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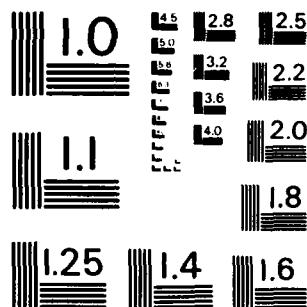
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ENDURANCE CAPACITY CHANGES FOLLOWING INDUCED ERYTHROCYTHEMIA – THE UTILITY OF FROZEN BLOOD COMPONENT TECHNOLOGY

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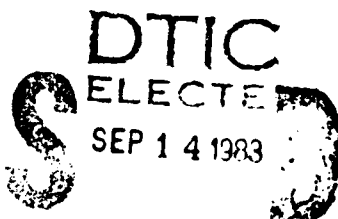
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ADMINISTRATIVE INFORMATION

This Technical Report describes work performed under Program Element 63706N, Project M0095-PN (NOSC 512 - FA24) between October 1979 and March 1980 for the Naval Medical Research and Development Command, Code 45. The work presented in this report was prepared by San Diego State University Foundation, under contract N66001-80-M-1664. Principal investigator was JA Hodgdon of the Department of Physical Education, San Diego State University, with collaboration of NL Campbell and HW Goforth (NOSC, Code 5143), under the direction of WT Rasmussen, Head, Bioengineering Branch (Code 5143).

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This report contains a literature survey investigating the enhancement of endurance capacity by induction of erythrocythemia through red blood cell (RBC) infusion and the possible importance of frozen RBC storage technology in effective erythrocythemia induction. The delivery of oxygen to the tissues appears to be the limiting factor for aerobic capacity in exercise employing most of the body muscle mass. Therefore, improvement of oxygen delivery might be expected to increase endurance. Infusion of RBCs appears to be an effective means of increasing oxygen delivery and endurance capacity. Such infusions increase the oxygen-carrying capacity of the blood and (Continued on reverse side)		

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promote increases in the cardiac output, which increase oxygen delivery. However, it appears that a critical volume of RBCs must be infused so that the blood volume, hence cardiac output, remains elevated after the body's plasma volume adjustments take place. Use of frozen-stored RBCs can increase the safety and effectiveness of induced erythrocythemia by allowing effective use of autologous transfusions with adequate recovery period following blood withdrawal. Development of a program of research to further explore the metabolic and hemodynamic consequences of induced erythrocythemia and the adaptation of this technique to the military setting is recommended.

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OBJECTIVE

Investigate possible applications of frozen blood component storage technology to increase physical work capacity by employing induced erythrocythemia.

RESULTS

1. Induction of erythrocythemia is an effective means of increasing endurance capacity, provided an adequate volume of RBCs is infused to result in a net blood volume increase.
2. Achieving this net increase appears to require approximately 322 ml (equivalent to 2 units of whole blood) of RBCs, although further work is needed to define this volume more precisely.
3. For induced erythrocythemia based on autologous transfusions, adequate time must be allowed for RBC volumes to return to prewithdrawal levels, otherwise an RBC volume greater than that withdrawn must be infused to get the same effect.
4. Glycerol frozen storage of RBCs appears to significantly enhance the chances for successful RBC loading. The increased viable storage life of frozen RBCs allows the successful use of autologous transfusion with minimization of risk. When frozen and thawed RBCs are used, the period of in vivo RBC rejuvenation is minimized and there is a decreased probability of pathogen survival and immunologic reaction to reinfusion.

RECOMMENDATIONS

1. Determine the relative risks associated with induced erythrocythemia and develop an optimum program for physical fitness augmentation in a military setting.

2. Conduct a program of research and exploratory development having the following purposes:

A. Clarify relationships between blood volume, hematocrit, and viscosity. It is unclear whether there is an optimum hematocrit or a range of optimal hematocrits, and existing information does not indicate whether an optimal hematocrit (or hematocrit range) varies with changes in blood volume.

B. Determine more closely blood volume changes which accompany infusion of differing RBC volumes, and confirm the performance changes accompanying such infusions.

C. Establish the time course of the blood volume and performance changes. How soon and for what duration can we reasonably expect to achieve benefits? Previous studies suggest a period of between 1 and 16 weeks.

D. Estimate the metabolic costs of RBC infusions.

E. Determine any other physiological effects (besides performance changes) of RBC infusions, such as changes in blood-flow distribution or alteration of heat-loss mechanisms.

3. Should this research indicate RBC infusions to be a safe, effective method of increasing work capacity, undertake exploratory development to design RBC infusion programs for military application, with due consideration for the logistics of employing this technology. From the standpoint of safety and flexibility, the use of frozen RBCs will be a necessary adjunct to such programs.

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TASK STATEMENT

This report is a literature survey which investigates possible applications of frozen blood component storage technology to increase physical work capacity by employing induced erythrocythemia.

