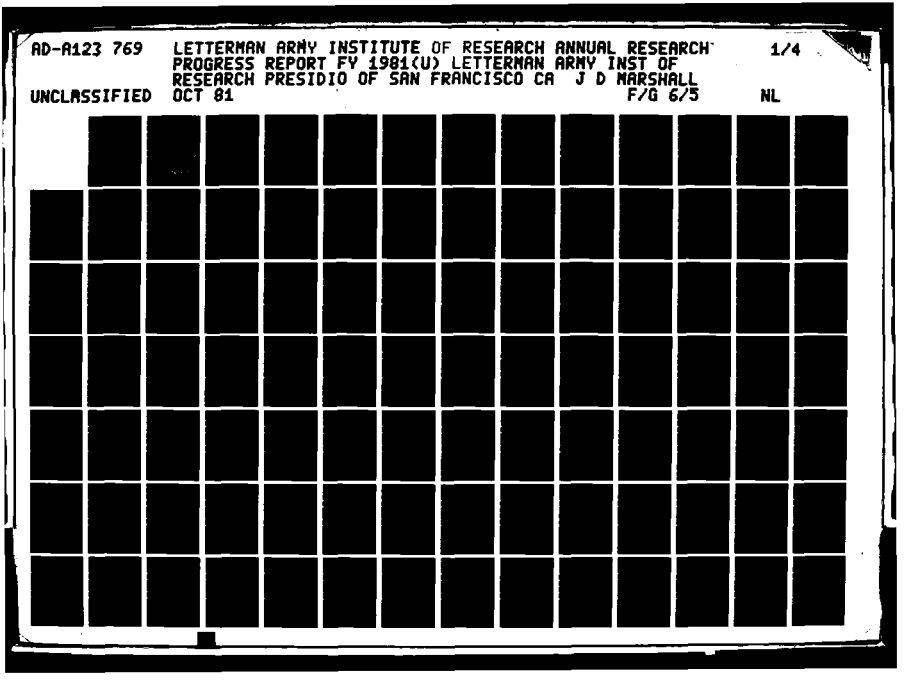
AD-A123 769
LETTERMAN ARMY INSTITUTE OF RESEARCH ANNUAL RESEARCH PROGRESS REPORT FY 1981(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA J D MARSHALL
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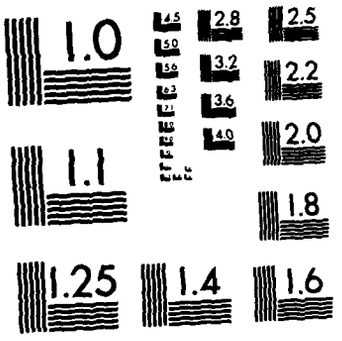

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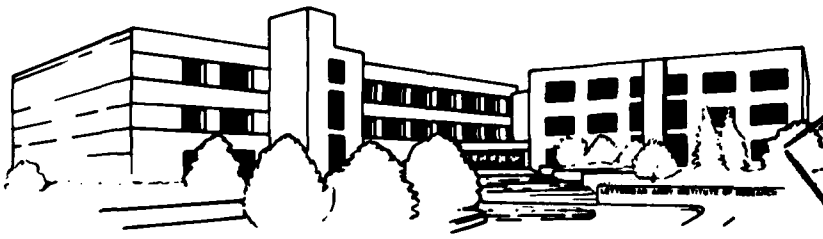
LETTERMAN ARMY INSTITUTE OF RESEARCH ANNUAL RESEARCH PROGRESS REPORT

FY 1981

RCS-MEDDH-288(R1)

30 SEPTEMBER 1981



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER RCS-MEDDH-288 (R1)	2. GOVT ACCESSION NO. AD-A123 769	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Letterman Army Institute of Research Annual Progress Report, FY 1981		5. TYPE OF REPORT & PERIOD COVERED Annual Research Progress Report, 1Oct80-30Sept81 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) John D. Marshall, COL, MS		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Letterman Army Institute of Research Presidio of San Francisco, CA 94129		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Ft. Detrick, Frederick, Maryland 21701		12. REPORT DATE Oct 81 13. NUMBER OF PAGES
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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)

During Fiscal Year 1981, progress was attained at Letterman Army Institute of Research in the following research areas: Basic and applied studies on blood, blood products and blood substitutes; Diagnosis and Treatment of Acute Laser injuries, Laser Technology - Ocular Bioeffects; Physiology of hemorrhagic shock, pharmacological intervention of shock; Immediate care of the combat wounded; Defense against chemical agents; and computer science. The progress made in this fiscal year is described in the reports of the work units presented.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

FOREWORD

The research conducted at the Letterman Army Institute of Research, Presidio of San Francisco, California was accomplished in Fiscal Year 1981 under the following Department of the Army projects:

3A161101A91C - In-House Laboratory Independent Research

3M161102BS02 - Basic Mechanisms of Recovery from Injury

3M161102BS10 - Research on Military Disease, Injury and Health Hazards

3M162770A871 - Prevention of Military Disease Hazards

3S162772A874 - Care of the Combat Casualty

3M162734A875 - Medical Systems in Chemical Defense

3E162777A878 - Health Effects of Military Lasers

Projects are subdivided into work units and studies, as appropriate, to accomplish project objectives.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

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Table of Contents (Cont)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 0178	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	H.Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	050 APC NL04
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)*

(U) Toxicology of Explosives and Explosive By-Products

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

016800 Toxicology; 005900 Environmental Biology; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 07	81 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	2.9	75
FISCAL YEAR CURRENT 82	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Hannon, J.P., DAC POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Military Toxicology, (U) Munitions Chemicals, (U) Carcinogenesis; (U) Teratogenesis; (U) Military Performance, (U) Laboratory Animals

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Under provisions of the National Policy Act of 1969 and all other Federal environmental laws, the U.S. Army is assigned responsibility for the protection of civilian and military personnel from chemicals generated by military and other activities during manufacture, training, and combat. Because of the markedly increasing requirements for toxicology testing by industry and government agencies, and a critical national shortage of facilities and trained personnel to address these requirements, the U.S. Army faces an untenable position in discharging its assigned responsibilities. The purpose of this work unit, therefore, is to establish and implement in-house toxicology programs specifically directed to the testing and evaluation of environmental chemical contaminants generated by munitions manufacture and use.

24. (U) Two areas of research will be pursued. The first will be concerned with the test and evaluation of chemicals for mutagenic, carcinogenic, reproductive, or teratogenic effects that may pose a health hazard to humans. The second reasearch area will be concerned with the impact of candidate chemicals on combat-related performance factors and the evaluation of treatment modalities when adverse effects are observed.

25. (U) 8010-8109. LD_{50} for 2-4 DNT for male and female rats and mice was determined to be 1427, 987, 1343, and 1080, respectively. Death occurred typically 3-5 days after dosing in rats, whereas death came generally within 24 hrs of dosing mice. Fourteen-day feeding study dose levels were 1.0, 1.5, 2.0, and 3.5 g 2-4-DNT per kg of feed. Feed intake and weight gain were inversely related to increased dose level. Directly proximal convoluted tubules in all groups and dose-related oligospermatogenesis in all male dose groups. No further research is planned.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	050	Toxicology of Explosives and Explosive By-Products

The following investigations have been conducted under this work unit:

STUDY NO. 1 Toxicology of 2,4-Dinitrotoluene (2,4-DNT)

The LD_{50} (median lethal dose) of 2-4 DNT required for male and female rats was determined to be 1427 and 987 mg/kg, respectively; the LD_{50} of 2,4-DNT for male and female mice was determined to be 1343 and 1080 mg/kg, respectively. Death typically occurred 3-5 days after dosing in rats, whereas death came generally within 24 hours of dosing mice. Fourteen-day feeding study dose levels were 1.0, 1.5, 2.0, and 3.5g 2,4-DNT per kg of feed. Feed intake and weight gain were inversely related to increased dose levels. Directly dose-related signs were eosinophilic absorption droplets in the epithelial cells of the proximal convoluted tubules in all groups and dose-related oligospermatogenesis in all male dose groups. Further analyses of data are in progress.

BODY OF REPORT

WORK UNIT NO.	050	Toxicology of Explosives and Explosive By-Products
STUDY NO.	1	Toxicology of 2,4-Dinitrotoluene (2,4-DNT)

PROBLEM

The U.S. Army Medical Research and Development Command is responsible for evaluating potential health hazards of all military high explosives and explosive by-products. Exposure to such hazards may occur among workers employed in munitions plants or in civilian populations as a result of environmental contamination associated with munition manufacture and assembly. Major concerns at the present time are the toxicologic effects of 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitrohexahydro-1,3,5 triazine (RDX) and their by-products. These chemicals are discharged into the environment, without significant treatment of waste waters, from loading shells with TNT and RDX mixtures. The waste waters are referred to as LAP (load, assemble, and pack) water, which contains a 1.6:1 blend of TNT and RDX, and condensate water which contains a blend of some 30 compounds produced by solar irradiation of TNT/RDX. This project is concerned with the acute, subacute, and subchronic toxicology of 2,4-dinitrotoluene (2,4-DNT), a major component (43% relative concentration) of condensate water. Prior studies by different organizations have addressed the toxicity of this compound, but the results are inadequate to establish comprehensive environmental standards. Thus, there is a need for verifying earlier findings. An LD_{50} study, using mice and rats and subsequent 14-day subacute and 90-day subchronic studies, will be conducted.

RESULTS AND DISCUSSION OF RESULTS

Initial efforts were directed toward determining acute oral LD_{50} in male and female rats and mice. Dosing animals with 2,4-DNT proved difficult because the compound is not readily soluble in the commonly used solvents; it was soluble in sufficient concentration in Tween 80 when heated to 70 $\pm$ 5C. The dosing solutions were maintained at 45 $\pm$ 2C and the syringes and dosing tubes at 37 $\pm$ 2C to prevent crystallization before dosing was completed. The LD_1, LD_{50}, and LD_{95}, with the 95% confidence interval for LD_{50}, are listed below for both sexes of rats and mice. Data are listed in mg/kg body weight based on calculated weight of 2,4-DNT dissolved in a measured volume of Tween 80; no allowance for volume change of dosing solution was made.

Values in mg/kg

	LD_{50}	95% Confidence Interval Low	High	LD_1	LD_{95}
Rats - male	1427	1118	1822	414	3426
female	987	712	1367	248	2622
Mice - Male	1343	1049	1719	410	3106
female	1080	856	1363	316	2575

Death in rats from 2,4-DNT occurred typically 3 to 5 days after dosage.

Before death, rats appeared to be extremely depressed; surviving animals appeared normal at the conclusion of the study, 14 days after dosing. Death in mice typically occurred within 24 hours; surviving animals appeared normal after 14 days.

Dose levels for the 14-day feeding study were 1.0, 1.5, 2.0, and 3.5 g 2,4-DNT per kg of feed. As dose levels increased, feed and weight gain decreased. The appearance of eosinophilic staining absorption droplets in the epithelial cells of the proximal convoluted tubules was dose-related and was seen in all treatment groups. Oligospermatogenesis in male treatment groups was also dose related. Analyses of additional collected data continues.

CONCLUSIONS

Although data analyses are not complete, it is obvious that 2,4-DNT has significant adverse health effects at relatively high dose levels.

Toxicology of Explosives and Explosive By-Products (Continued)

RECOMMENDATIONS

Long-range studies using reduced levels should be conducted to provide baseline data for developing environmental exposure criteria.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 3370	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	H. TERMINATION	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	053 APC LL01
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)*

(U) Immediate Care of the Combat Wounded

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012900 Physiology; 000800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 01	81 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	0.0	00
	CURRENT 82	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

POC: DA

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Hemostasis; (U) Abdominal Cavity; (U) Alginate; (U) Experimental Animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) If a liquid material could be infused into the belly to fill all of the dead space, and this liquid then could change its state to a gel, hemostasis would occur by virtue of the blood not having any place to flow. This material would have to be a thin liquid initially, and then be able to change its state to a gel very quickly without the generation of heat. In addition, the gel would have to be able to be placed into solution or be able to be "peeled off" the viscera when definitive treatment became possible at a hospital center. The purpose of this work unit is to find the best material to test the preceding theory, and to see what the physiological effect would be of filling the abdominal cavity of an experimental animal with gel Also to see if filling the belly with a gel will provide short-term hemostasis in an animal system.

24. (U) Alginate, the irreversible hydrocolloid used to prepare dental impressions, was tested at various concentrations. Alginate mixed 1:6 in normal saline and combined with Triton X-100, a surface tension agent, produced a foamy gel. When this gel was placed into the abdomen of laboratory rats, all of the animals died.

25. (U) 8010-8109 This study is terminated. No work has been done this fiscal year, and no further studies are contemplated. The toxicity of Triton X-100 and an adequate delivery system are insurmountable problems. The basic hypothesis of this study may not be valid.

* Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	053	Immediate Care of the Combat Wounded

Various concentrations of alginate, the hydrocolloid used for dental impressions, were tested. A 1:6 solution of alginate in normal saline produced a firm but heavy gel within two minutes. The same solution, when mixed with a surface tension agent, 1% Titron X-100, and stirred in a blender, produced a foamy but still heavy mass. If the alginate-Triton X-100 mixture was prepared with N_2 gas bubbled through the liquid as it gelled, a light, foamy mass was produced. In our preliminary studies in rats, we found that this last preparative process with the alginate-Triton X-100 mixture could fill the abdomen fairly well. If studies along this line are continued, we are going to need a delivery system which is capable of coating the entire cavity consistently each time the procedure is performed.

BODY OF REPORT

WORK UNIT NO. 053 — Immediate Care of the Combat Wounded

PILOT STUDY — Hemostasis in penetrating wounds of body cavities

PROBLEM

The combat medic on the battlefield is faced with a difficult situation when trying to stabilize the vital signs of a patient with a penetrating wound of the abdominal cavity. Although the medic has the capability of infusing blood replacement solutions to treat shock, he does not have the equipment and facilities to stem the flow of a major blood vessel bleeding into the abdomen. In this situation, all of his blood replacement solution may be, in fact, pouring through the damaged vessel into the abdomen. In future conflicts where air superiority may be lacking, evacuation of casualties may take longer than those of the Vietnam war. Those patients with major bleeding vessels of the abdominal cavity may never live to reach a definitive treatment facility.

Some method of temporary occlusion of major vessel bleeding in body cavities is needed. This method must be adaptable to a combat situation and not require sophisticated equipment.

If a liquid material could be infused into the abdominal cavity to fill all of the dead space, and if this liquid then could change its state to a gel, hemostasis might occur by virtue of the blood not having any place to flow. This material would have to be a thin liquid initially, and then become a gel very quickly without the generation of heat. In addition, medical personnel should be able to dissolve or remove the gel when definitive treatment becomes possible at a hospital center.

RESULTS AND DISCUSSION OF RESULTS

The irreversible hydrocolloid, alginate (Jeltrate, L.D. Caulk Co., Milford, DE), which is used to prepare dental impressions, was selected as the initial test material in this pilot study. Using normal saline solution as the diluent, we prepared varying concentrations of alginate (1:1-1:10). A 1:6 concentration of alginate powder in saline seemed to be most suitable. One excellent property of the material was its lack of heat production as it changed from a liquid to a solid state. It produced a firm rubbery gel within 2 minutes of mixing. When this material was instilled into the abdomen of two normal anesthetized rats, it filled the abdomen fairly well. It coated the viscera, producing a "mold" of the organs. When the animals were sacrificed, the gel could be peeled from the viscera easily.

The gel, if allowed to stand for 24 hours or more, began to lose water and to shrivel. When the entire mass had gelled in the abdominal cavity, the weight of the solid was noted. To be effective, such a mass would have to be much lighter to keep from compromising the venous circulation. Next, a surface tension agent, 1% isooctyl phenoxy polyethoxy ethanol (Triton X-100, Rohm & Hass Co., Philadelphia, PA), was combined with the saline/alginate. When mixed in a blender, a foamy gel was produced. However, with a similar water content as the first solution, this solution still produced a heavy gel. To lighten the mixture, nitrogen gas was bubbled through the alginate/Triton X-100 as it was stirred in a Waring blender. The result was a spongy light mass with an adequately firm consistency. This material was then infused into the abdomen of rats by using a perforated infant feeding tube to deliver the material. It gelled within 2 minutes. The animals were allowed to recover from anesthesia. All four animals died within 24 hours. On necropsy, the abdomen was partially filled with the gel. Some portions of the cavity did not have alginate present. The material held its texture well and could be peeled from the intestines, leaving a mold of the organs.

CONCLUSIONS

The basic hypothesis of this study needs further investigation. Better solutions and more effective delivery systems are necessary.

RECOMMENDATIONS

Triton X-100 may have itself been toxic to the animals. Solutions, such as dextrans or other materials should be evaluated in future experiments. In addition, a more effective method of delivery of the material while it is in its liquid state should be developed. A propellant-can arrangement would be ideal if the proper solution can be found. Because of other priorities of the principal investigator, this pilot study will not be continued at this time.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 3426	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	054 JL01
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

Isolation of Hematopoietic Stem Cells for Long Term Cryopreservation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 07	83 07	DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	3.4	65
FISCAL YEAR CURRENT 82	3.8	170

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Bolin, Robert B., LTC, MC
NAME: POC:DA :DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Stem cell failure; (U) Laboratory Animal; (U) Mononuclear cells; (U) Pheresis; (U) Radiation syndrome; (U) Cell harvest

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The development of radiation injury by a group of soldiers would result in definable morbidity and mortality according to the exposure. Acute radiation syndrome, above Level-1 severity, would require intensive medical support. The treatable and main syndrome is hematopoietic, where death can be attributed to hematopoietic stem cell failure. The ability to easily isolate stem cells, store them in the frozen state for indefinite periods and make them readily available to combat theater hospitals would drastically affect morbidity, mortality, and anomic states of irradiated soldiers. This work is directed at development of a rapid procedure whereby mononuclear cells can be frozen for long periods.

24. (U) Mononuclear cells will be harvested, preferably from peripheral blood, by several pheresis procedures. These cells will be evaluated for stem cell functions by feeder layer cultures and/or in vivo splenic implants in rodents. Harvest techniques will be optimized and attempts will be made to further isolate homogeneous stem cell populations by gradient techniques. Bone marrow stem cells will also be isolated. Freezing protocols will be examined for isolated stem cells. The final product(s) will be tested in aplastic dogs.

25. (U) 8010-8109 Pheresis procedures have been developed for dogs and monkeys using discontinuous and continuous flow equipment. Continuous flow proved four times more efficient for stem cells harvest. Citrate anticoagulant proved superior to others tested, but required close monitoring of ionized calcium in the blood to prevent citrate toxicity. With this protocol, about 0.24x10 stem cells/kg could be harvested in 90 minutes, a dose comparable to that needed for human marrow transplantation. Further processing of cells by gradients accomplished the separation of lymphocyte progenitors from the granulocyte/macrophage progenitor cells.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	054	Isolation of Hematopoietic Stem Cells for Long Term Cyropreservation

The following investigation has been conducted under this work unit:

STUDY NO.	1	Harvest Techniques

The development of radiation injury results in definable morbidity and mortality according to the degree of exposure. Acute radiation syndromes may require intensive prolonged medical support. The major treatable syndrome is due to bone marrow failure attributable to various degrees of injury to the hematopoietic stem cells (HSC). The ability to harvest, store, and engraft HSC would drastically affect morbidity, mortality and psychological states of irradiated soldiers. Attempts to harvest and store HSC conveniently are being investigated. Dogs are used to establish feasibility and techniques that can be applied to humans. Evaluation of HSC of bone marrow and circulating blood show they vary only in concentration. Although bone marrow has 20-fold greater stem cell content than blood, indications are that additional stem cells may be mobilized through prolonged pheresis or the addition of inert drugs. The feasibility of increasing yields in circulating blood is currently under investigation.

BODY OF REPORT

WORK UNIT NO. 054 Isolation of Hematopoietic Stem Cells for Long Term Cryopreservation

STUDY NO. 1 Harvest techniques.

PROBLEM

The development of a way to store and engraft hematopoietic stem cells (HSC) should have significant impact on military medicine as well as a psychological impact for involvement in military conflicts with potential nuclear warfare.

The development of ratiation injury by a group of soldiers would result, according to the radiation exposure, in a definable morbidity and mortality. The resultant acute radiation syndrome beyond Level I severity would require medical support of the majority of those exposed (>200R whole body radiation). Individuals with higher exposure rates (>600R) would require medical attention immediately after exposure whereas those with intermediate exposures would recover from the acute prodome, then develop a severe illness after a latent period of 1 to 3 weeks with hematopoietic failure in over half those exposed (Levels II and III clinical stages). Although half those receiving Level II doses would survive, the chance of survival would be greatest in those receiving medical palliation. The main syndrome in Levels II and III is the hematopoietic one and death can be attributed to hematopoietic stem cell failure. The $LD_{50/60}$ is estimated between 300 and 500 rads (50% deaths within 60 days) which characterizes the protracted nature of this syndrome as compared to the central nervous system syndrome ($LD_{50/2}$) or the gastro-intestinal syndrome ($LD_{50/8}$). Modern hemotherapy with red cells, platelets, and white blood cell transfusions along with isolation and antibitotics could support victims with the hematopoietic syndrome and could signficantly alter current LD_{50} estimates. However, such intensive support is beyond current combat zone capabilities and would be logistically difficult even in CONUS hospitals presently offering specialized hemotherapy. If several victims required simultaneous treatment, support would be impossible in military and most civilian hospitals.

Hematopoietic stem cell transfusions would offer two distinct advantages: 1) HSC transfusions could be given early in the disease. Engraftment would then limit the clinical course to a period of days rather than weeks. This would result in earlier return of survivors to duty, as well as increased numbers of potential survivors. 2) Conceivably, HSC transfusions given during the prodromal syndrome could eliminate the main phase (hematopoietic syndrome), thereby minimizing medical support requirements.

Isolation of Hematopoietic Stem Cells for Long Term Cryopreservation (continued)

The pyschological importance of this type of medical support is great. The agony of prolonged illness with a high probability of death has a great psychosocial impact on those associated with such experiences. The ability to treat and shorten the illness will lessen anomic behavior of associates who are otherwise well-bodied and capable. Hematopoietic stem cell transfusion could also be effective treatment for aplastic anemia due to toxic chemical exposure.

HSC transfusions require the development of technology to harvest and store HSC for future use. Bone marrow harvest and freezing for transplantation is one developing technology addressing the goals of HSC transfusion but is impractical for large scale military needs. The ability to harvest and isolate HSC from blood could provide the logistical technology for the military if harvest of a therapeutic dose can be obtained from a single donor.

RESULTS AND DISCUSSION OF RESULTS

Pheresis procedures have been developed for dogs. Two pheresis procedures have been compared. Continuous flow pheresis with the IBM 2997 has proved four times more efficient than batch processing with Model 30 Haemonetics. Animals tolerate these procedures, but prolonged cell harvest to increase yields has required the development of techniques to eliminate citrate toxicity. Mononuclear cell cohorts have been isolated and defined by isopyknic gradients. Cell densities studied (1.066 to 1.077) show granulocyte stem cell activity in the least dense fraction whereas lymphocyte stem cell appears in more dense fractions. Approximately 0.24×10^5 stem cell/kg can be harvested and concentrated 2000-fold. This dose is comparable to that needed for human marrow transplantation and shows that blood-derived stem cell harvest is feasible.

CONCLUSIONS

Through isopyknic gradient separations, stem cells may be isolated and harvested. In addition, lymphocyte stem cells, which cause hematopoietic stem cell graft failure, can be removed by gradient techniques. This latter procedure may eliminate the need for stem cell matching between donor and patient.

RECOMMENDATIONS

Further investigtions should be aimed toward establishing a uniform, viable dose of stem cells. Prolonged pheresis on the use of non-toxic agents should be tested for stem cell enhancement. After establishing dose uniformity, transplantation studies using irradiated dogs should be done.

Isolation of Hematopoietic Stem Cells for Long Term Cryopreservation (continued)

PUBLICATIONS

1. STEWART, D.A., R.B. BOLIN, B.A. CHENEY, J.T. HAWKINS, K.W. CHAPMAN, and D.R. TOMPKINS. Characteristics of lympho-myelopoietic stem cells isolated in canine peripheral blood. Cell Cult Congress Proc (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 3429	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	102 APC LL02
b. [redacted]	61101A	3A161101A91C	OO	057
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Effects of Sensory Denervation in the Care and Management of Traumatic Wounds

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 003500 Clinical Medicine; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 05	83 07		

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	0.1	40
FISCAL YEAR CURRENT 82	2.0	71

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Randolph, D., PhD, DAC POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Sensory denervation; (U) Resuscitation; (U) Domestic animal; (U) Combat injuries; (U) Laboratory animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The study of combat-related injuries requires the production of experimental wounds in animal models. For long-term studies, it is desirable to have a reliable animal model that can be completely deprived of sensation to a selected and isolated area of the body. The physiological effects of maintaining the pain-free (anesthetic) state for a prolonged period of time must be determined along with the physiological effects of chronic sensory denervation on the healing of experimental wounds.

24. (U) Swine will be used for these studies. Rhizotomy will be the preferred surgical procedure to denervate a hind limb, but other surgical techniques will be considered. If surgical denervation is not satisfactory, other methods of regional anesthesia will be evaluated. Determination of effects of sensory denervation will involve placement of chronic indwelling catheters, monitoring various neurophysiological factors, and histopathologic studies of the experimental wounds. Response testing will be performed on a regular basis to see if any return (or additional loss) of sensory function occurs.

