

Prolonged Partial Venoarterial Bypass: Physiologic, Biochemical, and Hematologic Responses

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Prolonged veno arterial extracorporeal bypass (50–80% of cardiac output) was evaluated in normal, unanesthetized sheep. The evaluation protocol included serial measurements of hemodynamics, pulmonary and renal function, serum enzymes to detect organ damage, and detailed studies of coagulation and platelets. Blood exposure surfaces were primarily polyvinyl chloride and silicone rubber. Gas interfaces were carefully excluded with the exception of four experiments utilizing bubble oxygenators. Heparin dose was titrated to maintain activated clotting to two to three times baseline. Characteristics of 48 hour uncomplicated extracorporeal circulation in 8 sheep included normal hemodynamics, mild respiratory alkalosis negligible hemolysis, slight gradual increase in heart, liver, and muscle enzymes. The most significant changes occurred in coagulation and platelets characterized by an initial reduction in coagulation factors with a continued reduction in platelet count and return to normal clotting factors during extracorporeal circulation. This is followed by a two times normal increase in platelets and fibrinogen following extracorporeal circulation.

PROLONGED EXTRACORPOREAL CIRCULATION with gas exchange through a membrane oxygenator is a radical but sometimes necessary approach to management of the patient with severe pulmonary insufficiency. Partial venoarterial cardiopulmonary bypass provides passive support of gas exchange during the period of pulmonary failure and affords active treatment of the lung by reducing pulmonary hydrostatic pressure. Extensive and orderly laboratory investigation in the physiology of prolonged bypass,^{3,5,6,10,14,15} membrane oxygenator design,^{2,14,15,17} anticoagulation control,^{3,10,12} responses in normal animals,^{2,3,6,8,10,14,17} and in patients during open-heart procedures,^{7,21} led to the first

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successful clinical application on this technique reported by Hill, O'Brien and others in 1971.¹¹ Since that report the technique has been used successfully in at least ten other clinical patients.¹² With widespread clinical application appearing feasible, it is increasingly important to establish the normal responses to prolonged venoarterial bypass in an animal model which resembles the human. This study was undertaken to evaluate and define the responses to prolonged extracorporeal circulation in normal sheep, and to identify the complications, potential risks, and best techniques for clinical application.

Materials and Methods

Partial veno-arterial bypass with a membrane oxygenator was carried out in eight awake normal 50–80 lb farm lambs. Bypass was carried out for 24–48 hours, terminated electively, and the animals followed for two weeks. Measurements were made at regular intervals and full autopsies performed. Data from these studies represent the response of the normal sheep to prolonged bypass.

Eight other sheep died of technical complications after 6–40 hours of bypass. Data from these studies are presented to illustrate the sensitivity of the testing protocol and the potential risks of the procedure. In four sheep prolonged bypass was attempted with a gas-interface (bubble) oxygenator. Data from these studies are presented to compare the variables of gas-interface exposure to the non-gas interface system.

Sheep were anesthetized with Ketamine, 10 mg/kg intravenously, with doses repeated as necessary during the cannulation procedure. Sheep do very well under Ketamine and awake promptly following anesthesia. Further sedation or analgesics were not necessary. After anesthesia ap-

Submitted for publication March 26, 1974.

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Supported by grants from the National Heart and Lung Institute, Donald E. Baxter Foundation, Orange County Heart Association, and the California Tuberculosis and Respiratory Disease Association.

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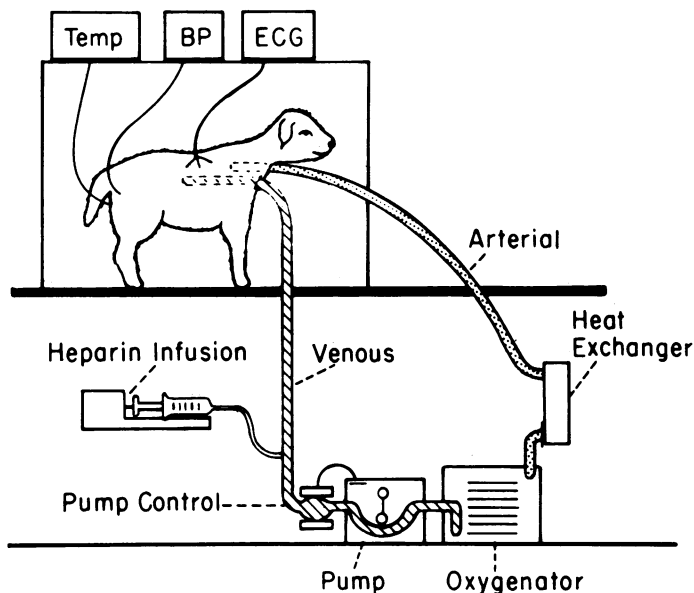


