P11

CERTIFICATE

I, Dr. K.S.V.K. SubbaRao .....hereby declare that I have actually performed all the procedures listed/carried out the project under report.

Signature.....

Place: Trivandrum

Name in K.S.V.K. SUBBA RAO

Date: 15-Nov. 85 · capital letters

Forwarded + Recommended

HOD 16/11/85

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM 695011

Name	KSVK SubbaRao
Page	of
Date	15- Nov. 1985

~~LIST OF PROCEDURES DONE~~

PROJECT REPORT

TITLE OF THE PROJECT:

Setting up and Standardisation of an experimental model to produce haemorrhagic shock in mongrel dogs so as to evaluate haemoglobin solution which is being developed in the division of Pathophysiology of BMT Wing

NAME: KSVK SUBBARAO

PROGRAMME: M.Ch (CCVTS)

MONTH & YEAR OF SUBMISSION: November 1985

Note:— (i) In the case compilation of procedures done, the contents and the subsequent pages should be made into different sections (a) Procedures done (b) Procedures assisted (c) Procedures participated (d) Procedures attended/participated etc in Other Centres. Each section should be preceded by a leaf carrying the name of the section that is succeeding.

(ii) The Contents page will carry into. as per model given under

PROCEDURES DONE

Closed Mitral valvotomy.....124 (say)
Patent ductus arteriosus-ligation.....10
Atrial septal defects.....20
.....
.....

PROCEDURES ASSISTED

Closed Mitral valvotomy.....100 (say)
.....

(iii) In the subsequent pages details of each procedure done/assisted should be given in the format given below:—

Heading: **Closed mitral valvotomy**

Date	Name of the patient	Age	Sex	Patient No.

(iv) In the case of Project Report in the page immediately following the Certificate page the under-mentioned details should be given:—

(a) Title
(b) Duration
(c) Aim and scope
(d) 50 word summary of work done

CONTENTS

ACTIVITIES DURING POSTING AT BIOMEDICAL TECHNOLOGY WING.

I Involvement in the ongoing research project.

Setting up and standardisation of an experimental model to produce haemorrajic shock in mongrel dogs so as to evaluate haemoglobin solution which is being developed in the division of patho-physiology of BMT Wing

II. Performance of the following surgical procedures in animals to improve surgical skill

A. Interposition grafting in Thoracic aorta of pigs using prosthetic vascular graft.

B. Establishment of Cardio pulmonary bypass in sheep to evaluate the hard shell oxygenator

---

Project report

Title of the Project:

SETTING UP AND STANDARDISATION OF AN EXPERIMENTAL MODEL TO PRODUCE HAEMORRAGIC SHOCK IN MONGREL DOGS SO AS TO EVALUATE HAEMO GLOBIN SOLUTION WHICH IS BEING DEVELOPED IN BMT WING OF OUR INSTITUTE.

INTRODUCTION:-

Clinical shock is a complex group of syndromes with various physiological patterns sharing in common reduced oxygen consumption in the early period. The primary goal of therapy therefore is the restoration of O_2 debts and maintanance of oxygen delivary. Restoration of blood volume, pressure flow and O_2 carrying capacity are only therapeutic means to this end. The ideal therapeutic agent is therefore is the one which accomplishes these four therapeutic goals most rapidly and effectively with the least incidence of complications.

Haemorragic shock has been produced in animals to study the patho physiology and to test the efficacy of various resuscitation fluids

Herein we have tried to establish an experimental model by producing reversible haemorragic shock in mongrel dogs so that the haemoglobin solution that is being developed can be evaluated in comparision to other resuscitation fluids.

