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Bansi L. Koul M.D.

ECMO
-Safety & Efficacy

ECMO - Safety & Efficacy

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ECMO - Safety & Efficacy

Abstract

Three adult patients with severe acute respiratory failure were treated with a conventional high flow veno-venous extracorporeal membrane oxygenation (ECMO), using heparin coated ECMO system and low dose of heparin (Study I). Two patients survived and are living a normal life. The third patient died of candida sepsis. This method of ECMO did not provide optimal respiratory support in the initial ECMO period when the lung functions were poor. Acute intermittent pulmonary hypertensive crises, bleeding diatheses, recurrent pneumothorax, liver dysfunction and plasma leak from the oxygenators were the main clinical problems during ECMO.

In study II, 6 healthy pigs were subjected to 24 hours of veno-right ventricular ECMO. The veno-right ventricular ECMO substituted the total lung function of the animals at extracorporeal blood flows amounting to 80% of the total cardiac output. Competence of the tricuspid valve is an important prerequisite for the success of this procedure.

In study III, 6 pigs were subjected to 18 hours of total veno-arterial ECMO. All the animals died within 4 hours of weaning from ECMO due to varying degrees of arterial hypoxemia, severe pulmonary hypertension and progressive lactacidosis. 80% of the lung tissue showed features of shock lung. Thus 18 hours of total veno-arterial ECMO is 100% fatal in healthy pigs on account of irreversible ischemic pulmonary damage.

In another study, 6 pigs were subjected to 18 hour partial veno-arterial ECMO during which 25% of the cardiac output was diverted through the pulmonary artery to the lungs (Study IV). 6 hours after weaning from ECMO, a slight but significant decrease in arterial oxygen tension, a significant increase in the pulmonary vascular resistance (mean = 76%) and a slight increase in the pulmonary clearance of ^{99m}Tc-DPTA was observed. 80% of the pulmonary parenchyma was normal. Thus 25% right cardiac output is the border-line safe pulmonary blood flow needed for preservation of adequate lung function during 18 hours of veno-arterial ECMO at normothermia in healthy pigs.

6 healthy pigs were subjected to 24 hours of heparin free total veno-right ventricular ECMO, using Carmeda heparin coated system (Study V). Deterioration in the arterial blood gases and in the pulmonary hemodynamics was not clinically significant. Total platelet count and plasma free hemoglobin remained unaffected. Fibrinogen, fibrin monomer and vonWillebrand factor alone were significantly altered after 24 hours of ECMO and is attributed to the surgical trauma and the sporadic formation of fibrin and blood clots in the membrane oxygenators. The heparin coated surface thus inhibits both the coagulation cascade and the platelet activation during a 24 hour heparin free ECMO in healthy pigs.

Key words

ECMO, ARDS, Heparin coated surfaces, Low dose heparin, Total artificial lung, Pulmonary sequelae, Safe ECMO, Right cardiac output, Heparin free ECMO

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ECMO
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Bansi L. Koul M.D.

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Cover : Position of cannulas for veno-right ventricular ECMO.
Artist, Ronny Lingstam, University Hospital, Lund

To
my mother

5/6

This thesis is based on the following papers, which will be referred to in the text by their roman numerals:

I Veno-venous ECMO for ARDS with a surface heparinized system. Bansi Koul, Torbjörn Wetterberg, Gun Öhqvist, Per Olsson. Scand J Thorac Cardiovasc Surg (accepted for publication).

II Veno-right ventricular bypass as total extracorporeal lung assistance - An experimental study. Bansi Koul, Torbjörn Wetterberg, Trygve Sjöberg, Per-Ola Kimblad, Jan Kugelberg, Stig Steen. J Thorac Cardiovasc Surg 1991;101:719-23.

III Pulmonary sequelae of prolonged total veno-arterial bypass Evaluation with a new experimental model. Bansi Koul, Helena Willen, Trygve Sjöberg, Torbjörn Wetterberg, Jan Kugelberg, Stig Steen. Ann Thorac Surg May 1991;51 (in press).

IV Veno-arterial ECMO - How safe is it ? Evaluation with a new experimental model. Bansi Koul, Per Wollmer, Helena Willen, Jan Kugelberg, Stig Steen. Submitted for publication.

V Twenty four hour heparin free veno-right ventricular ECMO - An experimental study. Bansi Koul, Ole Vesterqvist, Nils Egberg, Stig Steen. Submitted for publication.

ABBREVIATIONS (given in alphabetical order)

ACT	Accelerated Clotting Time
APTT	Activated Partial Thromboplastin Time
AT	Antithrombin
ARDS	Adult Respiratory Distress Syndrome
ARF	Acute Respiratory Failure
CBAS^{R}	Carmeda BioActive Surface
CO	Cardiac Output
^{99m}Tc-	Technetium-99m-labelled-
DPTA	Diethylenetriamine pentaacetate
ECLA	Extracorporeal Lung Assistance
ECMO	Extracorporeal Membrane Oxygenation
ELISA	Enzyme-Linked Immunosorbent Assay
FDP	Fibrin Degradation Products
FiO_2	Inspired Oxygen Fraction
FMM	Fibrin Monomer
GT	Glutamyl Transferase
Hg	Mercury
H_2O	Water
i.m.	Intramuscularly
i.v.	Intravenously
kPa	Kilopascals
LD	Lactate Dehydrogenase
LFPPV-	Low Frequency Positive Pressure Ventilation-
$ECCO_2R$	Extracorporeal Carbon dioxide Removal
$PaCO_2$	Arterial Carbon dioxide tension
PaO_2	Arterial Oxygen tension
PEEP	Positive End Expiratory Pressure
pH	Inverse log Hydrogen ion concentration
PIP	Peak Inspiratory Pressure
PT	Prothrombin complex
PVR	Pulmonary Vascular Resistance
SVR	Systemic Vascular Resistance
TAT	Thrombin-Antithrombin complex
TxA_2	Thromboxane A_2
TxB_2	Thromboxane B_2
V-A	Veno-Arterial
VAB	Veno-Arterial Bypass
V-V	Veno-Venous
vWF	von Willebrand Factor

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1. INTRODUCTION

1.1 ECMO

The term ECMO, refers to the prolonged use of extracorporeal membrane oxygenation of the venous blood for therapeutic purposes. This modality of treatment provides "lung rest" in patients with terminal acute respiratory failure, allowing lungs to recover from both the underlying disease and the deleterious effects of high pressure/high volume mechanical ventilation with high inspired oxygen fraction (FiO_2). ECMO is also being used for temporary support of patients with cardiogenic shock.

Membrane oxygenation has evolved as a sequel to conventional cardiopulmonary bypass and owes its existence to the discovery of heparin (McLean, 1916), the heart-lung machine (Gibbon, 1937), and the membrane oxygenator (Clowes et al, 1956) (1). The principle underlying membrane oxygenation is that when a membrane (polyethylene, silicone rubber) is intercepted in a b'ood/gas interface, gas exchange takes place across it. At present, microporous polypropylene membranes are used in almost all the oxygenators with the exception of a few like SciMed SM-35 (silicone rubber), and the Harvey HF-4000 (microporous polyethylene) (2). When used in the form of hollow fibers (25 micrometer thick, 200 micrometer internal diameter), microporous membranes have been shown to result in compact devices with high surface area/volume ratio.

ECMO was successfully performed for acute respiratory insufficiency first in 1967 (3). and by 1974 one hundred and fifty patients suffering from acute respiratory failure (ARF) had been treated with ECMO with varying results (4). The initial enthusiasm for ECMO diminished when the results of the multicentric, randomized, and prospective ECMO study (1973-1977) for ARF became known (5). A veno-arterial ECMO (V-A ECMO) was used in this study and it was undertaken to asses the beneficial effect of ECMO in severe ARF in adults. The study included 42 ECMO and 48 control (ventilator) patients. Four patients in each group survived and majority of the patients died of diffuse pulmonary inflammation, necrosis, and fibrosis. The interest in membrane technology, on the other hand, continued. Membrane oxygenators were used increasingly for routine open heart operations on account of reduced plasma protein denaturation, complement activation, hemolysis, gas embolism and vital organ dysfunction as compared to bubble oxygenators. Improvement in microporous membrane technology during the last decade, coupled with improvements in efficiency and reduction

in size of the integrated bypass circuits and their diminishing cost, has led further to their increasing use. While the use of V-A ECMO in the treatment of adult ARF suffered a set back, its use in the management of respiratory failure among neonates and infants continued because of improved survival in the latter group as compared to conventional ventilatory therapy (6-10).

Between 1973 and 1991, 4431 newborn patients had been treated with V-A ECMO for acute respiratory failure (11). 83% of these patients survived. The most common indications for ECMO in these patients were meconium aspiration (1698 patients, 93% survived), congenital diaphragmatic hernia (784 patients, 61% survived), respiratory distress syndrome (658 patients, 84% survived), persistent fetal circulation (573 patients, 88% survived) and sepsis (566 patients, 84% survived). A major problem in the non-survivors has been that of bleeding and neurological deficits.

Results of ECMO in cardiopulmonary failure in children have been relatively less rewarding (12,13). By Jan 1991, as per International ECMO Registry Report, 186 children were treated for pediatric cardiorespiratory failure with ECMO. 80 (43%) of these children survived (11).

There are some recent reports of V-A ECMO being used both in children and in adults for post cardiotomy cardiac support (14-18). According to the International ECMO Registry Report (Jan 1991), 366 neonates and children were supported with ECMO for post cardiotomy cardiac failure with 47% (173 patients) survival rate (11). These also include children who were supported by ECMO before and after cardiac transplantation. On the other hand, the survival rate in adult patients was poorer, 4 out of 18 patients (22%) survived (11). V-A ECMO is also being used to support adult patients with cardiogenic shock following a myocardial infarction or a failed percutaneous transluminal angioplasty (19-21). Some of these patients have subsequently been operated upon successfully or have been transplanted. Axelrod et al have demonstrated in their experimental studies that, contrary to a roller pump, an ECMO system based on a pulsatile pump, especially one that uses a diastolic counterpulsation, can significantly unload ischemic hearts and thus has a greater potential as a cardiac assist device (22-24). In a review of the use of ECMO in the adult north american population at 29 cardiac surgical centers performing 23,000 cardiac surgical procedures (9% of the total cardiac surgery performed in North America), 23 ECMO procedures

were performed during a one year period (25). While 52% of these procedures were performed for cardiopulmonary support, 22% were performed as bridge to cardiac transplant, 9% for cardiac support, and only 17% were performed for ARDS. Although V-A ECMO is now rarely used for ARF in adults, it is one of the few options available in treating adult patients with severe acute cardiopulmonary failure (26).