FIG. 1. Diagram of the extracorporeal circulation circuit.

appropriate catheters and electrodes were placed for femoral artery pressure, EKG, and temperature monitoring. The right atrium was catheterized using the largest polyvinyl catheter that would pass through the right internal jugular vein. Blood was returned to the carotid artery or the femoral artery which was ligated distally (Fig. 1). During bypass the sheep were allowed to sit and stand in a small restraining cage and ate and drank freely. A brief Ketamine anesthetic was given for decannulation at the termination of bypass. At the conclusion of bypass the cannulated vessels were ligated and incisions closed. Keflin® was administered intermittently during the procedure. No significant infections occurred in the catheterization sites. The bypass circuit was composed primarily of polyvinyl chloride $\frac{3}{8}$ " tubing and polycarbonate connectors. Venous blood drained directly to a servo-regulating ventricle or roller pump on the floor, and was pumped through the oxygenator (with the exception of the bubble oxygenator which was incorporated on the venous side of the circuit). The $.4m^2$ toroidal membrane oxygenator² (four animals), two $3m^2$ Landé-Edwards membrane oxygenators⁵ in parallel (four animals), the Bentley Pediatric Temptrol® oxygenator and the Travenol® bubble oxygenator were used in this system. The oxygenators were ventilated with 95% O₂, and 5% CO₂. The entire extracorporeal circuit was primed and rinsed twice with Ringer's lactate solution, then filled with freshly drawn non-hemolyzed heparinized sheep blood, warmed to 37C, and buffered to pH 7.4. The priming volume was approximately 2 L. Some crystalloid solution remained in the bypass circuit and the hematocrit of the primed extracorporeal circuit was 18–25%. Prior to placement of cannulas heparin, 2 mg/kg, was given as a single dose intravenously and bypass was instituted. Flow rates were gradually increased until the

desired flow rate was obtained, usually 80 cc/kg/min. All tubes were secured in place and the animal was returned to its cage and was allowed to awaken and move about during the remainder of the experiment. Heparin solution was infused continuously into the bypass circuit to maintain the activated clotting time between two and three times baseline. Whole blood activated clotting time (ACT)¹ was measured hourly and heparin dose adjusted appropriately. No drugs were given to reverse the heparin effect at the end of bypass.

Blood pressure, pulse, respiration, temperature, arterial, venous and oxygenator PO₂, PCO₂, and pH were measured and recorded at frequent intervals. Coagulation screening tests, specific factor assays, platelet counts, total plasminogen-activity, and fibrin (ogen) degradation products were measured prior to anesthesia, at regular intervals during bypass, and at up to two weeks following bypass. Total plasminogen was assayed by the caseinolytic method⁴ and the euglobulin lysis time.¹³ Fibrin (ogen) degradation products were determined by immunoprecipitin methods, utilizing anti-human fibrinogen.²⁰ Platelet adhesiveness, aggregation, and Factor III were measured in one experi-

