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STUDIES OF METABOLISM, FUNCTION AND MECHANISM OF DESTRUCTION OF--ETC(U)
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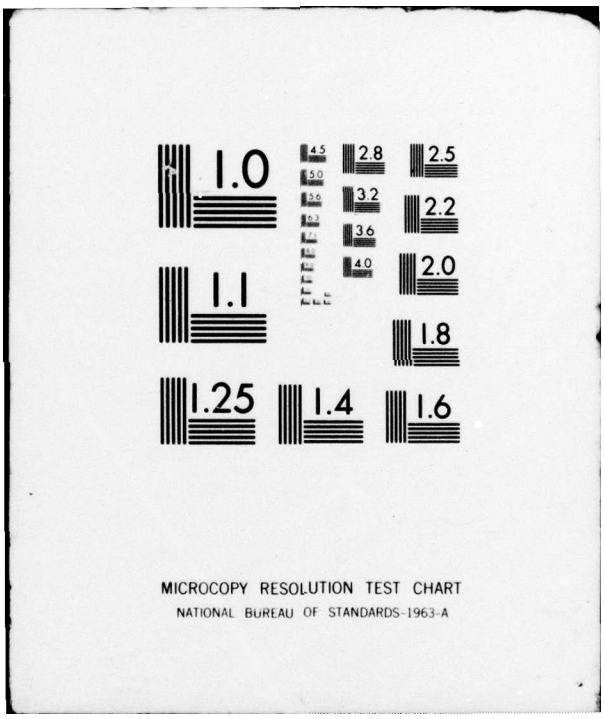
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STUDIES OF METABOLISM, FUNCTION AND MECHANISM OF DESTRUCTION OF RED CELLS

ANNUAL PROGRESS REPORT
April 1978
(November 1, 1976 to March 31, 1978)

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Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DA DA17-73-C-3135

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SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE *(and Subtitle)*
Studies of Metabolism, Function and Mechanism of Destruction of Red Cells.

5. TYPE OF REPORT & PERIOD COVERED
Annual Report.
1 Nov 1976 to 31 March 1978

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
Marshall A. Lichtman, M. D.
Jules Cohen, M. D.

8. CONTRACT OR GRANT NUMBER(s)
DADA 17-73-C-3135

9. PERFORMING ORGANIZATION NAME AND ADDRESS
University of Rochester School of Medicine and Dentistry
Rochester, New York 14642

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
~~62110A~~ 3A162102B71R, 01.072
62772A 3S762772A814 00.072

11. CONTROLLING OFFICE NAME AND ADDRESS
US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

12. REPORT DATE
April 1978

13. NUMBER OF PAGES
6 pages

14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*
6p.

15. SECURITY CLASS. *(of this report)*
Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*
Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*
Red cell, oxygen, radiographic contrast material, acidosis

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*
The effect of contrast materials on red cell membrane potential and on the pH of plasma and red cells have been studied. These studies indicate that contrast materials alter the red cell membrane potential and in so

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doing, cause a redistribution of protons such that plasma is acidified. This effect occurs in vivo as well as in vitro. However, the nature of the contrast material, the site of injection, and the transit time of the contrast material are important factors in determining the pH of blood in a regional capillary circulation. The writers

We have examined the role of oxygen hemoglobin affinity on oxygen delivery in patients with cardiac decompensation. A decrease in oxygen affinity occurs as arterial oxygen flow rate falls and this minimizes the reduction in venous Po_2 needed to maintain oxygen consumption. They partial pressure of O2

We have established a model for the study of oxygen transport using the gracillis muscle of the dog. Preliminary studies indicate that alkalosis does not impair resting or exercising oxygen consumption if flow rate is normal. If flow is reduced, alkalosis may impair oxygen consumption during exercise.

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Progress Report for Contract No. DA17-73-C-3135 November 1, 1976 to March 31, 1978

1. Effects of contrast materials on red cell membrane potential and the pH of plasma and red cells.

Radiographic contrast materials added to blood reduce the red cell membrane potential by balancing the internal impenetrable anions, hemoglobin and organic phosphates. In so doing, a redistribution of protons occurs such that plasma is acidified. The time course of acidification of plasma is measured in seconds, with a nadir of pH occurring 12 to 15 seconds after addition of Hypaque (1.5 to 3.0 ml/10 ml blood) and a half-time of acidification requiring about 6 seconds. The acidification process is slowed in part by an initial alkalosis due to Hypaque. The acidification of blood is more rapid after addition of Renografin (1.5 to 3.0 ml/10 ml blood) than after addition of Hypaque since the former solution is slightly acidic. The time course of plasma acidification indicates that a maximal reduction in blood pH may not occur in the capillaries of regional circulation following injection of contrast materials into its afferent vessel, since the transit time of the contrast material may be less than the time required for maximial acidification of plasma.

