IN VITRO STUDY OF THE EFFECT OF HYPOTHERMIA ON THE DISSOCIATION CURVE OF BLOOD

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Matthew D. Munding
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This thesis for honors recognition has been approved for the Department of Biology and Chemistry by:

John Addis 4/1/92

Dr. John Addis Date

Professor of Biology

Marilyn Schendel 4-1-92

Dr. Marilyn Schendel Date

Professor of Chemistry

Darrell Hagen 4-1-92

Mr. Darrell Hagen Date

Professor of Computer Science

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ABSTRACT

The aim of this study was to examine the change in the dissociation curve of oxygen from human hemoglobin at the temperatures of 37°, 25°, 18°, 12°, 7°, and 3° C. The partial pressures of oxygen and the calculated hemoglobin saturations were determined for samples at the various temperatures. From this data the partial pressures at 75% saturation were calculated, and the differences in the partial pressures at 75% saturation were compared. The dissociation curve was shown to shift to the left as the temperature was decreased down to 12°. Below 12°, the shift was not further observed. At the lower temperatures, the oxygen did not dissociate as well as it did at the higher temperatures.

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INTRODUCTION AND LITERATURE REVIEW

Hypothermia is a lowering of the normal body temperature. Although hypothermia is often detrimental, it can be manipulated in some situations to be a benefit to the body, as in cardiovascular surgery and neurosurgery. Hypothermia may be induced by surface cooling or cardiac bypass which involves shunting the blood of the patient out of the body and externally controlling the temperature, the blood-oxygen levels, and the flow rates (7). Under hypothermic conditions, all enzymatic activity is reduced resulting in lowered cellular metabolism. The hypothermic condition also reduces the vascular blood flow which decreases the amount of blood entering the surgical field (1). The delivery of oxygen (O_2) by blood to the vital organs, especially the brain, during a surgery using hypothermic conditions is essential for the prevention of neurological damage.

The purpose of this study was to examine how a decrease in temperature will affect the dissociation of O_2 from hemoglobin. In order to examine this effect, the relationship between the partial pressure of oxygen (pO_2) and the 75% oxygen saturation of the hemoglobin in blood was compared. The pO_2 and the 75% saturation were determined from samples of oxygenated and deoxygenated red blood cells. This process was repeated at six different temperatures.

In human adult erythrocytes, hemoglobin A is the principle type of hemoglobin (15). It is specialized to carry O_2 molecules. Each hemoglobin molecule is made up of four polypeptide chains; two are alpha chains, and two are beta chains (15). Each chain contains a heme group that can bind an O_2 molecule. The binding of O_2 in hemoglobin is cooperative. That is, the binding of one O_2 molecule facilitates the binding of another O_2 at a different heme site. The unloading of the O_2 is also cooperative. Unloading of O_2 at one site will facilitate the unloading of the other sites.

The dissociation of O_2 from hemoglobin can be represented by a graph called the O_2 dissociation curve. The shape of the curve is sigmoidal due to the cooperative binding of the heme groups in the hemoglobin. This graph plots the saturation of hemoglobin with O_2 (Y) versus the pO_2, as seen in Figure 1 (15).

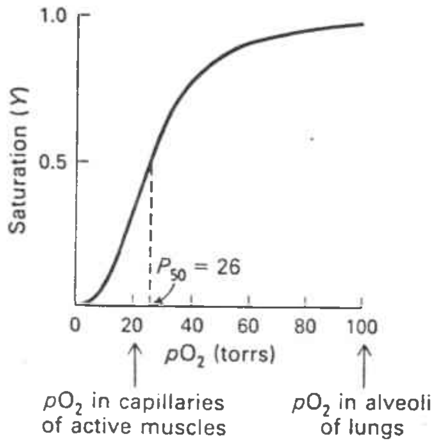

Figure 1. O_2 dissociation curve of blood under physiological conditions. (from Stryer, Biochemistry , 3rd ed., 1988)

The saturation or the fractional representation of the amount of heme binding sites occupied by O_2 molecules ranges from 0 to 1. A saturation value of zero indicates that sites are unoccupied. A saturation value of 1 indicates that all the sites are occupied. The higher the pO_2, the higher the saturation, and the lower the pO_2, the lower the saturation.