25. (U) 8010-8109. The technique of sensory nerve root rhizotomy was developed to produce sensory denervation to one hind limb. The technique allows direct surgical approach to the dorsal roots of the lumbar spinal nerves. Neurophysiological monitoring techniques to evaluate sensory evoked potential of the normal and operated sides are still under development. To this point in the study, we have not absolutely confirmed total sensory denervation of the hind limb.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 102 The Effects of Sensory Denervation In the Care and Management of Traumatic Wounds

The following investigations have been done under this work unit:

STUDY NO. 3 Measurement of sensory-evoked potentials in the pig.

Measurements of sensory-evoked potentials in pigs were performed using a nerve stimulator and a signal average computer. Sciatic and tibial nerves were exposed surgically in anesthetized pigs, and evoked potentials were recorded along the proximal portion of the isolated nerves, at the dorsal spinal nerve roots, and along the lumbar and thoracic spine. The evoked potentials were measured both before and after severance of the dorsal nerve roots. The majority of evoked response was believed to be muscle contraction artifact. A conclusive method to determine sensory denervation is still under investigation.

BODY OF REPORT

WORK UNIT NO.	102	The Effects of Sensory Denervation in the Care and Management of Traumatic Wounds
STUDY NO.	3	Measurement of sensory-evoked potentials in the pig

PROBLEM

The concept of pain, our ability to measure it, our ability to block it, and the effects of pain on wound healing are all crucial to these studies. If sensory innervation to a traumatized area could be blocked completely, would the physiology of wound metabolism be altered?

Pain response in animals is determined routinely with a standard neurologic evaluation. Unfortunately, pigs are not amenable to routine diagnostic neurological examinations as are other animals, such as dogs and cats. Pigs quickly become less cooperative and more aggressive toward handling and restraint unless they are tranquilized or sedated. A more reliable technique to evaluate pain might be the measurement of sensory-evoked potentials. This technique involves electrically stimulating a peripheral nerve and then recording the evoked response more centrally, i.e., on the spinal cord or brain. The sensory-evoked response can be produced in any peripheral nerve or anatomic structure supplied by a particular peripheral nerve with a sensory component. (This method should help in the evaluation of pain response with more certainty, but alone cannot provide absolute proof of sensory denervation.)

RESULTS AND DISCUSSION OF RESULTS

Limited work was completed during the past year due to the PCS transfer of the principal investigator. Nine pigs underwent anesthesia and surgical exposure of the tibial and sciatic nerves in one hind leg, and stimulating electrodes were placed in these nerves. Recording electrodes were placed proximally in the isolated nerves at the level of the dorsal spinal nerve roots and at various levels along the lumbar and thoracic spine. Based on recommendations from a clinical veterinary neurologist at the University of California, Davis, recording electrodes were placed percutaneously near the lumbar spinal cord. Electrical stimulation was applied peripherally, before and after severance of the dorsal nerve roots, and the measured sensory-evoked potentials were compared for the two situations at each recording level. In some, but not all of the experiments, the evoked

response was diminished after cutting the nerve roots. However, in none of the cases was the evoked response eliminated completely. Careful reevaluation of the sensory-evoked potentials from all previous studies suggests that most of what had been measured was muscle contraction artifact. Therefore, a conclusive method to determine sensory denervation has not been developed.

CONCLUSIONS

Further study is needed to develop more accurate methods of measuring sensory-evoked potentials. Complete sensory denervation of the hind limb has not yet been accomplished. Techniques used previously in this study will be reviewed, but different methods for sensory denervation and for evaluation of evoked response will be considered.

RECOMMENDATIONS

Investigation of surgical denervation of the pig hind limb should continue. Efforts also should continue to perfect measurement of sensory-evoked potentials from the spinal cord. New studies should be conducted to identify temporary regional neural blocking techniques. Consideration will be given to spinal catheters and administration of various chemical agents. In parallel to such studies, we believe that sensory denervation might best be measured through psychologic training techniques, i.e., classic conditioning. Consideration should also be given, not only to complete sensory denervation, but to certain other methods of pain control such as transcutaneous nerve stimulation.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG3431	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	058 APC EL11
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) An athymic nude mouse-grafted human skin model.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012900 Physiology; 007900 Indust (occupational) medicine; 017100 Weapons effects

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 06	82 01	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	1.4	137
FISCAL YEAR CURRENT 82	1.3	138

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Laboratory Animals; (U) Dermal;
(U) Nude Mouse; (U) Skin Permeability; (U) Percutaneous Penetration; (U) Xenograft

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The nude (nu/nu) mouse-grafted human skin model was established at LAIR and is being evaluated for its usefulness as a tool for investigating skin permeability, the physiology of dermal penetration, and mechanisms involved in sustaining or preventing injury to skin and in healing injured skin. This model will be particularly useful for investigating a problem like medical defense against chemical warfare agents, which has no civilian counterpart and which entails hazards that preclude use of human volunteers as investigational subjects.

24. (U) A colony of nude mice was established at LAIR. An investigator with established expertise in using the nude mouse-grafted human skin model was obtained on a mobility assignment for 9-12 months under the Intergovernmental Personnel Act of 1970 to supervise and participate in the investigation and teach the LAIR staff how to perform the grafting procedures and successfully maintain and use the experimental animals. The dermal penetration studies are performed using modifications of procedures that have been used already with man and laboratory animals.

25. (U) 80 10 - 81 09. An actively breeding colony of nude (nu/nu) and BALBc/nu mice has been established. Techniques for successfully grafting split-thickness human and pig skin have been acquired and refined. Full thickness eyelid skin has been successfully grafted with retention of sebaceous glands. A continuing source for human surgical and autopsy skin has been established. Animals with pig and human grafts have been used to study dermal penetration of several compounds. Results of these studies are reported under DAOG 2348. Nine animals with established human grafts were sent to the U.S. Army Medical Research Institute for Chemical Defense for evaluation of their response to vesicants.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A1611101A91C	In-house Laboratory Independent Research
WORK UNIT NO.	058	An Athymic Nude Mouse - Grafted Human Skin Model

The possible use of chemical weapons in future conflicts makes it imperative that the U.S. Armed Forces be prepared with prophylactic, decontamination, and therapeutic regimens to minimize the impact of such weapons on operations. In order to evaluate proposed treatment regimens, a clear understanding of the physiology of the skin and of dermal penetration is necessary. A model with extraordinary potential has been developed to explore basic skin physiology, the effects of chemical agents, and the efficacy of various treatment modalities. A breeding colony of athymic nude (nu/nu) mice which provides a sufficient number of experimental animals for ongoing studies has been established by the Division of Cutaneous Hazards, LAIR. Mechanisms have been established for the procurement of both human and pig skin samples. The techniques for successfully grafting these skin samples on the mice have been implemented. The grafted model allows the study of dermal physiology and of treatment strategies in living human skin without exposure of human subjects. It also allows the study of chemical agents and related materials whose nature would preclude study in humans. Studies comparing the penetration of substances with varying penetration characteristics have been conducted, and the data so collected using grafts of human and pig skin compared with previously obtained in vitro data. Histologic studies have been used to define and describe the development of grafts on the mice, the effects of various chemical agents on the skin, and the complex manner in which these agents interact with treatment regimens. Alternate methodologies to permit full- and partial-thickness grafts are being explored. Studies using radioisotope labelled materials will provide information on penetration, and on biochemical interactions occurring in the skin. This model will be used to explore the mechanisms by which chemical agents cause injury to the skin, and to evaluate a number of prophylactic, decontamination, and therapeutic regimens. It also allows the study of such unrelated problems as laser burns and disease-bearing insect bites. The use of full- and partial-thickness grafts will allow for studies to determine the role of adnexal structures in the penetration rates of various compounds. The feasibility of establishing pedicle grafts is being examined.

BODY OF REPORT

WORK UNIT NO. 058 An Athymic Nude Mouse - Grafted Human Skin Model

PROBLEM

The probable use of unconventional weapons in future conflicts makes it imperative that U.S. Armed Forces be prepared with: (1) prophylactic, (2) decontamination, and (3) therapeutic regimens to minimize the impact of these weapons on operations. With regard to chemical agents, the nude mouse-human skin grafted model presents potential usefulness to explore all three categories above without the exposure of human subjects to any agent.

RESULTS AND DISCUSSION OF RESULTS

An actively breeding colony of nude (nu/nu) and BALBC/nu mice have been established. Techniques for successfully grafting split-thickness human and pig skin have been acquired and refined. Full-thickness eyelid skin has been successfully grafted with retention of sebaceous glands. A continuing source for human surgical and autopsy skin has been established. Animals with pig and human grafts have been used to study dermal penetration of several compounds. Results of these studies are reported under Work Unit 301, Agency Accession No. DAOG 2348. Nine animals with established human grafts were sent to the U.S. Army Medical Research Institute for Chemical Defense for evaluation of their response to vesicating agents. Histologic studies have been used to define and describe the development of grafts on mice. Preliminary experiments have been conducted on long-term freezing. Alternate methodologies to allow for the grafting of larger and full-thickness grafts are being undertaken. The use of full- and partial-thickness grafts will allow for studies to determine the role of adnexal structures in the penetration rates of various compounds. The feasibility of establishing pedicle grafts is being examined

CONCLUSION

This model can be used to evaluate dermal penetration of compounds. Grafts can be established which are histologically normal and contain some adnexal structures.

RECOMMENDATIONS

The colony will be useful to evaluate a broad range of dermatologic parameters. This model will be used to explore decontamination efficacy and toxicology. The evaluation of dermal lesions can be documented and described by histologic and electron micrographic studies in this model. Proposals are being made to use this model to

evaluate the impact of agent on the biochemical status of the skin which will provide for a data base on which to propose new interdictive and therapeutic regimens.

PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 6278	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NL	X YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	059 APC JL10
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)*

(U) Improved Antidotes for Cyanide Poisoning

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002300 Biochemistry; 003500 Clinical Medicine; 012600 Pharmacology;

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	0.1	02
FISCAL YEAR CURRENT 82	1.2	36

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Cyanide Poisoning; (U) Methemoglobin; (U) Antidote; (U) Chemical Defense; (U) Laboratory Animals

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Currently accepted treatment for cyanide poisoning, a potential battlefield chemical hazard, has associated risks when used under medical supervision. Rapid, self-administration of nitrite and thiosulfate under battlefield conditions will magnify these risks and seriously degrade the safety and efficacy of this antidote combination. This effort explores alternative approaches for treating cyanide poisoning, particularly those offering increased margins of safety in a potential mass casualty situation. An additional objective will be to devise antidotes that can be used prophylactically. The overall objective is to minimize combat casualties in the event of cyanide exposure and to reduce any potential adversary's tactical advantage from dispersing cyanide.

24. (U) A rat model for cyanide poisoning will be developed using a gavage technique and continuous measurement of total oxygen consumption. Analysis of toxicity will be derived from measures of oxygen consumption and mortality. Exogenous methemoglobin will be administered at various times pre- and post-challenge with cyanide. Measured values for experimental groups will be compared with controls.

25. (U) 80 10 - 81 09 The title and approach to this work unit has been changed because of assignment of a new principal investigator. The research will be performed in the Division of Blood Research at LAIR. Preliminary experiments to develop a rat model for cyanide poisoning have been successfully completed and work to test the effects of exogenous methemoglobin has been initiated.

*Available to contractors upon originator's approval

DD, FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 059 — Improved Antidotes for Cyanide Poisoning

The following investigations have been conducted under this work unit:

STUDY NO. 1 Evaluation of exogenous methemoglobin for cyanide poisoning

Cyanide poisoning can be successfully treated with nitrite and thiosulfate, but the use of this approach, particularly when repeated doses are required, may lead to a dangerous lowering of cardiac output and tissue perfusion, as well as a pronounced decrease in blood-oxygen carrying capacity. Successful treatment, therefore, requires professional medical attention that is unlikely to be available in case of mass casualties or with isolated battlefield conditions. It is desirable, consequently, to improve the safety of antidotes used for cyanide poisoning when rapid, self-administration by inexperienced personnel is required. It is further desirable to provide a prophylaxis for protecting troops against the potential hazard of cyanide poisoning during hostilities. The present study seeks to evaluate the feasibility of providing exogenous pre-formed methemoglobin as a protective agent for cyanide poisoning, thus precluding the necessity of administering nitrite for producing methemoglobin from hemoglobin in vivo. Because exogenously provided methemoglobin will circulate in the plasma, it may provide a more efficient means of scavenging cyanide than does methemoglobin produced in circulating red cells from nitrate administration. Also, exogenous methemoglobin can dissociate into dimer and monomer states that can be excreted by the kidney and thus avoid the need for thiosulfate. Preliminary experiments to develop a rat model for cyanide poisoning have been completed and work to test the effects of exogenous methemoglobin has been initiated.