BACKGROUND

BLOOD AND ITS FUNCTIONS

Blood is the medium of transport for substances within the body, both between cells in the body and between body cells and the environment. It is responsible for transport of metabolic precursor substances to sites of metabolism and removal of metabolic waste products. Blood is necessary for conduction and removal of excessive metabolic heat from body interior to body surface; it also contains many elements which make up the body's defense against disease. Of particular interest for this report, blood transports oxygen, the proton acceptor required for the operation of metabolic processes. Maintenance of functional blood is clearly a requirement for life. Preservation and storage of blood to be given to people suffering from blood loss or impaired blood function has become an important part of medical practice.

Blood is composed of two phases: solid or formed elements and liquid medium plasma. In the solid or formed elements, red blood cells (RBCs) carry oxygen and participate in carbon dioxide transport, and white blood cells (WBCs) and platelets play a role in the body defense reactions. In the liquid medium, or plasma, are dissolved various ions, metabolic precursors and wastes, and assorted proteins necessary to transport nutrients and hormones, as well as the suspended formed elements.

STORAGE OF BLOOD ELEMENTS

There are two general methods of storing RBCs: refrigeration and freezing. Both storage methods require the addition of a liquid anticoagulant/preservative. The most commonly used preservative agents have been acid-citrate-

dextrose (ACD) and citrate-phosphate-dextrose (CPD).^{*} Recently, adenine has been added to CPD, creating CPD-A, to make it the most widely used anticoagulant (ref 1, 2). Blood may or may not be separated into its components prior to refrigeration at 4°C. Before freezing, the plasma is removed from the RBCs and a cryoprotective agent is added to prevent damage. The most widely used agent is glycerol, which appears to minimize intracellular ice crystal formation. Ice crystals can puncture cell membranes. Also, as ice crystals are being formed from intracellular water, the salt concentration increases inside the cell to levels which denature proteins. There are two widely used methods of freezing with glycerol: (1) Fast freezing the RBCs in liquid nitrogen after equilibrating the cells with a relatively low concentration of glycerol followed by storage in gas phase liquid nitrogen at -150°C. (2) Equilibrating the RBCs with a relatively high concentration glycerol solution followed by slow freezing and storage at -80°C in a mechanical freezer. Plasma is frozen without cryoprotectants and stored at -35°C. This report is concerned with the storage and usage of whole blood or of separated RBCs (often referred to as "packed" RBCs because the plasma has been removed, thereby packing the cells together).

Refrigerated storage of RBCs has two major limitations. First, the viability of RBCs, measured as the percentage of transfused cells surviving after 24 hours, becomes unacceptable after 21 days^{**} of storage (ref 2, 3). Second, after 7 days of storage, the ability of RBCs to deliver oxygen to the tissues begins to decrease. This decreased ability is associated with a decrease in RBC concentration of a compound called 2,3-diphosphoglycerate (2,3-DPG) (ref 3, 4). With decreased 2,3-DPG concentrations, oxygen binds more tightly to the oxygen-binding protein, hemoglobin, and is not released to the tissues as readily. Although the RBC 2,3-DPG levels are usually restored within 24 hours following transfusion, this decreased oxygen delivery in the interim might

^{*}Bently L and M Meyers, Thawing of Frozen Blood Components, NOSC Code 5143 introductory letter report, 10 December 1976.

^{**}This has recently been extended to 35 days with the use of CPD-A as the common anticoagulant. However, at the time this work was done, the limit was 21 days.

present a problem in clinical situations, where multiple-unit transfusions are required and the RBCs must function immediately [eg, for traumatic injury, hemolytic disease of the newborn, and cardiac decompression (ref 3)].

If RBCs are stored frozen, the storage time may be extended up to 3 years (ref 5). If blood cells are frozen within 1 day following collection, they do not (after thawing) show the decrement in 2,3-DPG levels that is seen with refrigerated RBCs after 1 week. Additionally, in frozen RBCs there appears to be a decreased transfer of pathogens such as hepatitis antigen when compared to stored refrigerated blood.* The current limitation to the usage of frozen RBCs is that thawing of the blood and removal of the cryoprotective agent is a time-consuming process. It takes up to 1 hour, following removal from frozen storage, before a unit of packed RBCs is ready for transfusion.* Additionally, using current blood-thawing techniques, there is some risk of contamination of the blood sample while thawing, due to leakage of contaminants from the water bathing the sample bag. Continued research at the Naval Ocean Systems Center on thawing of frozen blood components by microwave energy promises relief from this problem.

HEMODYNAMICS AND EXERCISE

Physical exercise places great demands upon the cardiovascular system. Increased metabolic activity of muscle cells requires an increased supply of oxygen and other nutrients, an increased rate of removal of CO₂ and other chemical wastes, and removal of heat generated by muscle metabolism. At maximal levels of exercise, heart rate increases about 3-4 times, cardiac output goes up by about 5 times, and blood flow is redistributed so that the flow through active muscles may increase 30-fold (ref 6,7).