Temperature and pH affect the dissociation curve in different ways. The lowering of the temperature of blood has been shown to shift the curve to the left, as seen in Figure 2 (5).

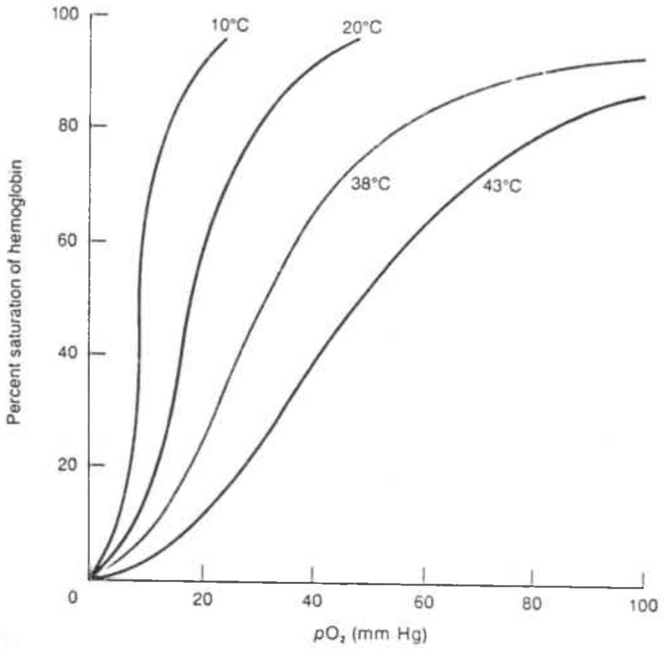

Figure 2. O_2 dissociation curves at different temperatures showing shift to the left. (from Tortora and Anagnostakos, Principles of Anatomy and Physiology , 4th ed., 1984)

At the same partial pressure, a decrease in temperature actually increases the saturation or decreases the dissociation. The pO_2 and percent saturation of the dissociation curve may change, but the sigmoidal shape of the curve does not change (9). At very low temperatures that decrease the ability of the hemoglobin to function properly, the shift to the left may not be seen.

In addition to transporting O_2, hemoglobin also transports hydrogen cations or protons (H^+) and carbon dioxide (CO_2). The presence of H^+ and CO_2 increases the acidity of the blood. This lowers the affinity of hemoglobin for O_2, so the release of the O_2 is easier. Where there is a high concentration of H^+ and CO_2, as in metabolically active tissues, hemoglobin releases O_2 readily. Similarly, when O_2 is present in high concentration as in the lungs, hemoglobin unloads H^+ and CO_2 more easily. The interaction of these gases and protons with hemoglobin is known as the Bohr Effect (15).

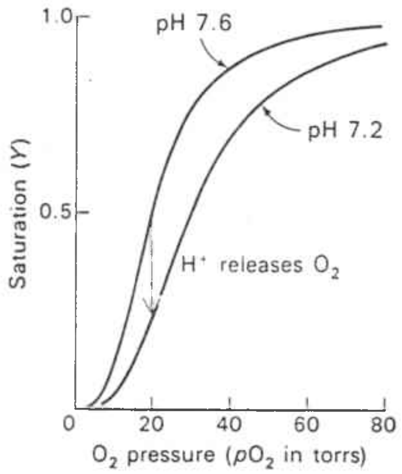

Figure 3. Effects of pH on the O_2 dissociation curve under physiological conditions. (from Stryer, Biochemistry, 3rd ed., 1988)

The shift to the left seen with decreasing temperature may be lessened by treating the blood or hemoglobin with CO_2 (5). This would tend to increase the partial pressure of CO_2 (pCO_2) and lower the pH. As seen in Figure 3, the lowering of the pH shifts the dissociation curve to the right. At the same partial pressure, a higher pH will have a higher degree of saturation while a lower pH will have a lower degree of saturation. In order to get the the same degree of saturation at a lower pH, there must be a higher pO_2.

