Intravascular and Extravascular Hemolysis Accompanying Extracorporeal Circulation

A Clinical Study

By Herbert W. Wallace, M.D., and William S. Blakemore, M.D.

SUMMARY

Eleven patients undergoing open heart surgery were studied by means of a technic previously developed by us to quantitate intravascular and extravascular hemolysis. Endogenous production of carbon monoxide, which correlates quantitatively with red blood cell and hemoglobin catabolism, was measured preoperatively, 5 hr postoperatively, and 24 hr postoperatively. Rates of plasma hemoglobin catabolism and intravascular hemolysis were calculated from serial plasma hemoglobin determinations. Extravascular hemolysis results from catabolism of erythrocytes damaged during perfusion and subsequently sequestered in the reticuloendothelial system. Intravascular hemolysis occurred only during perfusion and averaged $252.22 \pm SE 49.64 \mu$moles heme. Extravascular hemolysis measured for the first 5 hr after perfusion averaged $462.48 \pm SE 68.70 \mu$moles heme, or 64% of the total calculated red blood cell destruction. In all but one of the eight patients studied 24 hr after perfusion, heme catabolism remained elevated (mean, $46.76 \pm SE 13.05 \mu$moles heme/hr compared to a normal value of $\leq 22 \mu$moles heme/hr), indicating that extravascular hemolysis was still occurring. This extravascular destruction probably resulted from alterations in the cell membrane induced during the pumping procedure.

Additional Indexing Words:

Cardiac surgery         Hemoglobin

Red blood cell

Changes in blood induced by extracorporeal circulation are perhaps the most important factors hindering the clinical application of prolonged extracorporeal circulatory support, and one of the major ways of evaluating a pumping system has been to study its effect on erythrocyte destruction. The usual method is to use quantitative measurements of plasma hemoglobin to determine the intravascular hemolysis resulting from mechanical forces exerted on erythrocytes. However, this static measurement is influenced by the relationship of rates of production, metabolism, and excretion of plasma hemoglobin, and there is indirect evidence that many more erythrocytes are ultimately destroyed than plasma hemoglobin quantitation indicates.

We have developed a technic of quantitating intravascular and extravascular hemolysis based on the estimation of total rates of hemoglobin catabolism from measurements of endogenous carbon monoxide production. Carbon monoxide is an in vivo by-product of heme catabolism originating from the alpha methane bridge carbon atom, and in normal man and anesthetized dogs catabolism of 1 mole of heme yields 1 mole of carbon monoxide. In the present study we used this technic to assess intravascular and extravascu-
lar hemolysis in 11 patients before and after extracorporeal circulation. These patients had a variety of congenital and acquired heart diseases (table 1).

Methods

Clinical Procedures

The extracorporeal circuit was primed with 1,500 ml of whole blood, 1,500 ml of electrolyte solution,* 500 ml of 10% dextran in normal saline,† 2 g of ascorbic acid, and 25 mEq of sodium bicarbonate. Perfusion was conducted at flow rates ranging from 75 to 90 ml/kg/min for a mean duration of 90 min at 27 C during the first 75 min.

Measurement of Carbon Monoxide Production

We determined the rate of endogenous CO production (Vco) by the rebreathing method with a slight modification of the previously described apparatus.8 An airtight plastic hood fits over the patient's head and is sealed about his neck. The rebreathing system consists of a CO2 absorber, a demand valve connected to an oxygen supply, and a blower to insure adequate internal circulation. The entire apparatus is mounted on a cart so that it can be used at the bedside. For patients with a cuffed endotracheal or tracheotomy tube, we attached the tube to a similar closed system and inserted a one-way valve to insure adequate circulation of air. The PO2 of the inspired gas was monitored constantly and maintained at approximately 150 mm Hg. Venous blood samples were drawn at 20-min intervals for 2 hr beginning at least 15 min after the start of rebreathing. The body CO dilution was then determined by adding 10 ml of carbon monoxide and measuring the resultant increase in carboxyhemoglobin concentration. The blood carboxyhemoglobin concentration ([COHb]) was measured by an infrared method9 and Vco was calculated from the rate of increase of [COHb] and the body CO dilution, as described previously.4 Carbon monoxide production was determined preoperatively, approximately 5 hr after perfusion, and in eight of the patients 24 hr after perfusion.

