Influence of a Defoaming Agent upon the Hematological Complications of Pump Oxygenators

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SUMMARY

Measurements of plasma hemoglobin values were made at many different sites of a Cross-Kay disk pump oxygenator during extracorporeal bypass in patients undergoing reparative valve surgery. Hemoglobin values were always higher in the line of the aspirator and defoaming chamber than in any other part of the system. Sampling immediately proximal and distal to the defoaming chamber revealed significant increases in plasma hemoglobin values as a result of the defoaming process.

Studies of the hematological effects of the defoaming agent, polymethylsiloxane, which coats the disposable stainless steel sponge in the defoaming chamber, showed it greatly reduced the resistance of red cells to lysis by mechanical trauma. Similar studies of plasma showed the hemolytic effects of the defoaming agent are to some extent mediated by the plasma.

It is concluded that the widely used defoaming agent, polymethylsiloxane, contributes to the hemolytic action of the bubbling, suctioning, and defoaming processes during open heart surgery. The addition of a water soluble surfactant material, that stabilizes emulsions (Pluronic F68), blocked or prevented the hemolytic process without interfering with the action of the antifoam material.

Additional Indexing Words:
Polyethylsiloxane Hemolysis Cardiac surgery
Polyoxypolyethylene (Pluronic F68)

Extracorporeal pump oxygenators are widely used for reparative cardiac surgery and occasionally for support of patients during certain cardiopulmonary emergencies. While significant advances have been made in the safety and efficiency of these devices, the problem of both immediate and delayed red cell destruction remains to be fully explained. Technological improvements in the pump components and shorter duration of bypass time have led to considerably less hemolysis than was seen in the earlier experiences with these devices. However, the capabilities of prolonged assisted circulation and artificial heart systems will be jeopardized if the degree of hemolysis now tolerated in the 1-to-4 hour bypass procedures is not entirely eliminated.

During a study of rheological phenomena in pump oxygenators, it was noted that plasma hemoglobin levels were greater in the line of the aspirator and the defoaming chamber.
than in any other part of the system. Further
definition of this finding indicated that the
increased hemolysis was not due solely to the
trauma of suctioning, but was due also to the
defoaming agent, polymethylsiloxane (PMS),
which coats the stainless steel sponge in the
defoaming chamber. Because the reports of
Dobell and associates\textsuperscript{3} indicated that circulation
of cell-free plasma through an oxygenator
was as deleterious as the effects of using
whole blood, this study included an evaluation
of the hematological effects of defoaming
oxygenated plasma as well as whole blood.
Earlier reports indicated that the compound
polyoxypropylene-polyoxyethylene (Pluronic
*) had a protective effect against the forma-
tion of fat emboli\textsuperscript{4,5} and hemolysis\textsuperscript{5,6} in
cardiopulmonary bypass. Accordingly, studies
of the hematological effects of Pluronic were
also carried out.

**Methods**

The studies were divided into two categories:
(1) in vivo studies made before, during, and
after extracorporeal circulation from the patient and
the pump oxygenator, and (2) in vitro studies
carried out to determine the hematological effects of the
foaming and defoaming process.

The following measurements were conducted
in both the in vivo and in vitro studies: hematocrit
values were measured in a microcapillary
centrifuge at 10,000 mean G for 7 min; plasma
hemoglobin was measured photometrically by
the modified benzidine method\textsuperscript{7}; osmolality was
measured by the freezing point depression meth-
ood\textsuperscript{8}; pH was measured in a Beckman pH meter;
plasma surface tension was measured by means
of a Wilhelmy balance using a 2.5-cm platinum
strip\textsuperscript{9}; and red cell mobility was measured in
physiological saline by the method of Seaman
and Heard.\textsuperscript{10}

