Blood Damage in the Heart-Lung Machine (35651)

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(Introduced by J. M. McKibbin)

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Heart-lung machines can effectively oxygenate blood, but their prolonged use causes difficulties not directly related to the patient's heart condition or the specific procedures used to correct it. In extreme cases a patient may be seriously affected and may develop thromboses, abnormal permeability of the vascular system (particularly in the lungs), blood clots, and occasionally brain damage (1, 2). The results of several studies (3–6) suggest that denaturation of blood proteins at blood-gas interfaces in the heart-lung machine may be responsible for some of the postoperative difficulties.

We have found no specific studies of the effects of the gas/liquid interfaces in the heart-lung machine on individual blood proteins. Therefore, we undertook an investigation of the denaturation of gamma globulin, albumin, and their mixtures in the disc oxygenator. The advantage of studying individual blood proteins is that the results can be interpreted more easily than studies with whole blood. However, data obtained in this simple model system can, at best, tell only part of the story regarding the medical complications of open-heart surgery.

We chose to work with gamma globulin because of its instability at the gas/liquid interface. This instability is easily demonstrated. If a stream of air is passed through a clear solution of gamma globulin, a precipitate of the aggregated protein rapidly forms. Albumin was selected for study because it is one of the principal components of whole blood and because it is often added to solutions of labile proteins as a stabilizer.

The Complement System and Denatured Gamma Globulin. The complement system can be activated by interaction with antigen–antibody complexes or by interaction with heat denatured, aggregated gamma globulin. Activation is known to increase capillary permeability, to enhance phagocytosis, to initiate chemotactic migration of leucocytes, and to damage membranes (7, 8). Furthermore, when certain complement components are depleted, the loss of host defense mechanisms is known to occur (9, 10). If the heart-lung machine denatured gamma globulin in such a way that aggregates capable of activating the complement system were produced, these aggregates could become lodged in the small blood vessels of the lungs, the brain, and other organs and there initiate all of the potentially injurious effects associated with complement activation.

In some open-heart surgical patients we have found unexplained serum complement depletion persisting for periods greater than 24 hr after the operation. This observation has also been made by Hairston *et al.* (11). In addition to complement depletion, these authors reported that the sera of open-heart surgical patients had significantly lowered bactericidal capacity and reduced amounts of immunoglobulins after the surgery. These effects were absent in surgical patients who had not been exposed to oxygenators. Their data indicate that there has been extensive loss of gamma globulin from the sera of these patients. This loss would occur if gamma globulin were being denatured and aggregated by the oxygenator and subsequently lodged in small blood vessels or cell membranes, or taken up by the reticuloendothelial system and phagocytes. The observed loss of sera bactericidal capacity could be a direct result of depletion of complement and immu-

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noglobulins.

Subramanian and his colleagues have reported that bacterial phagocytosis by Kupffer cells in the liver is significantly reduced in rats following experimental cardiopulmonary bypass (12). The same effect was observed when livers were perfused with blood which had previously been circulated in the oxygenator. The lowered phagocytic index in the livers of these animals could be related to complement depletion or to competition between bacteria and denatured gamma globulin for the available Kupffer cells. An increased incidence of certain viral infections in heart-lung patients during the postoperative period has been reported by Kantor *et al.* (13). These observations could also be explained as a consequence of complement and immunoglobulin depletion by denatured gamma globulin.

From these reports and from our own experience, we were led to postulate that some of the complications arising from prolonged open-heart surgery are directly related to the denaturation of gamma globulin at blood-gas interfaces in the oxygenator.

Methods and Materials. Operation of the oxygenator. The liquid phase was not circulated outside of the machine. The gas section contained noncirculated ambient air. Total surface area produced during given time intervals was calculated from the area of the discs immersed in the liquid and the total number of revolutions during the interval. The trip switch was activated at each turn of the shaft by contact with the trip arm giving an exact count of the total number of revolutions.