Recognizing the barotrauma caused by prolonged mechanical ventilation at high air way pressures, Kolobow et al, advocated the use of apnoeic oxygenation in combination with extracorporeal removal of metabolically produced carbon dioxide (27). With this concept of ECMO, also known as low frequency positive pressure ventilation with extracorporeal carbon dioxide removal (LFPPV-ECCO$_2$R), clinical results in ARDS seem to have improved (28). Out of 55 patients treated with LFPPV-ECCO$_2$R in Milan, from July 1979 to December 1986, 26 (47%) survived (29). The LFPPV-ECCO$_2$R study has, however, been criticized for the lack of concurrent controls, and the randomized double blind allocation (30). By March 1988, more than 115 adult patients with ARF and fulfilling the ECMO entry criteria, were treated at various european centers with this modality of treatment, with 50% survival rate (31). One of the major changes in this new modality of treatment was a change over from the veno-arterial mode (V-A ECMO) used in ECMO study, to the veno-venous (V-V ECMO) one. This form of ECMO has recently also been employed for the treatment of non-cardiogenic pulmonary edema following coronary bypass surgery in adults (32).

When ECMO is applied in the veno-arterial mode, it is capable of supporting both the heart and the lung, whereas in the veno-venous mode it is applicable mostly for a pulmonary support. Based on these two basic modes of application, membrane oxygenation may be classified as in Fig.1. Several other forms of ECMO discussed in Fig.1 are the result of modification in the cannulation technique and/or the pump system.

A typical ECMO system consists of a membrane oxygenator with its built-in or a separate heat exchanger, a roller pump, a hypo-hyperthermia machine, a servocontrolled venous reservoir, tubing, connectors, and cannulas. For V-V ECMO, the draining cannula is placed either in the right atrium or in the inferior vena cava and the oxygenated blood from the membrane oxygenator is returned to the patient in the inferior vena cava or the right atrium respectively. For V-A ECMO in neonates and infants, cannulation is generally achieved with a venous line in the right internal jugular

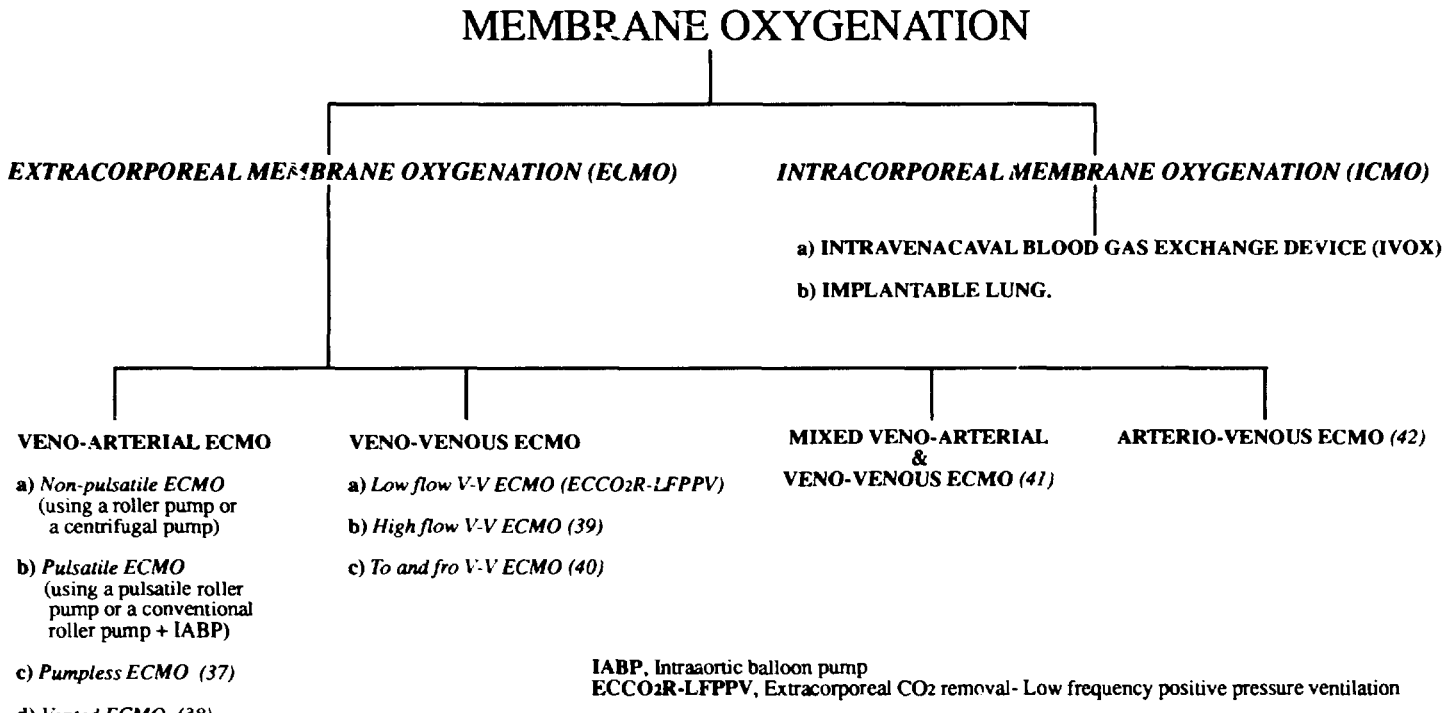

Fig.1. CLASSIFICATION OF ECMO

vein and an arterial cannula in the internal carotid artery. Collateral flow assures cerebral perfusion in these small children (33). At follow up, 70 to 75% of the neonates and infants treated with V-A ECMO are reported to be neurologically normal (34). For V-A ECMO in adults the popular approach for cannulation is femoral vein and the femoral artery.

The concepts of implantable lungs for chronic respiratory failure (35) and of intravenacaval blood gas exchange device (IVCBGE) (36) are relatively new and in a developing phase.

1.2 Heparin coated biomaterials

The method of binding heparin to rigid plastic surfaces was first reported by Gott et al (1963). The surface was prepared by dipping the material first in a colloidal graphite solution, and after drying it was dipped in benzalkonium chloride, a cationic surfactant. The graphite due to its absorptive properties held the positively charged surfactant firmly to the surface. This treated surface was subsequently dipped in a heparin solution (negatively charged) resulting in a graphite-benzalkonium-heparin (GBH) surface. This surface was shown to be thromboresistant (43,44). An improved method for the ionic binding of heparin, involving a pretreatment of the surface with tridodecylmethylammoniumchloride (TDMAC) in a lipophilic solvent, was reported by Grode et al in 1969 (45). When the plastic surface is dipped into this solution, the solvent etches the surface of the plastic, thereby allowing the TDMAC to be embedded into the wall. Advantages of TDMAC-heparin surface over GBH surfaces were; elimination of graphite, preservation of the transparency of the plastic surface, and relative resistance of the surface to the crushing damage caused by the application of metal clamps. TDMAC-heparin surface could be established on materials like polyvinylchloride, but biomaterials like silicone rubber (used in membrane oxygenators) and polycarbonate were seen to get damaged by high concentration of solvent (toluene) used to take TDMAC into solution. Another quaternary ammonium salt, methyltricapryl ammonium chloride (MTCAC), which could be taken in solution with a reduced concentration of toluene, was used instead to pretreat surfaces like silicone rubber and polycarbonate (46). Long term ECMO in experimental animals with TDMAC-heparin and MTCAC-heparin coated systems was attended with a syndrome of bloody vomits,

diarrhea and fluid balance shifts and was associated with a high mortality. This was shown to be due to the slow elution of both heparin and the surfactant into the blood (47). These ionically bonded heparin coated ECMO systems when stabilized further by cross linkage with, for instance glutaraldehyde (48), enabled successful long term heparin free bypass in experimental animals. However, a significant elevation of plasma free hemoglobin, SGOT and a significant decrease in blood platelets was observed in these experiments (47,49). Veno-venous and veno-arterial ECMO performed in baboons with similar systems produced contradictory results (50,51). Another method of preparing heparin coated surfaces involves blending of heparin into the polymer matrix, making heparin a permanent component of the biomaterial (surface grafting) (52,53).

Heparin has been bonded covalently to various surfaces using its hydroxyl and/or amino functions (54-57), carboxyl functions (58) and aldehyde functions (59). Partially degraded heparin (using nitrous acid) fragments with reactive aldehyde groups, and when coupled covalently by their end point to an aminated surface (60), yield a thromboresistant surface (Fig.2). This surface to a major extent satisfies the following criteria for an ideal thromboresistant surface (60-68);

a) Platelet compatibility (minimal platelet adhesion and activation).
b) Intact antithrombin binding sequence in the heparin fragments.
c) Surface adsorption of Antithrombin.
d) Surface adsorption of Thrombin and Factor Xa.
e) Inhibition of thrombin and factor Xa activity in presence of antithrombin.
f) Surface stability on exposure to blood over extended periods of time.
g) Maintained surface integrity on exposure to varying wall shear rates.

The end point attachment of heparin fragments not only leaves antithrombin binding sequence of these fragments intact but also provides them some degree of mobility in space, with the covalent binding imparting an additional stability. When similar heparin fragments are adsorbed covalently onto a surface as extended straight chains (multi point attachment - sterically restricted flat orientation), platelet compatible and thromboresistant properties of the surface are lost as exemplified in the Table 1 and Fig.3. The end point attached and covalently bonded heparin surface simulates the endothelial lining in its thromboresistance (Fig.3). This surface has also been shown to be

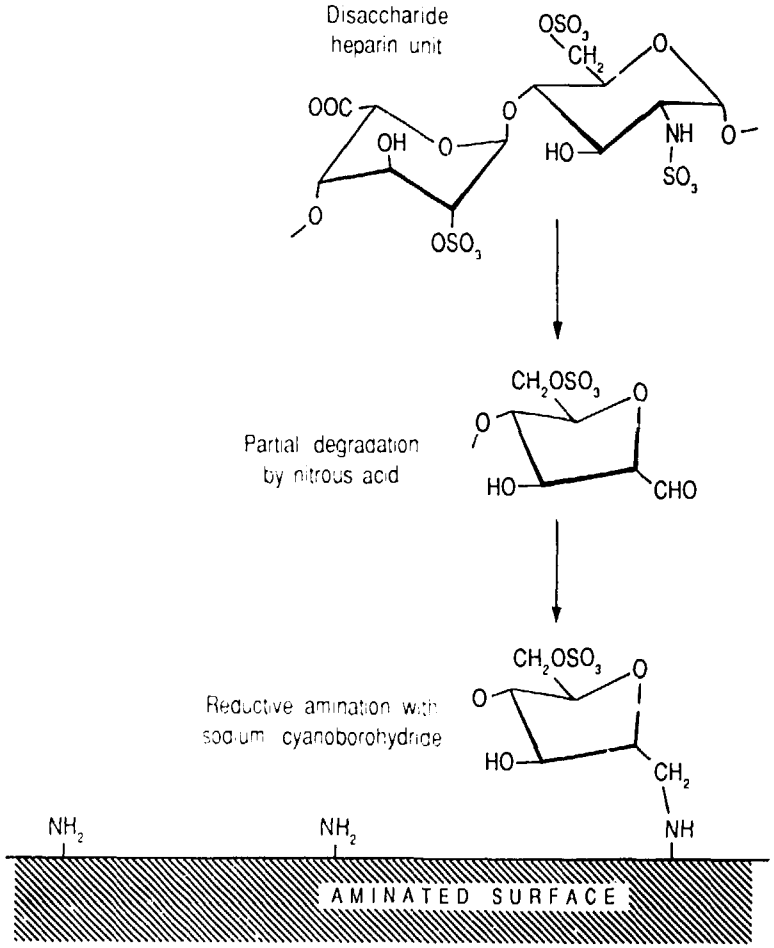

Fig. 2. End point attachment and covalent binding of the partially degraded heparin fragments to an aminated artificial surface.

compatible with granulocytes and macrophages (65), and is also more biocompatible in terms of complement activation (69).