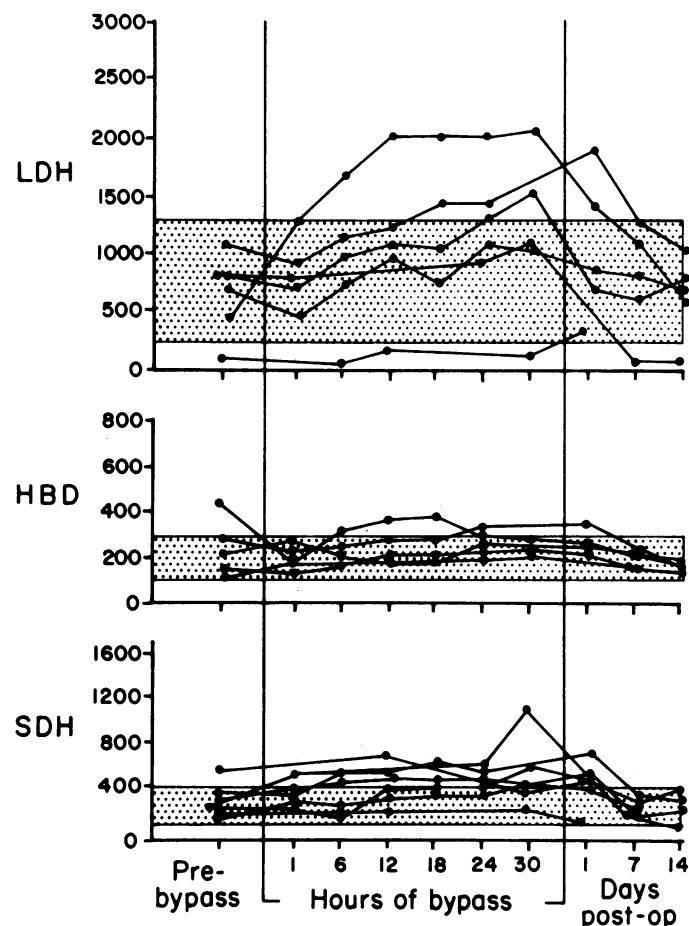


FIG. 2. Serum enzyme changes during uncomplicated extracorporeal circulation in eight sheep.

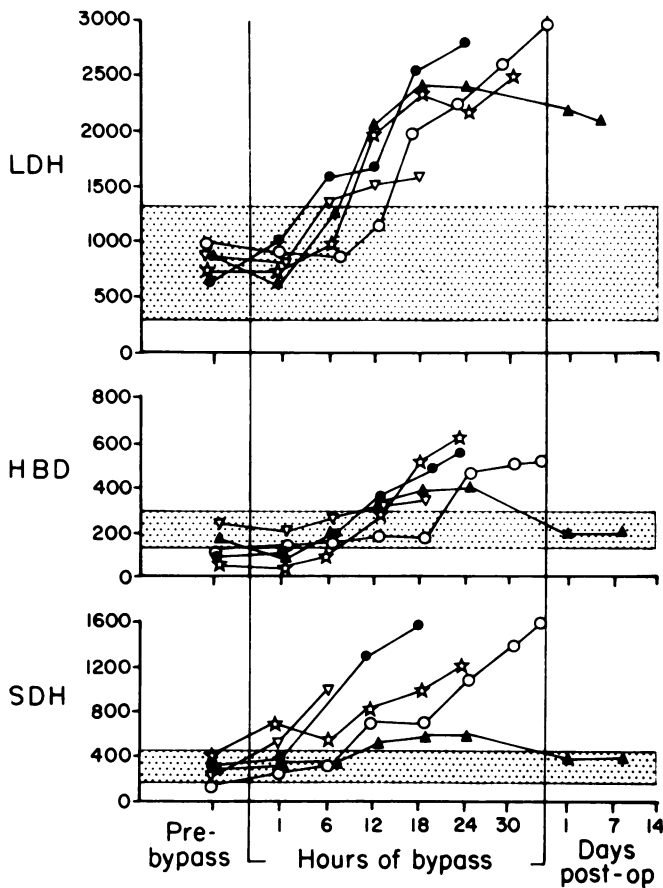


FIG. 3. Serum enzyme changes during extracorporeal circulation with complications associated with tissue damage (five representative sheep).

- = volvulus, intestinal necrosis
- △ = bubble oxygenator, survivor
- ▽ = low heparin, diffuse emboli
- = Cardiac damage from catheter
- ☆ = cardiac damage from catheter

ment. Specific organ perfusion and damage were assessed by a regular measurement of serum enzymes: sorbitol dehydrogenase (liver) a-hydroxy-butyrate dehydrogenase, (heart), and lactic dehydrogenase (most tissues). Blood urea nitrogen, glucose, electrolytes, and plasma hemaglobin were measured at regular intervals as were white blood cell count and differential, hemoglobin and hematocrit.

Results

During uncomplicated bypass in normal sheep hemodynamics and perfusion remained normal during extracorporeal circulation at 50-100 cc/kg/min. Normal sheep cardiac output is approximately 120 cc/kg/min. Mean arterial pressure stayed above 100 mm Hg and pulse ranged 90-116 beats per minute. The bypass flow was adjusted so that the left ventricle ejected and established a pulsatile flow contour with every beat. Other studies in our laboratory have shown that higher flows on bypass will result in incomplete emptying of the left ventricle with ejection occurring only occasionally. This may not be

necessarily detrimental. The bypass flows in this study were elected to maintain normal pulsatile peripheral perfusion and were calculated to approximate the level of bypass flow which would be necessary to supply total extracorporeal gas exchange in the face of pulmonary insufficiency. Hyperventilation with mild respiratory alkalosis occurred throughout the bypass in all animals. Hypotension with metabolic acidosis did occur in some lambs with complications associated with poor venous return and decreasing bypass flow. All the bubble oxygenator lambs developed progressive hypotension, three died during 12 hours, one survived 24 hours of bypass without residual damage.