Measurement of Plasma Hemoglobin Influx and Efflux

Plasma hemoglobin concentration was quantitated by the method of Crosby and Furth10 every 30 min during perfusion, immediately after perfusion, and at hourly intervals for at least 6 hr after perfusion. Urinary loss of hemoglobin was determined at intervals from the time extracorporeal circulation was stopped to the end of the CO study period.

Calculation of Intravascular and Extravascular Hemolysis

Intravascular hemolysis (IH) was defined as loss from the circulation of erythrocytes sufficiently damaged to destroy the integrity of the

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.K.</td>
<td>Mitral stenosis</td>
<td>Mitral valve replacement</td>
</tr>
<tr>
<td>L.T.</td>
<td>Atrial septal defect</td>
<td>Closure of atrial septal defect</td>
</tr>
<tr>
<td>W.P.</td>
<td>Aortic insufficiency</td>
<td>Aortic valve replacement</td>
</tr>
<tr>
<td>J.C.</td>
<td>Atrial septal defect</td>
<td>Closure of atrial septal defect</td>
</tr>
<tr>
<td>M.G.</td>
<td>Mitral stenosis</td>
<td>Open mitral commissurotomy</td>
</tr>
<tr>
<td>E.G.</td>
<td>Membranous subaortic stenosis</td>
<td>Excision of subaortic membrane</td>
</tr>
<tr>
<td>W.C.</td>
<td>Aortic stenosis, aortic insufficiency</td>
<td>Aortic valve replacement</td>
</tr>
<tr>
<td>R.S.</td>
<td>Prosthetic valve stenosis</td>
<td>Aortic valve replacement</td>
</tr>
<tr>
<td>O.S.</td>
<td>Mitral stenosis</td>
<td>Open mitral commissurotomy</td>
</tr>
<tr>
<td>J.B.</td>
<td>Aortic stenosis, mitral stenosis</td>
<td>Aortic valve replacement, mitral commissurotomy</td>
</tr>
<tr>
<td>H.H.</td>
<td>Atrial septal defect</td>
<td>Closure of atrial septal defect with patch</td>
</tr>
</tbody>
</table>

*Cromosol; Abbott Laboratories, Chicago, Illinois.
†Rheomacrodex; Pharmacia Laboratories, Inc., Piscataway, New Jersey.
IH is expressed as \( \mu \) moles of heme. The plasma hemoglobin compartment (\( H_b \)) is that body space through which acellular hemoglobin will diffuse. To avoid the use of radioisotopes with this group of patients, \( H_b \) was assumed to be 77 ml/kg, a mean value obtained in previous studies.\(^2\)

Since carbon monoxide is produced in a molar ratio to heme catabolism following both extravascular and intravascular hemolysis,\(^6,11\) \( V_{CO} \) is expressed as \( \mu \) moles of heme per hour; \( V_{CO_{excess}} \) represents that quantity of heme catabolism that cannot be accounted for by normal processes, as determined preoperatively:

\[
V_{CO_{excess}} = V_{CO_{postop}} - V_{CO_{prop}} \quad (2)
\]

\( V_{CO_{excess}} \) was shown in our previous studies\(^2\) to result from catabolism of erythrocytes damaged during perfusion and subsequently removed from the circulation or from catabolism of hemoglobin effluxing from the plasma hemoglobin compartment. If the amount of carbon monoxide that represents acellular heme catabolism is accounted for, extravascular hemolysis (\( E_H \)) is the loss from the circulation of erythrocytes that have been damaged but not lysed in the vascular tree and subsequently removed by the reticuloendothelial system and catabolized.