BODY OF REPORT

WORK UNIT NO. 059 Improved Antidotes for Cyanide Poisoning

PROBLEM

Although cyanide poisoning can be treated wih nitrite and thiosulfate, the use of this approach is not without some risks. Nitrites lower blood pressure, decrease heart rate, and reduce blood-oxygen carrying capacity. Effective release of cytochrome oxidase inhibition may require repeated doses of nitrite, leading to a dangerous lowering of cardiac output and tissue perfusion as well as a pronounced decrease in oxygen carrying capacity. In effect, the treatment substitutes one form of hypoxia (cytotoxic) with others (stagnant and anemic). Consequently, successful treatment requires a degree of judgment, experience and professional attention that is unlikely to be available under circumstances involving mass casualties or isolated battlefield conditions.

To overcome these deficiencies in the current therapeutic approach to cyanide poisoning, several alternatives have been suggested. For instance, cyanide can combine with the cobalt of vitamin B_{12}. This latter substance has received limited use in the treatment of cyanide poisoning, particularly that resulting from nitroprusside administration. It is also theoretically possible that sufficient stimulation of the rhodanese mechanism (which converts cyanide to thiocyanate, the latter being non-toxic and easily excreted) would provide the capability of quickly metabolizing large amounts of cyanide through this widely occurring but normally slow-acting biologic process. Neither of these potential alternative approaches has been widely studied but they illustrate the continuing effort to improve upon the treatment of cyanide poisoning.

The present study is designed to test the idea that methemoglobin could be supplied exogenously as an effective treatment for cyanide poisoning, thus avoiding the necessity of producing this material endogenously through administration of nitrites.

Theoretically, exogenous methemoglobin should provide the same competitive binding site for cyanide attached to cytochrome oxidase as that provided by endogenous methemoglobin; the undesirable effects of nitrites, however, would be avoided. Existence of the material in plasma rather than within erythrocytes would isolate it from the effects of methemoglobin reductase, conceivably prolonging the detoxifying action of a

Improved Antidotes for Cyanide Poisoning (Cont)

CONCLUSIONS

The model appears adequate for testing the efficacy of exogenous methemoglobin for cyanide exposure and those tests are presently underway.

RECOMMENDATIONS

The studies should be completed as originally planned.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 6276	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	060 APC NL05
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)*

(U) In Vitro Cell Toxicology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

016800 Toxicology; 005900 Environmental Biology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 01	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	0.8	101
FISCAL YEAR CURRENT 82	2.1	80

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Not Applicable

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NAME: McGown, E., DAC POC:DA

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Toxicity testing; (U) Chemicals; (U) Munitions manufacture; (U) Primary respiratory defense mechanism; (U) Cell and Organ Culture

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Occupational exposure to chemicals via inhalation occurs in military personnel in training and combat, and in civilian workers during manufacture of munitions and other military materials. Toxicity testing of inhaled materials using arimals is expensive and technically difficult. Rapid economical methods for initial toxicity screening of inhaled materials are needed. Since mucociliary clearance and alveolar macrophage activity are the primary respiratory defense mechanisms, in vitro systems using these cells and tissues may prove valuable.

24. (U) Techniques for using tracheal organ cultures and alveolar macrophages as in vitro toxicologic screening procedures will be developed and evaluated. Using these techniques, effects of known respiratory toxins will be compared with those of chemicals encountered in the military environment. Mechanisms of toxic cellular injury will be sought.

25. (U) 8010-8109. Viable tracheal organ cultures can be maintained for at least one month and cilia, which can serve as a useful index of functional ability, can be observed readily. Preliminary studies indicate that histologic and ultrastructural characteristics are sensitive indicators of non-optimal cultural conditions and, therefore, have potential to indicate subtle toxic effects. Phagocytic and microbicidal assays have been developed for alveolar macrophages and a commercial line of macrophages. These assays were used to test the toxic effect of nitrate ion in the two types of macrophages.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	060	In Vitro Cell Toxicology

The following investigations have been conducted under this work unit:

PILOT STUDY	Establishment and maintenance of hamster tracheal epithelium *in vitro*
STUDY NO. 1	Development and evaluation of *in vitro* assays for toxic compounds using pulmonary alveolar assays

PILOT STUDY. To determine the usefulness of hamster tracheal organ cultures in toxicologic assay systems, multiple series of tracheal rings were harvested and then studied by light and electron microscopy. The results indicated that ciliary motility was a useful index of explant functional ability and that histologic and ultrastructural alterations were reliable markers of *in vitro* toxicity. Although additional studies on the optimization of culture media and morphologic characterization need to be performed, results suggest that hamster tracheal organ cultures have considerable potential as sensitive indicators of toxic conditions.

STUDY 1. *In vitro* screening procedures are needed to detect toxicologic effects of airborne chemicals. One potentially useful system involves assays of alveolar macrophage function. We have established assays of phagocytic and bactericidal capabilities using rabbit alveolar macrophages and RAW 264 CELLS, a macrophage cell line. Current efforts are directed toward optimizing the systems, gathering data on reproductibility, and investigating effects of known toxic agents.

BODY OF REPORT

WORK UNIT NO. 060 In Vitro Cell Toxicology

PILOT STUDY Establishment and maintenance of hamster tracheal epithelium in vitro

PROBLEM

Occupational exposure to chemicals via inhalation occurs in military personnel in training and combat, and in civilian workers during manufacture of munitions and other military materials. Conventional inhalation toxicity testing using animal exposure is time-consuming, expensive, and technically difficult. Rapid, economical methods for the initial toxicity screening of inhaled materials and systems for defining biomolecular mechanisms by which chemicals exert their toxic effects are needed for developing effective antidotes, prophylactics, and chemotherapeutics.

An alternative to conventional animal testing methods is the use of tissue and organ culture techniques to assay the toxic potential of suspect agents. Culture systems are easily controlled and relatively inexpensive, but their usefulness in toxicology screening has not been well established. Therefore, it was the purpose of this pilot study to investigate the utility of one such system, hamster tracheal epithelium in organ culture.

This tissue was of interest because the mucociliary clearance mechanism of the tracheal epithelium is a principal defense system against inhalation of toxic materials. Any compromise of its functional abilities would have serious consequences for the organism as a whole. Therefore, we initiated studies on methods for harvesting, maintaining, and characterizing hamster tracheal organ cultures.

RESULTS AND DISCUSSION OF RESULTS

Tracheas from young adult Syrian hamsters were excised, sliced into thin rings, placed in various tissue culture media, and incubated at 37 C in a 5% CO_2 atmosphere for two to three weeks. Living cultures were regularly examined with an inverted light microscope for the continued presence of ciliary beating and periodically fixed with glutaraldehyde for light and electron microscopy. Preliminary data indicated that viable tracheal organ cultures could be maintained for at least one month and that ciliary motility could be observed readily and should serve as a useful index of the functional ability of the explant. The percentage of luminal cells that were ciliated could be

ascertained easily by microscopic examination of 1 um plastic-embedded sections and appeared to be a sensitive indicator of non-optimal conditions of culture. With the culture media employed, however, normal columnar morphology of the epithelium could not be maintained for more than a few days, and stratified squamous metaplasia was routinely observed. Preliminary studies indicated that these degenerative changes could be forestalled by the presence of retinyl acetate or by reducing the percentage of serum in the culture medium.

CONCLUSIONS

Hamster tracheal organ cultures can be harvested readily but require special attention for maintenance of morphologic normality. Their sensitivity to non-optimal conditions of culture and their compatibility with morphologic studies suggest that they have considerable potential as indicators of toxicity *in vitro*.

RECOMMENDATIONS

Additional studies should be undertaken to optimize the culture medium and characterize explant morphology by scanning and transmission electron microscopy. A known toxin should then be added to the medium and assayed for its effect upon epithelial structure and function *in vitro*.

PUBLICATIONS

None.

In Vitro Cell Toxicology (Continued)

STUDY NO. 1 Development and evaluation of in vitro assays for toxic compounds using pulmonary alveolar macrophages

PROBLEM

Inhalation toxicology studies with animals are extremely expensive and time consuming. There is an urgent need for sensitive, reliable, in vitro screening procedures to detect toxicologic effects of airborne chemicals. The purpose of this study was to establish and standardize in vitro assays for phagocytic and microbicidal capabilities of alveolar macrophages. These assays will then be evaluated for their ability to predict toxic effects in vivo.

RESULTS AND DISCUSSION OF RESULTS

The first procedure to be examined was that of Simpson et al (J Immun 29:221, 1979). In this assay, macrophages are challenged by Saccharomyces cerevisiae in the presence of methylene blue. Phagocytosis is measured by counting microscopically the number of ingested organisms. The intracellular bactericidal rate is assessed by comparing the number of organisms that took up the dye (dead organisms) with the number that excluded it. We found the phagocytic capacity (#yeast engulfed/100 macrophages) of (rat) alveolar macrophages to be approximately 250 after 1-hour incubation. The killing rate was 10-15%. Although these results are similar to those reported by Simpson et al, we are not satisfied with this assay procedure. Accurate counts are technically difficult, especially in those macrophages that contain more than three yeast cells. In addition, we found that methylene blue caused a 40% loss in viability in the macrophages themselves. Because of these drawbacks, we have abandoned the above procedure as a potential assay for use in in vitro toxicity testing.

The second assay system was similar to that of Mandell (Infect Immun 8:337, 1974). In this procedure, macrophages are suspended in an appropriate medium and challenged with Staphylococcus epidermis. After incubation for specified intervals, macrophages and bacteria are separated by differential centrifugation. Phagocytosis and bactericidal capabilities are then assessed by standard pour-plate techniques. We have applied these techinques to both rabbit pulmonary alveolar macrophages and RAW 264 CELLS (a well-characterized murine lymphoma cell line). Under conditions where alveolar macrophages killed 50% of the bacterial challenge, the killing rate of the RAW 264 cells was 95%. The reason for the lower bactericidal capability of the aveolar macrophages in not known. Possible explanations include: 1) inherent differences between cell types; 2) nonhomogeneity of the alveolar macrophage population; and 3) additional (unknown) factors in the medium required by the alveolar macrophages.

CONCLUSIONS

Assays for phagocytic and bactericidal capabilities of macrophages have been established using both pulmonary alveolar macrophages and an established macrophage cell line. They are now ready for further study as to their response to potential toxic agents.

RECOMMENDATIONS

Recommend that the above assays be explored further to gather data on their reproducibility and responses to known toxicants. Recommend also that the alveolar system be studied to determine if the in vitro bactericidal capability can be increased by changes in (optimization of) the assay medium.

PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 2375	80 10 05	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTC ACCESS	9. LEVEL OF SUM
80 10 01	H.TERMINATION	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	081 APC FL03
b. CONTRIBUTING	61102A	3M161102BS02	00	063
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Prevention and Treatment of Battlefield Infections

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10		DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	2.3	53
FISCAL YEAR CURRENT 81	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

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21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Skin; (U) Cutaneous; (U) Infection; (U) Immunity; (U) Iron; (U) Disease; (U) Models; (U) Battlefield; (U) Casualty; (U) Dermal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) New weapons systems, new options for combat casualty management, and alterations in battlefield evacuation times for casualties may alter the course and the hazards of battlefield infections. Studies are needed to assess the degrees of risk and types of infection likely to occur with different kinds of battlefield injuries or battlefield casualty management techniques and, where appropriate, to develop prophylatic measures to limit incidence and severity of those infections.

24. (U) Functional immune profiles will be used in animal models selected for each study on the basis of compatability of responses with those known to occur in humans. Animal models will be used to assess hazards of infections and efficacy of proposed preventive measures with major traumatic and minor wounds. Risk-benefit assessments will be made considering the type of injury, treatment or intended prophylatic measure.

25. (U) These studies were intended to provide information to aid in risk-benefit analysis of new combat casualty management options being developed in other research divisions at LAIR. Since the support programs will not need this information very soon, and resources are now quite limited these studies are being halted temporarily. Also, it has been determined that support of this type when resumed will be more effective and responsive if the people required are assigned directly to the division having responsibility for the support program.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M161102BS02 Mechanics of Recovery from Injury

WORK UNIT NO. 063 Prevention and Treatment of Battlefield Infections

The following investigations have been conducted under this work unit:

STUDY NO. 1 Establishment of the methods and baseline data required to conduct a normal host immune profile

EX-1 Establishment of the capability to perform a host immune profile in Sprague-Dawley rats

STUDY NO. 2 Care and management of contaminated and infected wounds in the combat soldier

EX-1 Development of an animal model for study of wound contamination and infection

STUDY NO. 1, EX-1. Histologic specimens were collected from 10 normal rats. Chemotactic assays were performed on mononuclear cell preparations from 25 rats. Zymosan-treated rat sera, Escherichia coli culture filtrate and lipopolysaccharide failed to demonstrate significant chemotactic activity. Smears of the cell preparations demonstrated the presence of 83±7% lymphocytes and 17±7% monocytes. Nonspecific esterase stains failed to demonstrate esterase activity in any of the cell preparations.