When physical performance requires energy release for time periods of several minutes to an hour or more, most of the energy demands are met by aerobic (oxygen-requiring) metabolism (ref 8). Furthermore, for exercise such

*See previous footnote: Bently and Meyers.

as running or swimming, which requires utilization of more than 50% of the body muscle mass, the maximum rate of oxygen utilization is determined by the ability of the cardiovascular system to deliver oxygen (central factors), rather than the metabolic capacity of the muscle cells (peripheral limitations) (ref 9). Under these conditions the measured maximal rate of oxygen consumption ($\dot{V}O_2$ max) is a measure of the capacity of the cardiovascular system to deliver oxygen to the working muscles. A corollary of this statement exists: if the oxygen-carrying capacity of the cardiovascular system is increased, $\dot{V}O_2$ max should rise and endurance work capacity (measured, for example, as time to exhaustion on an aerobic work task) should increase. It has been shown that increasing the oxygen-carrying capacity of the blood by having an exercising subject breathe pure oxygen increases $\dot{V}O_2$ max (ref 9). This finding has prompted exploration of other means of increasing blood oxygen-carrying capacity.

One method of increasing blood oxygen-carrying capacity that has been studied to a moderate extent over the past 30 years is infusion of additional RBCs into the circulatory system. This procedure may simply involve a transfusion of compatible RBCs (a homologous transfusion) or may involve withdrawal of blood from an individual followed by infusion of that individual's own RBCs (an autologous transfusion) after a period of recovery to allow the individual's circulating RBCs to build up to normal levels. This technique is often referred to as "blood doping" or "blood packing." The term blood doping suggests the use of pharmaceuticals, and the term blood packing might be confused with preparation of packed RBCs for transfusion. Therefore the term "induced erythrocythemia" will be used in this report to imply RBC infusion to increase circulating RBC levels.

HEMODYNAMICS AND RBC INFUSION

Infusion of whole blood or RBCs will increase the total blood volume of the circulatory system (at least transiently) and may lead to alterations in the proportion of blood volume occupied by RBCs. Each of these two effects alters circulatory hemodynamics differently, and the effect of such infusions upon oxygen delivery will be the result of several processes.

Total blood volume may be considered the sum of the plasma volume and the RBC volume. The percentage of the blood volume occupied by the RBCs is called the hematocrit. The infusion of whole blood or blood components will affect the plasma volumes and RBC volumes differently, depending upon the composition of the infusate. The infusion of packed RBCs leads primarily to increases in RBC volume with only small increases in plasma volume. There is, therefore, an increase in both blood volume and hematocrit. The infusion of whole blood will increase both plasma volume and RBC volume, and there will be little initial change in hematocrit. The infusion of resuspended RBCs will increase both plasma and RBC volumes; however, the resultant hematocrit will depend upon the initial blood hematocrit and the hematocrit of the resuspended RBCs.

Following an increase in blood volume by infusion of blood, homeostatic adjustments take place to return the blood volume to preinfusion levels. These adjustments take place primarily through changes in plasma volume (ref 10). Following infusion of whole blood, there is a reduction in plasma volume. Little adjustment is possible in the RBC volume; so as the plasma volume decreases, the hematocrit increases. Depending upon the volume of RBCs infused, plasma volume adjustments may or may not return the blood volume to preinfusion values. It is not clear whether plasma volume adjustments follow infusion of packed RBCs into a healthy individual who has a normal hematocrit.

Infusion of either packed RBCs or whole blood will then be expected to result in a net increase in hematocrit. As hematocrit increases, viscosity (the resistance of a liquid to flow) of the blood also increases. With increases in viscosity, blood flow is decreased, leading to decreased oxygen delivery to the tissues. Two competing processes thus take place following an increase in hematocrit: (1) An increase in RBCs leads to an increase in oxygen-carrying capacity, hence an increase in oxygen delivery per unit of blood flow. (2) The resulting increase in viscosity leads to a decrease in blood flow and decreased oxygen delivery.

Recognition of the two competing processes has led to the postulate that there is an "optimum hematocrit" at which the competing processes are balanced and oxygen delivery is maximized (ref 11, 12). For hematocrits below optimum,

the oxygen-carrying capacity of the blood is below optimum; and for hematocrits above optimum, the blood viscosity impairs blood flow sufficiently to compromise oxygen delivery. The existence of an optimum hematocrit thus implies that there is a limit to the gains that can be achieved by induced erythrocythemia.

However, there are additional factors operating. Increasing the blood volume brings into play a set of circulatory adjustments which promote increased oxygen delivery - increasing blood volume increases filling pressure at the heart. The heart responds automatically to such a situation by increasing stroke volume and rate, and as a result cardiac output is increased. Additionally, there appear to be adjustments in the peripheral circulation to accommodate the increased blood volume, such as relaxation of venous walls (ref 13). These adjustments result in a decreased peripheral resistance to flow. Therefore increasing blood volume results in increased blood flow through the tissues and hence increased oxygen delivery (ref 12).

The combined effects of varying both hematocrit and total blood volume have been studied by Murray et al (ref 14) and by Castle and Jandl (ref 13). The findings of Murray et al are summarized in figure 1. These investigators infused packed RBCs or 6% dextrose in saline into anesthetized splenectomized dogs to alter hematocrit. In one group of dogs, a volume of blood equal to the volume of the infusate was removed, so that normal blood volume (normovolemia) was maintained. In another group of dogs, blood was not removed during infusion, so blood volume was allowed to increase (hypervolemia) as a result of infusion. The investigators measured cardiac output and arterial oxygen content. They calculated the product of these values to estimate tissue oxygen transport. These results are shown in figure 1. The numbers in parentheses represent the sample size.