**In Vivo Studies**

Samples of whole blood were obtained from
patients before, during, and serially after extracor-
oreal circulation with a pump oxygenator of the
Cross-Kay type (110 disks) with DeBakey
roller pumps. The priming fluid consisted of
2,500 ml of whole blood, 1,500 ml of Ringer's
solution, 44 mEq of sodium bicarbonate, 12.5 g
of mannitol, 50 ml of 50\% dextrose, 100 g of al-
bumin, and 75 mg of heparin. The defoaming
chamber consisted of a 1,000-ml volume glass
and a steel container filled with stainless steel
sponge coated with PMS.\textsuperscript{*} The technique of
coating the sponge with PMS has been described
elsewhere.\textsuperscript{11} The defoaming chamber served
principally to defoam blood being aspirated from
the operative field. This blood resulted from
flow from the bronchial circulation, the coronary
sinus, and as backflow through incompetent
aortic valves. Pre-bypass samples were collected
from the priming fluid just prior to start of by-
pass. During bypass, samples were taken from
the venous inflow line (inflow to pump), the
coronary aspirator lines proximal and distal to
the defoaming chamber, and the arterial return
flow line (fig. 1). Samples were also taken from
the cardiac chambers and the pericardial well,
sites at which the aspirator was placed to keep
the operative field dry. Twenty-two patients
were studied, the majority of whom were under-
going reparative or replacement mitral or aortic
valve surgery, or both. The duration of the pro-
cedures ranged from 1 to 3\% hours.

**In Vitro Studies**

The mechanism of the hemolytic action of
the foaming-defoaming process was studied in
vitro by gently bubbling a humidified gas mixture
of 95\% oxygen and 5\% carbon dioxide through
an aliquot of freshly drawn blood (anticoagulant-
EDTA [ethylenediamine tetraacetate] or hepa-
rin) or through samples of bank blood collected
within 1 to 5 days of the study. The bank blood
anticoagulant was an acid-citrate dextrose mix-
ture. The gas mixture was introduced into the
sample via a no. 19 stainless steel needle at a
flow rate of 25 ml/min for periods up to 90 min.
Approximately 3 cc of PMS-coated stainless steel
sponge was placed in the test tube just above
the fluid surface. Control studies consisted of
similar samples that were bubbled without sponge
and with plain (uncoated) sponge. Controls also
included samples left sitting for the sample
period without bubbling, half with coated sponge
immersed in the sample and half without sponge.

Similar tests were conducted on plasma and
buffered saline. After bubbling of the plasma
or saline, red cells were added to the plasma
or saline at a normal hematocrit, and mixed in a
tube on a rotor at 60 rpm for 30 min. This

\*Wyandotte Chemicals Corporation, Wyandotte,
Michigan.

*Circulation, Volume XXXVII, April 1968
mixing was important for two reasons: (1) it imitated in part the agitation the cells experienced during circulation through pump and patient, and (2) it ensured more complete exposure of the red cell to the PMS. The effects of a gas mixture of 20% oxygen, 5% carbon dioxide, and 75% nitrogen were similarly studied. The effects of temperature were evaluated by carrying out the bubbling and mixing procedures at 4 C, 22 C, and 37 C. The effects of time were evaluated by repeated measurements of hemoglobin levels during the bubbling and mixing periods.

Studies of the effects of the native antifoam material, that is, not derived from the coated sponge were carried out by sonating PMS into a suspension in 5% glucose or physiological saline at final concentrations ranging from 20 to 100 mg%. Aliquots of packed red cells were then added to this suspension and the above procedures repeated. The choice of 20 mg% concentration was based on the following: at the end of a 2-hour pump run the defoaming capability of a standard PMS coated sponge was greatly reduced, suggesting that the majority of the PMS had been removed from the sponge or was inactivated. The standard commercial sponge contains approximately 1,500 mg of PMS. The priming volume used here was 2,500 and assuming a patient with a mid bypass hematocrit of 30, PMS would be mixed in an additional 4,200 ml of dilute plasma. This would result in 1,500 mg PMS in 6,700 ml of fluid for an equilibration concentration of PMS of 22 mg%, if there were no losses due to excretion, adsorption, or destruction.