STUDY NO. 2, EX-2. A teflon wound window was fabricated to provide a transparent access port to study growth of bacteria in experimental wounds. This window was implanted into 2 New Zealand White rabbits to see how the animals tolerated the device. One rabbit developed a spontaneous Pseudomonas fluorescens infection while the other developed a mixed infection following implantation of the device. Modification of the window in-house was not possible due to fiscal constraints and lack of manpower. The project was terminated in response to new mission guidelines.

BODY OF REPORT

WORK UNIT NO.	063	Prevention and Treatment of Battlefield Infections
STUDY NO.	1	Establishment of the methods and baseline data required to conduct a normal host immune profile.
EX-1		Establishment of the capability to perform a host immune profile in Sprague-Dawley rats

PROBLEM

The potential compromise of the soldier's immunity as a result of massive blood loss or resuscitation may increase short or long-term susceptibility to infection and may have major impact on patient management and the time required for soldiers to return to duty. Studies in animals may help identify those areas in which compromise of the immune system can be expected and may indicate management procedures or therapy to minimize the impact of such compromises. Efforts were undertaken to establish appropriate techniques for studying the immune cell functions in rats.

RESULTS AND DISCUSSION OF RESULTS

Histological specimens (liver, spleen, and thymus) were collected from ten rats and evaluated by a veterinary pathologist. All were assessed to be normal. Blood was collected from twenty-five rats and layered over Ficoll-hypaque. Mononuclear cells were collected from the Ficoll-hypaque-blood interface after centrifugation. The cells were suspended at 5×10^6/ml and used in the chemotactic assay. Several substances were used as chemotactants: Zymosan-treated sera (from two separate pools of normal rat serum), filtrate from E. coli cultures, and Salmonella lipopolysaccharide. Smears were prepared and examined after staining with Wright's stain and with nonspecific esterase stain. No consistent increase in the number of mononuclear cells migrating in the presence of any of these substances was seen. Nonspecific esterase stains failed to show esterase activity (α-naphthyl butyrate) in any of the cell populations tested. However, the Wright's stains demonstrated the presence of $17 \pm 7\%$ monocytes in these preparations, and the hemotoxylin stains of the chemotactic filters clearly demonstrated the presence of sufficient numbers of monocytes. The remainder of the cells ($83 \pm 7\%$) were lymphocytes. Large numbers of platelets were present in all preparations.

CONCLUSIONS

Lymphocytes and monocytes can be collected successfully from the blood of rats by layering over Ficoll-hypaque. Zymosan-treated rat sera, E. coli culture filtrate and Salmonella lipopolysaccharide are not good chemotactants for rat monocytes.

RECOMMENDATIONS

Dextran sedimentation of the blood before Ficoll-hypaque separation should lead to high harvests of monocytes. Other stimulants of chemotactic activity should be tried and lymphocyte function evaluated.

PUBLICATIONS

None

STUDY NO. 2	Care and management of contaminated and infected wounds in the combat soldier
EX-1	Development of an animal model for study of wound contamination and infection

PROBLEM

An animal wound model is needed to develop improved methods for treatment in combat casualties. This model should have the following characteristics:

- The animal should respond to wound infection in a manner which would allow the information gathered to be applied to human wound infection.

- A standard wound should be easily created, require a minimum of surgical equipment, and not require prolonged anesthesia.

- The wound model should allow the formation of environments conducive to study both aerobic and anaerobic infections.

- The animal model should provide a wound in which other variables important in the course of wound infection may be studied. Some of the variables are presence or absence of necrotic tissue, clotted blood, and foreign materials.

- The model should allow ready assessment of surgical treatment procedures (debridement, lavage, etc.)

- The wound should be accessible to direct visualization and sampling during the course of infection and treatment. (Other wound models currently in use are closed or covered after inoculation of organisms, are not visualized during the course of infection, and require necropsy for evaluation)

In this study a wound window will be developed for the investigation of infections. Attempts will be made to create reproducible wound infections with species of aerobic and anaerobic bacteria usually associated with wound infections in man.

Due to the unique nature of battlefield environments which may be contaminated by biological, chemical, or radiological warfare agents, and because tactical conditions such as lack of air superiority or interdiction of evacuation routes may dictate excessive delays in delivery of definitive treatment to wounded soldiers, some of the new methods that must be considered may be suboptimal and determinations of efficacy will have to be made under conditions approximating those we may expect to encounter on future battlefields. These features make clinical studies unacceptable. As the amount of information that can be derived from in vitro experiments is limited, reproducible wound infection models in animals will be essential to determinations of feasibility, efficacy, and safety during development of new methods for combat casualty management.

RESULTS AND DISCUSSION OF RESULTS

A 4 cm diameter teflon ring was fabricated. This ring had a wide rim to insert under the skin, and a clear top which could be screwed onto the ring. A second ring with teflon screws was available to slide over the first ring to maintain skin position. This ring was inserted into a male New Zealand White rabbit through a skin incision to the left of the dorsal midline behind the scapula. The window fit well and the animal recovered from the general anesthesia without complications.

The window was tolerated well for 3 days. On the 4th day a thickening of the fascia was noted and a creamy white exudate appeared. The reaction grew progressively worse and the animal was sacrificed on the eighth day after surgery. Microbiological culture of the exudate indicated that it contained a pure culture of Pseudomonas fluorescens.

A second rabbit was used to test the window. Within one week of insertion, an exudate developed, although it was not as severe as the one in the first animal.

We feel that the wound window is too heavy and rigid. Additional

fabrication using other materials, such as silastic, in conjunction with testing of the modified devices on normal animals will be needed before any wound infection can be examined. Because LAIR's fabrication facilities are limited, any modification would have to be performed by a private manufacturer. This, coupled with the termination of the wound infection mission in the Division of Cutaneous Hazards, dictates termination of the project.

CONCLUSIONS

Further work is needed to produce an inert wound window for study of wound infections.

RECOMMENDATIONS

This project should be continued in a division at LAIR where investigation of infections can be defended as one of the major problems that must be considered in management of traumatic injuries.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE 6104	80 12 24	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	H. Terminate	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AD	082 APC IL02
b. CONTRIBUTING	61102B	3M161102BS02	00	074
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Long Term Cryopreservation of Platelets for Immediate Field Use

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 012900 Physiology; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
7601	Terminate	DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	5.0	129
FISCAL YEAR CURRENT 81	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Cheney, Barbara A., MS, DAC

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Platelet Storage; (U) Crypreservation; (U) Blood Storage; (U) Massive Transfusion; (U) Platelet Transfusion; (U) Traumatic Hemorrhage

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The need for effective hemostasis in severe combat injuries requires the availability of timely, effective component hemotherapy with coagylation factors and platelets. The former can be provided with relative ease but the latter, being perishable (72 hours storage limit) are logistically difficult to provide to rear line (even CONUS) medical facilities, and even more so to forward resuscitation units. This study is designed to develop and test storage systems whereby effective clinical doses of platelets can be stored, frozen for long periods of time, then easily thawed ready for immediate or delayed transfusion.

24. (U) The objectives of this work are to develop feasible freezing techniques using in vitro and in vivo tests of platelet viability and function to determine storage induced cellular injuries; evaluate existing full size clinical freezing protocols as to military objectives, feasibility and necessary modifications; develop therapeutic dose single unit capability; develop post-thaw suspension medias whereby platelets can be stored beyond 24 hrs; evaluate clinically feasible products in vivo on humans; evaluate in vitro tests of platelet function and viability and correlate to in vivo results to develop a battery of in vitro tests for pre-clinical studies.

25. (U) 7910-8009 a. Clinical trails were performed with human volunteers to evaluate glycerol-glucose cryopreserved platelets. This protocol showed promise as a nowash post-thaw technique but both static rate freezing (N=5) and controlled rate freezing (N=7) had in vivo platelet recoveries below 20%. b. Pheresis harvested platelets were collected and stored for two weeks in one bag. Recoveries (in vitro) were 50% of harvest suggesting storage conditions are not optimal. c. Density separation studies on liquid stored platelets showed cells can be separated according to degree of storage injury and glycoprotein membrane changes can be determined. d. This work units is terminated because the feasibility of using this technique is impractical at this time.

*Available to contractors upon originator's approval.

DD, FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	074	Long-Term Cryopreservation of Platelets for Immediate Field Use

The following investigations have been conducted under this work unit:

STUDY NO. 1	Cryopreservation strategies
STUDY NO. 2	In vitro viability function testing

STUDY NOS. 1 and 2. Severe injury to combat soldiers requires large volume fluid therapy to sustain life. In this setting, clotting factors and platelets are depleted through losses in shed blood, consumption, and dilution due to transfusion, all of which act in combination to impair hemostasis. The hemostatic defects can be corrected with transfusions of plasma (rich in coagulation factors) and platelet concentrates. Since platelets for transfusion must be frozen for long-term storage to meet military logistical requirements, this division addresses practical methods whereby platelets can be easily frozen, stored for long periods, thawed, and made ready for immediate or delayed use. This strategy places emphasis on preparing a one unit therapeutic dose that can be processed with minimal delay after it is thawed. Phase I clinical trials, in conjunction with Letterman Army Medical Center's Clinical Investigation Service, were performed with a freezing protocol (4% glycerol-5% glucose as the cryoprotectant) that fulfilled the military strategy requirements. This evaluation revealed that although the protocol fulfilled logistical needs, the in vivo recoveries were inadequate to fulfill therapeutic needs. Techniques to evaluate platelet storage changes in vitro have been developed in this laboratory and are being correlated with in vivo viability. Transfused platelet recovery can be accurately predicted by these in vitro tests.

BODY OF REPORT

WORK UNIT NO. 074 Long-Term Cryopreservation of Platelets for Immediate Field Use

STUDY NO. 1 Cryopreservation strategies

PROBLEM

Massive transfusion of stored blood or blood substitutes following severe combat injuries leads to impaired hemostasis. This defect aggravates bleeding, and leads to an inability to resuscitate the wounded soldier successfully. The defect is due to many factors: trauma, dilution of blood with resuscitation fluids, and the lack of platelets and coagulation factors in stored blood products. Platelets can be prepared and given in massive transfusion situations to prevent and treat bleeding due to thrombocytopenia. Blood and coagulation factors are relatively easy to obtain and store for massive transfusion needs but platelets stored in conventional liquid storage systems are too perishable (72 hr storage period) for field use. Current freezing schemas for storing platelets are cumbersome and time-consuming. The platelets require extensive washing after thawing to eliminate possible toxic cryopreservatives and the procedures are not field adaptable. This study is aimed at evaluating simple cryopreservation processes in terms of the field adaptability as well as storability for 72 or more hours after thawing.

RESULTS AND DISCUSSION OF RESULTS

A cryopreservation protocol, based on the work of Drs. Pert and Dayian of Albany, New York, has been established. The cryoprotectant in this protocol is 4% glycerol and 5% glucose. Since these compounds are physiological in the final product (1% less than each glycercl and glucose), the procedure does not require extensive processing after the platelets are thawed, it requires only dilution of the platelets with acidified plasma. Tests in our laboratory show this procedure results in a product with acceptable in vitro recovery after freezing. In addition to in vitro studies, a protocol was established with Letterman Army Medical Center's Clinical Investigation Service to evaluate the product of the glycerol-glucose cryopreservation protocol in vivo. Normal volunteers (N=12) were given autologous s thawed platelets labelled with ^{51}chromium. Two freezing techniques for the platelets have been used: controlled rate (33 C/min) and static rate (liquid nitrogen plunge). Those platelets frozen by controlled rate (donors N=5) had in vitro recoveries of 17±9% whereas those frozen by static rate (donors N=7) had in vitro recoveries of 72±9% with in vivo recoveries of 20±4%. Both groups had normal in vivo lifespans (7.2±1.1 versus 8.4±1.7 days). These results show that static rate freezing is better than controlled rate freezing because of higher in vitro recovery. Static rate techniques are simple and adaptable to military needs. Unfortunately, the in vivo recoveries are less than current

Long-Term Cryopreservation of Platelets for Immediate Field Use
(continued)

cryopreservation strategies (using DMSO as the cryoprotectant, recoveries are reported in the literature at 35-40%). Function of previously frozen platelets is currently being evaluated in thrombocytopenic patients. At the present time two non-immunized patients, who have shown good response to previous platelet transfusions, have been given therapeutic doses ($<3.3 \times 10^{11}$) of glycerol-glucose preserved frozen-thawed platelets. One patient developed a fever after transfusion (103 F) but did not have a rise in the platelet count or a shortened bleeding time. These results suggest the frozen platelets were not viable and may not be functional.

CONCLUSIONS

Although the glycerol-glucose protocol fulfills military logistic needs, the in vivo studies do not support a conclusion that the procedure is adaptable for therapeutic needs for thrombocytopenic patients.