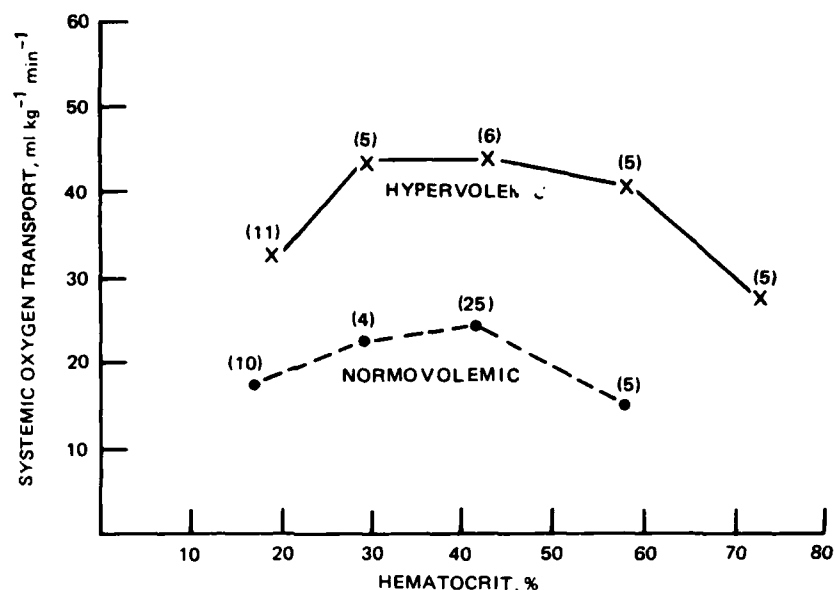


Figure 1. The relationship between oxygen transport and hematocrit for normovolemic and hypervolemic anesthetized dogs. [Redrawn from Murray et al (ref 14)].

It would appear from these data that the general form of the relationship between hematocrit and oxygen delivery is not altered by increasing blood volume. It is an inverted "U"-shaped curve, as would be predicted from the earlier discussion of hematocrit, O_2 content, viscosity, and oxygen delivery. However, increasing the blood volume does shift the curve upwards, so there is increased oxygen delivery at all hematocrits studied. Evidence suggesting that these general findings reported in dogs can be extrapolated to man is given in the paper of Castle and Jandl (ref 13).

The above discussion suggests that induced erythrocythemia should work to increase oxygen delivery to the tissues under certain conditions. Immediately following infusion, cardiac output (hence oxygen delivery) probably is elevated due to increased blood volume and venous return. However, following adjustment of plasma volume, which is thought to be complete in less than 24 hours (ref 15), infusion of red cells will increase oxygen delivery only if (1) the increase in RBC volume is sufficiently greater than the plasma volume adjustment and (2) the net effect of the blood volume increase overcomes the effect of increased hematocrit on blood flow.

This discussion has assumed that the phenomena described for the person at rest will still apply to an exercising person. Weisse et al (ref 16) have demonstrated that the inverted "U" relationship between oxygen delivery and hematocrit is true for normovolemic exercising dogs.

In summary, it appears that oxygen delivery and hence work capacity can be increased by inducing erythrocythemia by infusion of whole blood or packed or resuspended RBCs, provided a suitably large volume of RBCs is infused. An optimum volume of RBCs has not been determined.

APPROACH

In this report, literature relating to the effects of induced erythrocythemia on exercise parameters and to the effects of changes in blood composition on hemodynamics is reviewed. This review has three objectives:

1. To verify the benefits of induced erythrocythemia for physical endurance performance.
2. To ascertain the impact of frozen RBC technology on induction of erythrocythemia for increased work capacity.
3. To determine whether sufficient information exists to specify the safe limits of erythrocythemia.

The review of the literature on induced erythrocythemia covers 14 studies dealing with the infusion of either whole blood or packed or resuspended RBCs into human volunteers to modify performance of physical tasks (ref 17-30). While this may not be an exhaustive review, it is thought to be sufficient to identify the several critical parameters of induced erythrocythemia discussed in this report.

Twelve of the 14 groups of investigators attempted to effect erythrocythemia by phlebotomy (withdrawal of blood) followed by autologous reinfusion after a period of recovery. The other two used simple homologous transfusions

of cross matched packed RBCs (ref 24) or whole blood (ref 22). In 11 of these studies the investigators used $\dot{V}O_2$ max, endurance time, or performance time for some physical task as the measurement criterion. Some of these investigators also measured heart rate at submaximal work loads. In the other studies, the investigators used only indirect measures of endurance capacity — either heart rate at submaximal workloads (ref 22, 27) or the physical work capacity (PWC) estimated from heart rate at submaximal loads (ref 21). In general, heart rate at submaximal workloads is inversely related to $\dot{V}O_2$ max [correlation coefficient varies from 0.7 to 0.5 (ref 6)].