The first clinical V-V ECMO for adult respiratory distress syndrome (ARDS) with covalently bonded heparin coated system was reported in 1986 (70) and since then about fifty patients have been treated with covalently bonded heparin coated systems (71-76). In their series of 86 patients with severe ARDS treated with V-V ECMO, Knoch et al report a one year survival of 55% and among their last 15 patients treated with a heparin coated ECMO system the mortality was only 20% (76). Use of heparin coated ECMO systems in ARDS seems to have reduced the frequency of fatal bleeding,

resulting in the bargain in prolonged ECMOs. Very little is known about the problems, prognosis and the long term sequelae of these prolonged ECMOs.

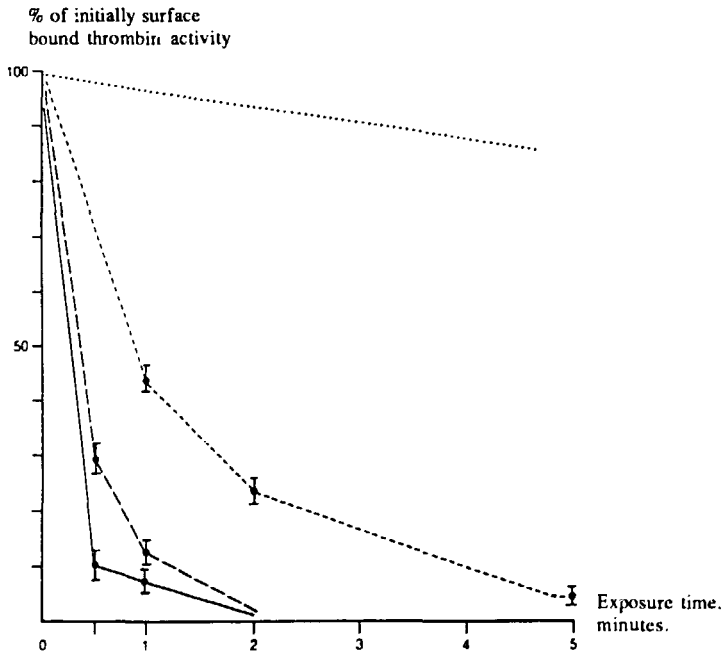

Fig. 3. Inhibition of surface confined thrombin activity on the endothelium and different heparin coatings. ——, Endothelium(n=12);– – –, End point attached and covalently bonded heparin coating(n=10); - - -, Glutaraldehyde-stabilized ionic heparin complex coating(n=6); ·····, Multi point covalently bonded heparin coating(n=6). (Reproduced with kind permission from the publishers, The Annals of New York Academy of Science, and the authors Olsson et al, from "Requirements for thromboresistance of surface-heparinized materials", 1983) (62).

A major problem in patients with advanced ARDS is that of systemic arterial hypoxemia. Despite high extracorporeal blood flow during a V-V ECMO, an optimal systemic arterial oxygenation may not be achieved in these patients (39,77). This is possibly due to partial recirculation of the oxygenated blood through extracorporeal circuit, thereby reducing the efficiency of this ECMO. A conventional V-V ECMO may therefore not be adequate as total extracorporeal lung assistance (ECLA). New methods are needed to improve the efficacy of the conventional V-V ECMO to make this treatment optimal. A high flow veno-arterial ECMO, on the other hand, provides an efficient and adequate gas exchange function, and supports the circulation as well. V-A

ECMO has however, for some unknown reasons, not improved the prognosis in respiratory distress syndrome in the adult patients. Results of V-A ECMO in neonatal

Table 1: *PLATELET ADHESION TO HEPARIN SURFACES (50 cm^2) AFTER EXPOSURE FOR 30 min TO HUMAN NATIVE BLOOD (about 1.5 ml)*

Surface	*Clotting*	*Number of adhering platelets (x $10^{-3}/cm^2$)*	*Platelet count in exposed blood (x 10^{-9}/L)*
Polyethylene	Yes	4960 ± 744	-
MPA	Yes	9 ± 7	-
EPA	No	8 ± 3	259 ± 29 (not different from pre-experimental value)

MPA: Multi point attachment of heparin
EPA: End point attachment of heparin

(Reproduced with kind permission from ISAO press and from authors Larm et al, from "An approach to antithrombosis by surface modifications", Progress in Artificial Organs, 1985:313-18 (ISAO Press, Cleveland, OH 1986).

ARF, on the other hand, have been better. One of the major autopsy findings in patients treated with V-A ECMO and those operated for heart under prolonged cardiopulmonary bypass, has been that of pulmonary damage (5,41,78-82). The genesis of this pulmonary damage is not clear. Complement activation is believed to be one of the factors (83). A V-V ECMO with supposedly an equipotent effect on the complement activation has, on the other hand, improved the prognosis in adult ARF. The extent to which exclusion of the pulmonary artery circulation plays a role in the genesis of the pulmonary damage is not known.

Bleeding is the major bypass related problem of both the adult and the neonatal ECMO (5,7,28). Heparin treatment in itself may induce thrombocytopenia (84). A conventional V-V ECMO, in spite of therapeutic doses of heparin, does activate platelets and the coagulation (85). V-V ECMO with covalently bonded heparin coated systems appears to produce minimal activation of platelets and the coagulation proteins in

experimental animals (86, 87). At present, V-A ECMO is the only method available for postcardiotomy cardiac support in children. ECMO in this clinical situation differs from the one used for pulmonary support in that these patients usually have previously undergone long runs of conventional cardiopulmonary bypass with its damaging effects (83). When these patients are switched over to a conventional V-A ECMO (requiring therapeutic doses of heparin), bleeding from the large operated area and other generalized bleeding diatheses pose a serious and often a life-threatening problem. Under these circumstances, a heparin free ECMO with a covalently bonded heparin coated system may be advantageous because the circulating heparin can be neutralized with protamine with an ongoing ECMO, without compromising the efficacy of the heparin surface (67). The extent to which a prolonged heparin free ECMO with a covalently bonded heparin coated system per se affects the in vivo activation of platelets and the coagulation proteins, and other vital organ function needs further evaluation.

2. AIMS

Clinical experience with three adult patients with severe acute respiratory failure (Study I) showed that a conventional high flow veno-venous ECMO may not provide an optimal respiratory support in the initial ECMO periods, when patients' pulmonary function is very poor. In all these three patients, ECMO was performed with covalently bonded heparin coated systems using low doses of heparin. Based on this experience, following studies were undertaken with the aims:

a) To develop a safe and effective total extracorporeal artificial lung. (Study II)

b) To assess the feasibility of veno-arterial ECMO for prolonged total extracorporeal heart and lung assistance. (Study III & IV)

c) To determine the pulmonary function and the in vivo activation of the platelets and the coagulation cascade, following the use of covalently bonded heparin coated system, after a twenty four hour heparin free veno-right ventricular ECMO. (Study V)

3. MATERIALS & METHODS

3.1 Patients (Study I)

Three patients, all males, with Adult Respiratory Distress Syndrome (ARDS), and fulfilling the entry criteria as recommended in the ECMO study (5), were treated with a heparin coated veno-venous ECMO for 8, 12, and 34 days (Tables I & II, Paper I). In two patients, ARDS developed as a sequel to trauma and in the third it followed a bilateral pneumococcal pneumonia.

3.2 Animal preparation (Study II-V)

For each study, six swedish native breed male pigs with a body weight in the range of 50 - 60 kg were used. The animals were put to sleep by i.m. injection of ketamine hydrochloride, 25mg/kg body weight. Pentothal sodium, 250-500 mg was given i.v. before a tracheostomy was done. An endotracheal tube was placed in the trachea and connected to a Siemens Elema Servo Ventilator 900. A volume-controlled ventilation (FiO_2 = 0.21-0.40) with a minute ventilation of 170 ml/kg body weight was instituted. Anesthesia and relaxation were maintained by continuous i.v. infusion of a mixture of ketamine hydrochloride (300 mg/h) and pancuronium bromide (20 mg/h). Central venous and arterial pressure lines were established through the neck vessels. An indwelling Foley catheter was placed in the urinary bladder through a suprapubic cystotomy. Prophylactic antibiotics (Penicillin-Streptomycin) were given 12-hourly. Four liters of i.v. fluids were given during the 24 hour period. A midline sternotomy was performed, and the pericardium opened by an inverted T-incision. Pressure lines connected to transducers were placed in the pulmonary artery and in the left atrium.

The experiments were conducted under regular monitoring of ECG, central arterial and venous pressure, pulmonary and left atrial pressure, urinary output, blood gas analysis and the body temperature. Cardiac output was measured either by a Swan-Ganz thermodilution technique (Study III,IV) or electromagnetically (Study II,V). Total body oxygen uptake was calculated from blood gas analysis of arterial and mixed venous blood, cardiac output and the hemoglobin concentration (Study III,IV). Lung compliance (open chest and pleura) was obtained from the ventilator. Functional residual capacity was determined both before and at the end of the experiment (Study III) by a computerized tracer gas wash-out technique (88). Blood samples were taken before and

at the end of the experiment for routine hematology, blood biochemistry, and for measurement of renal and liver functions. Only those animals with normal pre-experiment central and peripheral hemodynamics and blood gases were used in the studies.

3.3 Methods

End point attached and covalently bonded heparin coated ECMO systems (Carmeda, Stockholm, Sweden) were used in all the five studies. The system consists of;

a) Maxima hollow fibre oxygenator (Medtronic Inc., Minneapolis, Minn.)

b) Wire-reinforced 28F, 24F and 20F cannulas (Research Medical Inc., USA)

c) Polyvinylchloride tubing 3/8 inch (2 m x 3)

d) Venous collapsible balloon and its servoregulator (Carmeda)

e) Two-way, three-way and Y-connectors (3/8 inch) with and without Luer-lock sidearm.

To prevent mechanical tube rupture in the pump-house, about ½ meter long heparin coated C-flex tubing was interposed in the conduit part of the ECMO system in all the experimental studies (89). The ECMO system has a total surface area of about $2m^2$ and requires about one liter prime. For clinical ECMO (Study I), the system was primed with plasma while in the experimental studies (II-V) 5% albumin was used. The extracorporeal circuit comprised, in addition, of a roller pump and a hypo-hyperthermia machine (Gambro, Lund, Sweden). Low dose heparin was used during clinical ECMO, and activated partial thromboplastin time (APTT) was kept between 40 and 50 seconds. Levels of Antithrombin III (AT III) were kept above 80 %, and 500 - 1500 I.U. of AT III were administered i.v. when needed. All experimental ECMOs were carried out without any heparin or AT III administration. During experimental ECMO, oxygenators were run on 100% oxygen, and mechanical ventilation was readjusted so as to achieve normal PaO_2 and $PaCO_2$ in the effluent blood from the pulmonary veins. At the end of the experiments, the animals were sacrificed by an intracardiac injection of 20 mmol of potassium chloride and the relevant vital organs were autopsied.