RECOMMENDATIONS

The glycerol-glucose procedure, as designed, is inadequate and, therefore, a revision should be made so that further studies address optimization of yields after freezing of the platelets. Unless in vivo yields are greater than 30%, this procedure will not become therapeutically useful. Further investigation into the use of simple platelet harvest from donors and commercially adaptable blood platelet plastic bags (e.g. polyvinyl chloride) will also be needed to optimize military adaptability of glycerol-glucose protocol.

PUBLICATIONS

None

STUDY NO. 2 In vitro viability, function testing

PROBLEM

The development of frozen platelet protocols has had to rely on the ability to evaluate platelets by in vitro parameters. The tests currently available have not been reliable from laboratory to laboratory and have questionable value when the platelets are perturbed by the presence of cryoprotectants.

Long-Term Cryopreservation of Platelets for Immediate Field Use (continued)

RESULTS AND DISCUSSION OF RESULTS

Tests of platelet integrity and autologous function have been performed on platelets frozen, thawed then infused into the donor. Morphology score appears to be the best indicator of in vivo platelet recovery. Actual recovery, in vitro, does not correlate with in vivo results. Osmotic shock recovery was too insensitive a test to evaluate in vivo results. The in vivo recovery was measured by radiolabel techniques (^{51}Cr) in which labelling was done after thawing the platelets. This procedure, as compared to labelling the platelets before freezing may bias the results so that in vivo correlations cannot be accurately made.

CONCLUSIONS

Tests of platelets based on morphology are valid for predicting in vivo recovery.

RECOMMENDATIONS

Morphology tests should be used to evaluate cryopreserved platelets to determine the ability of these cells to tolerate freezing. Multiple variable analysis should be made on all tests to see if in vitro observations can be strengthened.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DAOG 2392	2. DATE OF SUMMARY* 81 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 80 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8A. DISP'N INSTR'N NL	8B. SPECIFIC DATA - CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	241 APC SL01
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)*

(U) Analytical Biochemistry Research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002300 Biochemistry; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
79 09	CONT	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	3.6	259
FISCAL YEAR CURRENT 82	2.3	138

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research

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21. GENERAL USE

Foreign Intelligence Not Applicable

20. PERFORMING ORGANIZATION

NAME:* Letterman Army Institute of Research
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Tillotson, J.A., DAC

NAME: Waring, P.P., DAC POC:DA

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Analytical Biochemistry; (U) Instrumentation; (U) Automated Analyses; (U) Clinical Chemistry

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The objectives are to develop and adapt new concepts in analytical biochemistry to provide reliable and advanced procedures and services to military-oriented research programs at LAIR and, on occasion, to approved cooperating agencies; to develop analytical procedures to meet specific needs for research as, for example, the development of micro-automated assay procedures for enzymes altered during traumatic or stress conditions; to develop procedures applicable to animal models and human subjects in various research programs and field studies.

24. (U) Analytical support will be provided to studies in military medicine requiring routine analyses in volume, or unique equipment and special techniques for assays of physiological specimens obtained during medical research and toxicology projects. Specific analyses will be originated or adapted as required to meet the needs of specific studies and to improve the economy and efficiency of laboratory operations. Research will be conducted on a continuing basis in support of objectives indicated to provide new methods. Whenever feasible and practical, methods will be automated and linked to computer systems.

25. (U) 8010-8109. Nontoxic vehicles were formulated to deliver non-water soluble test compounds to assay systems (SLRL mutagenicity assay and animal toxicity studies). A medium was designed to solubilize and stabilize organophosphinates. High performance liquid chromatographic assays were developed for 2,4-dinitrotoluene and dimercaptopropane sulfonic acid.

* Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO.	3M161102BS10	Research on Military Disease, Injury and Health Hazards
WORK UNIT NO.	241	Analytical Biochemistry Research

The purpose of this work unit is to provide analytical chemistry support for ongoing research projects. This involves setting up, refining, and automating published procedures as well as developing new techniques. Examples of new procedures developed during the past year are 1) formulation of nontoxic vehicles to deliver non-water-soluble test compounds to assay systems (SLRL mutagenicity assay and animal toxicity studies), 2) formulation of a medium to solubilize and stabilize organophosphinates, and 3) development of high performance liquid chromatographic assays for 2,4-dinitrotoluene and dimercaptopropane sulfonic acid.

BODY OF REPORT

WORK UNIT NO. 241 Analytical Biochemistry Research

PROBLEM

Ongoing research projects require analytical chemistry support. For maximum efficiency, analytical procedures must be simplified and automated. For accuracy and significance to the project, procedural quality with regard to target specificity and interferences must be defined and improved. Frequently, analytical procedures must be developed de novo.

RESULTS AND DISCUSSION OF RESULTS

The major research efforts of the Analytical Chemistry Services Group have been in response to problems arising in toxicology studies.

We were tasked with the problem of incorporating several non-water-soluble compounds into a suitable carrier medium so that they could be tested for mutagenicity by the drosophila sex-linked recessive lethal (SLRL) assay. First were several insect repellents. Although these amphiphilic compounds were easily incorporated into liposomes, this carrier was not suitable for the SLRL assay because of the low toxicity of the test compound. The doses became self-limiting because of caloric density resulting from the increasing level of phospholipids. The problem was ultimately resolved by incorporating the test compounds into a commercial lipid emulsion by sonication.

The next compounds examined were organophosphinates. Because of their highly nonpolar nature, attempts to incorporate them into liposomes or emulsions were unsuccessful. However, a satisfactory aqueous medium has now been devised which stabilizes these normally labile compounds and is nontoxic to drosophila. A system has also been devised to monitor the breakdown products. Current efforts are directed toward development and automation of assays for these organophosphinates.

An automated high performance liquid chromatographic (HPLC) procedure was developed for 2,4-dinitrotoluene. The capability includes assay of the compound in tissue and feed extracts. An HPLC method was also developed for dimercaptopropane sulfonic acid, a potential antidote for lewisite. Current efforts are directed toward recovery and analysis of this compound in tissue and body fluid extracts.

Publications this year represent primarily work which was completed during the transition period when nutrition functions were transferred to the Department of Agriculture.

CONCLUSIONS

The formulations of non-toxic vehicles for non-water-soluble compounds are significant advancements. As a result, SLRL tests of insect repellents were successfully completed. The stabilized phosphinate preparations were particularly important and SLRL testing is now ongoing. The information gained concerning chemical properties of the phosphinates will provide a basis for formulation of dosages for animal studies.

RECOMMENDATIONS

Recommend continued efforts to develop new relevant procedures and to automate existing assays.

PUBLICATIONS

1. TILLOTSON, J.A. and R.S. O'CONNOR. Steady-state ascorbate metabolism in the monkey. Am J Clin Nutr 34: 2397-2404, 1981

2. TILLOTSON, J.A. and E.L. MCGOWN. The relationship of the urinary ascorbate metabolites to specific levels of ascorbate supplementation in the monkey. Am J Clin Nutr 34: 2405-2411, 1981

3. MCGOWN, E.L., R.J. O'CONNOR, and J.W. NEHER. Erythrocyte filterability, fragility and membrane proteins in folic acid deficient guinea pigs. J Nutr (in press)

4. LEWIS, C.M., E.L. MCGOWN, M.G. RUSNAK, and H.E. SAUBERLICH. Interactions between folate and ascorbic acid in the guinea pig. J Nutr (in press)

5. OMAYE, S.T., J.A. TILLOTSON, and H.E. SAUBERLICH. Metabolism of L-ascorbic acid in the monkey. Adv Chem Ser (in press)

6. MCGOWN, E.L., C.M. LEWIS, A. ROBLES, P.P. WARING, J.H. SKALA, V. GILDENGORIN, and H.E. SAUBERLICH. Investigation of possible antivitamin B-6 properties in irradiation-sterilized chicken. Institute Report No. 87. San Francisco, California: Letterman Army Institute of Research, June 1981

7. KNUDSEN, J. and E.L. MCGOWN. A computer program to process spectrophotometric analytical data associated with curvilinear absorbance/concentration relationships. Institute Report No. [illegible]. San Francisco, California: Letterman Army Institute of Research, (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 6271	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	242 APC HL01
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with security Classification Code)*

(U) Aspects of Cardiopulmonary Resuscitation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012900 Physiology; 008800 Life Support; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	1.1	64
	CURRENT 82	0.5	33

19. RESPONSIBLE DOD ORGANIZATION

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NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Laboratory Animal;
(U) Cardiac Arrest; (U) Shock; (U) Heart Massage; (U) Blood Flow

23 TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective of the work unit is the development of therapeutic maneuvers, suitable for use on the battlefield, which will increase the effectiveness of cardiopulmonary resuscitation performed on gravely wounded soldiers.

24. (U) A cardiopulmonary resuscitation model using anesthetized pigs has been developed. Cardiac output and regional blood flow are measured with the radiomicrosphere technique. Neurologic status following arrest and resuscitation is assessed by means of a neurologic deficit score.

25. (U) 80 10 - 81 09 Blood flow determinations during cardiopulmonary resuscitation performed in the standard manner as approved by the American Heart Association has been compared to a number of suggested alterations. Only administration of epinephrine was found to improve blood flow to critical organs as compared with standard cardiopulmonary resuscitation. The ability of epinephrine, naloxone, and fructose-1,6-diphosphate to decrease neurologic injury sustained during cardiopulmonary resuscitation is now being determined.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO: 3M161102BS10 Research on Military Disease, Injury, and Health Hazards

WORK UNIT NO: 242 Aspects of Cardiopulmonary Resuscitation

The following investigations have been conducted under this work unit:

STUDY NO. 1 Blood flow during experimental cardiopulmonary resuscitation in pigs

STUDY NO. 2 Use of pharmacologic interventions to improve outcome in cardiopulmonary resuscitation

STUDY NO. 1. The radiomicrosphere technique was used to measure cardiac output (CO) and flow to the myocardium (MQ) and brain (BQ) during cardiopulmonary resuscitation (CPR) in anesthetized pigs. Manual closed chest massage, performed at 60 compressions per minute and maintained for a third of the massage cycle (standard CPR), was compared with six variations: compression phase of the massage cycle doubled in length (PC), 20 mmHg increase in airway pressure (IAWP), abdominal binding (AB), massage rate doubled (MRD), intra-arterial infusion of Ringer's lactate (IAF), and epinephrine 1 mg IV (EP). Four flow measurements were made in each animal. The first measurement was made when the circulation was supported by the beating heart. Following the induction of cardiac arrest by fibrillation, successive measurements were made during standard CPR, variation CPR, and again during standard CPR. Flow data during standard CPR, expressed as the fraction of flow, found when the circulation was supported by the beating heart were: CO-0.27, MQ-0.40, BQ-0.28. Flows were increased by EP: MQ-1.13, BQ-0.66. IAWP, AB, MRD, and IAF had little effect on flow. PC decreased MQ to 0.19, suggesting that inadequate myocardial perfusion may be a consequence of prolonged chest wall compression during CPR.

STUDY NO. 2. A recently completed LAIR protocol (Study 1, Work Unit 242) investigated ways of increasing blood flow during cardiopulmonary resuscitation. It seems reasonable to assume that an increase in flow to critical organs during cardiopulmonary resuscitation will be associated with a more favorable outcome, but proof of this assumption is lacking. Study 2 is designed to determine whether, in fact, ease of resuscitation, restoration of central nervous system function, and short-term survival can be improved both by increasing blood flow and by pharmacologic interventions.

BODY OF REPORT

WORK UNIT NO. 242 — Aspects of Cardiopulmonary Resuscitation

STUDY NO. 1 — Blood flow during experimental cardiopulmonary resuscitation in pigs

PROBLEM

Although cardiopulmonary resuscitation (CPR) has saved many thousands of lives since its introduction in 1960, there is no reason to believe that present techniques are optimal and cannot be improved. In fact, there is presently much interest in trying to increase the effectiveness of closed chest cardiopulmonary resuscitation. Unfortunately, evaluation of the proposed modifications of CPR is based upon indirect indices of blood flow, such as blood pressure. Data do not exist in the literature demonstrating the magnitude of blood flow during CPR. The purpose of this study was to rectify this deficiency.

RESULTS AND DISCUSSION OF RESULTS

Table 1 presents blood flow determinants made during CPR. Standard massage, as recommended by the American Heart Association, was compared to a variety of modifications. Only administration of epinephrine was found to significantly increase blood flow to the heart and brain.

CONCLUSIONS

Administration of epinephrine 1 mg IV should be standard practice during cardiopulmonary resuscitation performed on the battlefield.

RECOMMENDATIONS

The model should be appropriately modified so as to study the effect of putative therapeutic interventions on survival and function of critical organs, such as the heart and brain, post-resuscitation.

PUBLICATIONS

1. BELLAMY, R.F. Blood flow during experimental cardiac massage. (abstract) Circ Shock 8:191, 1981

TABLE 1. Blood Pressure and Flow† During Experimental Cardiopulmonary Resuscitation.