HAEMOGLOBIN SOLUTION:

Haemoglobin can be prepared from the outdated blood. Being a normal Constituent of blood it fulfills most of the requisites of a blood substitute the most important being capability of transporting oxygen. It also possess the oncotic activity and has a lower viscosity than blood. One of the advantages of haemoglobin solution is that it does not require cross matching or typing prior to use. It can be stored for long periods in solution in physiological saline

The high O_2 affinity and short intravascular halflife were drawbacks which prevented it being used clinically. Attempts to store haemoglobin solution at room temparature were futile. It could be stored for 2 years at $-20^{\circ}C$ and for 12 months at $4^{\circ}C$. This is a great

advantage in itself especially for army in comparision to the 21 days of shelf life of whole blood.

PREPARATION OF STROMA FREE HAEMOGLOBIN:

This was prepared from outdated red cells. The red cells were lysed and then the solution was centrifused at low speed. The solution was then dialysed against phosphate buffer. The crystals formed in the dialysis tubing were washed with phosphate buffer, dissolved in water and further dialysed against kidney dialysis fluid. The resultant solution was sterilised by gamma radiation.

The solution obtained at normal serum potassium and sodium spectral maxima and minima were characteristic of oxyhaemoglobin. No attempt was made at this time to Concentrate the haemoglobin solution.

The haemoglobin Content of this solution was about 5 to 6 gm%.

EXPERIMENTAL PROTOCOL:-

Twenty experiments were carried out using mongrel dogs weighing between 9 to 13.5 kgs. They were anaesthetised with Sodium pentobarbital, placed in a lateral position. Sodium heparin (1mgs/kg) was given at the begining of experiment to facilitate free bleeding. The dogs underwent haemorragic shock by bleeding through an arterial catheter so that the mean BP comes down to 20 mm/Hg in the initial experiments. Later the mean BP was brought down to 10mm/Hg and it was maintained at that pressure for about an hour. Small amounts of blood were reinfused as needed to maintain the mean BP at 10 mm/Hg. Throughout the experiment ECG CVP, arterial blood pressure (Systolic, diastolic and mean) Skin temparature and respiratory pattern were continuously monitored. The urine output was monitored in a few experiments but later stopped because of technical reasons.

The state of shock was also observed

by clinical parameters like rapid shallow breathing and sphincter relaxation apart from maintanance of mean arterial BP at 10 torr. The volume of blood that is bled varied in different animals from 350 cc to 770 cc. In one animal exchange transfusion was done by infusing normal saline as blood is being withdrawn. The Hb% and Hct. were measured in the blood that is obtained at various stages to determine the effect of splenic regeneration.

After keeping the animal in a shocked state for about an hour the animals were resuscitated with varying quantities of the following fluids

1) Normal Saline 2) Dextran alone 3) Dextran + Saline 4) Saline + Blood 5) Haemoglobin Solution.

The rate, time and type of recovery in these animals was observed immediately after reinfusion as well as for about 3 hours after words. The Hb% and Hct were measured next day morning also in surviving animals.