The results were evaluated statistically using Wilcoxon signed ranks test for paired data, and value of $p = <0.05$ taken as significant.

3.3.1 Study I

Venous drainage to the ECMO system was provided by a 28F wire-reinforced cannula placed in the right atrium via the right internal jugular vein and the arterialized blood was returned into the inferior vena cava by the 24F wire-reinforced cannula intro-

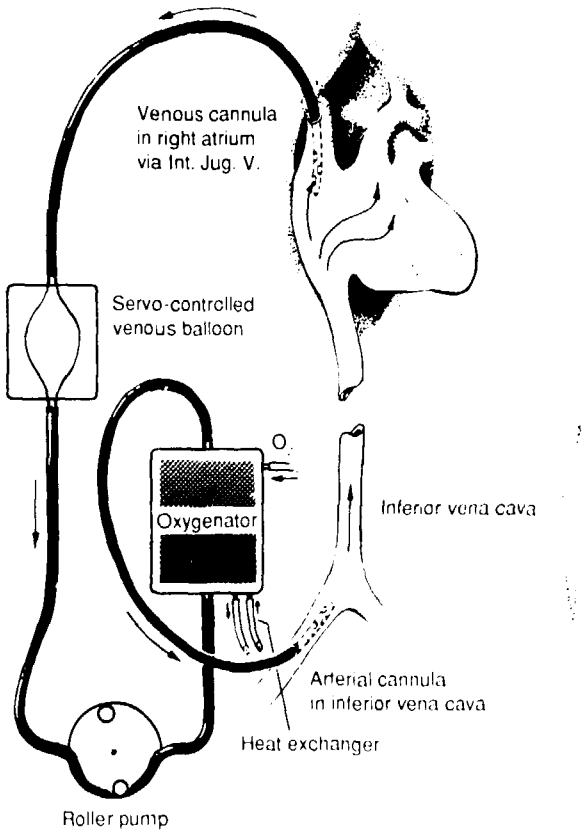

Fig. 4. Schematic diagram of conventional veno-venous (right atrium-inferior vena cava) ECMO. The arrows over the cardiac silhouette show the diversion of the arterialized blood from extracorporeal circuit to the lungs and to its recirculation back into the extracorporeal circuit via the venous cannula.

duced via the right femoral vein (Fig.4). As soon as a stable bypass was achieved, maximal possible blood flow was directed through the extracorporeal circuit. Oxygen

supply to the oxygenator was regulated according to the gas analysis of the arterialized blood leaving the oxygenator. The rectal temperature of the patients was lowered to about 34^0C in the initial phase of the ECMO in order to reduce oxygen consumption, thereby holding PaO_2 close to normal limits. Oxygen consumption by the patients was also kept low by muscle relaxation, heavy sedation and beta blockers. After the institution of ECMO, the peak inspiratory pressure (PIP) and the FiO_2 in the ventilator were reduced to lowest permissible levels, depending on systemic arterial PaO_2 and $PaCO_2$. Serum levels of fibrinogen and fibrin degradation products (FDP) and the blood platelet count were monitored daily. Patients were given prophylactic antibiotics during the entire period of ECMO. Oxygenators were changed in the event of plasma leakage. Weaning from the ECMO was conducted by reducing the gas flow to the oxygenator and/or extracorporeal blood flow. Decannulation was performed by a direct pull out technique. The patients received total parenteral nutrition during the entire period of ECMO. Two of these patients have been followed up for 22 months, with periodic lung function and stress tests.

3.3.2 Study II

A veno-right ventricular ECMO lasting for 24 hours was employed in this study (Fig.5). Three purse-string sutures were placed in the right atrium for cannulation. A 24F wire-reinforced arterial cannula was guided into the right ventricle across the tricuspid valve, aided by continuous pressure manometry. Venous drainage to the extracorporeal circuit was provided by two 28F wire-reinforced cannulas placed in the right atrium and in the inferior vena cava respectively. Veno-right ventricular bypass was started and maximal possible blood was diverted through the extracorporeal circuit. During the bypass, the ventilator was set to dead space ventilation (Table I, Paper II). Competence of the tricuspid valve was checked in two animals, both before and after the placement of the right ventricular cannula, by intraoperative Dopplerechocardiography. After 24 hours of ECMO, the lungs were ventilated again as before bypass and heparin administered intravenously (3 mg/kg body weight). Thereafter the animal was weaned off the bypass. Autopsy of the heart, lungs, liver and kidneys was performed and the pulmonary artery branches meticulously dissected. The extracorporeal circuit was examined grossly for clot formation and tube damage. The plasma volume of the animal was measured

with intravenous I^{125} Radioactive Iodine Human Serum Albumin, both before and at the end of ECMO.

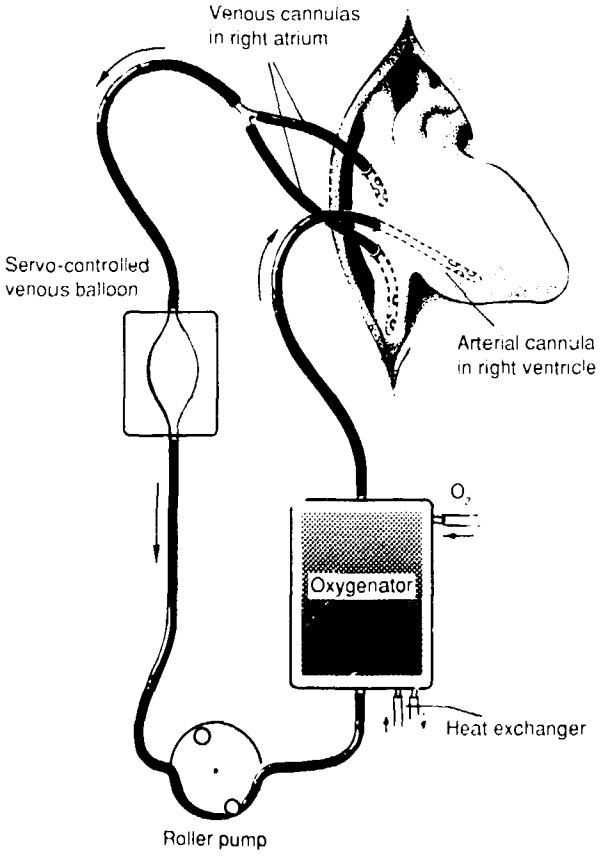

Fig. 5. Schematic diagram of veno-right ventricular ECMO. Cannulas for venous drainage were placed in the right atrium and in the inferior vena cava and the arterialized blood was returned by a cannula placed in the right ventricle across the tricuspid valve.

3.3.3 Study III

An 18 hour total veno-arterial bypass (VAB) was employed. Two purse-string sutures were placed in the right atrium for cardiac cannulation. Venous return to the heart-lung machine was provided by placing two separate 28F wire-reinforced cannulas in the inferior vena cava and in the right ventricle (across the tricuspid valve) respectively (Fig.6). Oxygenated blood from the heart-lung machine was returned to the

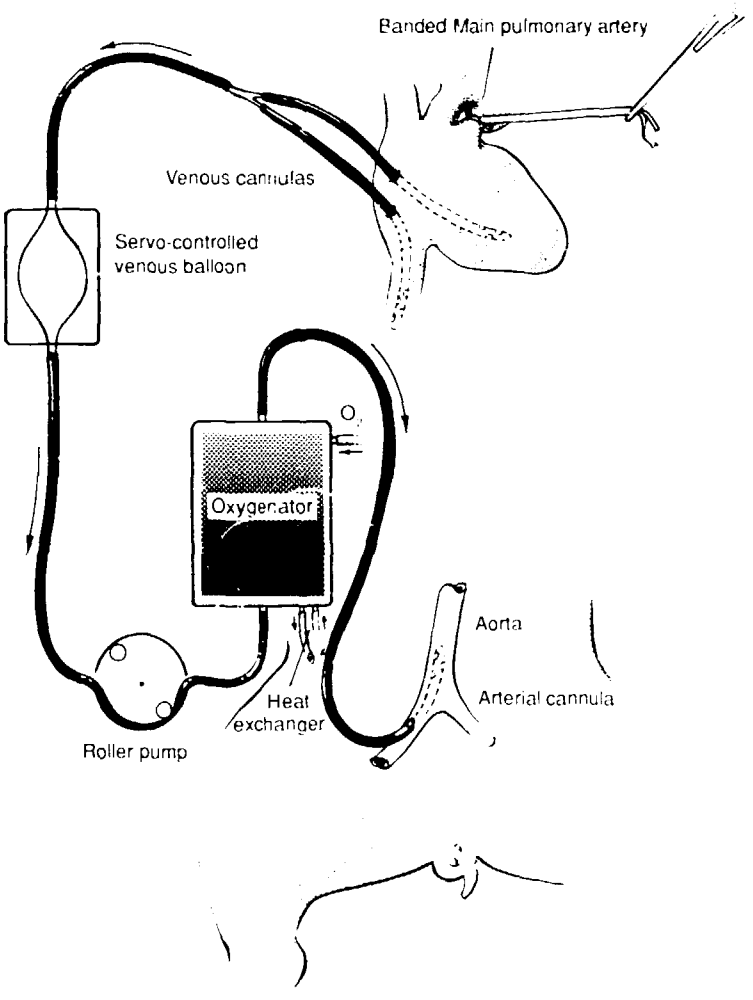

Fig. 6. Schematic diagram of total veno-arterial ECMO. Systemic venous return was drained into the extracorporeal circuit by means of cannulas placed in the right ventricle and the inferior vena cava. During 18 hours of ECMO, the main pulmonary artery was occluded by a snare and the heart was kept in a beating state.

animal through a 20F wire-reinforced cannula in the right femoral artery. Veno-arterial bypass was started and maximal possible venous return diverted through the extracorporeal circuit. The main pulmonary artery was thereafter occluded and the ventilator set to the dead-space ventilation. After 18 hours of total VAB, the main pulmonary artery was opened to the circulation again and the animal weaned from the

bypass over a period of one hour. During the period of weaning and following it, the ventilation was reset to parameters similar to those at the start of the experiment. At the conclusion of the experiment, autopsy of the heart and lungs was performed in all the animals. Histopathological examination of the lung samples was carried out under light microscope after staining with hematoxylin and eosin.

