WHEN DOES BLOOD HAEMOLYSE?

A Temperature Study

C. CHALMERS AND W. J. RUSSELL

SUMMARY

Incubation of blood in vitro for up to 1 hour at temperatures below 45°C caused no significant haemolysis as determined by a colorimetric method. The duration of storage of blood in CPD or ACD did not affect the sensitivity of the red cells to haemolysis at 50°C. Blood stored in ACD haemolyses less rapidly at 50°C than blood stored in CPD: but there is no significant difference in the rate of haemolysis at 45°C and below.

Rapid infusion of large amounts of cold blood into a patient produces a significant and possibly dangerous reduction of the body temperature. Cooling of the heart may provoke arrhythmias and even cardiac arrest. For this reason, it is now customary to warm stored blood before large transfusions. Ideally the blood should be heated to body temperature (37°C), but temperatures above 32°C are acceptable (Russell, 1974).

Most blood warmers operate by heat exchange with a water bath. The temperature of the blood at the outlet of the warmer depends on both the temperature of the heat source and the flow rate of the blood. The thermostat of the blood warmer should be set so that the highest possible output temperature is achieved at high flows: while at low flows, when the output temperature of the blood approaches the bath temperature, the red cells are not damaged. Temperatures above 50°C are known to damage red cells (Landois, 1875) but Howland and Schweizer (1965) showed that passing blood through a warmer at 40°C does not cause haemolysis. Most commercially available blood warmers have an operating temperature range between 37°C and 39°C. With the common polyvinyl chloride coil, the output temperature is 30°C or below with a flow rate of 150 ml/min through a bath at a temperature of 37°C (Russell, 1974). If the bath temperature could be raised without damaging the red cells, blood could be delivered to the patient at a higher temperature and the danger of a massive cool infusion of blood could be avoided.

This study was undertaken to see if stored blood haemolysed when incubated at temperatures between 40°C and 50°C.

METHOD

Blood was taken from volunteers into Fenwal packs containing either acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose (CPD) anticoagulant. Each pack was gently agitated throughout the bleeding to ensure complete mixing of blood and anticoagulant. The final volume was controlled by weight as specified by the manufacturer. The final gross weight was 575 g for CPD (450 ml) or 640 g for ACD (500 ml), that is, the anticoagulant formed about 12% of the pack contents in both cases. The blood was stored at 4°C until used. Electrophoresis was performed on the blood of each volunteer to ensure that no abnormal haemoglobin was present.

Before testing, the blood pack was gently agitated for 5 min when first removed from the refrigerator, and for a further 1 min before taking samples, to ensure a uniform haematocrit. The blood was returned to the refrigerator between samples.

For each temperature batch, the blood was allowed to flow freely from the pack into five 10-ml graduated centrifuge tubes. A temperature batch consisted of the blank and the four tubes heated for different periods at a single temperature. The centrifuge tubes were stoppered and four of these tubes placed in a Gallenkamp shaking reaction incubator which was set at either (1) ambient temperature, (2) 37°C, (3) 41°C, (4) 43°C, (5) 45°C, or (6) 50°C. The fifth tube was used as an
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unheated blank kept at ambient temperature until spun. One tube was removed from the bath at 15, 30, 45 and 60 min. Each tube was centrifuged at 2,500 r.p.m. for 10 min to pack the red cells. Following the method of Shaw (1967), the plasma was carefully removed with a Pasteur pipette and 0.25 ml Lissapol NDB (ICI) was added to 3 ml plasma to clear turbidity. The optical density (OD) of the cleared plasma was read directly in a colorimeter (Lars Ljungberg) against a 540-nm filter. The zero of the colorimeter was set with distilled water and the OD of the plasma blank was subtracted from all readings, so that the increase in OD was measured. A considerable variation between plasma blanks from any one bag on the same day was found, and a new blank was centrifuged with each change in temperature. The blank was kept in a covered cuvette and read before each reading. The readings at ambient temperature and at 37°C were made as a means of checking the consistency of the method and of estimating errors.

A standard haemoglobin solution was prepared from fresh red cells; 10 ml blood was centrifuged at 2,500 r.p.m. for 10 min. The plasma was removed and the cells resuspended in physiological saline; washing and centrifuge were repeated three times. The cells were then resuspended in saline, and lysed by repeated freezing and thawing. Cell debris was removed by centrifuging and the haemoglobin concentration of the remaining solution was measured by the cyanmethaemoglobin method (Drabkin and Austin, 1935). This standard solution was used to calibrate the colorimeter for the haemolysis measurements. Successive aliquots of the haemoglobin solution were added to plasma to form a series of standards. The whole procedure was followed with four different solutions of haemoglobin, and the four series of standards were used to calculate a regression line and to provide an estimate of the error at each value of haemolysis (table I).