Hypothermia reduces cellular metabolic requirements such as O_2 which is essential for cellular metabolism (1). By doing this, hypothermia's clinical value is to reduce the O_2 requirement of living tissue by reducing the cellular metabolic activity (5). Although the cellular metabolic activity has been decreased, the cells still require O_2 to survive.

In monkeys, a correlation between O_2 consumption and glucose metabolism has been shown (3). This is because O_2 is used in glucose metabolism. During hypothermia, the blood sugar levels rose irregularly. During rewarming, the levels of sugar returned to normal. The glucose was not being used as rapidly at lower temperatures as it was at normal temperature. It is not clear if the O_2 was not readily available for glucose metabolism or if the process of glucose metabolism was itself slowed down by the lower temperatures thus requiring less O_2. Both possibilities may have contributed to the decreased rate of glucose metabolism. If the glucose is not used, it will accumulate in the blood and can be measured. By examining and comparing O_2 consumption and sugar utilization, this study suggested that, if these two metabolic processes could be used as guides to the clinical use of hypothermia, the maximum benefits of cooling would be between the temperatures of 28° and 29° (3).

It also has been shown that a decrease in O_2 consumption is paralleled by a decrease in blood flow during hypothermia (5). During hypothermia, the circulatory blood volume decreases (4). This decreased blood volume is due to a decrease in the amount of plasma in the blood. The hematocrit, which measures the percentage of red blood cells in blood, increases during hypothermia (11). The increase is consistent with a decrease in plasma volume at lower temperatures (8). The blood does not flow as well with this loss of plasma. This contributes to the decrease in blood flow.

The loss of space occupied by the plasma is compensated for by the blood vessels reducing their diameters. This compensation is a passive vasoconstriction (8). An active vasoconstriction may be caused when the vessels are stimulated to contract because of a detected decrease in O_2 consumption (8).

Hypothermia has its greatest effect on circulation by affecting the heart. The heart rate decreases possibly due to the cooling of the pacemaker region of the heart. The time occupied by systole (contraction) increases as does the time for diastole (relaxation) of the heart (4). This increased time for contraction and relaxation decreases the cardiac output which is a measures of the amount of blood the heart pumps per minute. The decrease in cardiac output decreases the delivery of O_2 to the body. This prevents the delivery of more O_2 than is necessary.

Tissue damage, especially in the brain, is a great concern when using hypothermia. The brain responds to hypothermia in much the same way as the rest of the body tissues (14). Overall, the cerebral O_2 and glucose consumptions vary proportionately with the body temperature during hypothermia (5,10,13). Between 31° and 27°, there is a sudden decrease in the consumption of O_2 and glucose. Below 27°, however, the O_2 consumption does not correspondingly decrease with a decrease in temperature while the glucose does (3,14).

Cerebral circulation is different from general circulation in the fact that cerebral vasoconstriction is unlikely (10). Cerebral vasoconstriction has not been proven to occur, and a study of the muscular branches of vertebral arteries did not observe any constriction when cold was applied (10). However, experimentally, it has been shown that a rise in the level of the pCO_2 during hypothermia causes cerebral vascular dilation (4, 13, 14). This may increase the oxygen availability in two ways. The Bohr Effect facilitates the unloading of oxygen with an increase of pCO_2. Also, the dilation will allow more blood to flow through the vessel. This increased blood flow would not be favorable during a surgery because more blood woould be entering the surgical field. Under hypothermic conditions, the increased blood flow caused by increased pCO_2 causes an uncoupling of cerebral blood flow and metabolism. The blood flow increases, and the metabolism decreases. This uncoupling is not seen in general blood flow and metabolism.

Hypothermia is used in surgery to protect the brain or heart from periods of hypoxia or ischemia (8). In cardiopulmonary bypass and circulatory arrest, the depth of hypothermia is an important variable (12). Cardiopulmonary bypass is used in neurosurgeries to control blood flow and blood gases, and to reduce the body temperature. The ability of blood's hemoglobin to function properly at low temperatures is very important in a surgery utilizing hypothermia. With more research on the effects of hypothermia, the depth and length of time hypothermia can be used might be increased.