The rate of acidification of plasma is a function, primarily of the hydration of carbon dioxide to form carbonic acid. Since this is not enzymatically mediated in plasma, the reaction time is measured in seconds. Following addition of Hypaque to blood, the full decrement in pH required about fifteen seconds to occur at 37°C. The changes were complex in that pH rose initially due to the alkaline nature of Hypaque solution and subsequently pH fell. Major reductions (> 0.2 units) required about 6 seconds to occur following addition of Hypaque to blood. During this time pH actually changed by 0.3 units if one considers the initial alkalosis, when 3 mls of Hypaque were added to 10 mls of blood. Following addition of Renografin, the initial alkalosis was absent and the acidification of plasma occurred more rapidly and was intensified since it was the result of a slightly acidic solution coupled with the effect of contrast materials on the red cell membrane potential. Nevertheless, a reduction of >0.2 units of pH, in the presence of 3 mls of Renografin per 10 ml blood, required 3 seconds to occur.

These studies indicate that a) the nature of the contrast material, b) the site of injection and c) the transit time of the contrast material-blood solution, are important factors in determining the pH of blood in a regional capillary circulation. A bolus injection into an artery supplying an organ (eg. coronary or cerebral artery) may result in transit of much of the contrast material prior to achieving the nadir of blood pH.

2. The role of hemoglobin-oxygen affinity in oxygen transport during congestive heart failure.

We have examined the interrelationships among blood oxygen content, blood flow, oxygen binding by hemoglobin and oxygen consumption in cardiac patients with and without chronic cardiac decompensation. We have quantified the role that decreased oxygen-binding to hemoglobin may play in maintaining oxygen consumption in the presence of low systemic blood flow rates.

The volume rate of oxygen delivery to tissues was expressed as the arterial oxygen flow rate index (OFI_a), the product of oxygen content and blood flow. OFI_a varied from 738 to 262 mls $O_2 \cdot min^{-1} \cdot m^{2-1}$ whereas oxygen consumption ($\dot{V}O_2$) varied from 170 to 117 mls $O_2 \cdot min^{-1} \cdot m^{2-1}$. Thus, mean $\dot{V}O_2$ fell only 19% despite a mean decrease in OFI_a of 63%. $\dot{V}O_2$ was maintained because the extraction of oxygen rose from about 20% to 50% in close association with the decrease in OFI_a.

Oxygen binding to hemoglobin decreased as OFI_a decreased. At in vivo conditions of pH, P_{CO_2} and temperature, P_{50} in vivo rose; this facilitation of oxygen release at the P_{O_2} of tissue capillaries could explain about one third of the observed increase in oxygen extraction as OFI_a fell. An alternative interpretation is that an increase in P_{50} in vivo minimizes the reduction in mixed venous P_{O_2} needed to maintain $\dot{V}O_2$ when increasing proportional extraction of O_2 compensates for decreasing OFI_a.

3. Gracilis muscle model for studies of oxygen transport

We have developed an isolated muscle model to test the hypothesis that altered hemoglobin-oxygen binding can influence tissue oxygen uptake when blood flow and arterial blood oxygen content are held constant (Figure 9). The model is a variation of that described by Renkin in Acta Physiol. Scand. 54:223, 1962.

In our initial experiments, the dog gracilis muscles were isolated, the gracilis artery and vein were cannulated and the muscle was perfused with blood that had been collected earlier the same day from the same dog. The blood had been treated in one of several ways to modify $Hb\text{-}O_2$ affinity, then oxygenated and passed through a finger pump into the muscle. Various blood treatment modalities were tested, including (1) blood storage in ACD to reduce 2,3-DPG levels, (2) exposure to metabisulfite (3) treatment with potassium cyanate to carbamylate the hemoglobin. All such treatments appeared to produce red cell damage and perhaps sludging so that muscle vascular resistance rose dramatically during blood infusion, and interpretation of the data was difficult. These manipulations will nevertheless be pursued, as these findings may have an important bearing on oxygen transport when patients are transfused with stored blood.

At present, however, we are autoinfusing the muscle from the donor dog and are manipulating hemoglobin-oxygen affinity by inducing respiratory alkalosis (Bohr effect). In a typical experiment, the following protocol is followed after muscle isolation and establishment of controlled flow:

1. Control gracilis arterial (A) and venous (V) blood sampling.
2. Induction of respiratory alkalosis by hyperventilation
3. Repeat A, V sampling for determination of resting muscle $\dot{V}O_2$ and lactate production
4. Stimulation of the muscle for approximately one minute and measurement of $\dot{V}O_2$ and lactate production during exercise
5. Collection of blood samples during recovery period
6. Restoration of ventilation to normal is followed by a period to allow recovery of muscle to basal conditions
7. Repeat steps 3-5
8. Alteration of blood flow rate to the muscle and repeat of steps 3-7.

9. At each step blood samples are taken for determination of blood pH, P_{O_2}, Pco_2, $\%HbO_2$, %HbCO, Hb level, lactate concentration. Such sampling allows not only calculation of VO_2 and lactate production but of P_{50} at both standard and *in vivo* conditions.

10. At each step, the muscle is also subjected to a test for arterial occlusion to be sure that it has retained its capacity to autoregulate and is thus behaving physiologically.

The preliminary data suggests that in the range of normal flow rates, alkalosis does not impair either resting or exercise V_{O_2} in spite of the associated reduction in P_{50} *in vivo*. However, during alkalosis, the muscle appears to operate at lower levels of venous P_{O_2}. It is possible, then that with further reduction in arterial flow rate, or with more extreme exercise, induced affinity changes may have an impact on tissue O_2 uptake.

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