3.3.4 Study IV

A standardized V-A ECMO, lasting for 18 hours was employed (Fig.1, Paper IV). Venous return to the heart-lung machine was provided by two 28F wire-reinforced cannulas in the superior and the inferior vena cava respectively. Oxygenated blood from the heart-lung machine was returned to the animal through a 20F wire-reinforced cannula in the right femoral artery. V-A ECMO was started and maximum possible blood was diverted through the extracorporeal circuit. The superior and the inferior vena cava were taped and snared, thus diverting the entire systemic venous return into the extracorporeal circuit. 800 ml/min of the venous blood downstream to the servo-controlled collapsible balloon was redirected into the right atrium by a 1/4 inch PVC tubing and a 20F wire-reinforced cannula with the help of a pediatric roller pump (Stöckert, München, FRG). The left inferior vena cava was ligated at its entry into the coronary sinus, thereby allowing only the coronary sinus blood (approximately 400 ml/min) and the venous blood from the extracorporeal circuit (800 ml/min) to enter the pulmonary circulation. The total right cardiac output of about 1200 ml/min thus achieved, was confirmed at regular intervals by the thermodilution technique. After 18 hours of V-A ECMO, the caval snares were removed and the animal weaned from the bypass over a period of about 15 min. During the period of weaning and following it, the ventilation was reset to parameters similar to those at the start of the experiment. After weaning from the ECMO, the animals were observed for a period of six hours, following which the experiments were intentionally concluded.

The permeability of the alveolo-capillary barrier was studied, both before and after 18 hour of V-A ECMO, by measuring the pulmonary clearance of inhaled technetium-99m-labeled diethylenetriamine pentaacetate (^{99m}Tc-DPTA)(90).

3.3.5 Study V

A 24 hour Veno-right ventricular ECMO was employed in this study. The methodology used is similar to that described in study II (Fig.5). In addition to routine hematology, blood biochemistry and liver and renal function tests, following investigations were also carried out in this study.

2,3-dinor-thromboxane B_2 in urine

The in vivo formation of thromboxane A_2 (TxA_2) was monitored by mass spectrometric measurement of its metabolite 2,3-dinor-thromboxane B_2 (TxB_2) in urine (91).

Blood Platelet and coagulation studies

Platelet count was performed in an electronic cell counter (Coulter S 880; Coulter Electronics, Luton, England). Accelerated clotting time (ACT) was determined using Hemochron 400 (International Technidyne Co., Edison, NJ). Prothrombin complex (PT) and Activated partial thromboplastin time (APTT) were performed in accordance with the instructions of the manufacturer (Nycomed AS, Oslo, Norway). Antithrombin (AT) was determined by a functional chromogenic assay (92). Thrombin-Antithrombin complexes (TAT) were measured by an ELISA type assay (Behring, Marburg/Lahn, - Germany) (93). Fibrinogen was measured by polymerization-time determination (94). Fibrin monomers (FMM) were determined enzymatically (Kabi Pharmacia, Stockholm, Sweden) (95). Factor XII was measured by a chromogenic assay (96). von Willebrand factor (vWF) was determined in an ELISA system (97).

Plasma levels of AT and Factor XII were measured by employing pooled porcine plasma as calibration standard, while plasma levels of TAT,FMM and vWF were measured using antibodies against the human proteins and using human calibration standards.

4. RESULTS

4.1 Study I

In the initial part of the ECMO, the patients needed to be ventilated with relatively high FiO_2 and high PIP to achieve acceptable systemic arterial PaO_2 (Table III, Paper I). This was required in spite of satisfactory extracorporeal blood flow and gas exchange, hypothermia, skeletal muscle paralysis, and heavy sedation. Patients 1 & 3 had

relatively high cardiac output and both of them could subsequently be weaned from the ECMO after 8 and 34 days respectively. Patient 3 was, however, cannulated twice on account of an initial unsuccessful weaning from the ECMO on the 14th day. Patient 2 with post traumatic ARDS, was right from the outset hypokinetic with cold and cyanotic periphery. Despite aggressive medical and ECMO therapy, his general condition continued to be poor. He was subsequently diagnosed to suffer from candida sepsis and died after 12 days of ECMO. Autopsy showed heavy, hepatized lungs with bilateral intrapulmonary well circumscribed fleshy nodules of about 1 cm diameter and areas of liquefaction necrosis. Microscopy revealed features of shock lung.

Patients 1 & 3, while on ECMO, showed thrombocytopenia and bleeding diathesis (Fig.1, Paper I). One of them needed a thoracotomy for control of bleeding following insertion of an intercostal tube drain. Bleeding diatheses were corrected by transfusions of platelets and fresh frozen plasma. Levels of serum fibrinogen and AT III were normal. FDP levels were, however, moderately elevated in one of the patients. Prostacyclin (Flolan-Wellcome, 5-17 ng/kg/min) i.v. was started in Patient 3, with a view to stabilize the platelet counts. After termination of ECMO, the platelet counts returned rapidly to normal. On an average, between 675 and 970 ml of blood or blood products, were transfused every day to maintain a normal hemoglobin concentration. After deducting the blood loss on account of sampling, oxygenator changes, thoracotomy and the chest tube drainage, the average daily need for blood transfusion was still 350 ml. Hemolysis, as measured from serum haptoglobin and plasma free hemoglobin, was negligible. All the three patients showed elevation of serum bilirubin, glutamyl transferase (GT) and lactate dehydrogenase (LD) during an ongoing ECMO.

Oxygenators were changed on an average after 3 to 5 days of their use, on account of plasma leakage. During oxygenator change, two patients (Patients 2 & 3) repeatedly suffered from severe bradycardia and systemic arterial desaturation, and it could be documented and correlated to the acute onset of severe pulmonary hypertension in one of them (Patient 3). This patient received intravenous infusion of prostacyclin which seemed to blunt the severity of these crises. These crises were also accompanied by an abrupt drop in the lung compliance. Prior ventilation with 100% oxygen or a quick oxygenator change did not moderate these crises and it took several hours for the pulmonary artery pressure to revert back to the basal levels. Bilateral inter-

stitial emphysema, more marked on the right side, heralded the frequent onset of pneumothorax in Patient 3. However, air leakage following intercostal tube drainage lasted only for a day or two. All the oxygenators functioned satisfactorily. There were three incidents of tube rupture in the pump house with no serious consequences for the patients.

At 22 month follow up (Fig.2, Paper I), the patient with post pneumococcal ARDS (Patient 1), had regained his lung functions almost completely and was no longer dyspnoeic on exercise. The patient with post traumatic ARDS (Patient 3), on the other hand, did not show any substantial recovery in his lung volumes and capacities after the 12 month follow up. His lung functions continued to be poor at 22 month follow up although his exercise capacity was near normal. This patient became severely hypoxic at the peak exercise (Fig.2, Paper I). Both these patients are back on their jobs. At the time of discharge from the hospital, the patient with post traumatic ARDS treated with ECMO for 34 days was diagnosed to have right sided diaphragmatic paralysis and a Horner's syndrome on the left side. However, at six month follow up, the diaphragm had recovered completely.

4.2 Study II

The bypass remained stable for the entire period of 24 hours in all the animals (Figs.1-3, Paper II). In one animal, the bypass was allowed to continue for a total period of 72 hours, and the vital signs and the blood gas values remained stable. Pulmonary and systemic arterial oxygen tensions were identical and normal throughout the experiment. Carbon dioxide tensions in the oxygenated blood from ECMO and the systemic arterial blood were also identical and normal. Mean extracorporeal blood flow accounted for about 80 % of the cardiac output. Intraoperative Dopplerechocardiography in two animals not only confirmed an optimal positioning of the right ventricular cannula but also a relatively competent tricuspid valve.

4.3 Study III

During 18 hours of pulmonary artery occlusion, the VAB provided a stable hemodynamics in all the animals. Systemic and mixed venous blood gases remained within the normal limits in all the animals. After the cessation of VAB, all the animals

died within four hours (range: 0.5-4 hours). Death was preceded by massive pulmonary edema in two and cardiac arrest in the remaining four animals. All the animals showed a significant deterioration in the arterial and mixed venous blood PO_2 (FiO_2 = 0.21) in the immediate post bypass period (Table 2, Paper III). The PaO_2 values, however, improved spontaneously and successively in all the animals. The total body oxygen uptake in the post bypass period (prior to death) when compared to the prebypass values, showed an increase in three animals and a slight decrease in the other two animals (Table 1, Paper III). Arterial and mixed venous blood PCO_2 also showed a significant increase in the immediate post bypass period (Table 2, Paper III). The striking biochemical alteration in the post bypass period and preceding the death was a progressively increasing severe acidosis in all the animals. Lactates in the arterial blood rose in direct proportion to the increase in the negative base excess (Fig.1, Paper III). The pulmonary vascular resistance (PVR) increased in all animals except one and by an overall average of about 200% (Table 1, Paper III). The systemic vascular resistance (SVR) on the other hand, remained essentially unchanged. Lung compliance decreased significantly and the functional residual capacity also showed a decrease (Table 1, Paper III).

The microscopic changes in the pulmonary parenchyma corresponded to an exudative stage of diffuse alveolar damage (Fig. 2A, Paper III). Intra-alveolar hemorrhage, fibrin exudation and inflammation involved more than 80% of the pulmonary tissue examined (Fig.2B, Paper III). Multiple thrombi were seen in the small alveolar capillaries. Early necrotic changes were seen in the worst affected areas.

4.4 Study IV

During 18 hours of V-A ECMO, all the animals maintained stable hemodynamics with systemic and mixed venous blood gases remaining within normal limits. The pulmonary blood flow remained constant at 1.2 l/min during the entire period of V-A ECMO. After weaning from the V-A ECMO, although the PVR dropped successively in all the animals, yet it was still significantly higher at the 6th post ECMO hour by an average of 76% compared to the pre ECMO values (Table I, Paper IV). At this time interval, the cardiac output had also decreased significantly by an average of 25% (Table I, Paper IV). The systemic arterial and the mixed venous PO_2 also showed a significant

decrease at the end of the observation period by an average of 18% and 26% respectively. The lung compliance decreased significantly by an average of 31%. After eighteen hours of V-A ECMO, the rate of pulmonary clearance of ^{99m}Tc-DPTA had decreased significantly by 21% (Table I, Paper IV). The systemic arterial and mixed venous PCO_2, pH and the base excess remained unchanged and so was the blood pressure and the SVR (Figs.2 & 3, Paper IV).

Histopathology of the lung specimens revealed focal alveolar wall thickening and alveolar capillary congestion (Fig.4, Paper IV). Exudation of protein-rich fluid in the alveolar spaces was seen sporadically with occasional red cell migration. About 80% of the pulmonary parenchyma looked normal.