MATERIALS AND METHODS

Three cc of heparin, an anticoagulant, were taken up into a 60 cc syringe. This syringe was used to obtain about 60 cc of blood from six human donors. The blood from each donor was divided equally between two 50 ml centrifuge tubes and centrifuged for about 5 min at 2500 rpm. The plasma was taken off without disturbing the blood cells by using a syringe and needle. The amount of plasma that was taken off was replaced with a 0.9% sodium chloride (NaCl) saline solution. The saline was mixed with the blood cells by gently shaking. Blood cells were rinsed twice more as indicated in Figure 4. The contents of the centrifuge tubes (blood cells and saline) were combined into a 60 cc syringe case. Five cc of glucose and three drops from an eye dropper of simethicone, an antiflatulent, were added.

Once this was completed the 60 cc case was suspended in a Hemotherm heater/cooler pump bath of water. An electronic temperature sensor and an Aqua-F bubbling stone that had a gas tube attached to it were suspended through the lid of the case into the blood cell mixture. The gas tube could be attached at the other end to either an O_2 gas source or a nitrogen gas source. A tube attached to a cockstop was suspended into the mixture, and samples were taken through this with a 1 cc blood gas syringe. Six samples were taken at each of the following temperatures: 37°, 25°, 18°, 12°, 7°, and 3°. The first sample taken had pure O_2 bubbled through it for