		Massage Rate Doubled	Prolong Compression	Increase Intra-Abdominal Pressure	Increase Airway Pressure	Intra-Arterial Infusion	Epinephrine	Open Chest Massage‡
MEAN AORTIC PRESSURE mmHg	s	31.3±5.7	36.4±3.4	31.6±7.4	27.1±3.7	38.0±3.0	30.4±5.8	23.4±7.4
	v	44.4±7.2*	45.4±2.7*	40.2±9.4	33.6±2.6*	43.2±5.4*	59.6±18.4*	41.2±4.0*
CARDIAC OUTPUT	s	0.26±0.08	0.22±0.07	0.22±0.09	0.36±0.19	0.26±0.07	0.30±0.08	0.24±0.09
	v	0.31±0.15	0.19±0.09	0.09±0.01*	0.50±0.22	0.32±0.07	0.28±0.08	0.17±0.06
LEFT VENTRICLE	s	0.46±0.11	0.40±0.10	0.33±0.19	0.45±0.23	0.39±0.17	0.34±0.11	0.31±0.08
	v	0.65±0.26	0.19±0.08*	0.26±0.10	0.54±0.31	0.37±0.16	1.13±0.59*	0.64±0.13*
BRAIN	s	0.35±0.21	0.36±0.14	0.18±0.10	0.33±0.25	0.22±0.11	0.26±0.14	0.23±0.11
	v	0.30±0.12	0.37±0.17	0.10±0.06	0.30±0.14	0.21±0.12	0.66±0.40*	0.62±0.28*
HEPATIC	s	0.31±0.19	0.19±0.02	0.22±0.09	0.22±0.13	0.18±0.10	0.18±0.14	0.18±0.10
	v	0.27±0.10	0.11±0.06*	0.05±0.02*	0.18±0.10	0.19±0.12	0.27±0.06	0.01±0.02*
KIDNEY	s	0.17±0.04	0.11±0.05	0.14±0.04	0.19±0.07	0.14±0.09	0.17±0.11	0.12±0.05
	v	0.18±0.04	0.13±0.04	0.03±0.01*	0.23±0.16	0.23±0.11	0.06±0.07*	0.02±0.03*

s = standard massage; v = variant massage
Data are given as mean ± one standard deviation.

†Expressed as fraction of flow existing prior to cardiac arrest
‡The variation for open chest massage was occlusion of the descending aorta
*$p<0.05$, Student's t-test for paired measurements

Aspects of Cardiopulmonary Resuscitation (Cont)

STUDY NO. 2 — Use of pharmacologic interventions to improve outcome in cardiopulmonary resuscitation

PROBLEM

Methods are required to optimize survival and to minimize neurologic deficit following battlefield cardiopulmonary resuscitation. The cardiac arrest model developed in Study 1 has been modified and will be used to evaluate the effects of several potentially useful pharmacologic interventions on the ease of resuscitation, restoration of brain function, and short-term survival. Drugs to be tested are epinephrine, naloxone, and fructose-1,6-diphosphate. Comparison of postresuscitation neurologic states will be made among three treatment groups and two control groups. One control group will be animals that will be monitored with instruments but who have not sustained cardiac arrest. The second control group will be monitored by instruments and will undergo a cardiac arrest.

RESULTS AND DISCUSSION OF RESULTS

Work on Study 2 has just begun. Only the first control group has been completed.

CONCLUSIONS

None

RECOMMENDATIONS

Completion of Study 2 is indicated.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE 6078	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CF	245 APC EL09
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:4		

11. TITLE (Procede with Security Classification Code)*

(U) Physiologic Basis of Laser Effects

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

009600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 12	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	7.6	549
FISCAL YEAR CURRENT 82	10.3	542

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE

Foreign Intelligence not Applicable

ASSOCIATE INVESTIGATORS

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22. KEYWORDS (Procede EACH with Security Classification Code) (U) Laboratory Animal; (U) Laser Hazards; (U) Eye Damage; (U) Electrophysiology Test Battery; (U) Laser Systems Safety

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23(U) Evaluate, by ocular test battery and electrophysiologic methods, effects of low-level laser radiation on vision and ocular tissue as may be experienced from laser training simulators or under combat conditions.

24(U) Use a multidisciplinary approach to assess the effects of low-level laser radiation upon the ocular system. Primates are used to correlate low-level change in visual function and Visual Evoked Cortical Potential (VECP) with observed changes in retinal ultrastructure following laser exposures at levels at or above the ED_{50}.

25(U) 8010-8109 Studies with lower vertebrates on the possible mechanism of low-level laser exposures continues to support the notion that non-thermal alterations are directly related to retinal receptor photopigment and neural factors. Laser flash effects were observed immediately as transient events with a duration of 2 seconds. Late changes occurred 90 to 120 seconds after exposure, consisting of delayed effects in amplitude and latency of the VECP. Structural alterations at the fovea consist of an increase in striated rootlets and basal bodies of the pigment epithelium layer following laser exposure.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M161102BS10 Research on Military Disease, Injury and Health Hazards

WORK UNIT NO. 245 Physiologic Basis of Laser Effects

The following investigation has been conducted under this work unit:

STUDY NO. 1	Effects of laser irradiation on visual function
EX-1	Behavioral low-level exposure
EX-2	Morphologic/electrophysiologic study - primate
STUDY NO. 8	Laser flash effects on Rhesus visual function and performance
EX-1	Behavioral effects of single or repetitively pulsed laser exposure on Rhesus spatial vision

The effects of laser exposure on vision and visual processes were assessed in this study unit. Two types of military situations are addressed by this research. The first involves potential hazards from low-level laser exposure in training situations; the second involves the potential hazard from moderate to low-level laser sources on the battlefield. Will exposure that produces marginal morphological retinal change alter critical visual functions such as acuity and contrast sensitivity? We have examined these questions with animal subjects, largely with Rhesus monkeys, although to investigate the effects of laser exposure on basic visual processes lower vertebrates were used. Behavioral and morphological changes (changes in the frequency of occurrence of macular pigment epithelial striated rootlets and basal bodies) in foveal/macular visual function have been found for low-level chronic visible (514 nm) laser exposure. These exposure levels were well below the maximum permissible levels for extended source criteria. Transient changes in acuity and contrast sensitivity to acute visible laser small spot exposure were obtained. These investigations are in continuation with regard to increase in number of animals exposed and variation in visual function measured in animal subjects as well as in more detailed analysis of the morphology of both chronic and acute laser exposure effects on visual function.

BODY OF REPORT

WORK UNIT NO. 245 — Physiologic Basis of Laser Effects

STUDY NO. 1 — Effects of laser irradiation on visual function

PROBLEM

STUDY NOS. 1 & 8. The objective of this research is the evaluation of effects of low-level laser radiation on vision and ocular tissue. Research is conducted with specific military uses of lasers for both training and combat scenarios.

EX-1 — Behavioral low-level exposure

RESULTS AND DISCUSSION OF RESULTS

Two new behavioral animals were started in behavioral paradigm. These animals will be tested for spectral sensitivity with both stationary and moving acuity targets. In both animals, training has progressed to behavioral generalization.

RECOMMENDATIONS

Continue exposure of trained animals for both low-level chronic and acute exposure experiments. Measurements of acuity, spectral and contrast sensitivities, and dynamic acuity need further assessment with respect to exposure conditions presently employed to determine effects of low-level laser exposure on both battlefield and training scenarios.

Develop a personnel laser dosimeter to monitor cumulative laser exposure received by any individual. Such exposure history would dictate the necessity or schedule for individual laser ocular vision examinations or reexaminations. Supplemental AMRDC contract to evaluate effects of low-level prolonged laser exposure on wavelength discrimination should be established. These data will determine to what extent color vision is altered by low-level laser exposure.

EX-2 Morphologic/electrophysiologic study - primate

RESULTS AND DISCUSSION OF RESULTS

In previous work in this project area, we showed that low-level visible laser radiation produced prolonged changes in spectral sensitivity of the Rhesus, using foveal acuity criteria. This year we have been able to correlate these earlier findings with actual structural alterations observed at the fovea in animals that were behaviorally naive but exposed to nearly identical exposure regimens as originally employed where behavioral changes were produced. Foveal changes consisted of an increase in striated rootlets and basal bodies of the pigment epithelium layer. The present observations have not shown changes elsewhere in the retina that are clearly different from eyes that were patched. However, the more peripheral retinal areas have not yet been evaluated for rootlet and basal body changes.

Vertebrate electrophysiologic measurements for single extracellular preparations have continued to support previous data in which changes to low-level laser exposure were obtained. Multiwavelength exposures (514 + 633 nm), compared to equal energy exposures of either 514 or 633 nm alone, have been found to be more effective. These latter findings support the notion that low-level effects are in fact mediated beyond the photoreceptor/photopigment system in the inner neural retinal layers.

CONCLUSIONS

Changes in foveal spectral sensitivity measured behaviorally after low level laser radiation at 514 nm have been correlated with ultrastructural changes in the fovea after comparable low-level exposure. These data, therefore, indicate morphologic as well as previously suggested neural changes. At present it is impossible to indicate how neural and morphologic alterations are associated in producing behavioral change, but resolution of this problem will be a major objective in subsequent experiments.

RECOMMENDATIONS

Continue exposure of trained animals for both low-level chronic and acute exposure experiments. Measurements of acuity, spectral, and contrast sensitivities, and dynamic acuity need further assessment with respect to exposure conditions presently employed to determine effects of low-level laser exposure on both battlefield and training scenarios.

Develop several additional behavioral paradigms for future evaluation of laser operational exposure conditions. One such paradigm would involve Rhesus trained in a laser designator task. Preliminary work in this lab has been completed for such a task. Other important work might involve wavelength discrimination, dark adaptation, and increment spectral sensitivity. Such tasks may be useful in evaluating subtle change in visual function associated with low-level laser exposure. Develop a personnel laser dosimeter to monitor cumulative laser exposure received by any individual. Such exposure history would dictate the necessity for scheduling for individual laser ocular vision examinations or reexaminations.

STUDY NO. 8	Laser flash effects on Rhesus visual function and performance
EX-1	Behavioral effects of single or repetitively pulsed laser exposure on Rhesus spatial vision

RESULTS AND DISCUSSION OF RESULTS

Behavioral techniques for measuring the effects of brief laser flash exposure on contrast sensitivity and visual acuity in Rhesus have been developed. For exposure levels at or near the ED_{50} for retinal burn criteria, measurements of contrast sensitivity and acuity have been made. The preliminary results of these experiments suggest that levels of exposure at ED_{50} can produce transient changes in acuity and contrast sensitivity. With present measures of achromatic (white light) acuity and contrast sensitivity testing, we have only occasionally obtained losses lasting longer than one session. In previous work, we reported that white light measurements were less sensitive in detecting residual retinal alterations (Zwick, Bedell, and Bloom, 1972; Robbins, Zwick, and Hanelin, 1978). It is our expectation that residual visual function losses in spectral function (color vision) may be detected when spectral stimuli are employed in evaluation of transient losses.

CONCLUSIONS

Brief laser flash exposure, as may be expected under battlefield conditions, has been found to produce transient change in visual acuity and contrast sensitivity. While such exposure leads to foveal opacities, little evidence of permanent change has been detected under present test conditions. However, it is expected that detection of residual change in visual function correlating with foveal lesions will be obtained when spectral test conditions are employed.

RECOMMENDATIONS

Develop several additional behavioral paradigms for future evaluation of laser operational exposure conditions. One such paradigm would involve Rhesus trained in a laser designator task. Preliminary work in this lab has been completed for such a task. Other important work might involve wavelength discrimination, dark adaptation, and increment spectral sensitivity. Such tasks may be useful in evaluating subtle change in visual function associated with low-level laser exposure.