Purified gamma globulin and albumin were obtained from Pentex Corporation. Solutions were prepared in a buffer of pH 7.4, total phosphate concentration 0.004 *M*, and sodium chloride concentration 0.14 *M*. Gamma globulin solutions were centrifuged for 20 min at 27,000*g* before being placed in the oxygenator in order to remove insoluble material. The solutions were placed in the liquid section of the oxygenator and samples were withdrawn at timed intervals. The experiments were carried out at room temperature, 23–26°, with ambient air in the gas section.

Relative turbidity was measured in a Brice-Phoenix light scattering photometer model 2000 thermostated at 25°. The light source was a mercury lamp fitted with a monochromatic filter (436-mμ peak). Intensity of scattered light was measured at 90°.

Fluorescence spectra were recorded with an Aminco-Bowman spectrophotofluorometer. The fluorescent probe 1-anilinonaphthalene-8-sulfonate (ANS) was purified by recrystallization (14). Extrinsic fluorescence was measured by mixing an excess of the dye with protein solutions and measuring emission at 465 ± 10 mμ (excitation at 375 ± 10 mμ).

Results and Discussion. Although we use the term "oxygenator" with reference to the machine employed in these studies, it should be pointed out that the results obtained are not simply due to the oxygenation of the solutions but are a consequence of the interaction of the components with gas/liquid interfaces. The perturbing factors responsible for the alteration of protein structure are much more likely to be associated with the physical chemical discontinuities of the interfacial region rather than the presence of oxygen in the liquid phase. We have found that gamma globulin solutions subjected to the action of a disc oxygenator of the type often used in open-heart surgery (Fig. 1) show a concentration dependent increase in turbidity

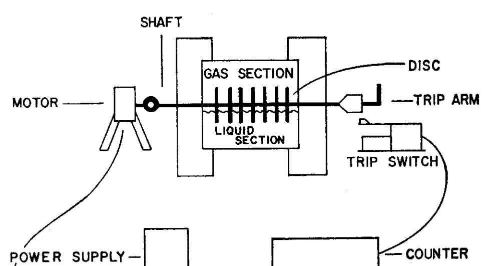

FIG. 1. The disc oxygenator. During open-heart surgery, the patient's blood is oxygenated as it continuously circulates from the patient to the liquid section of the oxygenator. Oxygen is supplied continuously to the gas section. Rotation of the shaft carries a film of blood up into the gas section on the surface of each disc. The extent of the interface is controlled by the number of discs and the speed of rotation. Oxygenators used for surgery have varying numbers of discs and liquid section volumes depending on the requirements of the patient.

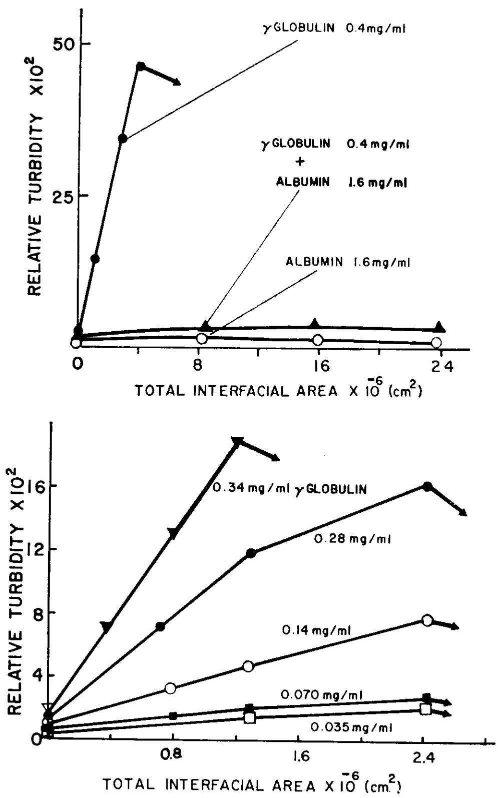

FIG. 2. Relative turbidity of protein solutions from the oxygenator. Data for gamma globulin solutions are shown in the top panel. The appearance of a visible precipitate is indicated by arrows in both panels. The total interfacial area is the total amount of air–liquid surface generated by the turning discs and was obtained by multiplying the time at which each sample was drawn by the total number of turns of the shaft up to that time and the total area of air–liquid film generated per turn of the shaft.