4.5 Study V

Clinical variables

During 24 hours of veno-right ventricular ECMO and in the period following it, all the animals maintained a stable systemic and pulmonary hemodynamics (Table 1, Paper V). The mean extracorporeal blood flow rate amounted to about 80% of the mean systemic cardiac output. The systemic arterial and the mixed venous blood gas analyses during and after the cessation of ECMO were also within normal limits (Table 1, Paper V). Bleeding from the open sternotomy wound and from the cardiac cannulation sites was negligible and no exogenous blood transfusion was given. Both plasma and the blood volume measurements showed a significant decrease (11% & 15% respectively) after 24 hours of ECMO (Table 2, Paper V). The plasma free hemoglobin levels remained within the normal limits. After the onset of ECMO there was an immediate drop (20% at 4th hour) both in the hematocrit and the hemoglobin concentration on account of hemodilution. Thereafter both these parameters showed no statistically significant change (Fig.1, Paper V). All the oxygenators functioned satisfactorily at blood : O_2 gas ratio of 1:1. At the conclusion of the experiments, all the oxygenators showed small, sporadic but definite areas of thrombosis with special affinity for areas of blood stagnation. At autopsy, there was no evidence of macroscopic thrombosis in the heart and the pulmonary artery branches nor any suggestion of pulmonary embolism. The liver and the kidneys looked normal.

Coagulation variables

ACT, APTT and PT remained unaltered during and after 24 hours of ECMO (Fig.1, Table 3, Paper V). The total platelet counts remained also unaltered after 24 hours of ECMO (Table 3, Paper V). Plasma levels of AT III decreased significantly after 24 hours of ECMO (Table 3, Paper V). The pre ECMO plasma levels of TAT and FMM were high, and FMM levels showed further increase ($p = <0.05$) after 24 hour ECMO (Table 3, Paper V). Plasma levels of fibrinogen remained unaltered and those of factor XII decreased significantly (Table 3, Paper V). Plasma levels of vWF also decreased significantly. When the measured levels of these coagulation factors were corrected for the blood loss in the respective animal, then fibrinogen, FMM and vWF alone were significantly affected by the 24 hour ECMO (Table 3, Paper V).

Urinary levels of TxB_2

Levels of TxB_2 in urine increased significantly in the pre ECMO period (Fig.2, Paper V). After the initiation of ECMO, these levels decreased successively, and in the last six hours of ECMO the levels were similar to those in the pre experiment period (Fig.2, Paper V).

5. DISCUSSION

5.1 Clinical V-V ECMO (Study I)

Systemic arterial hypoxia was a common feature in all the three patients and all of them fulfilled the ECMO entry criteria (5). Extracorporeal blood flow of 4 - 5 l failed to provide satisfactory systemic arterial oxygenation in the initial period of ECMO in all the three patients. Under such circumstances, mechanical ventilation with high PIP and/or high minute volumes had to be continued over a certain period of time. The aim of ECMO is to relieve the lungs from the deleterious effects of high pressure/high volume ventilation and of high FiO_2 - an aim which was only partially fulfilled in our patients. Two patients did have relatively high cardiac output prior to the initiation of ECMO and in these situations a veno-venous bypass with a blood flow rate of 4-5 l/min may not be able to overcome a right to left shunt across the pulmonary vascular bed. Moreover, it is possible that the right atrial-inferior vena cava ECMO per se, produces a significant extracorporeal shunt, making this bypass less efficient. It is also possible that an inferior vena cava-right atrial ECMO is more favorable in this regard. With venous

cannula in inferior vena cava, the extracorporeal blood flow has been shown to get limited when outer diameter of the cannula exceeds 50% of the caval diameter (98). Placement of large size (28F) venous cannula in the internal jugular veins was followed by diaphragmatic paralysis and Horner's syndrome in one of the patients. This could be due to a direct pressure of the venous cannulas against the phrenic nerve and the stellate ganglion. An inferior vena cava - right atrial veno-venous ECMO may also obviate these risks since arterialized blood can be returned to the patient through a smaller cannula without affecting the pressure downstream to the oxygenator (Patient 3).

Pulmonary hypertension is one of the features of severe ARDS (99). In addition to mildly elevated pulmonary artery pressure, an intermittent pulmonary hypertensive crisis was documented in Patient 3 and these crises possibly occurred in Patient 2 as well. These crises occurred whenever the oxygenators were changed. At present we do not know the pathophysiology of these crises. Prostacyclin moderated the severity of these crises.

Two of the three patients showed a bleeding diathesis, manifesting on the 3rd day in one and on the 14th day of ECMO in the other patient. APTT values were in the range of 35 - 45 sec and serum levels of fibrinogen were within the normal limits while levels of serum FDP were somewhat elevated. At the time of appearance of bleeding diathesis, blood platelet counts in one of these patients were unaltered, though low ($47x10^9$/l), and in the other patient these were in the range of 56-$100x10^9$/l. V-V ECMO using Carmeda heparin coated ECMO system for periods up to five days lead to a slight drop in blood platelet counts in the experimental animals (86,87). About 50% patients of clinically diagnosed ARDS have thrombocytopenia (100). All the three patients under discussion did have thrombocytopenia prior to the initiation of ECMO, and during the ECMO period these counts followed a variable course. After discontinuation of ECMO, the platelet counts shot up rapidly. During an ongoing bleeding diathesis, a thoracotomy was performed in Patient 3 with no unusual operative and postoperative blood loss. These observations suggest that ECMO and ARDS together lead perhaps to both thrombocytopenia and thrombasthenia. However, at slightly elevated APTT values permissible with the heparin coated ECMO system, likelihood of fatal bleeding might be reduced. We have at present no convincing evidence that treatment with prostacyclin did offer a protection to the platelets, although it has been shown to prevent platelet adhesion over

non-heparin coated biomaterials (101). Prostacyclin is known to produce effusion into the serous cavities (102-104) and the patient in question did suffer from cardiac tamponade after about a week's treatment. This complication ought to be borne in mind when treatment with prostacyclin is contemplated.

Patient 2 had a poor peripheral circulation from the very outset and he was treated with specific antibiotics against several pathogenic organisms isolated from time to time. This patient ultimately died of a verified candida sepsis. He became increasingly dependant on mechanical ventilation despite unaltered extracorporeal blood flow, satisfactory oxygenator function and maximal medical treatment, a development which seems to carry an ominous prognosis.

Appearance of interstitial emphysema on chest x-ray in one patient was followed by recurrent pneumothoraces which were self limiting. It appears that these result from the rupture of tension air sacs into the pulmonary interstitium, with the pocket of air subsequently dissecting to the surface of the lung to produce a self limiting pneumothorax.

Plasma leakage from the oxygenators occurred in all the three patients. Interestingly, they all had hyperbilirubinemia with elevated levels of serum GT and LD. It is possible that elevated levels of bile salts accompanying the hyperbilirubinemic states, reduce the surface tension of the blood and predispose the oxygenators to an early plasma leakage. The ECMO systems functioned satisfactorily and could be run with confidence by the routine intensive care personnel. The hemolysis was negligible. The problem of tube rupture in the pump house has now been solved with the introduction of C-Flex tube segment in the circuit (89).

Decannulation by a direct pull-out technique may be performed when heparin coated Systems are used (slightly elevated APTT values). This bedside procedure saves cumbersome transport of the patient to the operation theater. For this purpose, provision of a subcutaneous tunnel for the cannulas before their exit from the skin is mandatory, since it facilitates manual compression and local clot formation over the phlebotomy.

Adequate nourishment of these hypermetabolic patients is problematic, especially during long ECMO periods. On one hand there is need for restriction of extra supply of carbohydrates for restricting CO_2 production (especially in the pre weaning period) and on the other hand, there is the factor of uncertainty regarding the effect of intravenous lipids on membrane function. We have lately been providing these patients

with i.v. lipids about six hours before an oxygenator change is due. The oxygenator is ultimately changed when the plasma turbidity test has become negative.

The two surviving patients are living a normal life. At 22 month follow up, the lung volumes and the lung functions at maximal exercise in Patient 3 were still low. Survival rate in patients with post traumatic ARDS treated with low flow veno-venous ECMO (LFPPV-ECCO$_2$R) is lower when compared to other varieties of ARDS (28). Whether post traumatic ARDS per se carries a poorer long term prognosis as well is not known.

While treatment with ECMO is life saving, some vital questions however remain unanswered namely,

a) How to counteract acute pulmonary hypertensive crises effectively?

b) Which active modalities of treatment should be adopted to hasten lung recovery during ECMO?

c) Why do these patients need more than the estimated blood transfusion in the absence of a significant hemolysis and bleeding diathesis?

d) Are there any negative effects of prolonged hypothermia?

e) What are the effects of prolonged foreign body reaction (fever, leukocytosis, raised C-reactive proteins) and complement activation on the recovery of lung function?

5.2 Veno-right ventricular ECMO as total ECLA (Study II)

As was seen in the above ARDS study, a V-V ECMO, despite high extracorporeal blood flow, did not allow complete optimization of the ventilator treatment. Patients needed continued mechanical ventilation with relatively high PIP and high minute volumes to obtain adequate PaO_2. Recirculation of the oxygenated blood in the extracorporeal circuit is a possible explanation. The dead space ventilation applied during the 18 hour period of veno-right ventricular ECMO did neither contribute to the oxygenation nor to the carbon dioxide elimination. Further, like a conventional V-V ECMO, the veno-right ventricular ECMO provides a uniform oxygenation of the entire arterial system including the aortic root. The stable central hemodynamics and almost normal Dopplerechocardiography findings (in two animals) point to a competent tricuspid valve even when the right ventricular cannula is positioned across it. This study shows that a

veno-right ventricular ECMO, with extracorporeal blood flow equal to 80% of the systemic cardiac output, can provide normal oxygenation and normal carbon dioxide elimination in 60 kg-pigs with totally disabled lungs. A competent tricuspid valve is an important prerequisite for the success of this procedure.

Role of V-V ECMO in the treatment of ARF is mainly supportive. It is therefore important that consideration is given both to the immediate efficacy and to the long term safety, when an unconventional pulmonary support is contemplated. A veno-right ventricular ECMO is an effective alternative to a conventional high flow V-V ECMO, and its superiority in a patient with severe ARDS has already been verified (Wetterberg T, personal communication, 1990). Both right ventricular and inferior vena cava cannulation was performed by a percutaneous technique and the patient was successfully weaned from the veno-right ventricular ECMO after 35 days.

Recirculation of the oxygenated blood in the extracorporeal circuit may also be avoided by returning the oxygenated blood from ECMO system directly into the pulmonary artery. In an experimental study, in which the systemic venous return was bypassed directly into the pulmonary artery (roller pump) with a view to reduce the right ventricular pre load, the animals were seen to develop severe pulmonary hypertension and pulmonary damage after just a few hours of bypass (105). Moreover, this method of ECMO is less amenable to cannulation by the percutaneous technique.

