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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	26th December, 1994	Final, August 1991-October 1994

4. TITLE AND SUBTITLE
Effect of mild hydrostatic pressure on red blood cells

5. FUNDING NUMBERS
N00014-91-J-1880

6. AUTHOR(S)
Professor Saul Yedgar

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Hebrew University, Jerusalem, Israel 91904

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Dr. Constance Oliver,
Office of Naval Research,
800 North Quincy Street,
Arlington, VA 22217-5660.

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FEB 13 1995
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10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

We studied the effect of physiological levels of hydrostatic pressure, such as that applied in diving or hyperbaric treatment, on **1.** flow (rheological) properties and **2.** membrane fluidity and morphology of red blood cells (RBC). It was found that application of such pressure **1.** Enhances the aggregability of RBC (studied by a computerized image analysis which was developed for this purpose), and higher than normal flow rate was required to disperse the cells. **2.** Reduces RBC membrane fluidity (studied by fluorescence anisotropy of lipid probes and tryptophan), and changes the cell morphology (studied by scanning electron microscopy) from normal discocytes to stomatocytes. Physical and rheological properties of RBC, as well as their shape, play a major role in blood flow and in their cellular-biochemocal functions. Thus, the alterations in RBC reported here may be pertinent to the microcirculatory and physiological distorters observed among humans subjected to elevated pressure.

19950203 271

14. SUBJECT TERMS	15. NUMBER OF PAGES
Hydrostatic pressure, red blood cells aggregation, membrane fluidity and morphology, microcirculation diving, hyperbaric treatment.	3
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
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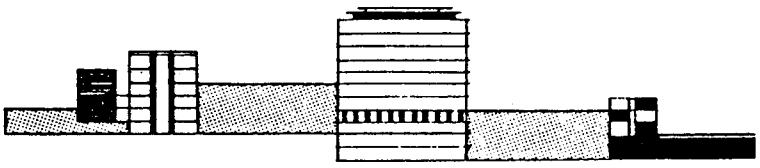

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Professor Saul Yedgar
Department of Biochemistry

Tel: 972-2-439218; 758306 (w); 520159 (h).
Fax: 972-2-757379; 784010.
E-mail: YEDGAR@MD2.HUJI.AC.IL.

FINAL REPORT, Grant No. N00014-91-J-1880.

EFFECT OF HYDROSTATIC PRESSURE ON RED BLOOD CELLS.

The following studies have been performed:

1. Monitoring of red blood cell aggregability in a flow-chamber by computerized image analysis: A system for direct observation and video recording of red blood cells (RBC) under flow, and computerized analysis of their aggregability was constructed. A narrow-gap flow-chamber was designed, in which a blood sample is subjected to controllable flow. Software was developed to provide the RBC aggregate size distribution at different shear stresses. From the size distribution curves the average or median size (or any other percentile) of the aggregate population as a function of shear stress, as well as the shear stress required for complete disaggregation, can be derived. The kinetics of spontaneous aggregation, in stasis, from singly dispersed cells, as well as the disaggregation kinetics at varying shear stress, can be derived from temporal monitoring of the cells' images. This system provides quantitative measures of RBC aggregability from direct visual monitoring of the aggregation and disaggregation processes. This study is described in detail in **Reference 1**.

2. Monitoring of erythrocte aggregate morphology under flow by computerized image analysis: The morphology of red blood cell (RBC) aggregates was studied by direct visualization of RBC aggregation at different flow conditions in a computerized image analyzer. The aggregate morphology is expressed by an Aggregate Shape Parameter (ASP), defined as the ratio of the aggregate projected area to its square perimeter. Aggregation was induced by either dextran-70 (m.w. 70,000) or dextran-500 (m.w. 500,000), and compared to that in plasma. It was found that the aggregate morphology is characteristic of the aggregating agent - in dextran-500 the RBC form rouleau aggregates as in plasma, while in dextran-70 they form clusters. In each system, while maintaining the overall typical morphology, the ASP decreases (i.e. the aggregate becomes longer) as the aggregate size is increased. The distribution of the ASP as a function of the aggregate size remains unchanged when the aggregate size is changed by modulation of the dextran concentration or the shear stress. Stretching of a rouleau aggregate by application of shear stress is reflected by a corresponding change in the ASP. It is suggested that the ASP is characteristic of the intercellular interactions. A theoretical model is proposed for evaluation of the deviation of aggregate shape from that of the rouleau structure. This study is described in detail in **Reference 2**.