\[
IH = \frac{\text{maximal plasma hemoglobin concentration}}{H_b} \quad (1)
\]

\[
E_H = \frac{V_{CO_{excess}} - V_{HB_{pl}}}{T} \quad (3)
\]

\( E_H \) is expressed as \( \mu \) moles of heme. Total plasma hemoglobin catabolism (\( V_{HB_{pl}} \)), expressed as \( \mu \) moles of heme per hour, is estimated from the rate of decrease of the level of hemoglobin (in \( \mu \) moles of heme per milliliter) determined from serial plasma samples, multiplied by the plasma hemoglobin compartment (in milliliters). The interval of measurement (\( T \)) is expressed in hours; in this study it was 5 hr.

**Results**

The preoperative rate of CO production was considered to be a base-line value indicative of the heme metabolic processes of the patient. The mean preoperative \( V_{CO} \) of these patients was 27.80 ± SE 7.35 \( \mu \) moles heme/hr (table 2). The upper limit of normal CO production in man is approximately 22 \( \mu \) moles heme/hr.\(^4\) Five hours after cessation of extracorporeal circulation the mean \( V_{CO} \) was 154.44 ± SE 15.56 \( \mu \) moles heme/hr. Total plasma hemoglobin catabolism during the first 5 hr after perfusion averaged 33.08 ± SE 6.90
μmoles heme/hr, and excess heme catabolism averaged 126.67 ± se 17.21 μmoles heme/hr. In two instances plasma hemoglobin could not be quantitated for technical reasons and a rate of loss of 0.69 μmole heme/100 ml/hr (mean of 17 comparable cases studied for other reasons) was assumed. Urinary loss of hemoglobin during the 5-hr period averaged 2.95 μmoles heme. An average of only 28.3% of the excess CO production could be attributed to catabolism of hemoglobin effluxing from the plasma hemoglobin compartment.

Intravascular hemolysis was calculated from data shown in table 2. The maximal plasma hemoglobin concentration was invariably found at the end of the perfusion period. In the two instances in which plasma hemoglobin could not be quantitated, a maximal value of 93.5 mg/100 ml (mean of 20 comparable cases) was assumed. IH averaged 252.22 ± se 49.64 μmoles heme (table 3).

EH measured for 5 hours after perfusion averaged 462.48 ± se 68.70 μmoles heme (table 3). During this 5-hr period extravascular hemolysis accounted for an average of 64% of the total erythrocyte destruction measured. In one patient 22 times more erythrocytes were damaged, sequestered, and catabolized extravascularly than were destroyed intravascularly during cardiopulmonary bypass.

Of the eight patients studied 24 hours after perfusion, all but one were found to have an elevated rate of heme catabolism, with a mean \( \dot{V}_{co} \) of 46.76 ± se 13.05 μmoles heme/hr (table 4). The one patient whose \( \dot{V}_{co} \) was within normal limits (fig. 1) had the shortest total bypass time of the entire group (33 min). Urinary loss of hemoglobin did not occur beyond 5 hours postperfusion.

Figure 2 illustrates the results of a typical study. Preoperatively, the \( \dot{V}_{co} \) was 21.95 μmoles heme/hr, and the plasma hemoglobin concentration was 1.5 mg/100 ml. The plasma hemoglobin concentration reached a maximum of 8 mg/100 ml at the end of the perfusion period and decreased at a mean rate of 5.7 ml/hr for the next 5 hr. Five hours after