The results of these studies are not consistent. Six of the 11 studies which measured $\dot{V}O_2$ max or endurance time found increases following reinfusion of whole blood or RBCs (ref 17-19, 24, 26, 30); the other five found no improvement (ref 20, 23, 25, 28, 29). Of the studies which employed indirect measures, two found decreases in submaximal heart rate (ref 22, 28), which were interpreted as an increase in endurance capacity, whereas four (ref 19, 21, 23, 27) found no significant change. In no case was there a decrease in work capacity following infusion of blood or RBCs. Further analysis of these 14 reports suggests that there are critical features of the experimental designs that explain the lack of consistency in results. These features are detailed in the next section.

EXPERIMENTAL DESIGNS

Designs and results of the 14 studies surveyed are summarized in tables 1, 2, and 3. Table 1 contains information about study designs and methods. Table 2 summarizes the results of the various studies. Table 3 shows hematocrits before blood withdrawal (control), prior to RBC infusion, and following RBC infusion. As is shown in table 1, whole blood or resuspended RBCs were infused in eight of the studies (ref 17, 21, 22, 25-27, 29, 30), packed RBCs were infused in five others (ref 18-20, 23, 24), and in one study, both whole blood and packed RBCs were used (ref 28). The choice to use whole blood or packed RBCs as the infusate appears to have no effect on the study results. When packed RBCs were used as the infusate, three investigations reported increases in $\dot{V}O_2$ max or work capacity (ref 18, 19, 24) and three reported no effect (ref 20, 23, 28). Considering investigations in which whole blood or resuspended RBCs were infused, a similar situation was found: four investigations reported improvement (ref 17, 22, 26, 30) and four reported no change (ref 21, 25, 27, 29).

As previously discussed, increased blood volume resulting from the transfusion is lost within 24 hours (see the discussion in Williams et al, ref 28). Addition of fluid to the blood space leads to an increase in capillary pressure, which leads to exudation of the fluid from the vascular system. The net result indicates that infusion of either one unit of whole blood or one unit of packed RBCs should result in approximately the same hematocrit following fluid adjustments.

Also included in table 1 is the method of preserving the withdrawn blood. For most studies, blood was preserved with ACD and refrigerated at +4°C. In two studies (ref 18, 19), the preservative was ACD plus adenine, and in four others (ref 17, 26, 29, 30) blood was preserved by glycerolization and freezing. As mentioned above, these latter two methods allow for a longer storage time between phlebotomy and reinfusion. Inspection of tables 1 and 2 shows that the storage mode per se does not determine whether endurance capacity is enhanced.

Reference Number	Control Group?	Single/Double Blind?	Autologous Infusion?	Volume Withdrawn (ml)	Storage Method*	Infusion Volume (ml)	Infusion Component	Time from Withdrawal to Infusion (days)
22	Yes	No	No	—	1	2000***	Resuspended RBCs	—
21	No	No	Yes	550-650	1	550-650	Whole blood	7
25	No	No	Yes	1000-1200	1	1000-1200	Whole blood	14
18	No	No	Yes	800	2	360	Packed RBCs	28
28	Yes	Double	Yes	460	1	460 260	Whole blood Packed RBCs	21
19	No	No	Yes	800	2	360	Packed RBCs	30-35
27	No	No	Yes	400-600	1	400-600	Whole blood	14-21
20	Yes	Single	Yes	460	1	260	Packed RBCs	17
29	Yes	Double	Yes	460	3	460	Resuspended RBCs	21
17	No	Double	Yes	900	3	900	Resuspended RBCs	42
24	No	No	No	—	1	1000-1500	Packed RBCs	—
23	Yes	Double	Yes	450	1	260	Packed RBCs	21
26	No	No	Yes	1200	3	800 1200	Resuspended RBCs Resuspended RBCs	**
30	No	Double	Yes	920	3	920	Resuspended RBCs	56

*1: preserved with ACD, refrig. @ +4°C

2: preserved with ACD + adenine, refrig. @ +4°C

3: protected with glycerol, frozen @ -80°C

** Period not specified but was long enough to allow reestablishment of baseline levels of Hct & Hb

*** Given as 500-ml infusions on each of four consecutive days

Table 1. Summary of experimental designs.

Ref	$\Delta \dot{V}O_2 \text{ Max}^*$	$\Delta \text{ Work Time}^*$	$\Delta \text{ Heart Rate}^*$ (sub max)
22			+ 9.6%
21			NS*** (measured PWC)
25	NS***		
18	+ 9%	+ 23%	
28		NS***	+
19	+ 8%		NS***
27			NS***
20	NS***		
29		NS***	
17	+ 5%	+ 39%	
24	+ 13%	+ 15.6%	
23	NS***		NS***
26	+ 5% (800 ml) NS*** + 7% (1200 ml)		
30		+ 3%**	

* Changes are the differences between preinfusion and postinfusion values

** Actually measured treadmill 5-mile run time

*** NS = not significant ($p > 0.05$)

Table 2. Summary of experimental results.

