Plasmas Protein Denaturation at the Blood Gas Interface

*In Extracorporeal Oxygenators**

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THE phenomenal advances that have occurred during the past 15 years in the surgical treatment of diseases of the heart and great vessels and recently in mechanical circulatory assistance can be directly traced to parallel developments in cardiopulmonary bypass systems. The virtues of the presently available heart-lung systems are generally appreciated, however, a broad spectrum of deleterious effects upon the blood have been recognized which at present significantly impede the use of these systems for prolonged circulatory support. Hemolysis, platelet destruction and microembolism resulting from extracorporeal circulation and oxygenation were recognized relatively early but only recently have the alterations that occur at the blood-gas interface received appropriate attention.

A variety of post-perfusion complications have been reported including hypotensive reactions[20], blood coagulation defects[12] and the "post-perfusion lung syndrome"[8]. In most instances these problems were attributed to traumatic alterations in the formed elements of the blood until plasma protein denaturation at the blood-gas interface was recognized[16]. These and many other related discoveries have provided a stimulus to increased investigations of the dynamics at the blood-gas interface and a wealth of pertinent information has become available.

The importance of continued progress in this area cannot be over-estimated since the key to revolutionary developments in mechanical circulatory and respiratory assistance probably lie in the resolution of the kinetics at the blood-gas interface. For this reason it seems pertinent to review and summarize the presently available information concerning plasma protein denaturation at the blood-gas interface in extracorporeal oxygenation systems.

Protein Architecture

The current concepts of protein structure are largely based on theoretical considerations and three levels of organization, primary, secondary and tertiary, are thought to exist in the complex architecture of a protein.[19] The primary structure involves the linking of amino acids in a unique sequence with peptide bonds to form the protein's characteristic polypeptide chain.[21]

The secondary level of organization is concerned with the maintenance of a helical configuration in the primary polypeptide chain. This helical arrangement is the most favorable energy form of the chain, but cannot be maintained in a natural environment without reinforcement. This configuration is stabilized by hydrogen bonding in a regular sequence between the carbonyl and imido groups of the amino acids in the polypeptide chain.

The tertiary organization is responsible for the overall shape of the protein and involves extensive coiling of the secondary structure. This configuration is maintained by both high energy bonds such as disulfide bonds, and lower energy bonds such as hydrogen, salt, phenolic, and hydrophobic links, and by Van der Waal's forces. These bonds and forces give the protein its globular or ellipsoidal shape with external polar groups and non-polar internal groups. The coiling and folding gives the protein its complex and rigid structure and is of prime importance to the unique properties of biologically active proteins.[5, 16]

Theoretical Considerations in Protein Denaturation

Disruptions of the second and third levels of the protein complex are integral to the denaturation process. Denaturation, as defined by Neurath[15], is "Any non-proteolytic modification of the unique structure of a native protein, giving rise to definite changes in chemical, physical, or biological properties". This definition excludes hydrolysis of peptide bonds and thus omits the first level of complexity from consideration.

Denaturing agents disrupt the bonds involved in the secondary and tertiary levels of protein structure without the lysis of peptide bonds or carbon-to-carbon linkage. The external polar groups of proteins are aligned at the blood-gas interface by strong electrostatic forces operating from the interface to a depth of 10 to 40 Angstroms.

This polarization exerts enough force to disrupt the intermolecular secondary and tertiary structures by breaking the previously described weak bonds. The net result is an unfolding of the protein, or parts of the protein

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TABLE 1. A comparison of blood alterations produced by blood-gas interface and membrane oxygenators.[11]

Test	*Blood-gas interface gas exchanger*	*Membrane Oxygenator*
Viscosity	Increased by 100%	Increased by 14%
Solubility, % Change denatured protein salted out	Albumin +89% Globulin +183%	Albumin +10% Globulin +8%
Electrophoresis	10% increase in Globulin	2 to 6% increase in Globulin
Turbidity, % light transmission	10 to 40% of control	90 to 98% of control
Cellular Changes	Coating of 75 to 100% RBC	
Biological Signs	Shock ..... 25% Death ..... 16% Decerebrate signs 48%	Shock ..... 0% Death ..... 0% Decerebrate signs 0%

structure, into a randomly coiled or linear configuration. These alterations vary with the pH, temperature, salt concentration of the solution, and the specific protein involved.[11]

Alterations Produced by Protein Denaturations

The changes which occur may be grouped into several categories as suggested by Neurath[15]:

1) Alteration of normal biological activity: Many enzymes lose their catalytic properties, antigen-antibody responses disappear, and hormones may lose their regulatory powers;

2) Increased reactivity of constituent groups: Unwinding of protein molecules exposes the sulfhydryl, disulfide or phenolic groups which are normally shielded within the protein structure. This exposure increases the availability of these groups for reaction with other plasma constituents. If the molecule transports lipids, these lipids may be released and conglomerate to produce fat emboli;

3) Decreased protein solubility: Spatial disturbances of external groups may result in exposure of the internal groups of the protein and lead to changes in solubility as evidenced by precipitation of denatured proteins;

4) Loss of crystallizing ability: A protein may lose its ability to crystallize or form its characteristic crystalline shape due to gross changes in the protein.

Lee and associates[11] studied denaturation of plasma proteins at the blood-gas interface and characterized the changes involved in protein denaturation as follows:

1) Changes in molecular configuration: The changes in protein configuration may produce an increase in intermolecular friction and thus explain the observed alterations in viscosity;

2) Alterations in reactivity and steric configuration: The blood-gas interface may rearrange the protein macromolecular structure resulting in flocculation, precipitation or gelling. The exposed non-polar hydrophobic groups may then be absorbed on surfaces with lipid components, such as erythrocytes. This reaction may explain the blood sludging occasionally observed in patients after perfusion and it has been suggested that this reaction may lead to agglutination[13];

3) Lipid release: Lipoproteins may release their contained lipids which may explain the 100% incidence of cerebral fat emboli found in post-perfusion patients that have died from other causes.[11]

Implication of the Blood-Gas Interface

Considerable evidence exists that blood undergoes harmful alterations during extracorporeal circulation.[13] Most of this evidence is based on complex determinations which are difficult to perform and require special techniques. It should be emphasized that the establishment of suitable parameters for accurately assessing the denaturing effect at a blood-gas interface must include considerations aimed at eliminating other possible contributing factors. For example, in experimental studies blood incompatability may be eliminated by priming the system with the recipient's own blood.[12]

DeWall and associates[3] demonstrated that many post-perfusion complications were related to the release of hemoglobin from lysed erythrocytes. The release of biologically-active substances from lysed cells may be controlled by either removing the cellular constituents[4] or by binding or inactivating the released substances with appropriate pharmacologic agents.[14].

The importance of selectively separating the contributing factors in order to permit valid conclusions was emphasized by Hollenberg and co-workers.[9] They demonstrated that many post-perfusion complications attributed to plasma protein denaturation were actually the result of serotonin and histamine release from destroyed platelets and that these effects could be averted by removing platelets from the system.

Martin et al[14] confirmed these observations by means of a different experimental approach which demonstrated that the vasoactive responses attributed to serotonin and histamine could be controlled by the serotonin-hystamine antagonist cyproheptadine.

If the blood-gas interface is to be implicated as the major source of plasma protein denaturation, the extracorporeal system must be free of any defect which would produce similar alterations. Meticulous attention must be given to cleanliness of every part of the extracorporeal circulation apparatus and control of temperature and acid-base balance is essential. Furthermore, the effects of hydrophobic surfaces, such as highly polished metal surfaces, tubing, and other nonwetable materials, must be considered. Only

experiments constructed with careful control of these variables can be expected to produce reliable results indicative of the damage occurring at the blood-gas interface.

Lee[11], Brinsfield[2], Neville[16], and Dobell[4] have compared the alterations in several characteristics of blood and plasma produced by oxygenators employing a blood-gas interface with membrane oxygenators. Results typical of those obtained in these studies are seen in the experiments of Lee and associates[11] (Table 1). They concluded that the membrane oxygenator was less traumatic to the cellular and plasma constituents of blood than conventional blood-gas interface oxygenators.

Dobell et al[4] compared animal survival following administration of blood or plasma circulated for prolonged intervals through a screen blood-gas interface oxygenator or a membrane oxygenator (Table 2).[17] Of particular significance was their observation that when plasma alone was circulated and reconstituted with its cellular elements just prior to administration the animal mortality was 100% indicating that this combination was the most lethal. They concluded that the toxic factor was primarily contained in plasma and assumed it to be a product of protein denaturation.[17]

In addition they found that the membrane oxygenator produced substantially less toxicity than the screen oxygenator. As a result of these and other studies it is now generally appreciated that membrane oxygenators are significantly less traumatic to blood than conventional oxygenators employing a blood-gas interface.