There was no significant change during ECC in serum sodium, potassium, chloride, carbon dioxide content, bilirubin, or total protein. Blood urea nitrogen increased from 39 to 59 mg% (average). Plasma hemoglobin at the end of bypass was 8 mg%, average (6 to 33 mg% range). The surviving animal with the bubble oxygenator developed azotemia (BUN 61 mg%) and hemolysis (plasma hemoglobin 104 mg%). Two lambs in the complication group had transfusion reactions as a result of using a donor that had been previously sensitized. Both showed hemolysis promptly following transfusion of donor blood (plasma hemaglobin 84 and 183 mg%).

Serial measurements of LDH, HBD, and SDH showed progressive rise during extracorporeal circulation to approximately twice baseline levels but generally remained below the upper limits of normal for sheep in our laboratory

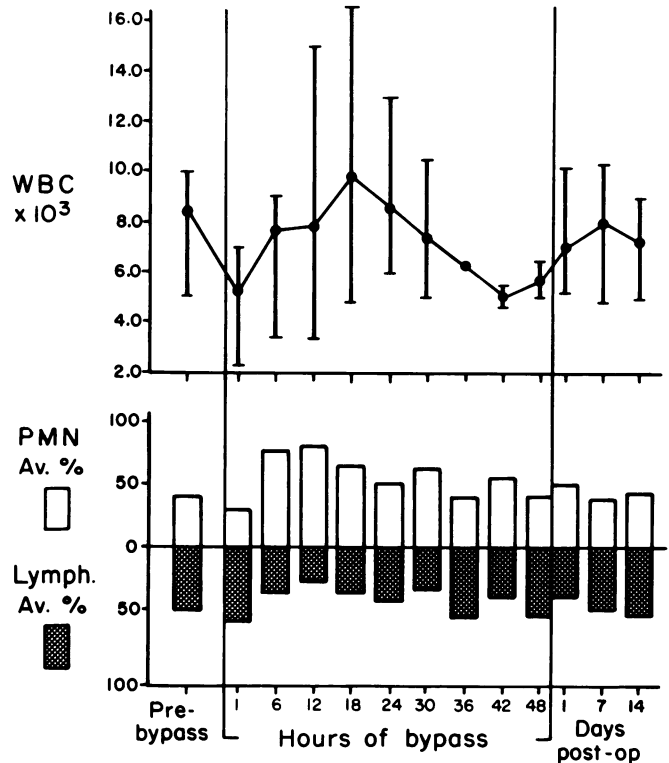


FIG. 4. Leukocyte changes during uncomplicated extracorporeal circulation (eight sheep).

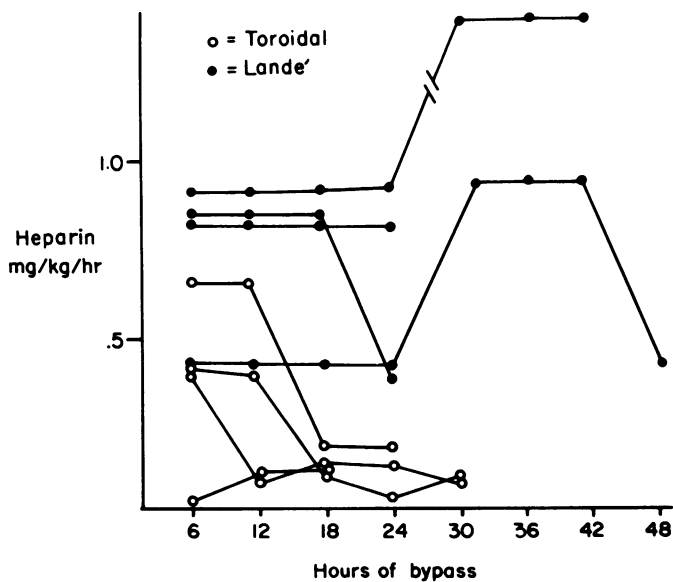


FIG. 5. Heparin dose during uncomplicated extracorporeal circulation in eight sheep.