OBSERVATIONS

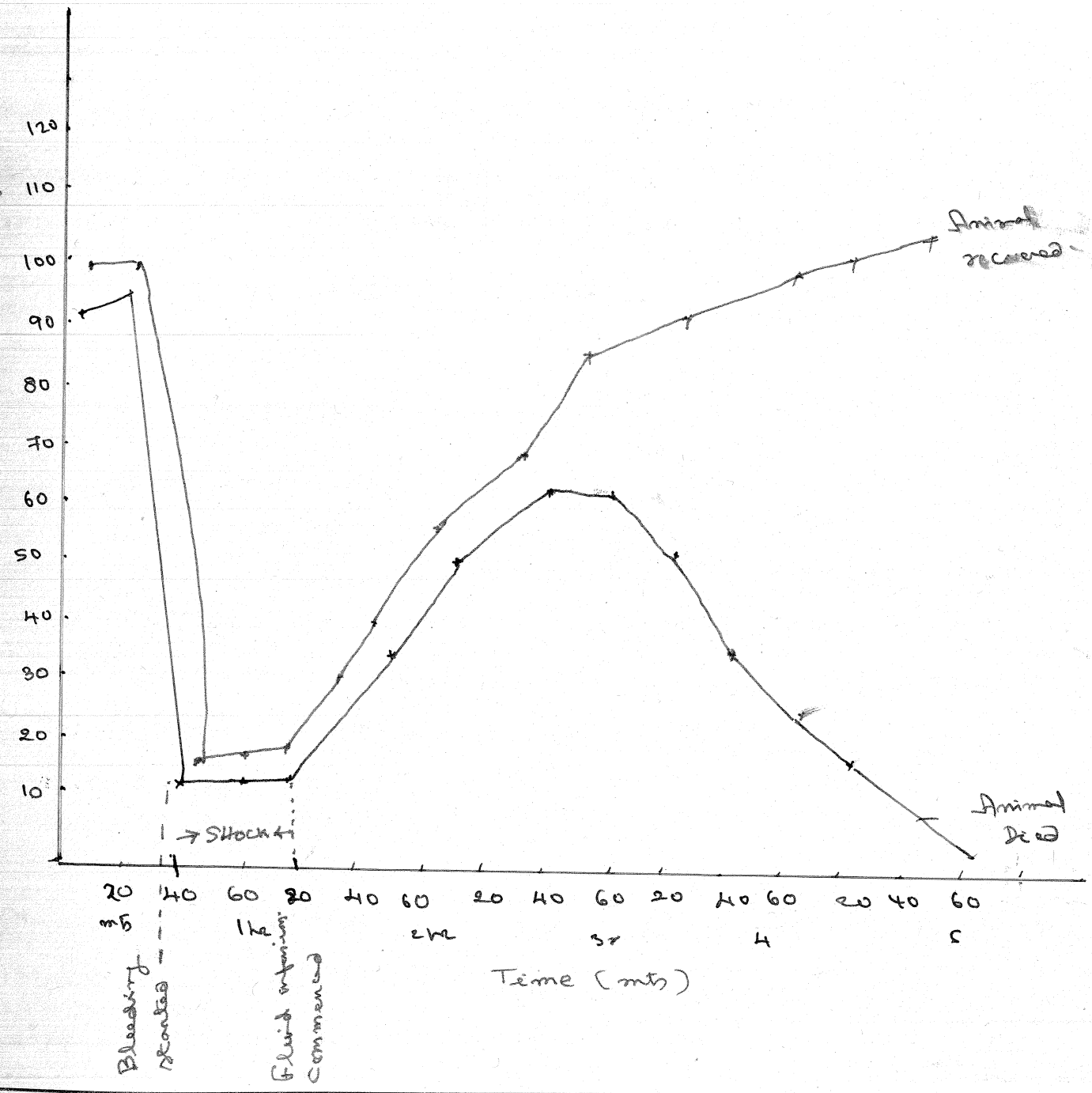
S.NO.	Weight of the dog (kgs)	Amount bled (ml)	State of shock. (MAP & Period)	Type and amount of fluid re-infused (ml)	Result.
1.	10.5	447	20 (MAP) and immediate resuscitation	Normal Saline 200.	Normally recovered.
2.	10.5	500	"	Normal Saline 100	"
3.	10.5	550	14 (MAP) "	Normal Saline 150	Died.
4.	11	500	10 (MAP) "	Normal Saline 100	Normally recovered.
5.	10	1450	20 MAP. Exchange transfusion was done using Nacl.	Normal Saline 1500	Recovered but drowsy Hb 6.74 Hct 19%
6.	9	550	20 (MAP) – 2hrs.	Normal Saline 250	Recovered normally.
7	10	450.	10 (MAP) – ½ hr	Normal Saline 500	Recovered normally.
8.	11	350	10 (MAP) – 1hr.	Normal Saline 350.	Died.
9.	10.2	590	10 (MAP) – 1hr.	Blood : 75 Nacl : 420	Died.
10.	12.5	710	10 (MAP) – 1hr	Dextran = 710	Recovered kept dull till next day Hb: 9.34.

