main way of controlling pH. Administration of sodium bicarbonate buffer solution had some effect on stabilizing the pH.

Each of these four parameters is related to the other three and constant awareness of this was important.

BLOOD COAGULATION

It is imperative that blood clotting does not take place in the oxygenator system and the patient. For this reason an anticoagulant, heparin, was used. The activated partial thromboplastin time test

is sensitive to heparin therapy (13). An activated partial thromboplastin time two to three times normal (12-20 seconds) was optimal in preventing clots, but at the same time preventing hemorrhaging internally. Heparin is metabolized by the liver at a rate of around 50% per hour. In order to maintain a constant level of heparin in the blood, an automatic syringe drive was used. Along with the heparin, fluids, electrolytes, and glucose were administered to maintain metabolic and electrolyte balance. The heparin dosage to continuously infuse was determined as follows:

Body Weight (kg) X 0.44 cc heparin/kg/hr = __cc heparin/hr
 __cc heparin/hr + glucose normal saline = 20 cc of solution in syringe.

Sample Calculation for a 2 kg rabbit:

2 kg X 0.44cc heparin/kg/hr = 0.88cc heparin/hr
 0.88cc heparin/hr + glucose normal saline = 20cc solution in syringe.

75 units/cc of heparin-normal saline was used. The syringe drive was turned on one hour after an initial dosage of heparin was given the rabbit via the cannulation technique. The activated partial thromboplastin time test (see Appendix) was periodically performed to insure that the desired clotting time of two to three times normal was maintained. Heparin could be neutralized with protamine sulfate.

ELECTROCARDIOGRAM

The standard lead II configuration was used for the electrocardiogram. Subcutaneous needle electrodes were used. The electrocardiogram was monitored on the ECG-EEG Fetal Monitoring System- Medical Development Corporation, model 73-1-1, and recorded on the Sanborn eight channel recorder. The shift created in the baseline of the ECG was enough to detect respiratory rate. Respiratory rate was calculated from the trace on the chart recorder.

TEMPERATURE

In line temperature measurements were made by use of a thermister. These values were recorded on the Sanborn chart recorder. Body temperature was monitored by use of a rectal thermometer.

Normal body temperature was maintained in several ways. The radiant heat created by the surgical light was directed toward the entire system. The oxygen was heated before being passed into the oxygenator. A heating pad was placed under the rabbit and warm towels were placed over the rabbit. A plastic cover was placed over the towels to conserve heat.

BLOOD PRESSURE

The pressure of the blood entering and leaving the rabbit was monitored during the first few trials. This was done by using Statham strain gage transducers and the General Electric Patient Monitor. These values were recorded on the Sanborn recorder.

ANESTHESIA

During the entire duration of the trials, surgical anesthesia was maintained. Sodium pentobarbital (64.8 mg/cc) was injected into the extracorporeal circuit at the first sign that the rabbit was awakening. Usually 0.25 cc of sodium pentobarbital was administered at one hour intervals.

C. INDIVIDUAL TRIALS

TRIAL #1

The main objective of this trial was to practice placement of the cannulas and to accustom the team to the entire operation. In this experiment a plastic bag was placed over the head to prevent ventilation. A misjudgement in the placement of the syringe drive was disastrous. The heparin glucose-normal saline solution was sucked into the system because the syringe drive was connected on the negative pressure side of the pump. One and a half hours worth of heparin solution was infused in a matter of minutes. The rabbit died minutes later of massive hemorrhage. Valuable PCO_2 and pH data and knowledge about the dynamics of the system were gathered and are shown in Table 2.

TRIAL #2

Although little data was collected in this trial, the presurgical and surgical portions of this experiment were much shorter in time.

Several refinements, such as the placement of the syringe drive on the positive pressure side of the pump were tested. The cannula assembly shown Figure 15, was first used in this experiment. Again, as in Trial #1, a plastic bag was placed over the rabbit's head to prevent ventilation. Data from this trial is presented in Table 3.

TRIAL #3

In this trial it became quite evident that there were several things which could be done to improve chances of the rabbit surviving for a longer period of time. It was in this experiment that the flow rate of 225 ml/min was determined to be the optimal flow rate for total support of respiration by use of the oxygenator. In this experiment it was found that administration of heparin to the donor rabbits supplied us with more blood per rabbit. The use of the face mask supplying carbon dioxide and nitrogen was initiated in this experiment. The cause of death in this experiment was the loss of flow from the carotid cannulas. The blood in the buffer bag was pumped into the animal thereby increasing its blood volume to perhaps one and one half times normal. This resulted in massive hemorrhage. Data from this trial is presented in Table 4.