Reference	Control Hct (%)	Preinfusion Hct (%)	Postinfusion Hct (%)	RBC Vol Infused (ml)
22	46.3		58.3	435 ***
21	39.7 *	38.0*	40.0 *	218-258
25		41.7	43.7	430-516***
18	42.3 *	38.3 *	43.2 *	338
28				196 ***
19	40.3		41.5	322
27	42.2	38.7	43.3	168.8-253.2
20	**	↓	↑	196 ***
29	42.5	43.0	44.8	196
17	43.8	44.0	47.2	394
24				783 ***
23				196 ***
26		45.6	49.2 (800 ml)	364
			50.5 (1200 ml)	547
30	46.2	45.6	48.4	425

* Estimated from Hb concentration values

** Only relative changes reported

*** Estimated assuming a control Hct = 43.5

Table 3. Hematocrits and estimated RBC volumes infused.

Comparison of the study design information given in table 1, the results given in table 2, and the RBC volume information given in table 3 suggests that the volume of RBC infused is one major factor determining whether or not induced erythrocythemia leads to an increase in $\dot{V}O_2$ max or endurance performance. The importance of this factor was discussed in a previous section. A second factor, which may be important in studies utilizing autologous infusions, is the period of time, following phlebotomy, allowed for recovery before reinfusion.

FACTORS AFFECTING ENDURANCE ENHANCEMENT

PACKED CELL VOLUME

It would appear that a successful increase in endurance capacity requires that the infusion of an RBC volume be equivalent to that found in 800 ml of whole blood and that the recovery period between phlebotomy and reinfusion be at least 28 days in length. The separate importance of each of these factors for success following autologous transfusion cannot be determined from this sample of studies. In each of those studies where a recovery period of at least 28 days was used, the investigators also infused at least 800 ml of blood or the RBC volume equivalent in 800 ml of blood (ref 17-19, 26, 30).

Hematocrit values are listed (when available) in table 3 for control (prephlebotomy), preinfusion, and postinfusion (usually 2 hours after infusion) periods for these 14 studies. Also listed in table 3 is an estimation of the RBC volume infused. For studies which used autologous transfusions, this volume was calculated by multiplying the control hematocrit fraction ($Hct/100$) by the volume of blood withdrawn. For studies in which the control Hct was not provided or for which a homologous transfusion was used, an estimated control Hct of 43.5% (the mean of all the provided control Hct values) was used. Comparison of the information in table 3 with the results given in table 2 reveals that improvement in endurance capacity is found only if the estimated RBC volume infused is equal to or greater than 322 ml, which is the RBC volume equivalent calculated from the 800 ml of whole blood used in the study of Ekblom et al (ref 19).

The study of Robinson et al (ref 25) is the only one listed here in which an RBC infusion volume greater than 322 ml is used but in which no indication of improved endurance capacity (in this case $\dot{V}O_2$ max) was found. However, the technique these investigators employed for assessing changes in $\dot{V}O_2$ max would not necessarily allow them to detect $\dot{V}O_2$ max increases. Robinson et al used a graded treadmill protocol to determine the speed and grade at which their experimental subjects achieved $\dot{V}O_2$ max. This determination was done prior to any blood volume intervention. They then withdrew 1000-1200 ml of whole blood from their subjects, allowed them to recover for 2 weeks, had them run for 5 minutes at the same speed and grade on the treadmill that had previously elicited a maximal response, and performed $\dot{V}O_2$ measurements. The 1000-1200 ml of whole blood was then reinfused, and $\dot{V}O_2$ at the same speed and grade was measured again. The differences in $\dot{V}O_2$ between these two treadmill runs were not significant at $p < 0.05$. However if $\dot{V}O_2$ max were increased as a result of RBC infusion, the speed and/or grade at which the subject would be expected to have to run to achieve $\dot{V}O_2$ max would be greater than the speed and grade used for the postphlebotomy run. The $\dot{V}O_2$ measured following RBC infusion, if submaximal, would simply be the equilibrium $\dot{V}O_2$ for that subject at that speed and grade and would not be expected to be markedly different from previous values (maximal or submaximal) at that speed and grade. The authors do not report prephlebotomy $\dot{V}O_2$ max values for their subjects; therefore, the effect of RBC removal and RBC infusion cannot be determined.

For all other studies, infusion of 322 ml or more of RBCs resulted in some indication of enhanced endurance capacity: either increased $\dot{V}O_2$ max (ref 17-19, 24, 26), increased work time (ref 17, 18, 24) or work pace (ref 30), or a decrease in heart rate at a submaximal work load (ref 22). One study, that of Spriet et al (ref 26), provides measures of cardiac output at maximal exercise following infusion of 800 ml and 1200 ml of resuspended RBCs. The results are consistent with the described effect of blood volume increases on cardiac output. Cardiac output was increased by 28% 2-7 days following the 800-ml infusion and by 37% 2-7 days following the 1200-ml infusion.

Robinson et al (ref 25) measured cardiac outputs at rest and during exercise. Cardiac output following reinfusion increased significantly ($p < 0.01$) (27% at rest) compared to preinfusion values, but these investigators found no changes at exercise. As discussed, the exercise task may not have represented maximal exertion at both preinfusion and postinfusion trials.

