# PROPOSAL FOR CLINICAL INVESTIGATION

1. TITLE: Plasma Protein Denaturation during Cardiopulmonary Bypass and the Influence of the Reticuloendothelial System upon these Changes.

2. PURPOSE: To determine the degree of plasma protein denaturation produced at the blood-gas interface of a bubble-oxygenating system and the influence of the reticuloendothelial system as a "biological filter" for these changes during cardiopulmonary bypass.

3. BACKGROUND: Extended cardiopulmonary bypass, especially with non-membranous oxygenators, is poorly tolerated, presumably due to blood cell destruction[1-3] and serum protein denaturation[4-7] occurring at the blood-gas interface of the oxygenator. Recently, extracorporeal recirculation has been shown to interfere with the phagocytic capacity of the reticuloendothelial system (RES).[8] Interference with clearance of a specific colloid challenge was considered secondary to RES blockade by destroyed cells and denatured proteins in the recirculated blood.

While most studies of recirculated, oxygenated blood have dealt with hemolysis, interest in protein denaturation has stemmed from the observation that recirculated plasma has produced significant morbidity[4] and mortality[9] in homologous recipients. However, the studies reported on serum protein changes thus far have been relatively non-specific and at times contradictory.

Lee and co-workers[4] first described changes in serum proteins resulting from in vitro recirculation in disc, screen and bubble oxygenators. They observed increased viscosity and turbidity, increased yields of "salted out" globulin and albumin, slightly increased sulfhydryl reactivity, and, on paper electrophoresis, decreased albumin with increased $alpha_2$-, beta- and gamma-globulin fractions. In comparing the screen and membrane oxygenators in vitro, Wright et al[5] found a slight increase in viscosity but observed no gross turbidity. In addition, they observed no increase in sufhydryl reactivity and with starch gel electrophoresis found an increased mobility of an $alpha_2$-globulin fraction. In all instances, changes were uniformly less with the membrane oxygenator than with the screen oxygenator.

Dimililer and Trout[6] found hyperamino-acidemia following in vitro recirculation in the disc oxygenator as shown by thin-layer, silica-gel chromatography. The chromatographic profiles were non-specific and the increased hemolysis observed was not linearly related to the increase in free aminoacids.

Using $I^{131}$ tagged albumin, gamma-globulin and fibrinogen in the priming volume of a bubble oxygenator, Siltanen et al[7] observed an increased degree of reticuloendothelial clearing of the re-circulated, tagged albumin and fibrinogen fractions in recipient rats. The latter

response was felt to represent a consequence of denaturation of these fractions.

While these data are collectively non-specific, they do at least indicate that some degree of macromolecular alteration probably results from recirculation of blood through extracorporeal circuits.

With the current, widespread clinical use of disposable bubble oxygenators, with their greater potential for producing blood damage(10) it would seem important to define the changes in serum proteins more specifically in an in vitro system by using more precise methods of analysis.(11,12) In light of the recent observations concerning the activity of the RES during bypass, it would also be important to compare these in vitro results with an intact and subsequently blocked RES using an autologous in vivo preparation.

## 4. BIBLIOGRAPHY:

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## 5. TECHNICAL APPROACH:

### A. Introduction:

1. In vitro as well as in vivo studies are required in order to best derine macromolecular changes in extracorporeal systems and to resolve the differences reported to date.

In vitro studies should include prolonged recirculation in order to exaggerate effects and thus direct attention to components which might be altered during clinically comparable bypass times. Also, in vitro studies should be made on both plasma and whole blood in order to determine the influence of hemolysis upon the methods used to detect changes in serum proteins.

In vivo studies can be carried out in dogs and should involve extracorporeal recirculation times comparable to clinical conditions (e.g., two to three hours only). The canine RES can be suitably blocked

in order to modify its role as a "biologic filter" in clearing serum protein degredation products evolved in extracorporeal recirculation.

Since in vivo studies will be carried out in dogs, in vitro studies should be made on canine blood during the non-clinical phase of this project.

The following general methods of analysis are proposed for characterization of various blood samples:

a. DEAE cellulose column chromatography.[11,13]

b. Cellulose acetate electrophoresis.

c. G-200 Sephadex column gel filtration.[12]

In vitro recirculation as well as cardiopulmonary bypass in dogs would be carried out with the use of disposable 2-liter flow bubble oxygenators (Temptrol), from which timed samples will be aseptically collected for subsequent analysis. The priming volume (900 ml) for the 2-liter flow oxygenators shall conform to clinical hemodilution wherein colloid (500 ml of blood or plasma) is diluted with crystalloid (400 ml of Normosol solution) roughly equivalent to 20 ml crystalloid per kilogram of body weight. Timed blood samples (10 ml) will be centrifuged to sediment cells. Resultant supernates plus the 5 ml plasma-prime samples will be dialyzed against column-equilibrating buffer prior to DEAE cellulose chromatography and G-200 Sephadex gel filtration. Protein samples applied to DEAE cellulose columns will be eluted with a system providing both pH and concentration gradients of phosphate buffer.[13] Samples applied to Sephadex columns will be eluted with 1M Na Cl in 0.1 M tris-H Cl buffer, pH 8.0.[12] Eluates from either column, where recirculated whole blood is concerned, will be read at both 280 and 405 mu in the Beckman DU Spectrophotometer. Readings at 405 mu will detect the Soret band of hemoglobin. Where recirculated plasma samples are concerned, eluates will be read at 280 mu only.