TRIAL #4

In this experiment similar results were obtained as in trial #3. Problems with flow from the carotid arteries again developed. As long as the blood flow remained close to 225 ml/min the PCO_2 and pH were

controlled. The control of coagulation time was attempted with the use of protamine sulfate. Data from this trial is presented in Table 5.

TRIAL #5

This last experiment had the greatest potential for success. There was better control of the body temperature and the coagulation time was within desirable limits. Upon autopsy the rabbit was found to be pregnant and it had an infection of one of the uterine horns. There was no indication of hemorrhage. Stress placed on an already infected, pregnant animal was believed to be the cause of death. Data from this trial is presented in Table 6.

D. DISCUSSION OF IN VIVO TESTING:

OXYGENATION

As can be seen from the data in each of the five tables, there is no problem in oxygenating the blood with this system. Blood entering the oxygenator with a PO_2 of 15 torr, exited the oxygenator with a PO_2 of 475 torr. Considering that blood normally enters the lung with a PO_2 of 40 torr and exits the lung with a PO_2 of 104 torr, it is quite clear that the problem of oxygenation does not exist in this system. In all of the trials the percentage of hemoglobin saturated with oxygen went to 100% with just one pass through the oxygenator.

Table II. Trial #1 Data

Time (min)	Rabbit	PO ₂ (mm Hg)	PCO ₂ (mm Hg)	pH	O ₂ Sat (%)	Hb. (gm/100 ml)
15	OUT	15	40	7.34	23	7.4
	IN	475	28	7.5	100	7.4
30	OUT	27	52	7.15	23	7.4
	IN	475	37.5	7.27	100	7.4
45	OUT	28	55	7.1	23	7.4
	IN	625	40.5	7.17	100	7.4
60	OUT	28	55	7.04	58	7.4
	IN	625	40.5	7.115	100	7.4
75	OUT	27	60	6.98	43.1	6.2
	IN	525	37.4	7.11	100	6.2
90	OUT	35	69	6.87	37.2	6.0
	IN	575	44.0	6.96	100	6.0

Table III. Trial #2 Data

Time (min)	Rabbit	PO ₂ (mm Hg)	PCO ₂ (mm Hg)	pH	O ₂ Sat (%)	Hb (gm/100 ml)	Coag Time (sec)	Blood Flow (ml/min)
20	OUT			7.185		10.0		
	IN			7.3		10.0		
40	OUT			7.11		10.0		
	IN			7.17		10.0		
60	OUT			7.07		10.0		
	IN			7.13		10.0		90

Table IV. Trial #3 Data

Time (min)	Rabbit	PO ₂ (mm Hg)	PCO ₂ (mm Hg)	pH	O ₂ Sat (%)	Hb (gm/100 ml)	Coag Time (sec)
0	OUT IN		44	6.8	100	11.3	113.7
15	OUT IN		44 44	6.8 6.8	64 100	10.0 10.0	
25	OUT IN		52.5		49.9 100	9.9 9.9	
40	OUT IN		50	7.3	60 100		113.8
80	OUT IN		60 48	6.8 6.8	26 100	10.6 10.6	

Table V. Trial #5 Data

Time (min)	Rabbit	PO ₂ (mm Hg)	PCO ₂ (mm Hg)	pH	O ₂ Sat (%)	Hb (gm/100 ml)	Coag Time (sec)	Blood Flow (ml/min)	Blood Temp (C°)	O ₂ Flow (l/min)	O ₂ Press (psi)	Heart Rate (beats/min)	Resp Rate (breaths/min)
0	OUT IN	110	42	7.485	100	8.9		250	32.5	0	0	36	
30	OUT IN	120	29.5	7.5	100	10.2	85.8	145	34.8				
60	OUT IN	25 585		7.42 7.46	58.4 100	10.2 10.2		255	31.9	4	15	150	50
80	OUT IN	29 525		7.3 7.3	43.8 100	10.2 10.2		225	31.25	4	15	124	
110	OUT IN	34 440	38 31		44.6 100	10.8 10.4	52.4	240	31.5	4	15		25
155	OUT			7.106 7.106			96.4	190				210	25