followed by precipitation of the denatured protein (Fig. 2). Furthermore, gamma globulin solutions exposed to the oxygenator are highly reactive with guinea pig complement when compared to control solutions which have not been subjected to the oxygenator. In other experiments, we found that less than 100 μg/ml of gamma globulin which had been repeatedly exposed to an air-solution interface were able to fix 50% of the complement units (CH_{50}) offered. The specific complement fixing activity of these gamma globulin samples is similar to that found for heat denatured gamma globulin by Ishizaka and Ishizaka (15) and by Christian (16).

Further indication that gamma globulin is denatured in the oxygenator is found in the recent report that the sera of many patients who have had open-heart surgery contain antibodies to gamma globulin (17). We interpret these results as suggesting that gamma globulin altered by the oxygenator, is subsequently treated as a foreign protein by the patient's immune system. That autologous denatured gamma globulin is antigenic has been shown in other experimental systems (18).

The effect of albumin. When we subjected human plasma to the action of the disc oxygenator under the same conditions used for the experiments shown in Fig. 2, we did not observe the same large increases in turbidity. Apparently gamma globulin is more stable in plasma than in the buffer solution. We reasoned that the high concentration of albumin in plasma might be stabilizing gamma globulin at the air-liquid interface.

To test this hypothesis we exposed mixtures of albumin and gamma globulin to the disc oxygenator. The results, given in Fig. 2, show that the presence of albumin significantly reduced the turbidity. The slight increase in turbidity which was observed for the albumin–gamma globulin mixture is experimentally significant even though it is small compared to the increase for gamma globulin alone at the same concentration. Thus, although the presence of albumin prevents the extensive aggregation of gamma globulin, the protein mixture is still altered in some way by the action of the oxygenator.

The mechanism of surface denaturation of gamma globulin. The solubility of gamma globulin in ammonium sulfate solutions has been used as an indication of both its ability to activate complement and its state of aggregation (16). We used solubility changes to follow the kinetics of gamma globulin denaturation in the oxygenator. The preparations of gamma globulin which we used were soluble in solutions which were approximately 30% saturated with ammonium sulfate. However, if these preparations were placed in the oxygenator and samples taken at timed intervals, the solubility in the 30% solution de-

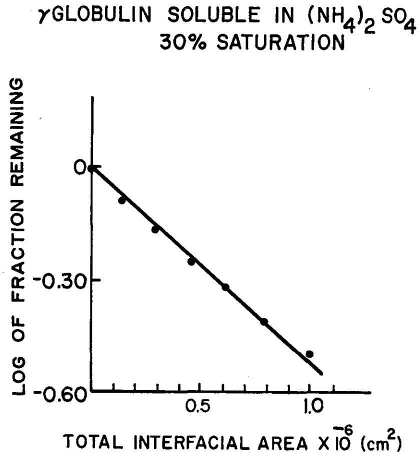

FIG. 3. Effect of oxygenator on gamma globulin solubility in ammonium surface solutions. Gamma Globulin solution (0.1 mg/ml) was placed in the oxygenator and samples were removed at timed intervals. Each sample was brought to a final concentration of ammonium sulfate which was approximately 30% saturated. The precipitated gamma globulin was removed by centrifugation and the concentration of the remaining solution was determined by measuring the optical density at 280 mμ. Each value on the graph represents an average from five similar runs. Maximum deviation from the mean was less than 5% for each observation.

creased with time. Soluble gamma globulin disappeared from the solution by a process consistent with first order kinetics (Fig. 3).