B. In vitro studies:

1. Two hour recirculation studies at 37°C in disposable bubble oxygenators using 3L oxygen flow per minute and 30 minute samples.

a. System primed with whole canine blood (500 ml) diluted with Normosol (400 ml).

b. System primed with canine plasma (500 ml) diluted with Normosol (400 ml).

2. Six hour (exaggerated) recirculation at 37°C in disposable bubble oxygenators using 3L oxygen flow per minute and one hour samples:

a. System primed with canine plasma (500 ml) diluted with Normosol (400 ml).

3. Two-hour recirculation at 37°C in disposable bubble oxytenators with no oxygen flow and 30 minute samples (non-bubbling control):

a. System primed with canine blood (500 ml) diluted with Normosol (400 ml).

b. System primed with canine plasma (500 ml) diluted with Normosol (400 ml).

4. Non-recirculating controls:

a. Static, autologous plasma-red cell hemolysate control, incubated at 37°C for two hours with 30 minute samples.

C. In vivo studies:

1. In order to study the influence of the RES on the pattern and quantity of serum protein denaturation, dogs (60-75 pounds) will be subjected to sequential cardiopulmonary bypass at 37°C with and without RES blockade using 2-liter flow oxygenators primed with previously drawn autologous blood (500 ml) diluted with Normosol (400 ml) with 30 minute samples taken for analysis.

a. Prior to the first cardiopulmonary bypass without RES blockade, each animal will donate 500 ml of blood for hemodilution oxygenator priming for the autologous in vitro recirculation control.

(1) Immediately after blood donation, the dog will receive 500 ml Normosol, intravenously and be returned to his cage for recovery from anemia prior to donating blood for the first cardiopulmonary bypass experiment.

(2) During the anemia-recovery phase, the diet will be supplemented with Fe $SO_4$, 15 grains daily.

b. For the cardiopulmonary bypass experiments, the dog will be connected to the oxygenator within the first week after autologous blood prime donation, even though somewhat anemic, since it will receive its own blood back during bypass.

c. RES blockade will be accomplished with Trypan-blue[14] given as 25 mg/kgm intravenously 6 and 20 hours prior to bypass.

2. In these 3-phase autologous experiments, the first, in vitro phase will define the pattern of serum protein change for a given animal as determined by the methods of analysis proposed. The first cardiopulmonary bypass with intact RES should show the degree to which the quality and quantity of the basic serum protein profile is modified by RES activity. Theoretically, active RES phagocytosis of altered blood elements evolved in bypass should result in a serum protein profile more comparable to the in vitro pre-circulation control sample, while with RES blockade, the same animal should then show an altered serum protein profile more comparable to the in vitro recirculated samples.

While most intact dogs can easily tolerate two hours of well-controlled normothermic cardiopulmonary bypass, it is entirely possible that animals with a blocked RES (? loss of critical "biological filter") will succumb with two hours of bypass.

6. EQUIPMENT:

a. Equipment on hand or available:

(1) Extracorporeal pump.
(2) Two-liter flow bubble oxygenators (Temptrol).
(3) Oxygenator connecting tubing.
(4) Fraction collector.
(5) Beckman DU ------ spectrophotometer.
(6) Beckman Microzone electrophoresis apparatus Model R-100.
(7) Water bath.

b. Equipment required:

(1) Sample applicator (Stock No. 324399) for Beckman Microzone Electrophoresis system, Model R-100, 2 each, @ $33 -------$ 66.00

c. Supplies and expendables:

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|---|---|---|
| (1) | Glass columns for chromatography ---------- | $ 400.00 |
| (2) | Stock chemicals ---------- | 500.00 |
| (3) | Standard laboratory glassware ---------- | 600.00 |
| (4) | Special chemical reagents ---------- | 500.00 |

d. Animals:

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|---|---|---|
| (1) | Dogs, 60-75 pounds, 10 each (@ $45 each) ---------- | 450.00 |

e. TDY ---------- 500.00

7. INVESTIGATION SCHEDULE:

a. Study to begin 15 July 1970 with anticipated completion date 31 December 1970.

8. EXPERIMENTAL SUBJECTS: AFR 169-8 pertaining to use of experimental animals will be followed.

9. USE OF DRUGS: Not applicable.

10. PERSONNEL DATA:

a. Medical Facility Commander: Brig. General E. H. Underwood, Jr.

b. Principal Investigator: Melvin D. Smith, Major, USAF, MC.

c. Associate Investigator: Raymond G. Armstrong, Lt Col, USAF, MC

11. MANPOWER:

a. One AF Major, AFSC 9411, 960 man hours.

b. One AF Sgt, AFSC 90450, 960 man hours.

c. One Lt Colonel AF, AFSC T9416A, 100 man hours.