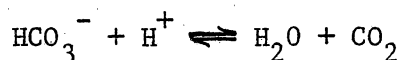
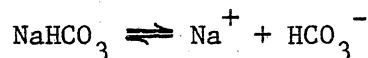
Table 6. Trial #5 Data

Time (min)	Rabbit	PO ₂ (mm Hg)	PCO ₂ (mm Hg)	pH	O ₂ Sat (%)	Hb (gm/100 ml)	Coag Time (sec)	Blood Flow (ml/min)	Blood Temp (C°)	O ₂ Flow (l/min)	O ₂ Press (psi)	Heart Rate (beats/min)
0	OUT IN	24	66	7.21 7.46	35	8.6	28.5	175	35	5	16	215
30	OUT IN	32 760			48.7 100	8.7 8.9		175 175	37			

CARBON DIOXIDE AND pH

Reduction of the PCO_2 of the blood took place in all trials. Through trial and error it was found that a blood flow rate through the oxygenator of 225 ml/min or more is required to provide adequate removal of carbon dioxide. As can be seen from Figure 16, the PCO_2 had a significant decrease as the blood passed through the oxygenator.

pH is directly affected by the PCO_2 in the blood. As can be seen in Figure 17, the pH dropped as the PCO_2 increased as seen in Figure 16. Administration of sodium bicarbonate was not enough to compensate for the shift in the pH in most instances. The carbon dioxide formed when the equations shown were pushed to the right was not removed by the oxygenator.



E. CONCLUSIONS: PRELIMINARY IN VIVO WORK

It has been demonstrated that the new blood pump and oxygenator system is applicable to in vivo work. Oxygen and carbon dioxide transfer are acceptable. The blood pump and oxygenator were not the cause of death in the five different trials. The problems which did lead to death are not insurmountable and further testing should prove this to be true.

In an earlier visit, personnel from the National Institute of Health pointed out that it was impossible to support a rabbit while it