Since the defoaming action of PMS is accomplished by a variety of physical and chemical processes rather than surface tension effects per
se, changes in the foamability of blood or plasma were tested by bubbling 1 ml of the fluid in a specially made glass column, 2.5 cm in diameter, 32 cm tall, and graduated in 1-cm increments. The base was tapered to form a well so that all the fluid could be bubbled. Bubbling was accomplished by metered gas flow through a no. 19 stainless steel needle inserted through a rubber stopper fitted over a glass side arm opening just above the base of the unit. Foam height was measured after 30 sec of gas flow, a period in which normal plasma would be converted entirely into foam. The unit was made in two parts to facilitate cleaning. Since the PMS antifoam is soluble only in certain organic solvents, the unit was washed first with ether, then with dichromate cleaning fluid, and flushed generously with distilled water. All other glassware used in this study was discarded after a single use. The stainless steel mesh was handled with clean chrome steel tweezers and scissors.

Studies of the effects of Pluronic were carried out by adding it to plasma and whole blood before the bubbling process and at various intervals during the mixing period. The effects of concentration were studied over a range of 20 to 200 mg%.

Results

In Vivo Studies

Plasma hemoglobin values rose progressively throughout the bypass procedure at all sampling sites. The highest plasma hemoglobin value recorded was 153 mg% and in the longest pump-bypass time was 215 min. The venous inflow line hemoglobin was 103 mg%. Comparison of plasma hemoglobin values at different sites revealed that hemoglobin concentrations were always greater in the line distal to (beyond) the defoaming chamber (fig. 1, site 2B) than in the venous inflow line (fig. 1, site 3 and fig. 2). Since the aspirator took up blood originating from the arterial system as well as from the coronary veins, comparisons of hemoglobin values were made of the arterial samples (fig. 1, site 5) and those of the aspirator line beyond the defoaming chamber (fig. 1, site 2B). Comparisons of 16 paired samples revealed that those of the aspirator line were greater in 13 instances and equal in three (table 1). Comparisons of hemoglobin values of samples taken from the aspirator line before and after the defoaming chamber with its PMS-coated sponge (fig. 1, sites 2A and 2B) showed greater values beyond the chamber in nine of 11 paired observations (fig. 3).

pH values monitored routinely during operation averaged 7.46 units (range, 7.40 to 7.55). Evidence of a falling pH led to either increased oxygen flow through the disk oxygenator or to the addition of sodium bicarbonate solution. Osmolality, which the surgical team had repeatedly monitored in over 200 prior cases, was spot-checked during these studies and was always found to be in the range of 275 to 315 mOsm. Hematocrit values in the preoperative period ranged from 37 to 49. Those taken from patients (venous inflow site) at the start of bypass ranged from 25 to 33 and at the end of bypass, 28 to 35. These variations are accounted for by whole blood transfusions administered during the operative procedure when necessary to correct for major blood loss. There were no significant changes in red cell charge between the beginning and the end of the bypass procedure.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma hemoglobin (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arterial line</td>
</tr>
<tr>
<td>A.B.</td>
<td>69</td>
</tr>
<tr>
<td>L.C.</td>
<td>51</td>
</tr>
<tr>
<td>V.B.</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>103</td>
</tr>
<tr>
<td>F.R.</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>36</td>
</tr>
<tr>
<td>J.W.</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>69</td>
</tr>
<tr>
<td>J.M.</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>26</td>
</tr>
<tr>
<td>M.O.</td>
<td>48</td>
</tr>
<tr>
<td>J.B.</td>
<td>58</td>
</tr>
<tr>
<td>P.U.</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

*Multiple observations on one subject represent sequential studies during one pump run.
in rapid and marked hemolysis, reaching 532 mg% in 90 min (fig. 5). Hemolysis was considerably less when blood was similarly bubbled without the PMS-coated mesh and when PMS-coated sponge was immersed in the blood without bubbling. The former had a peak hemoglobin value of 70 mg% after 60 min and the latter 52 mg% after 90 min of immersion. Foaming, of course, was considerably greater in the tube bubbled without the coated sponge. Stainless steel sponge without PMS coating had no significant defoaming capability and led to no more hemolysis than the bubbling process per se.