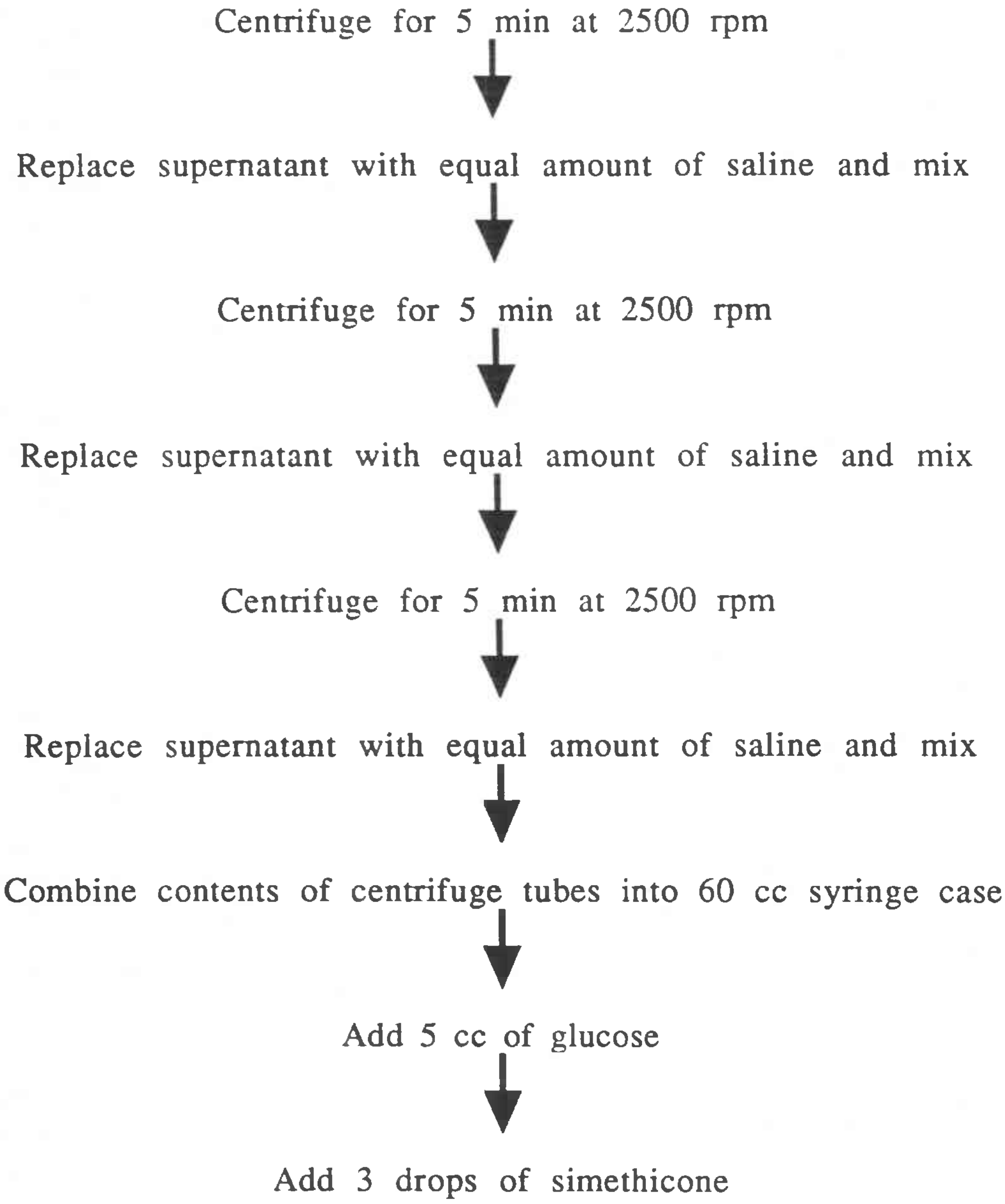

Figure 4. Flow chart of rinsing procedure.

a few minutes to allow 100% saturation. The bubbling was controlled manually, and simethicone was added as needed to prevent frothing of the blood. The O_2 was then replaced with nitrogen, and the bubbling rate was adjusted manually so that the same rate was maintained with nitrogen as with O_2. The rate was approximated by eye. Nitrogen bubbling began the desaturation of the hemoglobin. After two minutes of nitrogen bubbling through the sample, the second sample was taken which was the first sample to have nitrogen bubbled through it. A sample was taken every two minutes until a total of five samples with nitrogen bubbled through them were taken. (Each temperature then had one sample with only O_2 bubbled through and five with nitrogen bubbled through them.) After a sample was taken, any air bubbles in the syringe were purged out, and the syringe was capped. While the samples were being taken, they were stored in a cup full of ice.

Beginning at 37°, the temperature was decreased to the next lower temperature. The temperature of the contents of the 60 cc syringe case was monitored with the electronic temperature sensor. The water bath temperature was adjusted to maintain the temperature of the 60 cc syringe case to within ± 1° of the temperature being sampled. Hematocrits, using a microhematocrit centrifuge, were taken during sampling of the higher temperatures to check for lysing. If lysing was seen, the lysed blood was no longer used in the study.

The pO_2 and calculated Hb saturation of the samples were determined by the ABL-30 Acid Base Analyzer blood gas machine, and this data was recorded.

For each set of six samples at each temperature, the partial pressure at 75% saturation (P_{75}) was calculated using data from a sample that had a saturation above and a sample that had a saturation below 75% saturation. Each sample was considered a point on a graph with the saturations on the vertical axis and the partial pressures on the horizontal axis. The slope between the above values and the below values was calculated. Using this slope and the equation “Y=MX+B”, the value for the P_{75} was calculated. The P_{75} for each donor’s blood at each temperature was averaged with the other P_{75}’s of the same temperature from the other donor’s blood, and the standard deviation of each average was calculated.

If a partial pressure of one of the numbers used in the calculations was 100 or more above the other partial pressures and 15% above 75% saturation, it was excluded. Also, if there was an insufficient amount of sample for the blood gas machine to analyze or the blood gas machine rejected the sample, the data for that sample was excluded.

RESULTS AND DISCUSSION

The P_{75}'s calculated are shown in below in Table 1.

Table 1. Average partial pressures at 75% saturation for different temperatures.