We found that our preparations of albumin were soluble in solutions approximately 75% saturated with ammonium sulfate and that this solubility was not altered by the action of the oxygenator. Gamma globulin was completely precipitated by this concentration of the salt. Mixtures of albumin and gamma globulin exposed to the oxygenator and then made up to 75% saturation with respect to ammonium sulfate had solubility properties intermediate between those of solutions of the individual proteins. The change in solubility properties of the albumin–gamma globulin mixture occurs within the first few minutes of oxygenator operation. The rapidity of this change is consistent with the small but rapid increase in the turbidity of albumin solutions exposed to the oxygenator (Fig. 4). The fluorescence spectra of the supernatant and the precipitate (redissolved in buffer) in the presence of 1-anilino naphthalene sulfonate (ANS) were analyzed. (We find that ANS will fluoresce intensely in the presence of albumin but only weakly in the presence of gamma globulin. The presence of gamma globulin does not alter the fluorescence of albumin–ANS solutions. Thus, the analysis of fluorescence is specific for albumin in this case.) Albumin was found in both the precipitate and the supernatant.

These results indicate that the oxygenator produces some species in mixtures of albumin and gamma globulin which is not produced when either solution alone is subjected to the oxygenator. They also show that the new species produced contains both albumin and gamma globulin.

As a mechanism consistent with our results we propose that the first step in oxygenator denaturation is unfolding of the gamma globulin molecule in the liquid–air interface, and that the second step is the interaction of the unfolded molecule with another gamma globulin molecule (either native or altered). In subsequent steps, unfolded gamma globulin

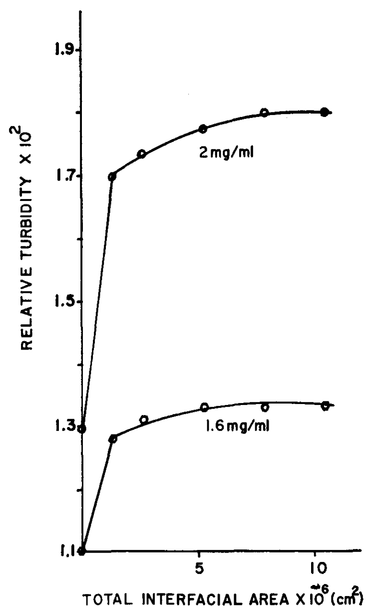

FIG. 4. Effect of oxygenator on albumin solution turbidity. Data were collected as in Fig. 2. Points shown are averages for three runs. Maximum deviation from mean value was less than 5% for each observation.

molecules interact with other aggregates to produce the observed large increases in turbidity and, ultimately, precipitation. We further propose that when albumin is present in large excess, the unfolded gamma globulin molecules interact with albumin molecules to produce a complex which does not aggregate further. The formation of this complex would account for the observed slight increase in the turbidity of mixtures of these proteins and their altered solubility in ammonium sulfate solution.

Implications for open-heart surgery. On the basis of these results we suggest that long-term operation of the heart-lung machine may produce biologically active quantities of denatured gamma globulin or gamma globulin-albumin complexes and that these altered proteins, particularly the denatured gamma globulin, may activate the complement system and thus cause some of the observed medical complications following surgery.

Since complement components are themselves subject to surface denaturation, it is possible that complement would be depleted by direct surface denaturation as well as by interaction with denatured gamma globulin.

Our data suggest that the presence of albumin reduces gamma globulin aggregation at the liquid–air interface. However, prolonged operation of the oxygenator may still produce significant quantities of altered protein even in the presence of albumin.

Gamma globulin denatured in the oxygenator in the presence of albumin has altered cryogenic properties which may also be related to the surgical complications. We find that gamma globulin–albumin mixtures exposed to the oxygenator have a greater tendency to form precipitates at lower temperatures than do control samples. The reduction of a patient's temperature during surgery may thus increase the potential difficulties from cryoprecipitable, denatured gamma globulin.

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