(LDH 1350, SDH 450, HBD 292) (Fig. 2). No organ damage was detected at autopsy in these animals.

In the complication group, when liver damage from embolization occurred in one lamb, SDH levels rose from 300 to 1600 in 12 hours. When cardiac damage from a catheter inadvertently placed in the coronary sinus occurred HBD levels rose from 75 to 600 units in 24 hours. Levels of LDH, HBD and SDH had increased five fold during a 40 hour bypass which appeared to be uncomplicated. Autopsy disclosed a volvulus with extensive bowel infarction. The bubble oxygenator experiments showed a two fold and three fold rise in all enzyme levels. These changes in tissue specific enzymes are outlined in Fig. 3. These findings demonstrate that tissue specific enzymes are a helpful retrospective analysis of the bypass course and identify the time sequence of early organ damage which can be correlated with other events.

Hematological studies showed a moderate leukocytosis during the first 24 hours of bypass which was characterized primarily by increase in the granulocytic series as shown in Fig. 4. There was a gradual drop in hematocrit and hemoglobin contraction which was related to sampling. Freshly drawn sheep blood was used for transfusion on several occasions when hypovolemia occurred due to sampling. Bleeding from the animal did not occur except in the bubble oxygenator experiments.

The heparin in dose required to maintain the ACT two times baseline varied between 0.2 and 1.0 mg/kg/hr. (Fig. 5). Dosage was somewhat less for the toroidal oxygenator during the second 24 hours of bypass. When the ACT was inadvertently allowed to fall to normal levels in two animals, clotting, diffuse embolization and death occurred.

The most striking changes during long term bypass occurred in the coagulation system. The changes in coagula-

tion are outlined in Figs. 6-8. In order to study conventional screening tests and specific factor assays it was necessary to inactivate the heparin in the blood samples with (Polybrene®) (hexamethadine). This was done by adding 50 μ gm of Polybrene® for each 1 cc of blood sample. We have previously demonstrated that this amount of Polybrene® neutralized the amount of heparin expected relative to the infusion rates. An excess of Polybrene® in this dosage does not affect coagulation assays by itself. In our studies it was found that all the coagulation factors decreased by 30-40% promptly after institution of bypass. Much of this is due to dilution effect. Coagulation factors in the prime were approximately 30% of control sheep levels. After this initial decrease the clotting factors gradually returned toward normal, reaching normal values by 24 hours and remaining at that level throughout bypass. These moderate changes in clotting factors were not detected by conventional screening tests (Fig. 6). The platelet count also dropped by 30% at the institution of bypass and continued to drop slowly at the rate of approximately 10% of the remaining platelets per hour as long as bypass was continued. Platelet function was studied in one animal. Platelet Factor III activity⁹ and platelet adhesiveness to glass beads¹⁹ were both lost during bypass. This appears to be an effect of heparinization and these assays can be returned to normal by addition of Polybrene® to the blood sample. Heparin appears to be an inhibitor of platelet adhesiveness and Factor III release, or at least an inhibitor of the conventional assays of platelet function. These interrelationships are undergoing further investigation in our laboratory. Assays of total plasminogen activity showed no significant levels of fibronolytic activity in normal sheep blood or no significant increase during prolonged bypass. The sheep is known to have a very inac-

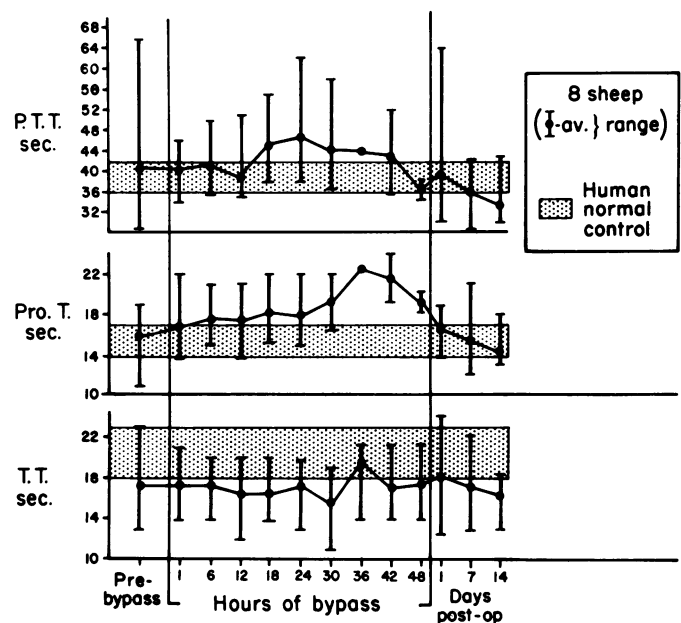


FIG. 6. Coagulation screening tests during uncomplicated extracorporeal circulation (eight sheep)