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### Table 4

<table>
<thead>
<tr>
<th>Patient</th>
<th>( \dot{V}_{co} ) (μmoles heme/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.T.</td>
<td>17.56</td>
</tr>
<tr>
<td>W.P.</td>
<td>39.07</td>
</tr>
<tr>
<td>J.C.</td>
<td>30.80</td>
</tr>
<tr>
<td>M.G.</td>
<td>35.12</td>
</tr>
<tr>
<td>E.G.</td>
<td>26.34</td>
</tr>
<tr>
<td>W.C.</td>
<td>74.63</td>
</tr>
<tr>
<td>R.S.</td>
<td>127.30</td>
</tr>
<tr>
<td>O.S.</td>
<td>23.27</td>
</tr>
</tbody>
</table>

| Mean    | 46.76 (SE 13.05)                  |

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### Table 3

<table>
<thead>
<tr>
<th>Patient</th>
<th>IH (μmoles heme)</th>
<th>EH T = 5 (μmoles heme)</th>
<th>Ratio of EH/total hemolysis (%)</th>
<th>EH/IH</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.K.</td>
<td>257.65</td>
<td>200.95</td>
<td>43.8</td>
<td>0.8</td>
</tr>
<tr>
<td>L.T.</td>
<td>128.38</td>
<td>731.86</td>
<td>85.1</td>
<td>5.7</td>
</tr>
<tr>
<td>W.P.</td>
<td>269.05</td>
<td>494.34</td>
<td>64.8</td>
<td>1.8</td>
</tr>
<tr>
<td>J.C.</td>
<td>14.32</td>
<td>322.15</td>
<td>95.7</td>
<td>22.5</td>
</tr>
<tr>
<td>M.G.</td>
<td>259.01</td>
<td>188.52</td>
<td>42.1</td>
<td>0.7</td>
</tr>
<tr>
<td>E.G.</td>
<td>162.76</td>
<td>145.57</td>
<td>47.2</td>
<td>0.9</td>
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<tr>
<td>W.C.</td>
<td>281.22</td>
<td>421.75</td>
<td>60.0</td>
<td>2.0</td>
</tr>
<tr>
<td>R.S.</td>
<td>173.25</td>
<td>557.95</td>
<td>76.3</td>
<td>3.2</td>
</tr>
<tr>
<td>O.S.</td>
<td>679.16</td>
<td>810.91</td>
<td>54.4</td>
<td>1.2</td>
</tr>
<tr>
<td>J.B.</td>
<td>260.77</td>
<td>671.22</td>
<td>72.0</td>
<td>2.6</td>
</tr>
<tr>
<td>H.H.</td>
<td>288.81</td>
<td>542.05</td>
<td>65.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Mean</td>
<td>252.22</td>
<td>462.48</td>
<td>64.2</td>
<td>3.9</td>
</tr>
<tr>
<td>SE</td>
<td>49.64</td>
<td>68.70</td>
<td>5.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Abbreviations: EH = extravascular hemolysis; IH = intravascular hemolysis.
HEMOLYSIS WITH EXTRACORPOREAL CIRCULATION

Figure 1
Heme catabolism of the one individual (L.T.) in the study whose CO production returned to normal by the first postoperative day. This patient had a total bypass time of only 33 min.

Figure 2
Carbon monoxide production was normal in this individual (J.C.) before perfusion; 5 hr after perfusion it had increased more than fourfold, and it remained slightly elevated 24 hr postoperatively. This pattern of CO production was typical of the patients studied.

Discussion
The principal finding of the study was that a far greater number of erythrocytes were traumatized during extracorporeal circulation and removed from the bloodstream during the first 5 hr after perfusion than were mechanically disrupted during surgery (presumably by the forces of the perfusion apparatus). The increased quantity of CO produced during this postoperative period was from two to 17 times greater than could be attributed to catabolism of hemoglobin effluxing from the plasma hemoglobin compartment. That this finding resulted from an underestimation of rates of catabolism of the hemoglobin-haptoglobin complex, or “free” hemoglobin, is unlikely since results of previous studies have indicated that plasma hemoglobin is catabolized slowly and CO production arising from this catabolism is small compared to that resulting from extravascular destruction of erythrocytes. Rates of decrease of plasma hemoglobin agreed with those found in previous studies. Our previous animal stud-
ies^2 indicated that circulatory changes induced by extracorporeal circulation did not increase the rate of catabolism of other heme compounds, nor did relative hypoxia or hypotension, and extracorporeal circulation induced no alteration in the reticuloendothelial system that might influence CO production. We, therefore, concluded that excess heme catabolism unaccounted for by catabolism of hemoglobin effluxing from the plasma hemoglobin compartment resulted from catabolism of hemoglobin from erythrocytes that were damaged intravascularly and sequestered by the reticuloendothelial system.