Plasma obtained by centrifugation was heated to 43°C and 50°C for 60 min to see if any change in OD occurred that was not associated with haemolysis.

ACD and CPD blood were tested for haemolysis by elevating the temperature when it was 1 day, 1 week, 2 weeks and 3 weeks old. Most packs had sufficient blood for two full tests. A test consisted of one batch at each of five different temperatures. One test was made at either 1 day or 1 week and another at either 1 or 2 weeks. The 3-week blood which was tested was 22-day CPD blood selected from the hospital blood bank.

Sources of error.

With the method used, the possibility of haemolysis from factors other than heat was small. However from preliminary studies, it was found that several causes of haemolysis must be avoided or controlled carefully if consistent results are to be obtained. These causes are:

(1) Removing blood samples by syringe, which produces an unpredictable amount of haemolysis.

(2) Water loss by evaporation, which increases the haemoglobin concentration.

(3) Condensation in the tube, which runs back into the blood. This causes haemolysis by creating a hypotonic environment locally.

(4) Drying of blood on the side of the tube: this causes red cell destruction and if the sample is contaminated with this dry blood, haemolysis is found.

The volume of detergent was also important as 20% more Lissapol caused a decrease in OD equivalent to approximately 1.5 mg/100 ml haemolysis.

The wavelength used (540 nm) is not specific for haemoglobin and each unit of blood required its own plasma blank. Thus this method does not measure absolute levels of free haemoglobin but only the increases.

RESULTS

Reproducibility and reliability of the method.

Variability and bias in the method were most relevant to this study as we were interested in determining the temperature at which haemolysis begins to occur. Seven batches of blood were studied at ambient temperatures. The means at 15,

<table>
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<tr>
<th>Haemolysis (mg/100 ml)</th>
<th>Tolerance limit</th>
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<tr>
<td></td>
<td>Upper</td>
</tr>
<tr>
<td>10</td>
<td>12.3</td>
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<tr>
<td>20</td>
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<td>50</td>
<td>52.3</td>
</tr>
<tr>
<td>60</td>
<td>62.3</td>
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30, 45 and 60 min were all within 1.9 mg/100 ml and so were pooled to form a single estimate of \(-1.0 \text{ mg/100 ml} \pm 2.77 \text{ (mean \pm SD)}\). Neither the value at each time nor the pooled estimate differed significantly from the estimates made from the plasma blanks. The four batches studied at 37°C gave means at each of the four times which differed by only 0.25 mg/100 ml, so again a single estimate was made. The level of haemolysis was 3.05 mg/100 ml \pm 5.5 \text{ (mean \pm SD)} which is significantly different from the mean value at ambient temperature \((P<0.005)\). Thus, although progressive haemolysis did not occur, a small amount of haemolysis occurred in some batches from the first sample at 15 min. The mean value of 3 mg/100 ml represents haemolysis of about 2.5 \(\mu\)l of blood. Such a small amount could have occurred from early condensation. The cells may be affected only in the first few minutes because sedimentation would give a protective top layer of plasma during the later part of the incubation. The variability of the method over most of the range used can be estimated from the four series of standards. Table I shows the 95% tolerance limits of the regression line of haemolysis and OD, the overall estimate of error should include the basal (37°C) haemolysis and its variation.

Heating plasma to 45°C for 1 hour caused no change in OD. After 1 hour at 50°C, protein coagulated and there was an increase in OD. When this plasma was centrifuged at 2,500 r.p.m. for 10 min, a clear supernate was obtained which showed an increase in OD equivalent to 10–20 mg/100 ml haemolysis.

### The effect of temperatures between 40°C and 50°C.

Incubation at temperatures up to 45°C for periods up to 1 hour caused less than 4 mg/100 ml haemolysis which was within the potential experimental error (table II). Also at temperatures of 45°C and below, the apparent haemolysis did not progressively increase from 15 to 60 min incubation. The blood incubated at 50°C showed haemolysis which increased linearly with the time of incubation (fig. 1). The mean rate of haemolysis in 22 batches was 2.26 mg/100 ml/min, with a range 1.04–4.21 mg/100 ml/min. At 1 hour the level of haemolysis for 22 samples was 131 ± 46 mg/100 ml (mean ± SD).

### The effect of age of the blood.

The rate of haemolysis at 50°C was not influenced by the age of the blood. This was true both for blood stored for as long as 2 weeks in ACD or as long as 3 weeks in CPD (fig. 2).