Hemolysis was greatly reduced by the presence of Pluronic, as the whole blood sample containing 100 mg% of Pluronic showed hemoglobin values of 82 mg% after 90 min of bubbling in the presence of PMS-coated stainless steel sponge.

Surface tension measurements were carried out on samples from seven patients. The mean value of these was 57.6 dynes/cm at beginning of bypass to 50.5 dynes/cm at end of bypass (fig. 4). Spraying of antifoam upon the surface of the start of bypass plasma samples resulted in a reduction of surface tension values to a range equal to those of the end of bypass samples. The mean foam height of plasma bubbled in the foam tester for 30 sec was studied in five patients. Foam height was reduced by an average of 30% between samples taken at the start and at the end of bypass.

**In Vitro Studies**

Bubbling (oxygenation) of whole blood with 95% O₂ and 5% CO₂ in the presence of PMS-coated stainless steel sponge resulted in rapid and marked hemolysis, reaching 532 mg% in 90 min (fig. 5). Hemolysis was considerably less when blood was similarly bubbled without the PMS-coated mesh and when PMS-coated sponge was immersed in the blood without bubbling. The former had a peak hemoglobin value of 70 mg% after 60 min and the latter 52 mg% after 90 min of immersion. Foaming, of course, was considerably greater in the tube bubbled without the coated sponge. Stainless steel sponge without PMS coating had no significant defoaming capability and led to no more hemolysis than the bubbling process per se.

Hemolysis was greatly reduced by the presence of Pluronic, as the whole blood sample containing 100 mg% of Pluronic showed hemoglobin values of 82 mg% after 90 min of bubbling in the presence of PMS-coated

**Figure 3**

*Plasma hemoglobin values before and after passage of blood through the defoaming chamber.*

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**Figure 4**

*Surface tension of plasma obtained from pump immediately after start of bypass and at end of bypass.*
sponge; a difference of 450 mg% of the values obtained without Pluronic. Pluronic when used with the 20% O₂, 5% CO₂, and 75% N₂ gas mixture protected against hemolysis as effectively as it did with the 95% O₂ and 5% CO₂ gas mixture. The effects of the differences in gas mixtures and the effects of Pluronic thereon are shown in table 2. The presence of Pluronic did not appear to alter greatly the defoaming action of the PMS-coated steel sponge. Bubbling whole blood with 20% O₂, 5% CO₂, and 75% N₂ in the presence of PMS-coated sponge induced slight but significant decreases in red cell mobility, 1.06 to 0.99 μ/sec/volt/cm. Our laboratory normal of 1.06 μ/sec/volt/cm is in precise agreement with the normal values reported from other laboratories.¹⁰,¹² There was no change in mobility when Pluronic was present in the sample similarly bubbled.

Results of the studies of the effects of bubbling plasma in the presence of PMS-coated sponge and then reconstituting it with its native red cells and mixing these for 30 min are shown in figure 6. The most marked hemolysis was seen with the plasma that had been bubbled with 95% O₂ and 5% CO₂ for 60 min, noting that the majority of the effects had been induced within the first 15 min. The control samples consisted of plasma that had not been bubbled and were reconstituted at the same intervals. These showed no significant increases in hemoglobin values with time. The addition of Pluronic in a concentration of 100 mg% completely prevented the hemolysis seen when plasma was bubbled in the presence of PMS-coated sponges. A PMS-coated sponge immersed in standing (nubbled) plasma for 60 min led to moderate hemolysis with a peak hemoglobin value of 65 mg%. The addition of Pluronic to separate blood samples bubbled in the presence of PMS-coated sponge for 15 min and for 30 min immediately aborted the rising plasma hemoglobin values found in the similarly bubbled control samples without added Pluronic. The inhibition of hemolysis by Pluronic was dose related. The

![Figure 5](http://circ.ahajournals.org/)

**Figure 5**

Hemolytic effects of bubbling whole blood with 95% O₂ plus 5% CO₂ with and without PMS as a function of time.