OTHER FACTORS

There are other experimental design factors which effect the results of these investigations, at least for those using autologous infusions. The most important factor appears to be the period of recovery allowed between phlebotomy and reinfusion. If the period is not sufficient to allow RBC mass to build up its prephlebotomy values, the net gain in RBCs from the infusion may not be large enough to lead to an increase in endurance capacity relative to prephlebotomy measurement. Because most investigators make their measurements prior to phlebotomy and after infusion, the direct effect of the infusion of RBCs upon endurance capacity is not determined.

Consideration of the prewithdrawal (control), preinfusion, and postinfusion hematocrit values from the studies that showed no change in endurance capacity (ref 21, 27, 29) illustrates this problem. [Frye et al (ref 20) provide only relative changes in hematocrit for these times, so their data are not considered.] Gullbring et al (ref 21) allowed only 7 days of recovery following blood withdrawal before reinfusion. As can be seen from table 3, the hematocrit had not returned to the control value prior to reinfusion. Reinfusion of RBCs establishes hematocrit levels which are only slightly different from control values. This is also the pattern seen for the results of Videman and Rytomaa (ref 27), but these investigators allowed 14-21 days to pass between blood withdrawal and reinfusion.

In contrast, Williams et al, in their second study (ref 29), allowed 21 days between phlebotomy and reinfusion, but the hematocrits they measured show return to control values within 21 days. The difference between Williams' work and that of the Gullbring and Videman groups is that on the average Gullbring and Videman withdrew a slightly greater amount of blood and allowed slightly less time for recovery. A period of 21 days would appear to be the

minimum required for regeneration of 196 ml (1 unit) of RBCs. For greater volumes more time should be allowed. In fact the minimum recovery time for which a significant result is found for induced erythrocythemia is 28 days (ref 18).

It would appear from Williams' results (ref 29) that the minimum volume of RBCs which must be infused is greater than 196 ml. Infusion of this volume led to no improvement in endurance capacity, despite the finding that the hematocrit had returned to control levels. However, because those studies which did not reinfuse at least 322 ml of RBCs are also those which did not provide a recovery time greater than 21 days, a more precise estimate of the critical RBC volume ($196 \text{ ml} < \text{RBC volume} < 322$) or of the possible individual variation which may influence individual critical RBC volumes cannot be determined.

Many of these studies (ref 18, 19, 21, 24, 25, 27) have been criticized (ref 17, 28, 29) for having inadequate controls, either because the subjects were not blind to the treatment, and therefore could not serve adequately as their own controls, or because training efforts invalidated the control measures — as appears to be true for the study of Gullbring et al (ref 21). Buick et al (ref 17), in their study using highly trained runners, seem to have avoided these problems. They used a double-blind, counterbalanced design (treatments were infusion of resuspended RBCs and sham infusion of saline), an autologous infusion of 900 ml of resuspended, previously frozen RBCs, and a postphlebotomy recovery period of at least 42 days. Using this design, they found a 5% increase in $\dot{V}O_2$ max and a 39% increase in endurance time for working at an intense work rate (exhaustion reached in 3-5 min). Williams and his associates also used a double-blind administration of either RBCs or a saline sham, but used a separate control group rather than a counterbalanced design. However, their negative results when compared with the positive results of Buick et al clearly indicate that it is not a question of whether induced erythrocythemia can increase endurance capacity but rather what are the critical values of the involved parameters needed to generate that increase.

SAFETY OF INDUCED ERYTHROCYTHEMIA

None of the cited reports deals directly with the safety of induced erythrocythemia procedures. Pace et al (ref 22) report that one subject suffered mild itching as a result of a homologous transfusion. There are no comments from other authors. Most studies used autologous transfusions and this, of course, greatly reduced chances of a reaction to transfusions.

Only Robinson et al (ref 25) reported blood pressures in conjunction with their experiments. They measured central venous pressures at the right atrium and arterial pressures at the brachial artery. Central venous pressure measured within 2 hours of infusion increased significantly over preinfusion values both at rest (change = 1.9 mm Hg; $p < 0.05$) and during exercise (change = +7.4 mm Hg; $p < 0.01$). This finding is an expected outcome of increasing blood volume (ref 15). They did not find significant changes in arterial pressure, either at rest or during exercise, as a result of the transfusions. While these findings are encouraging, they suggest a low risk of hypertension-related complications. Robinson et al do not indicate whether RBC volume had returned to control values for their study participants. It is, therefore, not clear how well these findings would extrapolate to the case where full RBC volume recovery has taken place. Additionally, Robinson et al took their measures within a few hours of the reinfusion. This means that plasma volume adjustments may not have taken place. The extent to which such adjustments might affect the resultant pressures also needs to be determined.