5.3 V-A ECMO as total heart & lung assistance (Study III & IV)

A total VAB of 18 hour duration resulted in 100% mortality in six healthy pigs within four hours of the cessation of bypass (Study III). Two animals died in the early post bypass period of a combined respiratory and metabolic acidosis. In the remaining four animals, the blood gas analysis was dominated entirely by metabolic acidosis. Blood lactates rose in direct proportion to the increase in negative base excess (n=3). There was a significant drop in the PaO_2 and PvO_2 in the early post bypass period. However, the post bypass total body oxygen uptake showed only a slight variation from the one in the prebypass period. It was in fact only two animals (no 3 & 5, Paper III), who registered a slight decrease in the post bypass total body oxygen uptake and both these animals died in the early post bypass period (60 & 30 min respectively) of severe pulmonary edema. The serial blood gas analysis in the remaining four animals was

dominated almost entirely by metabolic acidosis. One of the animals, who maintained a normal PaO_2 and $PaCO_2$ through the entire post bypass period, succumbed to death four hours after the cessation of VAB on account of increasing negative base excess (-21.5) and decreasing pH (7.06). In the presence of relatively normal systemic hemodynamics and a relatively unaltered total body oxygen uptake, the development of severe and rapidly progressive metabolic acidosis in the post bypass period can not therefore be totally explained on the basis of mild systemic hypoxia. The fact that acidosis manifested itself first after the cessation of VAB is suggestive of ischemic damage of the pulmonary parenchyma and a reperfusion phenomenon wherein, besides other toxic products, lactates from the ischemic lungs are washed out into the systemic circulation.

Light microscopy of the lungs showed morphological changes varying from frank pulmonary edema to intra-alveolar hemorrhage and pulmonary parenchymal necrosis in different stages of development in all individual specimens. More than 80% of the pulmonary parenchyma showed these changes. These histopathological changes can explain the significant drop in the pulmonary compliance and the decrease in the functional residual capacity. A two fold increase in the pulmonary vascular resistance may partly be ascribed to the massive pulmonary parenchymal damage with thrombotic obstruction of the pulmonary capillary bed. The form and the extent of these pathological pulmonary changes together with rapidly progressive metabolic acidosis culminating in the death of all animals would suggest that the pulmonary parenchymal changes induced were of irreversible type. The deterioration in the arterial blood gases was, however, not of such magnitude as to explain an early death of all the animals. Hypoxia, on the other hand, has been reported to be the principle alteration in the resected warm ischemic lungs preserved for periods beyond four hours (106). It is possible that the bronchial blood supply alone is sufficient to maintain the integrity of the alveolar gas exchange apparatus over long periods of pulmonary artery ischemia.

Functional and histopathological changes occurring in lungs as a sequel to conventional cardiopulmonary bypass have been ascribed to complement activation (107). It may be pointed out that the heparin coated system used in this study has been shown to be more biocompatible compared to non-coated system (69). It is doubtful that the pulmonary changes encountered in this study were, entirely or to a major extent, due to

complement activation since a 24 hour total veno-right ventricular ECMO in pigs was followed by normal pulmonary gas exchange and normal pulmonary hemodynamics (*Study V*). *This study has thus shown that an eighteen hour total VAB at normothermia* in healthy juvenile pigs is followed by pulmonary oedema of varying intensity, pulmonary hypertension, severe metabolic acidosis and global microscopic pulmonary parenchymal damage which is attended with 100% mortality.

In order to find out the minimal right cardiac output needed for optimal preservation of the pulmonary function during an 18 hour long V-A ECMO, the right cardiac output was increased step wise beginning with diversion of coronary sinus blood alone (about 400 ml = 5% of the cardiac output) in the pulmonary arterial system

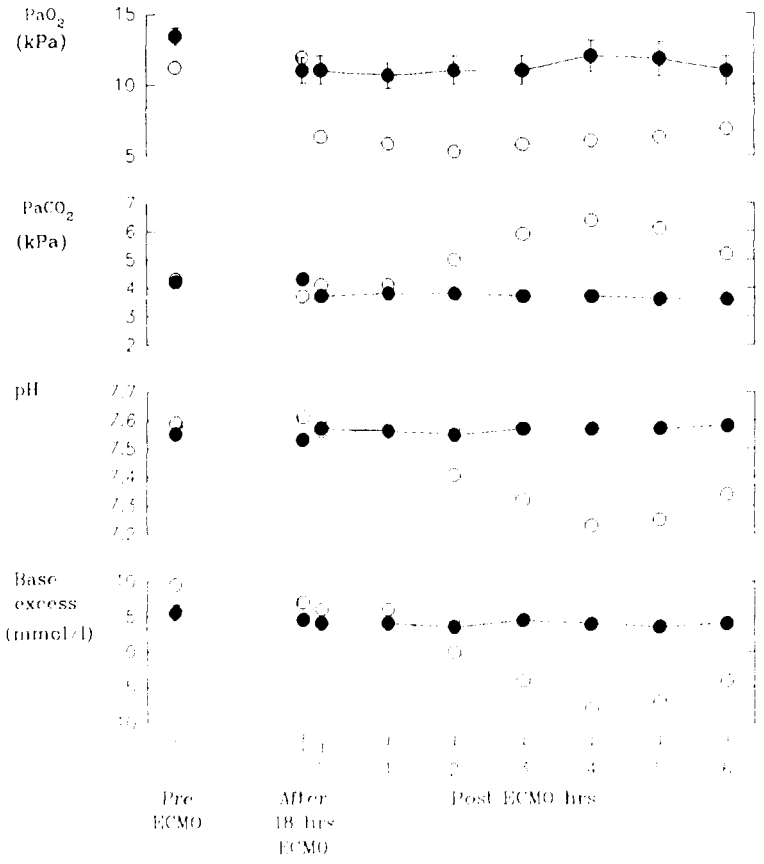

Fig. 7. Oxygen and carbon dioxide tensions and the acid-base balance in the systemic arterial blood following 18 hours of veno-arterial ECMO with 25% (closed circles, n=6, mean ± SEM) and 5% (open circles, n=1) right cardiac output.

(n = 1). This was achieved by separate caval cannulation and by snaring the tapes around the cavae, as in a conventional total cardiopulmonary bypass. After weaning from 18 hour V-A ECMO, the animal manifested in a manner similar to those with zero ml right cardiac output i.e. systemic arterial hypoxemia, severe pulmonary hypertension and progressive metabolic acidosis (Figs.7 & 8). This animal, however, recovered spontane-

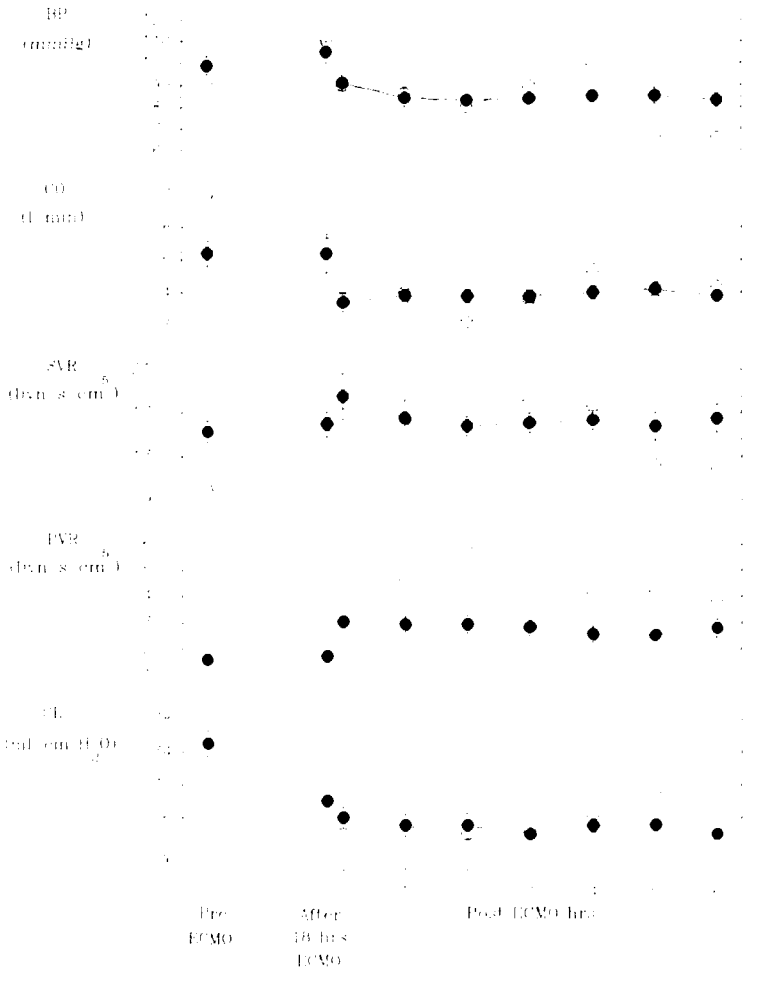

Fig. 8. Systemic and pulmonary hemodynamics and pulmonary mechanics following 18 hours of veno-arterial ECMO with 25% (closed circles, n = 6, mean ± SEM) and 5% (open circles, n = 1) right cardiac output. (BP, blood pressure; CO, cardiac output: SVR & PVR, systemic and pulmonary vascular resistance respectively; CL, lung compliance

ously from the metabolic acidosis after 10 hours of weaning, but his hemodynamics was very unstable with severe tachycardia, low cardiac output and severe pulmonary hypertension. Pulmonary clearance of ^{99m}Tc DPTA was grossly deranged and the pulmonary histology was similar to those with zero ml right cardiac output. Given enough of observation time, this animal would also have probably succumbed to death.

In another 18 hour V-A ECMO study (Study IV), in which 1200ml/min of venous blood was delivered to the lungs during the ECMO period, all the six animals survived the post ECMO six hour observation period, when the experiments were intentionally ended. This 1200 ml/min right CO corresponds to about 25% of the systemic CO measured in these animals. None of these animals exhibited either respiratory or metabolic acidosis. There was, however, a significant drop in the systemic arterial PO_2 to a mean of 83 mm Hg at the end of the observation period (Fig.3, Paper IV). This decrease in PaO_2 with an FiO_2 of 0.21 may clinically be expected on account of focal atelectasis and a small right to left shunt. Pulmonary vascular resistance, just prior to the cessation of V-A ECMO was markedly elevated due mainly to the reduced right cardiac output (1.2 l) during 18 hours of V-A ECMO, since it decreased abruptly and successively after the animals were weaned from the ECMO. A significant and variable increase in the pulmonary vascular resistance (mean = 76%, range = 30-163%, at the end of the six hour observation period, together with 20% microscopic involvement of pulmonary parenchyma, however, suggests that some pulmonary capillary endothelial damage may follow V-A-ECMO with a right cardiac output of about 25%. The pulmonary clearance of ^{99m}Tc-DPTA was also increased after 18 hour V-A ECMO. The mild, but statistically significant, increase of ^{99m}Tc-DPTA clearance in the present study is probably of less clinical significance keeping in view that the lungs were not only subjected to low right cardiac output but also to constant PEEP and low minute ventilation and no intermittent sigh or bronchial toilet was performed during the 18 hour ECMO.

Although the pulmonary function changes observed in this study are mostly reversible, yet it appears that 25% right cardiac output is the border-line safe pulmonary blood flow needed for preservation of adequate lung function during eighteen hours of V-A ECMO at normothermia in healthy juvenile pigs. These experimental studies therefore not only qualify right cardiac output as one of the important determinants in

the safety of a V-A ECMO but also specify the minimal right cardiac output needed for the safe conduct of prolonged V-A ECMO for cardiac or cardiopulmonary assistance.