<table>
<thead>
<tr>
<th>Gas mixture</th>
<th>Plasma hemoglobin (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95% O₂ &amp; 5% CO₂</td>
</tr>
<tr>
<td>Without Pluronic</td>
<td>23 38 50 68 168 328</td>
</tr>
<tr>
<td>With Pluronic, 100 mg%</td>
<td>26 37 38 39 59 82</td>
</tr>
<tr>
<td>20% O₂, 5% CO₂ &amp; 75% N₂</td>
<td>23 66 86 109 419 564</td>
</tr>
<tr>
<td>Without Pluronic</td>
<td>20 43 41 43 54 70</td>
</tr>
<tr>
<td>With Pluronic, 100 mg%</td>
<td>20 43 41 43 54 70</td>
</tr>
<tr>
<td>Bubbling time (min)</td>
<td>0 10 20 30 60 90</td>
</tr>
</tbody>
</table>

*Circulation, Volume XXXVII, April 1968*
maximal effect was attained at concentrations of 75 mg% for bubbled plasma and 100 mg% for bubbled whole blood.

Bubbling saline in the presence of PMS-coated sponge with careful control of pH did not lead to any hemolysis when subsequently mixed with red cells. pH and osmolality were not significantly altered by any of the bubbling or mixing procedures involving plasma or whole blood. The effects of temperature revealed no significant differences in defoaming action or hemolysis between 22°C and 37°C. At 4°C hemolysis was negligible but also the defoaming action of PMS was much less effective. Bubbling with either gas mixture did not induce any significant alterations in pH or osmolality.

Results of studies of the effects of mixing sonated PMS in saline (20 mg%) with red cells are shown in figure 7. Mixing the suspension for 60 min revealed that virtually all hemolysis occurred within the first 15 min. Without mixing, significant hemolysis did not occur nor did the sonated PMS in saline induce any significant hemolysis when Pluronic was present. Controls of mixing red cells in buffered saline in the same manner without PMS showed only minimal changes in hemoglobin values with time. Higher concentrations of PMS, up to 100 mg%, created only slightly greater hemolysis. The lowest concentration studied was 20 mg% (fig. 7.)

Discussion

The morbidity and mortality of cardiopulmonary bypass has decreased progressively since the inception of this mode of therapy. This is due largely to the better understanding of the physiology of mechanical circulatory support. However, certain problems persist. These are hemolysis due to red cell injury and pulmonary insufficiency. When bypass extends beyond 2 to 4 hours, these problems...
INFLUENCE OF A DEFOAMING AGENT

become the principal causes of postoperative morbidity and mortality. Red cell injury is due primarily to the mechanical trauma associated with the aspiration of blood from the operative field.\textsuperscript{12-15} The relatively large volumes of blood foam created by aspiration or bubbling are effectively controlled by rapidly acting defoaming agents. The most widely used and most potent defoaming agents are the polymethylsiloxanes. The present study indicates that, during defoaming, PMS significantly magnifies the hemolytic phenomenon by decreasing the resistance of the red cell to mechanical stress. The most logical explanation for this effect is that the chemical or biophysical phenomena involved in the destruction of foam also attack the lipids or lipoproteins of the red cell membrane. The two principal mechanisms by which PMS operates to destroy a foam are a reduction in the surface tension of the foam material and by dispersible surface layering. (S. Bradley and J. Cheka, personal communication.) The surface tension of this group of silicones is approximately 18 ± 2 dynes/cm while that of plasma is 56 ± 3 dynes/cm. Dispersible surface layering describes the characteristic of the PMS to spread itself over the surface of the foam. As it is strongly hydrophobic, it repels the water in the film surface thus destroying the stability of the foam. A number of studies were made of red cell and plasma lipids before and after the defoaming process with PMS. These included lipid electrophoresis, serum cholesterol, thin layer and gas chromatography, and protein electrophoresis on starch gel and cellulose acetate. No reproducible changes could be demonstrated. A more exhaustive investigation must be conducted before accepting the possibility that the defoaming action of PMS does not influence the lipids of the erythrocyte.