A factor that needs to be considered is the possible change in heart work load required to accommodate the higher cardiac output that results from increased blood volume and viscosity. Jan and Chien (ref 31) have looked at myocardial oxygen consumption in anesthetized dogs following normovolemic changes in hematocrit. They found the myocardial oxygen consumption to be unchanged for hematocrits between 20 and 60%. The cardiac output did decrease somewhat as the hematocrit increased, so the energy cost for the myocardium per unit of cardiac output rose with increasing hematocrit. The change in relative energy cost of cardiac output appears to be no greater than 10%, for hematocrits between 40 and 50%. [Normal for a dog is 45% (ref 13).] Clearly,

additional work needs to be done to expand these findings to the human organism as well as to the hypervolemic condition.

IMPACT OF FROZEN BLOOD TECHNOLOGY

The development of frozen blood component technology has had a profound impact upon induced erythrocythemia research. By storing frozen RBCs, investigators have had the time available to allow study participants to restore their RBC volumes to control levels prior to reinfusion. This has allowed researchers to identify at least one crucial factor in induced erythrocythemia: the critical RBC volume. In addition, longer storage times provided by frozen storage allow practical use of autologous transfusions in induced erythrocythemia research. Use of autologous transfusions has the obvious advantage of minimizing the possibility of transfusion reactions (ref 1, Sect IV).

Prior to development of frozen blood component technology, the user was limited to a storage time of 21 days for refrigerated blood. This period has now been extended to 35 days (ref 1, p 52). However, even 35 days may be too short a time for full RBC volume recovery in the donor. Even in the studies of Ekblom et al (ref 18, 19), donor-blood hematocrit levels had not recovered after 28 to 35 days. Use of frozen/thawed blood allows the storage time to be increased to more-than-adequate periods.

ADVANTAGES OF FROZEN BLOOD TECHNOLOGY

With the possibility of storing blood up to 3 years, the user is offered a great deal of flexibility in timing reinfusion. This flexibility would be critical should RBC loading prove to be a viable method of increasing work capacity in military maneuvers. Blood could be withdrawn during a training period or other downtime and preserved in frozen storage until time for mission deployment, when the user could be reinfused.

As stated under Storage of Blood Elements, not only do the thawed RBCs have the same or greater viability as refrigerated RBCs, they do not show the depletion of 2,3-DPG exhibited by refrigerated cells. This means that the

thawed RBCs are nearer to their maximal tissue oxygenation capacity immediately upon reinfusion than are refrigerated cells. Therefore the need to wait for RBC rejuvenation by the body resources is minimized, if not eliminated.

The use of frozen RBCs also provides an added element of safety in that it appears that pathogens such as hepatitis antigens are likely to be removed during separation and washing procedures. In addition, since blood components are separated prior to freezing, most of the white blood cells and platelets are removed, minimizing the chance of clotting and immunologic response to blood reinfusion (ref 32, Chap 10).

CONCLUSIONS

The results of the studies reviewed here are consistent with the position that induction of erythrocythemia is an effective means of increasing endurance capacity, provided an adequate volume of RBCs is infused to result in a net blood volume increase. Achieving this net increase appears to require approximately 322 ml (equivalent to 2 units of whole blood) of RBCs, although further work is needed to define this volume more precisely. Furthermore, for induced erythrocythemia based on autologous transfusions, adequate time must be allowed for RBC volumes to return to prewithdrawal levels, otherwise an RBC volume greater than that withdrawn must be infused to get the same effect.

Glycerol frozen storage of RBCs appears to significantly enhance the chances for successful RBC loading. The increased viable storage life of frozen RBCs allows the successful use of autologous transfusion with minimization of risk. The period of in vivo RBC rejuvenation is minimized and there is a decreased probability of pathogen survival and immunologic reaction to reinfusion when frozen and thawed RBCs are used.

RECOMMENDATIONS

Induction of erythrocythemia by RBC infusion appears to increase work capacity. There are special groups of Navy and Marine Corps personnel (eg, UDT and SEAL team personnel and Marine RECON personnel) whose covert mission performance might benefit from such increases in work capacity. Studies are

needed to determine the relative risks associated with induced erythrocythemia and to develop an optimum program for physical fitness augmentation in a military setting.

A program of research and exploratory development is needed, having several purposes:

1. To clarify relationships between blood volume, hematocrit, and viscosity. It is unclear whether there is an optimum hematocrit (ref 15) or a range of optimal hematocrits (ref 31), and existing information does not indicate whether an optimal hematocrit (or hematocrit range) varies with changes in blood volume.

2. To determine more closely blood volume changes which accompany infusion of differing RBC volumes, and to confirm the performance changes accompanying such infusions.

3. To establish the time course of the blood volume and performance changes. How soon and for what duration can we reasonably expect to achieve benefits? The studies of Buick et al (ref 17) suggest a period of between 1 and 16 weeks.

4. To estimate the metabolic costs of RBC infusions.

5. To determine any other physiological effects (besides performance changes) of RBC infusions, such as changes in blood-flow distribution or alteration of heat-loss mechanisms.

Should this research indicate RBC infusions to be a safe, effective method of increasing work capacity, exploratory development should be undertaken to design RBC infusion programs for military application, with due consideration for the logistics of employing this technology. It is clear from the standpoint of safety and flexibility that the use of frozen RBCs will be a necessary adjunct to such programs.

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