OBSERVATIONS Contd.

S.NO.	Weight of the dog. (Kgs)	Amount bled (ml)	State of shock (MAP & Period)	Type and amount of fluid re- (ml) infused	Result.
11.	12	500	10(MAP) – 1hr.	Whole blood 150 + Lomodex 340 + 5% DW 110	Recovered.
12.	12	500	10 (MAP) – 1hr.	Blood 140 Nacl 350	Recovered Malena ++
13.	10	665	10 (MAP) – 1hr.	665 – 5% DW	Recovered.
14.	10.	Died during induction.			
15.	10.5	1150	10 (MAP) – 1hr.	810 – DW5%	Died. Hb% 3gm%
16.	9	752	" "	600 – "	Died.
17.	11	1200	" "	540 – Hb Solution + 100 – 5% DW	Died.
18.	13.5	850	" "	Animal died before infusion could be given.	
19.	10.	Animal died during bleeding.			
20.	12	500	10(MAP) – 1hr.	500 ml of Hb Solution	Died. Haemoglobinuria Rectal bleeding

GRAPH SHOWING EXPERIMENTAL PROTOCOL

- Recovery after infusion of blood.
  Death after Saline infusion



CONCLUSIONS:

1. It is very difficult to produce a reversible state of shock during which time the animal cannot survive without infusion of resuscitation fluids. Either they recover spontaneously if the shock is not severe or die if it is mild severe and prolonged. irrespective of the type of infusion. We have found that if the mean BP is brought down to 10 mm/Hg and kept at that level for a period of 1 hour the animal goes into a state of shock from which recovery is possible only when resuscitation is done with fluid infusions.

2. It has been observed that if the resuscitation is attempted immediately after haemorrage the recovery is possible even with a very minute quantity of normal saline as resuscitation fluid.

3. To observe the recovery the animal should be kept in a state of shock atleast for about an hour. On such a case the recovery depends upon the type of fluid and rate of

administration

4. If the fluid infused has got sufficient Colloid osmotic pressure the recovery is possible even though Hb% drops down to a very low level. But the animals remain drowsy and lethargic.

5. Rapid or Slow bleeding has got no effect on the recovery so long as the resuscitation is attempted before it goes into an irreversible state of shock.

6. The infusion of Haemoglobin Solution produced death in the animals with haemoglobinuria and bleeding per rectum suggesting that further modifications are required in the Haemoglobin Solution before it can be tried experimentally.

FUTURE PLAN:

The stroma free haemoglobin solution used for this experiment was a first stage experimental product prepared during standardisation of methods for initiation of the project. It was prepared in kutty.

Sterility during various procedures though attempted was not very successful. The final product was however sterilised by gamma radiation.

Haemoglobin solution as such is known to have a very high affinity for oxygen thus limiting the release of O_2 at tissue level. It also has a very short intravascular retention time. These two limitations are very important and are major causes of failure to resuscitate the dogs from shock.

Pyridoxilation of this solution has proved to reduce the oxygen affinity and polymerisation improves intravascular retention time dramatically.

The haemoglobin solution is now being further modified by pyridoxilation followed by polymerisation and then will be evaluated in future.

Acknowledgements:

I am thankful to Dr. Meera Mohanty Prof. G.Y. Iyyer and Dr. Arthur Vijayam Lal for their help.

II

SURGICAL PROCEDURES PERFORMED IN ANIMALS AT BMT WING.

A. INTERPOSITION GRAFTING IN THORACIC AORTA IN PIGS WITH A PROSTHETIC VASCULAR GRAFT.

SURGICAL PROCEDURE

Pre operative management:

The animals were kept off feed 18 to 24 hours before surgery without restriction of water intake. Each animal was premedicated with atropine sulfate (0.04 mgs/kg) Promethazine Hcl (1mgs/kg) Pentazocine lactate (1mgs/kg) and diazepam (1 to 1.5 mgs/kg). Thirty to 45 mts after the injection the animal was laid on its back and its legs tied up manually.