Temp.	**Ave. P_{75} (torrs)**
37°	65.7 ± 11.7
25°	63.7 ± 36.3
18°	57.7 ± 11.5
12°	56.9 ± 7.9
7°	62.9 ± 15.8
3°	50.6 (estimate)

The P_{75} was calculated because the saturations often did not decrease below 50% at the lower temperatures, so the P_{50} could not be used for a comparison between temperatures. Some samples were excluded from calculations. The samples used as the "above" saturation in the calculations may have been a significant percentage higher than the 75%. At the higher temperatures where the hemoglobin desaturated rapidly, the large percentage difference in saturation corresponded to a large difference in partial pressure.

A dissociation curve could not be generated from this study because of lack of data at different temperatures and because there was not complete dissociation at lower temperatures. However, by comparing the values of the results with a dissociation curve, the shift to the left that the curve shows at lower temperatures can be seen between 37° and 12°. The P_{75} decreased between the temperatures of 37° and 12° from 65.7 to 56.9 torrs (respectively). At 12°, the P_{75} no longer decreased with a decrease in temperature. The values calculated for 7° and 3°, however, were obtained from two and one samples, respectively. The standard mean or average P_{75} for 7° was obtained from only two numbers and may not be reliable. The 3° value was an estimate because only one donor's blood sample dropped below 75% saturation, and the sample above 75% saturation was excluded because it had an insufficient sample.

The decreasing ability of the hemoglobin to desaturate below 75% as the temperature decreased, and the fact that below 12° the saturations usually did not drop below 75% may indicate the hemoglobin was not functioning properly. Below 12°, the hemoglobin did not desaturate to 0%. The lowest saturation measured at 12° was 11.5%. This is in contrast to Figure 2 which showed the hemoglobin at 10° to desaturate to 0%. This would mean the hemoglobin was functioning properly at lower temperatures. Possibly, our study may not have allowed enough time for the O_2 levels to decrease to a point that would allow the hemoglobin to unload the O_2 completely. At this time, the results of this study and the differences seen in Figure 2 cannot be reconciled.

The pO_2's in this study were higher than found in other studies. The pO_2 ranged from zero to nearly 1000 torrs. This may be attributed to the fact that this was an in vitro study, and 100% O_2 was being bubbled through the first sample taken at each temperature. The blood serum may have held more O_2 than it normally would. This may have caused the unnaturally high pO_2.

Other studies referred to may have had lower partial pressures because of differences in methods. Barcroft, et. al and Reeves controlled the rates at which the O_2 levels in the blood were decreased. They also had lower levels of O_2 to begin with because other gases, such as CO_2 and nitrogen, were also in solution with the O_2 to begin with (2,9). Different methods of removing O_2 from solution were also used. In Lawson's et. al study, sodium dithionate ($Na_2S_2O_4$) was used to actively take the O_2 out of the solution (6). Sodium dithionate binds to the O_2 making it unavailable for binding to hemoglobin. Our study replaced the O_2 in solution with nitrogen. As the nitrogen was bubbled through the solution, the increasing amounts of nitrogen forced the O_2 that was still present out of solution. Eventually, the nitrogen in solution should have replaced most of the O_2. As the nitrogen was bubbled through the blood, it became darker in color. The darkening indicated a decrease in O_2. When the hemoglobin unloaded the O_2, the increasing nitrogen levels reduced the chance of the hemoglobin loading another O_2 molecule. This method may not be as effective as the use of sodium dithhionate in reducing levels of O2 and cooould also explain the higher pO_2's found in this study.

This study was limited in identifying a lower temperature at which the deoxygenation of blood readily occurs as it does at higher temperatures. To find this temperature, a study using more temperatures should be conducted. Also, to make this study more accurate, the pH of the samples should be maintained at 7.4, the normal physiological level (15). If any change in pH occurs, the sample should be examined to account for any effects due to a change in pH rather than temperature. This study did show how a decrease in temperature affects the dissociation curve and saturation of blood.

The delivery and dissociation of oxygen in the blood during a surgery using hypothermia is of vital importance. Oxygen must be supplied to the tissues to prevent damage to them. The decreased ability of blood to unload oxygen at lower temperatures that was experienced in this study makes it important for researchers and surgeons to understand the functioning of blood at lower temperatures.

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