The degree of plasma protein denaturation has been found to be directly proportional to increasing time of extracorporeal oxygenation and since prolonged periods of bypass oxygenation are required for mechanical circulatory assistance or respiratory support it is evident that membrane oxygenators are the gas exchange device of choice at this time.

Methods of Modifying Protein Denaturation

Although the membrane oxygenator holds promise of being the ideal blood oxygenating system, from a practical standpoint their use is fraught with many serious disadvantages at this stage in their development. As a result of the delay in the development of practical membrane oxygenators several approaches have been investigated in an effort to protect blood and its constituents from the denaturing process, with varying degrees of success.

Hemodilution has provided some protection from protein denaturation[4] presumably because with dilution the blood protein concentrations are decreased hence less protein is present for reaction at the blood-gas interface per unit of time. Low molecular weight dextran is said to reduce blood sludging[1] and decrease protein denaturation. The mechanisms underlying its beneficial effects are poorly understood and while it has been postulated that low molecular weight dextran provides electro-negatively at the blood-gas interface on erythrocytes it is equally likely that all of its benefits may be attributed to its hemodilution effects.

Hypothermia may also be useful in reducing protein denaturation. Wilder et al[22] found that profound hypothermia (4° C) stabilized all blood constituents during extracorporeal oxygenation for as long as 15 hours. An equal consideration is the lower flows required during hypothermia thus reducing the overall exposure of blood to the gaseous interface.

Adrenal corticosteroids may provide significant protection to blood elements during cardiopulmonary bypass by lysosome stabilization.[18] Lysosomes are cytoplasmic organelles containing many acid hydrolytic enzymes within a lipoprotein membrane. These complexes are present in essentially all body cells and the integrity of the lipoprotein membrane may be compromised by hypoxia, hemorrhage, endotoxic shock or other toxic agents, resulting in release of the contained enzymes and cellular destruction.

Replogle and associates[18] administered massive doses of corticosteroids to patients undergoing surgical procedures on cardiopulmonary bypass and found a decrease in serum lysosome concentrations. They concluded that adrenocorticosteroids stabilized cellular and lysosomal membranes and thus decreased damage to the blood elements at the blood-gas interface.

A New Concept

Several other approaches to these problems are being pursued including new oxygenator designs. An interesting concept of blood oxygenation has been reported by Howlett and co-workers.[10] They obtained efficient blood oxy-

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TABLE 2. Animal survival following transfusion with blood or plasma circulated for extended periods through screen or membrane blood oxygenators.

Group	*Type of Experiment*	*No. of Exper.*	*Lived*	*Died*	*Time of death after bypass (hours)*				
A-1	Screen Oxygenator fresh blood	16	12	4	1	26	10–20	36–48
A-2	Screen Oxygenator blood circulated six hours	10	8	2	–	–	4	--
A-3	Screen Oxygenator blood circulated fifteen hours	26	4	22	2	7	13	–
A-4	Screen Oxygenator plasma circulated fifteen hours, mixed with stored cells	11	0	11	2	5	4	–
B	Membrane Oxygenator blood circulated twenty hours	20	18	2	–	–	–	2

genation with fluid fluorocarbon. The oxygenating system consists of two upright concentric cylinders. The inner cylinder is open at its top and contains fluorocarbon while the inner cylinder contains blood which enters at the bottom. As the blood flows upward it extracts oxygen from the fluorocarbon which is not miscible with blood.

Fluorocarbon is relatively inert, lacks biologic activity, has high oxygen solubility, low surface tension and has little adverse physiologic effects. A great deal remains to be learned about this technique of blood oxygenation however, and the development of more efficient and practical membrane materials may be a more realistic approach. The use of homologous lungs as an oxygenator[6] continues to reappear in the literature in spite of many inherent problems which make it unlikely that this method will ever be of practical significance. Many other areas such as hyperbaric oxygenation[7] are fertile grounds for investigation and one can be reasonably sure that the solutions to many of the problems presented here are impending.

This Study . . .

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Summary

The denaturation of plasma proteins at the blood-gas interface is clearly responsible for many problems occurring during cardiopulmonary bypass and post-perfusion complications. Research and experience has provided a better understanding of the pathophysiologic mechanisms involved and several protective measures have been developed. It has been demonstrated that blood oxygenators employing the blood-gas interface principle are inherently injurious to blood elements while membrane oxygenators are more physiologic and obviate many of these problems. The development of an efficient and practical membrane oxygenator is eagerly awaited and will be accompanied by remarkable developments in clinical medicine.

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