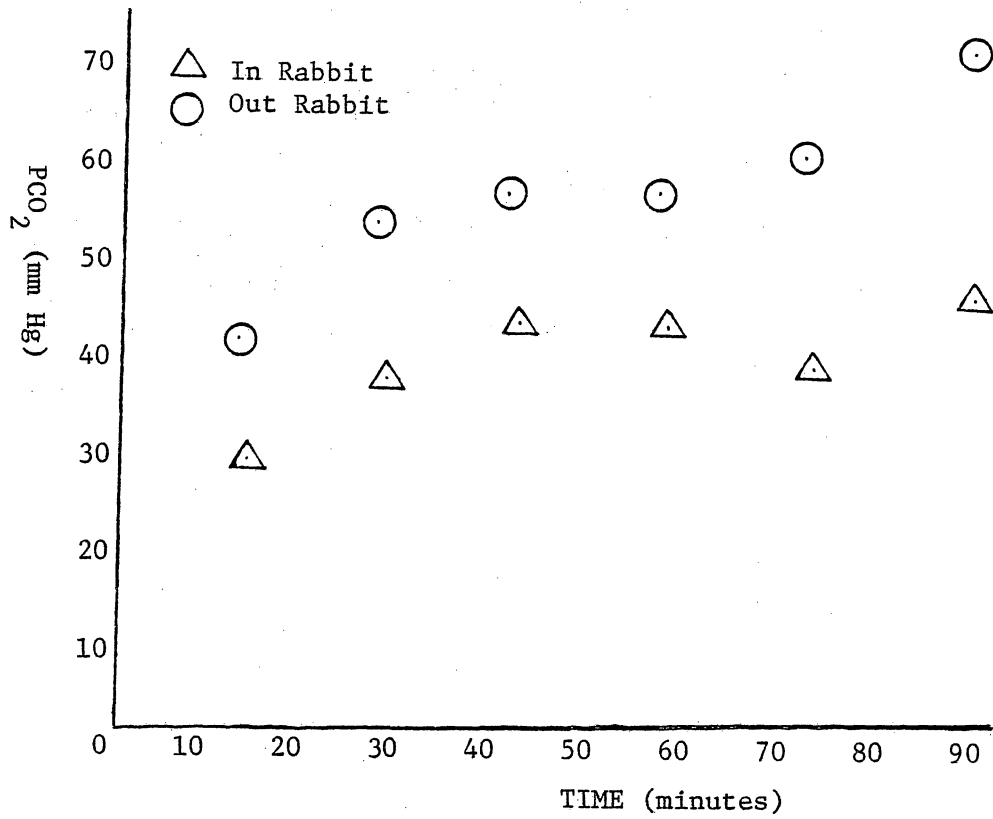


Figure 16. PCO₂ vs. Time

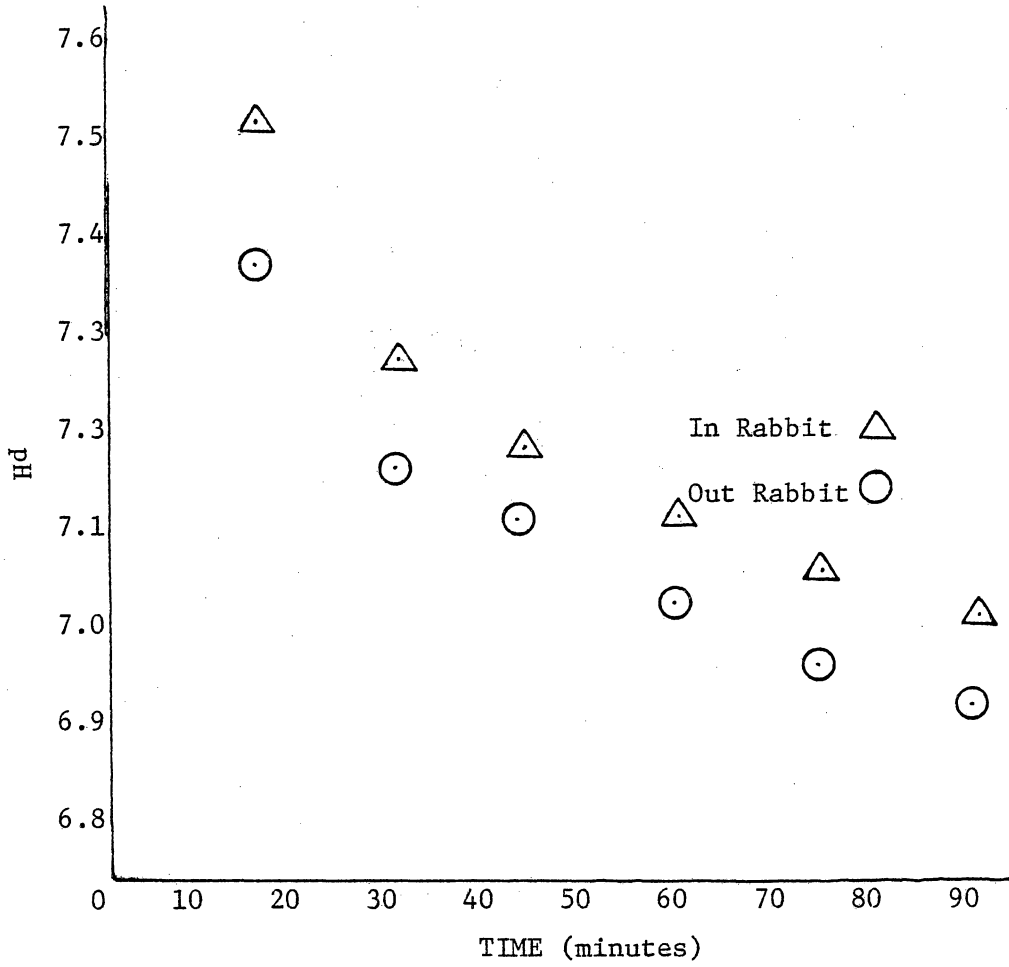


Figure 17. pH vs. Time

was breathing an atmosphere of carbon dioxide and nitrogen. In these in vivo experiments their assertions were not verified. The system quite easily compensated for any oxygen lost across the lung.

One must be reminded that the system used in these experiments was designed for infants weighing up to 2 kilograms. All of the rabbits used in the experiments weighed more than 3 kilograms.

Most important in drawing conclusions from the in vivo work is the concept of total support (as we did in these experiments) vs. partial support as would be found in a clinical situation where a ventilator would be used in conjunction with this blood pump and oxygenator. If one can support a 3 kilogram rabbit that is breathing carbon dioxide and nitrogen surely there can be no doubt that the system can easily supply the required partial support for an infant with neonatal respiratory distress.