Anaesthesia:

Anaesthesia was induced with halothane (4-5%) and N_2O (4:4) administered by a face mask attached to Boyle's anaesthetic apparatus with a halothane vaporiser. Endotracheal intubation was done and anaesthesia was maintained with a gas mixture of $N_2O : O_2$ (2:1) with IPPV using

a Ventilator. Tubarine (0.3 mgs / kg IV) and 15 mgs of Pentazocin lactate were administered 5 mts before skin incision. If the animal moved during surgery a repeat dose of muscle relaxant and analgesic were given. The esophageal temparature was monitored. In some animals arterial pressure also was monitored with an indwelling Cannula connected to a transducer and a pressure monitor.

Operation:

The anaesthetised animal was placed in the right lateral decubities position and the left hemithorax was prepared and draped. The left fore limb was pulled cranially and the Cartilagenous border of Scapula was palpated for locating the site of 5th intercostal space. The skin incision was made from the distal border of the Vertebra to the Costochondral junction of the Sternum. The thoracic muscles were incised with a Cautery and Chest was entered through intercostal space. The Chest retractor was placed. The lung was retracted

downwards and anteriorly exposing the thoracic aorta. The mediastinal pleura over the aorta was incised and stay stitches were applied. The descending thoracic aorta was mobilised from the level of left brachial artery to the point of origin of 1st pair of intercostal arteries. 4 to 5 cms of aorta was excised after applying cross clamps above and below. As far as possible intercostal vessels were not sacrificed. The gap was bridged by using a suitable length of prec clotted woven dacron vascular graft. Both the proximal and distal anastamosis were carried out using 3/0 Tevdek & 3/0 Prolene in a continuous fashion. After release of cross clamps careful haemostasis was secured. Fluid was administered during releasing of cross clamp to maintain the pressure. $NaHCO_3$ was administered intravenously to correct acidosis. Mediastinal pleura was approximated

The thoracic wound was closed in layers after applying a suction to intra thoracic catheter.

Recovery:

The effect of muscle relaxant was reversed with atropine and neostigmine. Animal was turned every ½ hour to 1 hour. When it regained full Consciousness Extubation was Carried out.

Analgesics and antibiotics were given for 5 days post operatively.

Five such operations were done.

B. ESTABLISHMENT OF CARDIO PULMONARY BYPASS IN SHEEP TO EVALUATE OXYGENATOR.

After premedication with atropine sulfate and promethazine hydrochloride anaesthesia was induced using thiopentone sodium and intubated after giving a muscle relaxant. The anaesthesia was maintained with N_2O and O_2. CVP line was placed in the external jugular vein and arterial pressure line was placed in the left carotid artery. Both vessels were exposed by a making a neck incision. Temperature was monitored with an esophageal probe.

The animal was placed in right lateral decubitus position and skin incision was made from the vertebral border to sternocostal junction. Thoracic muscles were incised with an electrocautery and the chest was entered through 4th intercostal space. Pericardium was incised and manipulated. Heparin (3mgs/kg) was given. Two purse strings were placed over the descending thoracic aorta and

controlled with rubber snares. Aortic cannulation was performed and connected to arterial line. A purse string stitch was placed over the pulmonary artery and venous cannula was introduced through it and placed in the RV outflow tract. It was connected to venous line and CP bypass was established. Animal was placed on bypass for about an hour. At the end of one hour rewarming was done to bring up the temperature. During bypass pH, blood gases and other haematological parameters were measured periodically to evaluate the functioning of oxygenator.

Then bypass was discontinued. Decannulation was done. Protamine was administered Chest was closed in layers after leaving one intercostal tube drain.

Waking up time was noted. and when the animal recovered completely from the effect of relaxant and anaesthesia a trial of spontaneous respiration was given. Once the animal is alert and blood gases are satisfactory

Extubation was carried out and the animal is shifted to recovery room and observed.

One such operation was done.