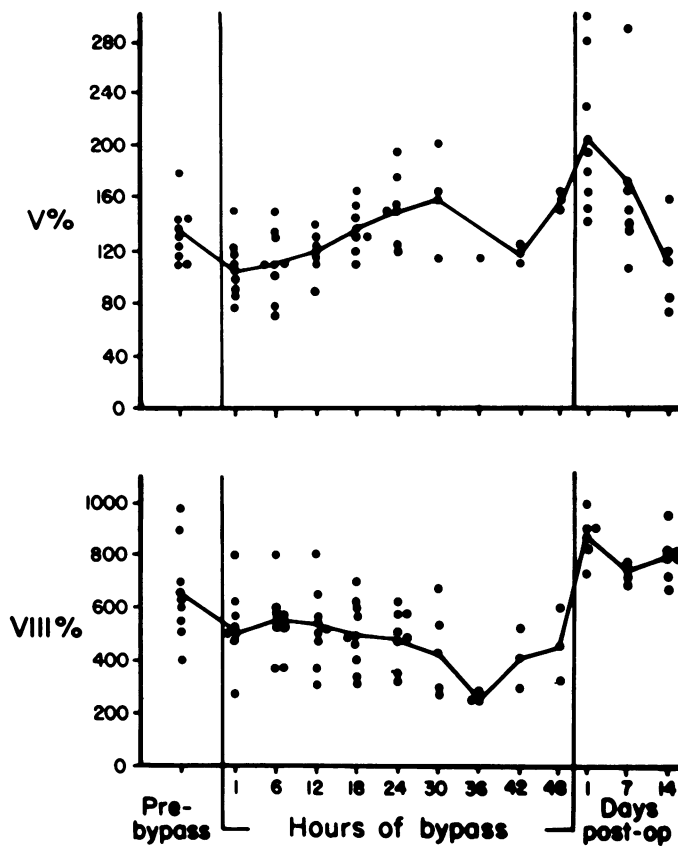


FIG. 7. Factor V and VIII during uncomplicated extracorporeal circulation (eight sheep)

tive fibrinolytic system¹⁶ and our data corroborate those observations. Fibrin(ogen) degradation products were not detected to a significant degree. Animals perfused with a bubble oxygenator showed similar, but more exaggerated changes in coagulation (Fig. 9). Platelets were nearly absent from the circulating blood within 12 hours of the institution of bypass with a bubble oxygenator and remained at very low levels during the next 12 hours in the one animal that survived.

Following bypass a significant hypercoagulable state occurred, with platelet count and fibrinogen level rebounding to two to three times baseline levels at one week after bypass.

The bypass system used in these experiments was safe and reliable. No significant mechanical malfunctions occurred in any of the experiments. Oxygenator performance with either the Landé-Edwards or the Toroidal membrane oxygenator did not change during these bypass experiments. Blood leaving the oxygenator was always fully saturated and CO₂ removal was excessive unless 5% CO₂ was used as the ventilating gas.

On autopsy the lungs, heart, brain, kidney and liver of surviving uncomplicated animals were normal. In two animals whose heparin dosage was inadvertently omitted and clotting time returned to normal, systemic embolization

was found. In other animals in which the venous catheter was inadvertently placed in the coronary sinus, myocardial hemorrhage associated with low cardiac output was found.

Discussion

Although we³ and others^{6,17} have previously used the dog as an experimental model for prolonged extracorporeal circulation, the active nature of that animal and the very active fibrinolytic system prompted the search for a more sensitive and a more human-like experimental preparation. The sheep has served this function extremely well. In addition to being anatomically and physiologically similar to the human, the coagulation mechanism of the sheep is quite valid for studies of extracorporeal circulation. One could imagine that the relatively high numbers of platelets and low fibrinolytic activity have been selectively bred into sheep during annual shearing over the last several centuries. At any rate, the sheep provides a severe test of thrombogenicity in an extracorporeal circuit. In addition sheep are docile and extremely easy to work with in a prolonged unanesthetized state.