The data obtained in the preliminary in vivo evaluation of the blood pump and oxygenator system fully justifies further evaluation.

4. RECOMMENDATIONS

The data obtained from the in vitro and in vivo evaluations suggest that long term support of a neonate is feasible with this system. More long term animal work should be the next step in the evaluation of the new system.

One of the main problems faced in this research was the lack of trained medical laboratory technicians. Much more data could have been obtained with trained personnel. Any further testing of the

scope that was performed in the preliminary testing should not be attempted without adequate laboratory support personnel.

Use of this system without systemic heparinization may be possible. Coating of the extracorporeal circuit with heparin may be possible and should be pursued.

Although gross anatomical autopsy was performed, microscopic tissue studies at autopsy should be performed. It is suspected that little change will be shown to occur in the body.

The membrane stabilizing ability of oxygen is an interesting phenomenon and should be investigated thoroughly.

The nature of this system makes it easily adaptable to computer control. The technology available today makes this a relatively easy task.

This system should be scaled up for use in children and adults. It has the potential for reducing the risks involved in open heart surgery.

With the use of different membrane material this configuration could be used as a dialysis unit as well as an oxygenator.

This system could be used for perfusion of organs awaiting transplant.

This research has the potential to arrive at the technology to produce an artificial implantable lung. Well funded and expedient thrusts forward are highly recommended in the continued evaluation of this system.

5. LITERATURE CITED

1. Dawes, Geoffrey S., "Changes in Circulation at Birth", *Anesthesiology*, 26, pp. 522-530, 1965.
2. Arp, Leon J., Dillion, R. E., Humphries, T. J., Pierce, D. E., "A New Approach to Ventilatory Support of Infants With Respiratory Distress Syndrome, Part I: The Arp Infant Respirator." *Anesthesia and Analgesia - Current Researches*, Vol. 48, No. 3, pp. 506-513, 1969.
3. Gluck, Louis, "Newborn Special Care." *Pediatric Therapy*, Ed. H. C. Shirkey, C. V. Mosby Co., p. 341, 1972.
4. Avery, M. E., Fletcher, B. D., *The Lung and Its Disorders in the Newborn Infant*, W. B. Saunders Co., p. 194, 1974.
5. Nelson, W. E., *Textbook of Pediatrics*, W. B. Saunders Co., p. 379, 1969.
6. Arp, Leon J., Dillon, R. E., Humphries, T. J., Pierce, D. E., "A New Approach to Ventilatory Support of Infants With Respiratory Distress Syndrome, Part II: The Clinical Application of the Arp Infant Respirator." *Anesthesia and Analgesia - Current Researches*, Vol. 48, No. 4, pp. 517-528, 1969.
7. Kuserow, B. K., Machanic, B., Collines, F. M., Clapp, J. F., "Changes Observed in Blood Corpuscles After Prolonged Perfusion With Two Types of Blood Pumps," *Trans. Soc. Art. Inter. Organs*, 11, pp. 122-126, 1966.
8. Longmore, Donald, *Machines in Medicine*, Doubleday and Co., p. 104, 1970.
9. Lester, Randall Vaughn, "Analysis of a Membrane Type Blood Oxygenator and a Ventricle Type Blood Pump," Thesis, Virginia Polytechnic Institute and State University, August, 1973.
10. Bentley, D. J., Biouna, J. G., Pasupathy, C., Sawyer, P. N., Stanczewski, B., "Development and Evaluation of a New Pulsatile Ventricle Pump for Use in Cardiopulmonary Bypass", *Biomaterials, Medical Devices, and Artificial Organs*, Marcel Dekker, Inc., p. 151, 1973.
11. Chamberlain, Geoffrey, "An Artificial Placenta," *American Journal of Obstetrics and Gynecology*, pp. 100, 5, 615-626, 1968.

12. Guyton, Aurthur C., Textbook of Medical Physiology, W. B. Saunders, Co., p. 110, 1976.
13. Degnan, T. J., Karasik, S., Lenahan, J., "Laboratory Control of Heparin Therapy with the Activated Partial Thromboplastin Time Test," Current Therapeutic Research, pp. 11, 6, 390-396, 1969.
14. Zucker, S., Cathey, M. H., "Control of Heparin Therapy," J. Lab. and Clin. Med., Feb., pp. 320-326, 1969.
15. Lumb, W. V., Small Animal Anesthesia, Lea and Febiger, p. 285, 1963.
16. Pitts, Robert A., "The investigation of a non-invasive oximeter and the further development of an extracorporeal oximeter. Thesis, Virginia Polytechnic Institute and State University, 1976.