Our animal studies^2 indicated that in the early postperfusion period carbon monoxide may shift from blood into other body stores. We were not able to determine whether such a shift occurred in the present study, since an evaluation of this event would have required the use of radioisotopic technics, which we wished to avoid. It is very unlikely that such a shift would occur 5 hr after perfusion; however, if it did, the \( V_{\text{CO}} \) measurement would be reduced and the true quantity of extravascular hemolysis would be greater than that measured.

Carbon monoxide production can be measured with an error of \( \pm 0.1 \text{ ml, STPD} \). An error in determining the rate of loss of plasma hemoglobin may arise, but it is probably less than 10%. These errors are of little significance in calculating EH since the rate of loss of hemoglobin from the plasma is small compared to the total rate of hemoglobin catabolism. Most of the possible sources of error in the technics used in this study would tend to minimize the value of EH. Urinary loss of hemoglobin was measured but was found to be insignificant compared to the amount of heme catabolism occurring, averaging only 2.95 \( \mu \text{moles of heme in the 5-hr postperfusion period.} \)

To rule out the possibility that the anesthetic technics significantly influenced the results, we measured CO production in two surgical patients who were anesthetized in a similar manner to the open-heart patients but who were not subjected to extracorporeal circulation. Carbon monoxide production was within normal limits both preoperatively and postoperatively. Other control studies provided no evidence that the anesthetic agents in the blood affected our analytic procedure for the measurement of carboxyhemoglobin.

It should be stressed that IH was calculated from data obtained only during perfusion, whereas EH was calculated from postperfusion data. Since the results obtained 24 hours after perfusion indicated that EH was still occurring, the percentage of total erythrocyte destruction occurring extravascularly would probably have been much greater if the investigation had been continued for a longer period. Four of the patients were studied periodically for more than 24 hr postoperatively. Two of them exhibited an elevation in heme catabolism on the second postoperative day and one on the third day; all three returned to normal rates of CO production by the fifth postoperative day. The fourth patient was studied only on the fifth day, when his \( V_{\text{CO}} \) was normal. Since the plasma hemoglobin concentrations of all 11 patients were normal by the first postoperative day and there was no urinary loss of hemoglobin beyond the 5-hr postperfusion period, we have little doubt that the number of erythrocytes destroyed extravascularly markedly exceeded the quantity destroyed intravascularly.

In several patients CO production was elevated preoperatively. The relationship of this increased heme catabolism to the hemodynamics of cardiac disease should be investigated.

Our technic of estimating intravascular and extravascular hemolysis by measuring CO production is as sensitive as labeling technics of determining erythrocyte destruction and is less time-consuming.\(^2\) It will undoubtedly be useful in the evaluation of prosthetic valves, artificial hearts, and mechanical devices for assisted or total extracorporeal circulation. In addition, this methodology allows quantitative determination of the influence of such variables as pressure, shear, and surface interaction upon erythrocyte membrane damage.
Acknowledgment

The authors gratefully acknowledge the technical assistance of Maria Leskiw, Barbara Bruser, Michael McPeck, and Darwin Kenepp, and the encouragement and suggestions of Dr. Ronald F. Coburn.

References

Intravascular and Extravascular Hemolysis Accompanying Extracorporeal Circulation: A Clinical Study
HERBERT W. WALLACE and WILLIAM S. BLAKEMORE

_Circulation_. 1970;42:521-527
doi: 10.1161/01.CIR.42.3.521

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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