Most of the findings in this study are similar to those previously found in patients,^{11,12,16,18} and other sheep experiments.^{8,14} In essence there are no major changes in hemodynamics, organ function, hemolysis, fluid and electrolyte balance, and no evidence of tissue damage measured by enzyme assay or at autopsy. Major changes with prolonged partial bypass occurred almost exclusively in the

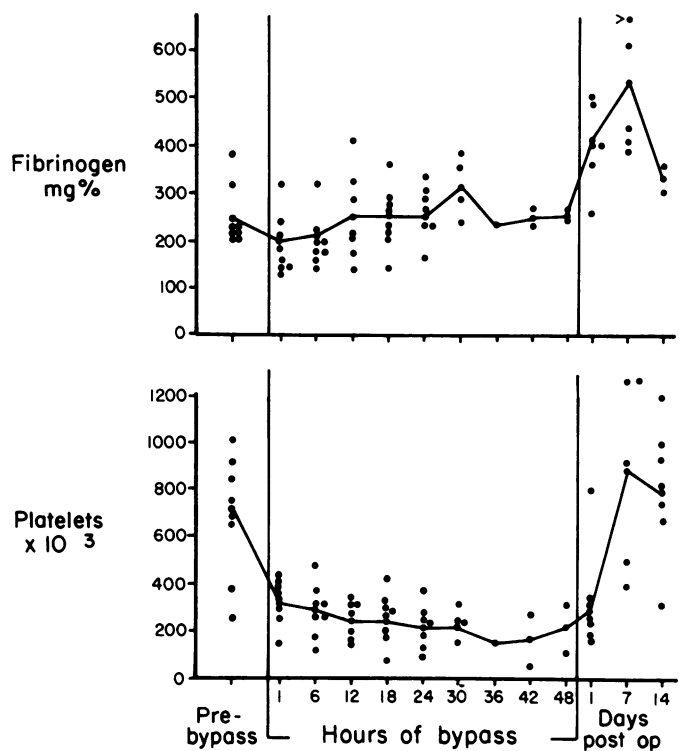


FIG. 8. Fibrinogen and platelet count during uncomplicated extracorporeal circulation (eight sheep)