3. Red blood cell aggregability is enhanced by physiological levels of hydrostatic pressure: The effect of hydrostatic pressure at physiological levels, such as that applied in diving or hyperbaric treatment (up to atm), on the aggregability of rat and human red blood cells (RBC) (i.e. their capability to form aggregates) was studied using the computerized image analysis system described above. The aggregate size distribution was determined under ambient pressure following application of hydrostatic pressure for various durations up to two hours. It was found that RBC aggregability markedly increases, up to three-fold, as the pressure which had been applied was increased. Accordingly, higher shear stress is required for dispersing the aggregates of pressure-treated RBC than those of untreated cells. The median size of human RBC aggregates was about three times higher than that of rat RBC, and this ratio was maintained following pressure treatment. RBC aggregability is a major determinant in blood flow, especially in the microcirculation. Pressure at the levels used in this study occurs in physiological states such as hyperbaric treatment or diving. The enhanced aggregability induced by application of such pressure implies that blood flow in microvessels might be altered under conditions associated with elevated hydrostatic pressure. This study is described in detail in **Reference 3**.

4. Membrane fluidity and shape of human red blood cells are altered by physiological levels of hydrostatic pressure: In this study we investigated the effect of hydrostatic pressure at physiological levels, as noted above, on human red blood cell (RBC) membrane fluidity and morphology. Membrane fluidity was determined by fluorescence depolarization (FA) of lipid probes, mainly diphenyl hexatriene (DPH) and of tryptophan ,as well as by energy transfer from the tryptophan to the lipid probes, in ghosts prepared prior to or after application of pressure to intact RBC. The morphology of intact RBC, prior to or after application of pressure, was evaluated by scanning electronmicroscopy. It was found that 1. The FA of DPH was increased as a function of the pressure applied and the duration of the treatment. At 15 atm he FA increased by 50%, reaching a plateau after 60 min of application of pressure. 2. Increased FA, to various extents, was observed also with lipid probes which reside in the membrane lipid core, but not with probes which monotor the polar/apolar phospholipid interface or the cell surface. 3. The same treatment increased tryptophan FA by about 20%. 4. Tryptophan energy transfer to lipid probes which resides in the lipid core was increased to various degrees, which were related to the increased in FA of these probes. 5. Following application of 15 atm for 1 hour more than 60% of the RBC changes their shape from discocytes to stomatocytes. These results demonstrate that physiological levels of hydrostatic pressure, such as that applied in diving or hyperbaric treatment, might reduce the membrane fluidity of RBC and alter their morphology. RBC membrane fluidity and shape play an important role in and cellular rheological functions. Thus, the pressure-induced alterations in RBC properties might be pertinent to the microcirculatory disorders observed in humans subjected to elevated hydrostatic pressure. This study is described in detail in **Reference 4.**

PUBLICATIONS:

1. Chen,S., Barshtein,G. Gavish,B., Mahler,Y., Yedgar,S. Monitoring of red blood cell aggregability in a flow-chamber by computerized image analysis.
   Clin. Hemorheology, 14:497-508, 1994.

2. Chen,S., Gavish,B., Mahler,Y., and Yedgar,S. Monitoring of erythrocyte aggregate morphology under flow by computerized image analysis.
   Biorheology, in press.

3. Chen, S., Gavish,G., Barshtein,G., Mahler,Y., and Yedgar,S. Red blood cell aggregability is enhanced by physiological levels of hydrostatic pressure.
   Biochim. et Biophys. Acta, 1192:247-252, 1994.

4. Barshtein,G., Bergelson,L., Dagan,A., Gratton,E., Yedgar, S.Membrane fluidity and shape of human red blood cells are altered by phyisological levels of hydrostatic pressure.
   Submitted for publication.

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