6. APPENDICES

CYANOMETHEGLOBIN METHOD FOR PLASMA HEMOGLOBIN DETERMINATION

Using the Coleman Spectrophotometer follow directions for free plasma hemoglobin. Two milliliters of plasma is used per five milliliters of Drabkins reagent to detect the small amount of hemoglobin present. The blood specimen is spun at 4000 rpm for 20 minutes. Percent transmittence is read at 540 millimicrons and milligrams of hemoglobin per 100 millilieters (mg%) are determined from a calibration curve.

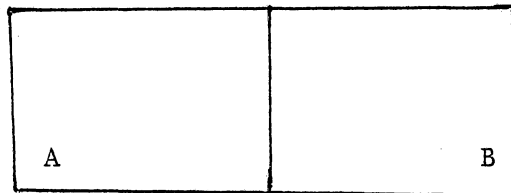
TESTS AND MACHINE USED

<u>Test</u>	<u>Units</u>	<u>Machine</u>	<u>Model</u>
PO ₂	torr	IL pH/Gas Analyzer	113
PCO ₂	torr	IL pH/Gas Analyzer	113
pH		IL pH/Gas Analyzer	113
% Hb Sat with O ₂	%	IL Co-Oximeter (®)	182
% Hb Sat with CO	%	IL Co-Oximeter (®)	182
Hemoglobin-blood	gm/100 ml	IL Co-Oximeter (®)	182
Hemoglobin-plasma	mg/100 ml	Coleman Junior II (®)	
		Spectrophotometer	6/20
Blood Flow	ml/min	Statham Macroflow	E 3000
O ₂ Flow	l/min	Puritan O ₂ Flowmeter	B
O ₂ Pressure	p.s.i.	Beckman O ₂ Pressure Regulator	

BLOOD TYPING

A. ABO typing

1. Divide a clean glass slide in the center using a marking pencil and label:



2. Place one drop of Anti-A serum on one side and one drop of Anti-B serum on the other.
3. Add to each side a volume of fresh blood obtained by venipuncture of a marginal ear vein equal to approximately $\frac{1}{4}$ - $\frac{1}{2}$ the volume of the antiserum used, or an amount sufficient to produce a final cell concentration of 10-15%. Using separate clean applicator sticks, mix each side over an area about one inch in diameter.
4. Tilt or rotate the slide and examine macroscopically for agglutination over a period not to exceed TWO minutes.

5. Interpretation:

Reaction With

<u>Anti-A</u>	<u>Anti-B</u>	<u>Blood Group</u>
-	-	O
+	-	A
-	+	B
+	+	AB

+ = agglutination; - = no agglutination

B. Rh Factor

1. Place one drop of Anti-Rh serum on a clean glass slide.
2. Add two drops of whole blood, each of equal size to the drop of antiserum.
3. Mix thoroughly with a clean applicator stick, spreading mixture over most of the slide.
4. Place slide on glass plate (45-50°C) of a view box.
5. Rock gently back and forth and examine for agglutination over a period not to exceed TWO minutes.
6. Interpretation: Agglutination - Rh positive
No agglutination - Rh negative

INJECTION AND PROCEDURE AND SHAVING

The injection procedure for withdrawing blood samples and administering drugs is shown:

- 1) Place rabbit in restraint box.
- 2) Using a sharp scalpel blade, shave the ear along the edge. This accomplishes three things: It exposes a clear view of the vein to be injected, it makes a more sterile field, and irritation of the shaving causes a local vasodilatation.
- 3) Liberally wipe the ear with 95% ethanol.
- 4) Grasp the ear, between the thumb and second finger, at its distal end. Insert a 25 gauge needle, bevel up, at a slight angle, into the lumen of the vein. Slowly withdraw the syringe plunger to see if blood enters the syring. If it does, proceed with injection. Always make the first injection distal in case another injection must be made. The next injection should be made more proximal.
- 5) Before withdrawing the needle, place a dry cotton ball over the site of injection and apply light pressure. Withdraw the needle and maintain the pressure on the site for a few minutes.
- 6) Remove animal from restraint box.