These studies may partly explain the unsatisfactory results encountered following the use of V-A ECMO in patients with acute ARDS (5). In patients with severe ARDS and especially those with an associated pulmonary hypertension, a V-A ECMO is likely to steal maximum amount of the systemic venous return from the right heart, rendering V-A ECMO almost "total". Under these circumstances, prolonged use of V-A ECMO is likely to make the already existing lung damage worse. It is possible that in these situations even a slight decrease in the right cardiac output is not conducive for the recovery of severely diseased lungs. A V-V ECMO, irrespective of low or high extracorporeal blood flow rates, does not affect the right cardiac output, and this may be one of the explanations for its improved survival rates in acute ARDS, as compared to V-A ECMO. On the other hand, the results of V-A ECMO in neonatal respiratory distress syndrome are satisfactory. How are the neonatal lungs protected during a V-A ECMO? This can partly be explained on the basis of special anatomical connections, specific for this age group. In an echocardiographic study aimed at assessing the left ventricular function in neonates on ECMO, contrary to the authors' expectations, none of the infants showed a significant decrease in their left ventricular dimension (108). 9 out of 12 infants on ECMO demonstrated a left to right shunt at the level of patent ductus arteriosus. Two of them needed ductal ligation before they could be weaned from the ECMO. 9 out of 11 neonates on ECMO demonstrated, by contrast study, a right to left shunt at the atrial level. The authors suggested that the decrease in pulmonary blood flow when a neonate is placed on ECMO is not as great as would be predicted in the adult patients and that the decrease in the pulmonary venous return is also not of the same magnitude. A similar trend was observed by Martin and Short in their Doppler-echocardiographic studies in 19 infants with persistent pulmonary hypertension, during treatment with V-A ECMO (109). In their report of 100 cases of neonatal respiratory failure, Bartlett et al, describe 16 patients who needed a ductal ligation while they were on ECMO (7).

In a recently published article, Rossi et al have demonstrated that full recovery of heart and lung function is possible after as many as 3 days of ventricular fibrillation and a conventional cardiopulmonary bypass aided with partial decompression of the left

heart (110). In this study, the cardiopulmonary bypass was conducted on sheep weighing between 14.5 - 17 kg. Throughout the bypass, heparin was administered in the form of a continuous infusion to keep the activated clotting time between 200 and 250 seconds. In this study, the authors have not discussed in detail the post bypass pulmonary hemodynamics and the pulmonary histopathology of the animals. Moreover, information about the presence or absence of various systemic-pulmonary anatomic shunts in these young animals is also lacking. Specie variations as regards the pulmonary tolerance to ischemia, can not however be ruled out.

5.4 Heparin free ECMO (Study V)

Bleeding is the major bypass related complication of a V-V ECMO (28). A heparin coated ECMO system may minimize or obviate the use of heparin and thereby prevent bleeding. However, the surface per se may promote disseminated intravascular coagulation and the platelet dysfunction to a degree that no clotting occurs in the extra-corporeal circuit (50,51). It is therefore imperative that activation of coagulation proteins and thrombocytes is tested in vivo by sensitive laboratory and clinical tests. In this study pulmonary functions were also investigated, since during a veno-right ventricular ECMO lung is the only relevant vital end organ that can get affected by the thrombosis in the extracorporeal circuit.

The 24 hour veno-right ventricular ECMO did neither affect the pulmonary gas exchange function (with 40% oxygen ventilation) nor the pulmonary hemodynamics to an extent that would clinically be considered as significant. No macroscopic pulmonary artery thrombosis or embolization was seen in any of the animals and the lungs appeared normal. 15% of the total hemoglobin was lost during the 24 hour ECMO and about 1/3rd (5%) of this was lost on account of bleeding from the operated sites. Most of the remaining loss could be attributed to the frequent blood sampling during the 24 hour observation period. Since the hemoglobin and the hematocrit values also remained essentially unaltered after an initial hemodilution, possibility of a significant blood loss and a serious platelet and coagulation dysfunction is small.

The plasma free hemoglobin levels also remained normal after 24 hour ECMO which would suggest that the heparin coating per se does not predispose the red blood cells to hemolysis. Unaltered ACT and APTT values and a clean segment of PVC tubing

in the roller pump house would suggest that there was no significant elution of heparin from the surface and that the surface is stable. Both blood and the plasma volumes showed a significant decrease after 24 hours of ECMO, therefore various coagulation parameters including the hemoglobin and the platelets were corrected for these volume variations (Tables 2 & 3, Paper V). There was no significant elevation of TAT complex after 24 hours of heparin free ECMO suggesting that the heparin coated surface not only catalyses inhibition of thrombin by antithrombin but also inhibits the coagulation cascade at one or several steps prior to the generation of thrombin. Inhibition in the activation of factor X has been postulated as one such step in the mechanism of action of end point attached and covalently bonded heparin coated surface (66). Inhibition in the activation of factor XII may be yet another mechanism.

A significant elevation of FMM may, on the other hand, be explained on the basis of sporadic clot formation in the membrane oxygenators. A similar isolated increase in the plasma fibrinopeptide A has earlier been reported with this surface during heparin free thirty hour veno-venous ECMO in dogs (86). Significant reduction in the levels of vWF was unexpected and is difficult to explain. A significant increase in plasma fibrinogen is suggestive of an inflammatory reaction. Fibrinogen levels do increase following a major surgery (111) and represent an acute phase reaction.

Studies on urinary excretion of TxB_2 in humans strongly indicate that this metabolite is a good indicator of the in vivo synthesis of TxA_2 (112) - a metabolite produced almost entirely from platelet cyclooxygenase-arachidonic acid pathway. Surface induced activation of platelets leads both to platelet aggregation and to their release resulting in the formation of TxA_2 (113,114). Alterations in the in vivo synthesis of TxA_2 have been shown to be reflected by a change in the excretion of the urinary metabolites within one hour (115). Therefore, measurement of urinary TxB_2 enables us to study the dynamics of the in vivo synthesis of TxA_2 and thereby platelet activation in a continuous fashion. In this study there was a progressive decrease in the urinary excretion of TxB_2 after first six hours of the initiation of ECMO which would suggest that the heparin coated system did not perpetuate the pre ECMO activation and the release of platelets. Unaltered levels of this urinary metabolite during the first six hours of ECMO does not however exclude an initial (first passage) activation and release of platelets. Platelet activation and its subsequent release has been shown to lead to the activation of the

coagulation system (116). Since coagulation system in this study showed no significant activation and the total platelet counts remained unaltered, a significant initial thromboxane mediated platelet activity and release is unlikely.

These clinical and laboratory investigations suggest that the covalently bonded heparin coated ECMO system used in the studies discussed above, does not significantly activate the coagulation cascade and the blood platelets in healthy juvenile pigs during a 24 hour heparin free ECMO. Further, the end point attached and covalently bonded heparin coated surface inhibits the coagulation cascade at one or several steps prior to the generation of thrombin. Moreover, for the evaluation of coagulation and platelet activation during a major extracorporeal circulation, blood and plasma volume changes ought to be taken into the consideration.

5.5 Heparin coated surface - Future clinical implications

Freedom from systemic heparin, associated with the use of covalently bonded heparin coated ECMO systems, facilitated smooth conduct of prolonged ECMOs in the experimental studies discussed (Study II - V), with the following advantages. (a) On account of minimal blood loss from the sternotomy and other operated sites, no blood transfusion was needed in any of the experimental animals. This in turn facilitated realistic calculations of (b) total oxygen consumption and (c) the blood volume estimations in the animals. (d) After initial hemodilution, the hemoglobin concentration and the hematocrit values remained essentially unaltered, which in turn facilitated (e) stability in central hemodynamics and other vital organ functions.

To supplement study V with a control group of animals, V-V ECMO was conducted in three pigs using non-coated systems and employing therapeutic doses of heparin (ACT = 180-240 sec). Two of the three animals died before 18 hours of ECMO was completed, on account of continual blood loss from the operated sites and the development of severe anemia. One of these two animals also developed severe hemolysis soon after the initiation of ECMO. The third animal showed severe circulatory instability after 16 hours of ECMO, again on account of severe anemia.

No plasma leak occurred in any of the oxygenators used in the experimental studies. All the oxygenators functioned satisfactorily and none was changed during an ongoing ECMO. On no occasion did the C-flex tubing in the pump house rupture.

However, development of sporadic thrombosis in the oxygenators is disturbing and needs attention and so does the plasma leakage from the oxygenators. Development of a reliable heparin coated cardiotomy reservoir is still in its evolution.

The heparin coated extracorporeal bypass system may also find a potential clinical use in the following:

a) The treatment of ARDS associated with trauma including head injury.

b) The treatment of acute traumatic and non-traumatic aneurysms of the descending aorta.

c) The routine open heart surgery in patients sensitive to heparin and those belonging to jehovah witness etc.

d) The emergency open heart surgery in patients with concomitant active bleeding e.g. bleeding peptic ulcer etc.

e) The emergency resuscitation of patients in cardiogenic shock (e.g. failed percutaneous transluminal angioplasty), while the patient is being prepared for surgery under cardiopulmonary bypass.

f) The heparin free short term postcardiotomy cardiac support.

g) The support of circulation during a complicated pericardiectomy.

h) The temporary support in patients waiting for heart, lung, or heart-lung transplantation, provided a potential prospective donor is available in the near future.

i) The temporary support in heart and/or lung transplant patients during the treatment for a severe and otherwise fatal acute rejection.

j) The non operative treatment of massive acute pulmonary embolism in conjunction with available fibrinolytics.

6. GENERAL CONCLUSIONS

a) A high flow V-V ECMO may not adequately support patients with severe acute respiratory failure.

b) Heparin coated ECMO systems in patients with severe ARDS may reduce the risk of fatal bleeding mainly on account of use of low dose heparin with only slightly elevated APTT values.

c) Acute intermittent pulmonary hypertensive crises, recurrent pneumothorax and plasma leakage from the oxygenators pose serious problems during treatment with ECMO in patients with severe acute respiratory failure, and merit further investigations.

d) A veno-right ventricular ECMO serves as an effective total extracorporeal artificial lung in 60 kg healthy pigs. A competent tricuspid valve is an important prerequisite for the success of this procedure.

e) An 18 hour long heparin free total V-A ECMO is followed by varying degrees of systemic arterial hypoxemia, severe pulmonary hypertension and progressive metabolic acidosis which in an uncorrected state is 100% fatal in healthy juvenile pigs.

f) A minimum of 25% right cardiac output is essential for preservation of adequate pulmonary function during an 18 hour long V-A ECMO in healthy juvenile pigs.

g) A covalently bonded heparin coated ECMO system neither activates the coagulation cascade nor the platelets during a 24 hour heparin free veno-right ventricular ECMO in healthy juvenile pigs.

h) The end point attached and covalently bonded heparin coated surface inhibits the coagulation cascade at one or several steps prior to the generation of thrombin.

i) In the assessment of coagulation and platelet activation during a prolonged and major extracorporeal circulation, consideration should be given to the blood and plasma volume changes.

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