Measurements of plasma hemoglobin values from the other sampling sites shown in figure 1 revealed no other localized area of increased red cell destruction. This is in agreement with the observations of others regarding the relative lack of mechanical trauma to red cells by the other pump components.\textsuperscript{1, 2, 16} Hemolysis, as indicated by the plasma hemoglobin values, was always greatest after exposure of the blood to the PMS-coated sponge in the defoaming chamber. While plasma hemoglobin levels rose progressively throughout the procedure at all sampling sites (fig. 2), it was always greatest just beyond the defoaming chamber (fig. 1, site 2B). The contribution of PMS to the hemolytic process was further supported by studies of two pump runs carried out for 1 hour with identical equipment in the dog laboratory. Suctioning and defoaming were completely avoided. Plasma hemoglobin values were slightly elevated but considerably less than in the pump runs of the same duration in which the antifoam agent was employed.

Other factors such as exposure of blood to the pericardial surfaces have been previously incriminated and may account for some of the changes found in the samples analyzed from that site.\textsuperscript{17} However, simultaneously obtained samples from the operative field (fig. 1, site 1) and the line distal to the defoaming chamber (fig. 1, site 2B) always revealed a step-up in plasma hemoglobin values after defoaming.

Osmolality and pH were not altered significantly intra-operatively and could not account for the changes seen.\textsuperscript{18, 19} The results of these clinical pump studies clearly suggest that the defoaming process magnifies the hemolysis commonly attributed to suctioning per se.

The validity of these clinical observations was extended by the in vitro studies. The combination of bubbling whole blood with exposure to PMS-coated sponge produced considerably more hemolysis than either bubbling or exposure to PMS alone. These observations support the concept that exposure to PMS increases the hemolytic effects of trauma to red cells. This finding is contrary to the impressions gained from earlier studies that PMS was without significant effect on red cells. In those studies the effects of mixing and PMS exposure were not quantified independently.\textsuperscript{20}
To evaluate whether these hemolytic actions were mediated by direct effect upon the red cell membrane or through some intermediate plasma component, studies identical to those on whole blood were carried out on plasma. Hemolysis occurred when red cells were subsequently mixed with this bubbled and defoamed plasma and thus indicated that the PMS effect could be mediated through plasma. However, the effects of PMS transferred by or through plasma are considerably less than those occurring directly in whole blood. This suggests that the action of PMS is principally a direct one upon the red cell membrane.

The effects of mixing sonated PMS in saline with red cells confirms the suggestion from the plasma studies that the effect of PMS upon red cells is primarily a direct one (fig. 7). Use of bubbling saline in the presence of PMS-coated sponge conferred no hemolytic activity when red cells were subsequently mixed with it. This was expected as PMS is insoluble in saline and was the reason for the sonation of the PMS as a technique of study. This is of significance to those who use only saline solutions as their priming fluid.

The addition of Pluronic at plasma concentrations of 100 mg% blocked virtually all of the hemolytic effects of PMS in both bubbled and defoamed whole blood and plasma. The defoaming process did not appear to be greatly affected by the presence of Pluronic.

Pluronic F68 is a condensate of polyoxypropylene and polyoxyethylene and has a molecular weight of 8,350. It can best be described as an emulsifier with fat and emulsion stabilizing properties. On the basis of these properties the conjecture can be made that Pluronic F68 may have acted to stabilize the lipids and lipoproteins of the red cell membrane. It may have a similar effect upon the lipid and lipoproteins of plasma, preventing their breakdown to nonesterified fatty acids, which in high concentrations are believed to be hemolytic.21

Acknowledgment

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References


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The Majesty of the Unknown

...In our moods of abstract theorization we tend to forget how great and how diverse are the functional commitments of biological macromolecules. They insulate, they fill out; they fetch and carry; they prevent the Organism as a Whole from falling apart or from dissolving in water; they prop up, they protect; they attack and defend; they store energy and catalyze its transfer; they store information and convey messages, and sometimes they themselves are messages. The successful prosecution of all these activities depends upon properties more complex, various and particular than can be written down in the language of energetics or information theory. —P. B. Medawar: *The Art of the Soluble*. London, Methuen & Co. Ltd., 1967, p. 57; also distributed by Barnes & Noble, Inc., New York.
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