The abdomen and neck region of each rabbit was shaved. In order to prevent discomfort to the animal 0.5 cc of sodium pentobarbitol (64.8 mg/cc) was injected in the marginal ear vein to sedate the

animal. The rabbit was then secured on its back for shaving. Animal clippers of good quality are a requirement for shaving the rabbits.

ACTIVATED PARTIAL THROMBOPLASTIN TIME TEST

Materials: Crushed ice, Activated Platelet Factor Reagent, 0.02 M CaCl₂ stop watch, plastic pipettes, cuvettes, plasma pipette, acid citrate dextrose.

- Procedure:
- 1) Draw sample: 0.9 cc blood/0.1 cc acid citrate dextrose
 - 2) Centrifuge at 2500 rpm for 20 minutes
 - 3) Take freshly centrifuged plasma and store in crushed ice until required.
 - 4) Reconstitute activated platelet factor reagent according to directions and maintain at room temperature.
 - 5) Prepare a 0.02 M solution of calcium chloride; transfer required amount with the analyzer; warm to 37^oC in the incubation block.
 - 6) Pipette 0.1 ml of the activated platelet factor reagent into a 70 X 7 mm cuvette and place in the incubation block for two minutes.
 - 7) Pipette 0.1 ml of the patient's plasma to the tube of step 6. Note time of mixing.
 - 8) After 5 minutes of incubation of the plasma/platelet mixture place the cuvette in the reading block.
Pipette 0.1 ml of calcium chloride using the automatic start feature of the analyzer.
 - 9) Clot formation will stop the timer. Read and record.

MATERIALS REQUIRED FOR ONE TRIAL

1. Blood Typing

clean glass slides - 3 per rabbit

anti Rh serum

anti A serum

anti B serum

wooden applicator sticks

25 gauge needles

1 cc tuberculin syringes

cotton balls

95% ethanol

scalpel blades

2. Shaving

animal clippers

restraint box

scalpel

sodium pentobarbitol (64.8 mg/cc)

25 gauge needles

tuberculin syringes

3. Sacrifice

sodium pentobarbitol (64.8 mg/cc)

25 gauge needles

tuberculin syringes

cotton balls

scalpel blades

scissors - skin

heparin (1000 units/cc and 75 units/cc)

sterile normal saline

500 ml flask - 1/ rabbit

large plastic funnel

4. Priming

large flask - 1000 ml

nylon mesh filter material

sylastic tubing

5. Surgery

sodium pentobarbital (64.8 mg/cc)

25 gauge needles

tuberculin syringes

cotton balls

scalpel - assorted blades

small curved clamps

medium straight clamps

surgical scissors

00 cotton-nylon ligatures

4X4 sponges

sterile normal saline - 2000 ml

cannulas - assorted sizes

tubing adapter assembly (see Figure 16)

surgical gloves

6. Data collection and maintenance

glucose-saline (commercially prepared D5W-normal saline or
100 gm dextrose and 8.5 gm of sodium chloride added to distilled
water to make one liter of solution)

sodium bicarbonate 7.5% (3.75 gm bicarbonate of soda in 50 ml of
distilled water)

acid citrate dextrose (sodium citrate 2.2 gm, citric acid 0.73 gm,
dextrose 2.45 gm and distilled water to make 100 ml)

10 cc syringes - 20

50 cc syringes - 10

2.5 cc syringes - 50

heparin (500 cc of 75 units/cc)

protamine sulfate - 10 cc

sodium pentobarbital (64.8 mg/cc)

subcutaneous ECG electrodes - 6

centrifuge tubes - 50

activated platelet factor reagent - 6

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A NEW BLOOD PUMP AND OXYGENATOR SYSTEM
FOR SUPPORT OF INFANTS WITH
NEONATAL RESPIRATORY DISTRESS:
PRELIMINARY IN VITRO AND IN VIVO EVALUATION

by

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(ABSTRACT)

A clinical need exists for a blood oxygenator and pumping system for the support of neonates with respiratory deficiencies. Such systems now available for support of adults are not suitable for neonatal patients. In vitro evaluation of a new blood oxygenator and blood pumping system was performed. The data obtained suggested that this system may be applicable to neonates. In vivo studies with rabbits to further analyze the new system were done. Preliminary data from these studies indicate that the new blood oxygenator and blood pump system may be applicable to supporting neonates with respiratory deficiencies. Suggestions for future development of this system are presented.