### The effect of storage in ACD or CPD.

At 50°C a faster rate of haemolysis was seen for

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time of incubation (min) 15</th>
<th>30</th>
<th>45</th>
<th>60</th>
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</thead>
<tbody>
<tr>
<td>41</td>
<td>2.0 ± 0.5</td>
<td>3.3 ± 0.7</td>
<td>2.7 ± 0.7</td>
<td>3.0 ± 0.4</td>
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<tr>
<td>43</td>
<td>2.8 ± 0.7</td>
<td>2.8 ± 0.9</td>
<td>3.0 ± 0.8</td>
<td>2.7 ± 0.7</td>
</tr>
<tr>
<td>45</td>
<td>2.1 ± 0.6</td>
<td>2.8 ± 0.7</td>
<td>2.8 ± 0.7</td>
<td>3.7 ± 0.9</td>
</tr>
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**TABLE II.** Mean haemolysis \(\pm SD\) at 41°C, 43°C and 45°C. The values at each temperature are derived from 27 estimates.

**FIG. 2.** The haemolysis after incubation at 50°C for 1 hour is plotted against age of blood stored in ACD (triangles) and in CPD (dots). No significant increase in haemolysis with age is seen for either group.
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blood stored in CPD than for blood stored in ACD. The difference between the two regression lines of the amount of haemolysis related to the incubation time was statistically significant (P<0.01) (fig. 3). At 45°C and below, the difference in haemolysis was within the variation of the method and cannot be considered significant. Figure 4 shows the results for 45°C.

![Graph showing haemolysis at 50°C for blood stored in ACD (10 batches) and in CPD (12 batches). The difference between the two linear regression lines is statistically significant (P<0.01). Means ± SEM.](https://example.com/graph1.png)

**Fig. 3.** The individual rates of haemolysis at 50°C for blood stored in ACD (10 batches) and in CPD (12 batches). The difference between the two linear regression lines is statistically significant (P<0.01). Means ± SEM.

![Graph showing mean haemolysis ± SD against time of incubation at 45°C for blood stored in ACD (12 batches) and in CPD (15 batches). The level of haemolysis in all cases is within the error of the method.](https://example.com/graph2.png)

**Fig. 4.** The mean haemolysis ± SD against time of incubation at 45°C for blood stored in ACD (12 batches) and in CPD (15 batches). The level of haemolysis in all cases is within the error of the method.

**DISCUSSION**

It has been known since the work of Landois (1875) that temperatures above 50°C damage blood. Our results confirm that haemolysis occurs at 50°C and show that the amount of haemolysis increases linearly with time of incubation. However, incubation for up to 1 hour at 45°C and below caused no significant haemolysis (fig. 5). This corresponds with the finding of Karle (1969) that rabbit blood incubated at 41.5°C does not haemolise in less than 10 hours.

Blood which had been stored for as long as 2 weeks in ACD or as long as 3 weeks in CPD did not show a significantly higher haemolysis at 50°C than blood which was stored for only 1 day. It would seem safe, therefore, to use a blood warmer to transfuse bank blood which had been stored for up to 3 weeks.

![Graph showing the level of haemolysis after 1 hour of heating at various temperatures. Each value is a mean of at least 22 values ± SEM. Only the value at 50°C exceeds the methodological errors.](https://example.com/graph3.png)

**Fig. 5.** The level of haemolysis after 1 hour of heating at various temperatures. Each value is a mean of at least 22 values ± SEM. Only the value at 50°C exceeds the methodological errors.

Blood stored in ACD had a significantly lower rate of haemolysis at 50°C than blood stored in CPD. This is of little practical consequence as blood for transfusion should not be heated to 50°C. However, it may be an indication that the red cells of blood stored in CPD are more fragile than those stored in ACD. Neither group showed significant haemolysis at 45°C or below.

The results of this study show that blood does not haemolise if warmed to 45°C for 1 hour. Red cells incubated at temperatures above 37°C may be damaged but not haemolysed. If such a state were possible, cells could have a decreased viability. In-vivo survival studies would be required to determine this. Karle (1969), using rabbit blood, found no decrease in survival 24 hours after reinjection of red cells which had been incubated in vitro at 41.5°C for 4 hours. In-vivo survival is probably the crucial test for red cell damage but, theoretically, important functions of the red cell, such as oxygen
transport, could be upset without a change in survival time. Our present study on haemolysis does not exclude more subtle changes at the temperatures where no haemolysis occurred.

At present the maximum working temperature for blood warmers is 39°C. However, no haemolysis occurs if blood is warmed to 45°C for 1 hour. If a 2°C or 3°C margin is provided for the safety thermostat, a warming bath could operate at 42°C without danger of haemolysing the blood it is warming. This small increase in bath temperature would be advantageous where fast infusion rates are used, as it would increase the temperature of the blood leaving the bath.

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REFERENCES