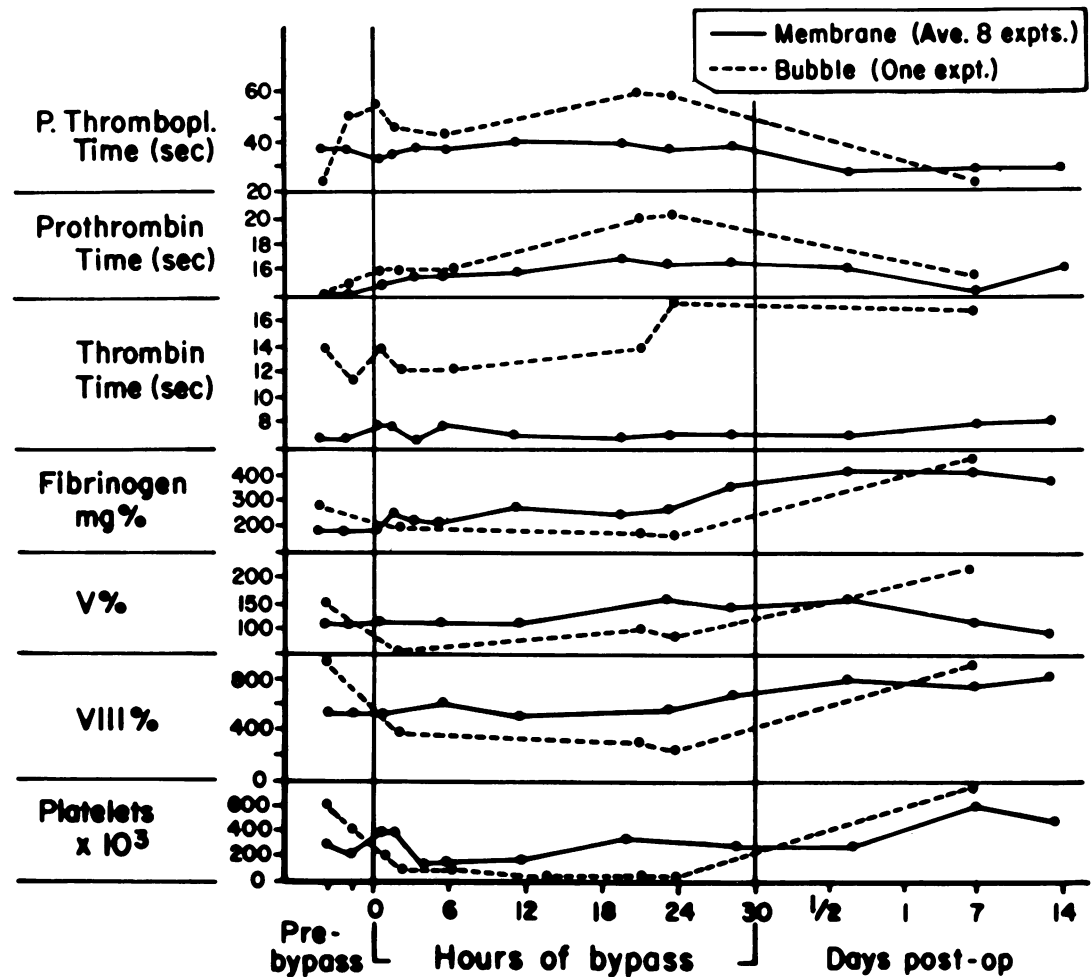


FIG. 9. Coagulation changes during extracorporeal circulation with a gas interface (bubble) oxygenator. Values for one 24-hour bypass sheep are compared to average values for eight sheep perfused with membrane oxygenators.

coagulation system and specifically in platelet numbers and function. Control of coagulation in the extracorporeal circuit has been very satisfactory with the heparin titration method proposed by Bartlett and Drinker in 1969.⁹ The heparin dose required to maintain the activated clotting time between two and three times normal was approximately 0.5 mg/kg/hr. This was the same for all types of oxygenators tested although the heparin requirement for the toroidal oxygenators appeared to be somewhat less than the Landé-Edwards (Fig. 5). The continuous gradual diminution in platelet count has been reported by other investigators.^{5,6,10,14,17} The fate of the disappeared platelets is open to question. There were no grossly obvious fibrin or platelet accumulations in the extracorporeal circuit at the end of bypass. We have no evidence to support or deny the observation of de Leval and others that platelets may be trapped in the liver.⁵ Hicks¹⁰ suggested that platelets aggregate in response to any foreign surface, are bound in the reticuloendothelial system, disaggregate and recirculate. The rebound effect in platelet count and fibrinogen level is quite marked and suggests a propensity to thromboembolism after a period of prolonged bypass which might be important clinically.

Use of venoarterial bypass is definitely preferable to veno-venous bypass for pulmonary or cardiac support. Pulmonary artery pressure significantly decreases during veno-arterial bypass facilitating removal of fluid from the lung interstitium. The high flow rate required for extracorporeal gas exchange support can be achieved without the possibility of re-circulation which invariably occurs at high flow rates with a veno-venous bypass.⁸ The use of venoarterial bypass also permits a variety of techniques for active treatment of the lung during bypass such as inflation and lavage with antibiotics or mucolytic containing fluids and continuous positive pressure inflation without ventilation.

Conclusions

Partial veno-arterial bypass using these techniques can be done safely and routinely for 48 hours in normal sheep without significant blood or organ damage. The awake sheep model and evaluation protocol described here provides an effective model for the study of prolonged extracorporeal circulation. Characteristics of 48 hour uncomplicated extracorporeal circulation in normal sheep include normal hemodynamics, mild respiratory alkalosis, negligible hemolysis, slight gradual increase in heart, liver,

and muscle enzymes. There is an initial reduction in coagulation factors with a continued reduction in platelet count and a return to normal clotting factors during extracorporeal circulation, followed by two times normal increase in platelets and fibrinogen after extracorporeal circulation. Initial studies indicate that platelet aggregation is unchanged during bypass; platelet adhesiveness and platelet Factor III release are both inhibited by low doses of heparin.

Acknowledgments

The authors are grateful for the assistance of Drs. Bruce Achauer, Julian Fraille, John German, and Michael Tavis; Extracorporeal circulation laboratory technicians, Nick Haiduc, Tamar Geraghty, Nancy Wetmore, James Thornton, Ken Proctor; and coagulation technicians, Gillian Williams, Paul Harris, James Hardeman, Wayne Anderson and Christine Woldanski.

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