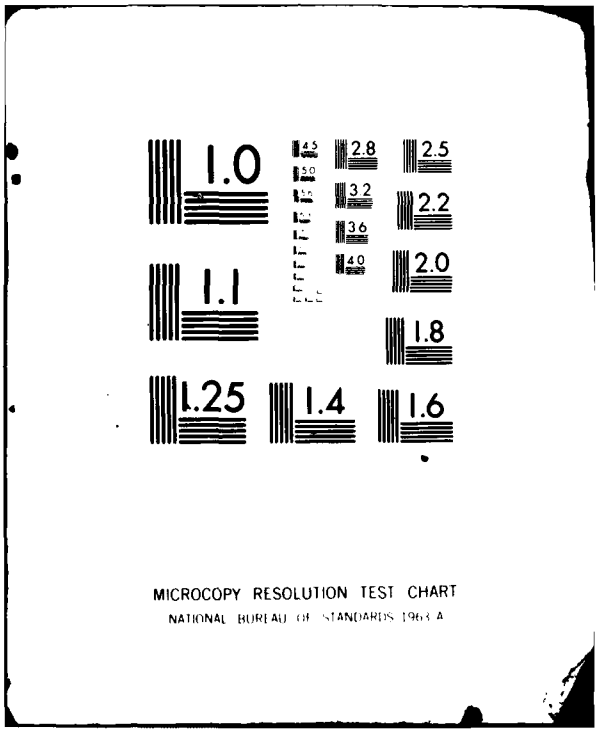
AD-A110 776 CENTER FOR BLOOD RESEARCH BOSTON MA F/G 6/5
CRYOGENIC PRESERVATION OF GRANULOCYTES AND MONOCYTES.(U)
JAN 82 F J LIONETTI, F W LUSCINSKAS N00014-80-C-0239
UNCLASSIFIED NL


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963 A

LEVEL II

13

OFFICE OF NAVAL RESEARCH

CONTRACT N00014-80C-0239

FINAL REPORT

CRYOGENIC PRESERVATION OF GRANULOCYTES AND MONOCYTES.

Fabian J. Lionetti
Center for Blood Research
800 Huntington Avenue
Boston, MA 02160

Summary of Research under Contract N0014-80C.

The goals of these studies:

1) to establish a clinically effective procedure for the long term storage of granulocytes, monocytes and stem cells of human blood;

2) to elucidate mechanisms of cryogenic injury to white cells;

3) to evaluate complement and immunoglobulin dependent phagocytic mechanisms of granulocytes and monocytes.

Granulocytes and monocytes of high purity were obtained by counterflow centrifugation. They were preserved with a combination of extracellular (hydroxylstarch, 6%) and intracellular (dimethylsulfoxide, 5%) cryoprotectants in a hyperosmolar (314 mOsM) balanced salt buffer containing bovine albumin (4%). Cells were cooled at 4°C to -80°C and stored in tubes in liquid nitrogen. Granulocytes of dog, guinea pig, baboon, and man were investigated. Monocytes were obtained only from human whole blood or pheresis cellular residues. Morphological recoveries ranged upwards of 90% in all species. Functional recoveries of thawed washed granulocytes in terms of membrane stability and phagocytic indices ranged from 80% in guinea pig and dog, 70% in baboon to 40% in human. Leukapheresed dog granulocytes were stable in liquid nitrogen for two years.

High yields (1×10^9) monocytes were obtained from plateletpheresis bags and preserved with the granulocyte protocol. All cells were recovered after 3 months storage in liquid nitrogen with 94% phagocytic index.

Technical Reports.

Cryogenic preservation of monocytes from human blood and plateletpheresis cellular residues. December 20, 1980.

Long term cryopreservation of dog granulocytes. December 22, 1981.

Publications.

Lionetti, F.J., Luscinskas, F.W., Hunt, S.M., Valeri, C.R., and Callahan, A.B.: Factors affecting the stability of cryogenically preserved granulocytes. Cryobiology 17:297-310, 1980.

Hunt, S.M., Lionetti, F.J., Valeri, C.R., and Callahan, A.B.: Cryogenic preservation of monocytes from human blood and plateletpheresis cellular residues. Blood 57:592-598, 1981.

Arnaout, A.A., Luscinskas, F.W., Lionetti, F.J., Alper, C.A., and Valeri, C.R.: Alternative complement dependent pathway ingestion of Fluolite particles by human granulocytes. J. Immunol. 127:278-281, 1981.

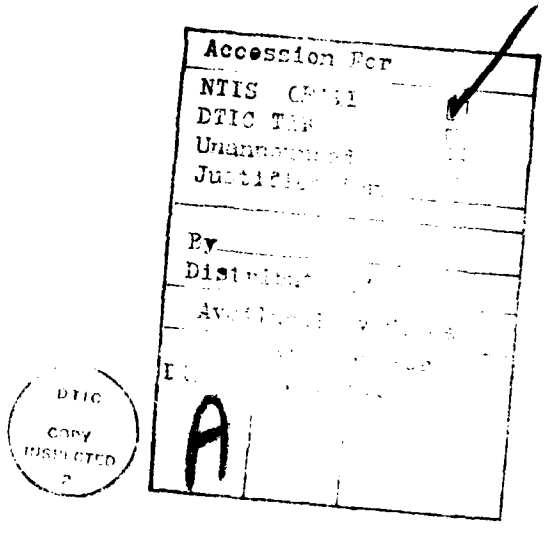
Luscinskas, F.W., Lionetti, F.J., Melaragno, A.J., and Valeri, C.R.: Long term cryopreservation of dog granulocytes. (submitted for publication to Experimental Hematology).

Conclusions:

The feasibility of long term cryopreservation of granulocytes and monocytes has been established. Granulocytes of lower animals (guinea pig, dog) may be preserved for one to two years with small losses of cells and functionality. A species difference in stability exists in which baboon and human cells are the least stable.

Human monocytes may be isolated in large (1×10^9) numbers as by products cells of plateletpheresis. They are stable and functional after months in liquid nitrogen.

A protocol for granulocyte freezing containing a combination of extracellular (HES) and intracellular (DMSO) cryoprotectants is applicable with good results to all white cell types in animal blood including B and T lymphocytes.



SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 3 Final	2. GOVT ACCESSION NO. AD-A110 776	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Cryogenic Preservation of Granulocytes and Monocytes.		5. TYPE OF REPORT & PERIOD COVERED Final 12-1-79 - 11-30-81 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) F.J. Lionetti F.W. Luscinskas		8. CONTRACT OR GRANT NUMBER(s) N00014-80C-0239
9. PERFORMING ORGANIZATION NAME AND ADDRESS Center for Blood Research 800 Huntington Avenue Boston, Massachusetts 02115		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NR 105-707
11. CONTROLLING OFFICE NAME AND ADDRESS Office of Naval Research		12. REPORT DATE January 25, 1982 13. NUMBER OF PAGES 5
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) DCASR Boston Army Base 666 Summer Street Boston, Massachusetts 02210		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Distribution of this report is unlimited within the government.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) Same as 16.		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Granulocytes and monocytes of high purity were obtained by counterflow centrifugation. They were preserved with a combination of extracellular (hydroxylstarch, 6%) and intracellular (dimethylsulfoxide, 5%) cryoprotectants in a hyperosmolar (315 mOsM) balanced salt buffer containing bovine albumin (4%). Cells were cooled at 4°C to -80° and stored in tubes in liquid nitrogen. Granulocytes of dog, guinea pig, baboon, and man were investigated. Monocytes were obtained only from human whole blood or pheresis cellular residues. Morphological recoveries ranged upwards of 90% in

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-LF-014-6601

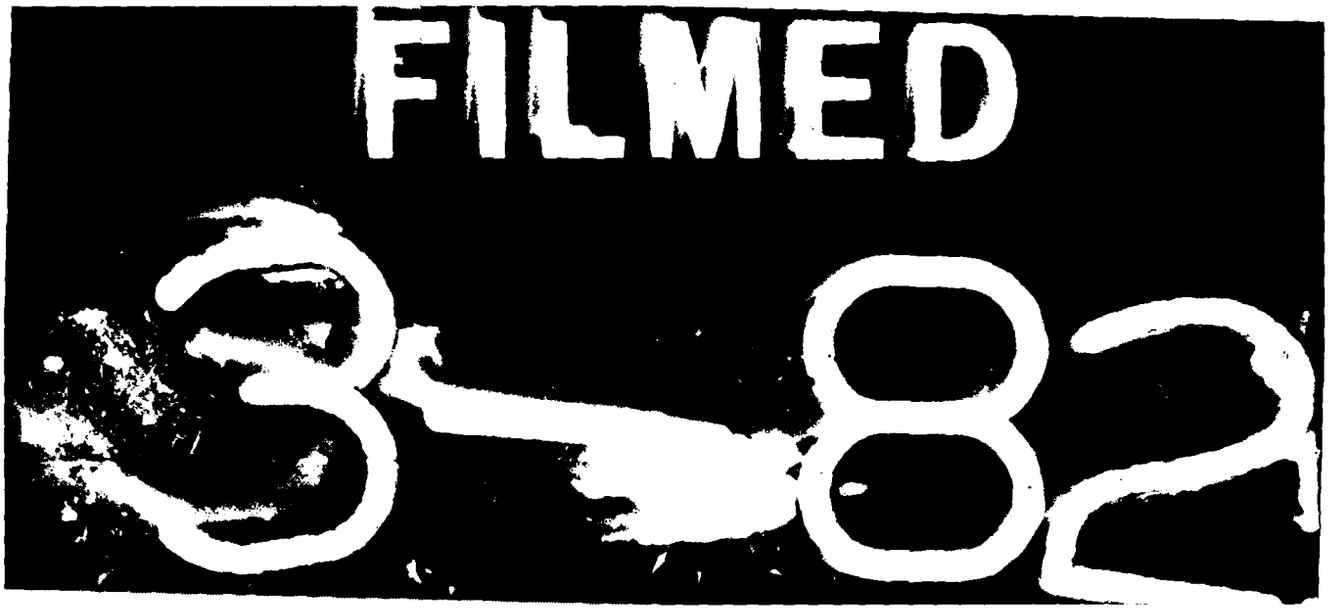
389700

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ABSTRACT (Continued).

all species. Functional recoveries of thawed washed granulocytes in terms of membrane stability and phagocytic indices ranged from 80% in guinea pig and dog, 70% in baboon to 40% in human. Leukapheresed dog granulocytes were stable in liquid nitrogen for two years.

High yields (1×10^9) monocytes were obtained from plateletpheresis bags and preserved with the granulocyte protocol. All cells were recovered after 3 months storage in liquid nitrogen with 94% phagocytic index.


FILMED
3-82