PUBLICATIONS

1. ZWICK, H., B.E. STUCK and E.S. BEATRICE, Low-level effects on visual processing. In: ProceedingsofSociety of Photo-Optical Instrumentation Engineers, April 1980

2. SCHUSCHEREBA, S.T., and H. ZWICK, The striated rootlet system of primate rods: A candidate for active photoreceptor alignment. In: Proceedings of Optical Society Meeting on Recent Advances in Vision, PTHA 11, 1980

3. BLOOM, K.R. and H. ZWICK Rhesus spectral dynamic visual acuity. In: Proceedings of Optical Society, Topical Meeting on Recent Advances in Vision, p WA3, 1980

4. ROBBINS, D.O., and H. ZWICK Long wavelength foveal insensitivity in Rhesus monkey. Vision Research 20, No. 11, p 1027-1031, 1980

5. ZWICK, H., B.E. STUCK, AND E.S. BEATRICE Low-level laser effects - long term effects. In: Proceedings of the Human Factors Society, V 24, p 151-156, 1980

6. ROBBINS, D.O., H. ZWICK, and M. HANELIN Changes in spectral acuity following laser irradiation. In: Proceedings of the Human Factors Society, V 24, p 162-166, 1980

7. ZWICK, H., D.O. ROBBINS, K.R. BLOOM, and D.J. LUND. Temporary and residual laser flash effects. In: Proceedings of the Aerospace Medical Association. p 92-93, 1981

8. BLOOM, K.R., and H. ZWICK. Spectral dynamic visual acuity. In: Proceedings of the Aerospace Medical Assoc., p 160-161, 1981

Physiologic Basis of Laser Effects (Cont)

9. ROBBINS, D.O., H. ZWICK, and M. HANELIN. Changes in spectral acuity following laser irradiation (Abstract). Investigative Ophthal Suppl, p 92, 1980

10. STUCK, B.E., G. DEVILLEZ, E.S. BEATRICE, and H. ZWICK. Microscopic evaluation of Rhesus retina after repeated low-level exposure to diffuse argon laser radiation (Abstract). Investigative Ophthal Suppl, p 189, 1980

11. BLOOM, K.R., and H.ZWICK. Rhesus spectral sensitivity for dynamic visual acuity criteria (Abstract). Investigative Ophthal Suppl, p 286, 1980

12. ZWICK, H., D.O. ROBBINS, and A. KNEPP. Effects of multi-wavelength coherent exposure on optic tectal neuronal activity in Pseudemys (Abstract). Investigative Ophthal Suppl, p 80, 1980

13. SCHUSCHEREBA, S.T., H. ZWICK, B. E. STUCK, and E.S. BEATRICE. Macular (foveal) RPE differences after low-level exposure to diffuse argon laser radiation (Abstract). Investigative Ophthal Suppl, p 80, 1981

14. ZWICK, H., D.O. ROBBINS, K.R. BLOOM. and D.J. LUND. Temporary and residual laser flash effects (Abstract). Investigative Ophthal Suppl, p 239, 1981

ABSTRACT

PROJECT NO. 3M161102BS10 Research on Military Disease, Injury and Health Hazards

WORK UNIT NO. 245 Physiologic Basis of Laser Effects

The following investigation has been conducted under this work unit:

STUDY NO. 2 Completed field portable dark adaptometer

Preliminary analysis of the data involved in human volunteers tested with a solid state LAIR LED adaptometer indicated that the red and green LEDs can be used to separate rod and cone functions with a rapid and simple automated technique. Thresholds were obtained without fixation over a non-specified 20-degree retinal area every 1.25 minutes. Results are reproduced as log units of brightness of the specified LED. Basic shapes of these curves are dissimilar and are indicative of the ability of the eye to adapt successfully to less light while perceiving light in the red and the green areas of the spectrum. Spectral dark adaptation measurements in the red region should be more rapid and shallow than measurements obtained in the green area of the spectrum. These measurements are easily made with this adaptometer. The variability obtained with this dark adaptometer is similar to that obtained from conventional devices. Using this dark adaptometer to evaluate patients with known visual abnormalities, results were similar to those obtained with conventional test devices.

BODY OF REPORT

WORK UNIT NO. 245 Physiologic Basis of Laser Effects

STUDY NO. 2 Completed field portable dark adaptometer

PROBLEM

Many of the field exercises conducted within the Army involve extensive night maneuvers. Such maneuvers place large numbers of personnel and millions of dollars of sophisticated weaponry into a combat scenario. No accurate measurements exist to assess the ability of these troops to adapt to low-level light or perform in night operations.

It is estimated that 15% of the "normal" population has some difficulty in altering light sensitivity in darkness. If the military has within its ranks a similar percentage of adaptation problems, there can be any number of leaders who have minimum ability to adapt to low-level light environments. Without actual intent, these individuals may jeopardize the lives of other military and friendly personnel and destroy equipment because either the affected individuals are not aware of this deficiency or cannot compensate for it.

Quantitative measurement of the process of adaptation has traditionally been a complex problem. The technique involves various light sources, filters, optics, and graphic data reduction. The process is a two-step procedure: 1) the visual system must be brought to a standard level of adaptation, and 2) the temporal course of visual threshold in a dark-adapting eye can then be measured over a subsequent time period.

The development of LEDs that function in the long (red) and intermediate (green) spectral regions has made it possible to measure spectral adaptation in a simplified manner.

RESULTS AND DISCUSSION OF RESULTS

Dark adaptation functions for 19 human volunteers were obtained using red and green LEDs. Data were obtained without fixation over a nonspecific 20 degree retinal area. Average threshold values were calculated every 1.25 minutes for 20 minutes. Results were graphically displayed using log units of brightness of the LEDs. The basic shapes of these functions are dissimilar, indicative of the photoreceptors used during the adaptation period.

Results may be summarized as follows:

Physiologic Basis of Laser Effects (Cont)

1. It is expected that dark adaptation measurements in the red region of the spectrum should be more shallow and rapid than measurements made in the green area. This is supported by our research with this device.

2. Traditional dark adaptometry in individuals with peripheral retinal disease reflects greater loss in rod than in cone adaptation. This is supported by mesurements with this device.

3. Variability of this device is similar to that obtained from conventional dark adaptometers.

CONCLUSIONS

It appears that the most routine screening for military assignment requiring night-vision can detect those individuals with severe night vision deficiencies and underlying retinal diseases. Routine use of dark adaptometry has been hampered by the complexity of the procedure and the instrumentation associated with the simplest dark adaptometry mesurement. The field portable, spectrum dark adaptometer eliminates these problems, and the computer automatically displays the data and provides options for measurements.

The associated problem of selecting those individuals who may adapt most rapidly and achieve the lowest thresholds is best approached with this type of automated system. The complexity of determining which night vision functions are essential to night vision performance can best be assessed with a device that offers maximum complexity of visual measurement with maximum variability in experimental design.

RECOMMENDATIONS

A field portable, spectrum adaptometer can greatly aid the applied visual scientist in resolving the present problems of night vision performance. Concurrently, it satisfies a need by the clinician for a device that can be rapidly and easily used to detect night vision deficiencies in the military population, and as a diagnostic tool in treating them.

PUBLICATIONS

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2. O'MARA, P.A., H.J. ZWICK, C.W. VAN SICE, A microcomputer-controlled solid state dark adaptometer. Behavioral Methods and Instrumentation, April 1981

Physiologic Basis of Laser Effects (Cont)

3. O'MARA, P.A., H.J. ZWICK, E.S. BEATRICE, D.J. LUND, Microprocessor controlled light emitting dark adaptometer. Medical Biological Electronics and Computing, March 1981

4. ZWICK, H.J., P.A. O'MARA, E.S. BEATRICE, A solid-state dark adaptometer, the LAIR dark adaptometer. In: NATO/AGARD, October 1980

ABSTRACT

PROJECT NO. 3M161102BS10 Research on Military Disease, Injury and Health Hazards

WORK UNIT NO. 245 Physiologic Basis of Laser Effects

The following investigation has been conducted under this work unit:

STUDY NO. 5 Electrophysiologic evaluation of retinal alterations following laser irradiation

Preliminary analysis of the data from experiments involving determinations of spot size and intensity of laser exposure indicated that little or no measurable change occurred immediately after the foveal exposures when amplitude, latency, phase, and frequency components of the Visual Evoked Cortical Potential (VECPs) were examined and compared to pre-exposure criteria. Specifically, a series of laser exposures at the ED_{50} and at twice the ED_{50} for a 50 micron spot centered on the fovea produced no measurable changes in the VECP at either spatial frequency used. When the grating size was reduced to 1.8 cycles per degree, no immediate effects were seen following a single exposure at twice the ED_{50} with a 500 micron spot. Delayed effects were noted one minute after exposure. Amplitude was reduced and a large phase shift of he main frequency components was observed in the waveform.

The conclusions reached in this series of experiments are:

1. Flash effects of a Q-switched ruby laser aimed directly into the foveal area of the retina were not observed immediately after the exposures, using changes in the VECP as the response criterion.

2. Longer term perturbations in the visual system, as measured by changes in the VECP, occur at time intervals consistent with the development of edema in the retina, but not consistent with visual flash effects.

3. In the light-adapted eye, it may not be possible to measure temporarily impaired (flashblinded) vision involving only the fovea since either the system recovers too rapidly to measure or the effect is not present with discrete nanosecond pulses.

BODY OF REPORT

WORK UNIT NO. 245 — Physiologic Basis of Laser Effects

STUDY NO. 5 — Electrophysiologic evaluation of retinal alterations following laser irradiation

PROBLEM

Laser rangefinders, ground locator-designators, and other devices capable of short, high intensity flashes of light have become operational and are currently being utilized by troops. The effects upon vision of these short (20 nanosecond), small retinal spot size flashes are currently being studied. In order to determine the effects upon vision and subsequent performance, it is necessary to study the physical parameters of the laser together with both the state of the visual system and the available methods for measuring it. Once these effects have been determined and the parameters specified for their production, protective devices or avoidance tactics can be developed.

The Visual Evoked Cortical Potential (VECP) is an electrical response of neural tissue measured at the occipital pole of the skull in response to stimuli presented to the visual system. This response generally reflects the state of the visual system, specifically the ability of neural elements (rods and cones) to transduce photochemical events into an electrical transient at the retina, and transmit these transients to the occipital cortex.

If the central visual area of the retinal macula (foveola and fovea) were affected by laser flashes, these disturbances should yield quantifiable changes in the VECP which could be related to the concept of flashblindness (the short-term, reversible change in visual function following sudden exposure to light at levels greater than the present light adaptional state of the eye).

RESULTS AND DISCUSSION OF RESULTS

The parameters that were studied were: Retinal spot size (50 and 500 microns); stimulus spot size (30 and 3.6 degrees); stimulus bar width (1.6 and 2.8 cycles per degree); and total intraocular energy (TIE) at either the ED_{50} (that amount of energy that produces an ophthalmoscopically visible lesion 50% of the time), or two times this amount ($2ED_{50}$). A series of exposures was made at different combinations of these parameters. The stimulus consisted of an oscillating grating projected onto the retina, centered on the fovea and operating at seven revolutions per second. The grating size subtended 30 degrees on the retina. With the insertion of a circular aperture it could be reduced to a 3.6 degree field.

Physiologic Basis of Laser Effects (Cont)

Cynomologus monkeys were administered a paralytic agent and their respiration was monitored and controlled. A ruby laser, operating at 694.3 μm, provided a 20-nanosecond pulse directed to the center of the fovea.

The results are summarized as follows:

1. No immediate effects were quantifiable when the 30-degree stimulus field, 50-micron spot size at both the ED_{50} and $2xED_{50}$ conditions were presented in a single 20-nanosecond flash.

2. A delayed effect was observed in one animal who received an exposure at $2xED_{50}$ with a 500 μ spot size and the 2.8 cycles/degree oscillating grating with a full 30 degree field of view. This change in the potential occurred 60 seconds after the exposure and consisted of an abrupt phase shift with a decrease in the amplitude of the waveform.

3. In order to stimulate only the central area, an aperture was placed into the fundus camera which produced a 3.6 degree field centered in the fovea. The oscillating grating produced a reduced (50%) amplitude VECP but the phase remained stable. Under this condition, no immediate effects were quantifiable. However, delayed effects occurred in all four animals tested. A single 500 μ exposure at $2xED_{50}$ caused large phase shifts with decreasing amplitude of the P1 wave component at 90 seconds after exposure and lasting 30 to 60 seconds. Following these perturbations, the visual system exhibited increased variability in both the amplitude and phase of the VECPs, and it continued until the experimental session was terminated (up to one hour exposure).

4. The 500 μ spot size, at the ED_{50} for the 3.6 field condition, also showed the phase shift together with a decrease in the amplitude at 120 seconds post exposure. As before, the variability increased following these initial large changes in phase and amplitude.

5. Another Cynomologus was sham-exposed and the VECPs recorded exactly as in the exposure cases. Phase and amplitude remained constant over a 2-hour period.

Analysis of these data is continuing, especially in the 2- to 5-second period immediately preceding and following the laser exposure. Transient changes apparently occurred immediately in response to the flash (within 2 seconds). However, with our present analytical techniques we are unable to quantify these observations. Auto- and cross-correlation techniques, as well as fast Fourier analyses, are being conducted to better isolate both the immediate and delayed effects.

CONCLUSION

Based upon the observations to date, little immediate (within the first 2 to 5 seconds) quantifiable change occurred which could be directly attributed to laser flash effects. In those instances in which an observable lesion (at both ED_{50} and at $2xED_{50}$) was produced, VECP changes were delayed. The changes are consistent with the production of edema within the affected area. Two explanations for these overall findings are offered. First, the Q-switched ruby laser operating at 20 ns does not produce flash blindness as we have defined this phenomenon, either because of the wavelength (red) or the shortness of pulse. Second, the measuring technique (the VECP) may not be sufficiently sensitive for detecting changes when, in fact, they do occur.

RECOMMENDATIONS

A variety of approaches should be attempted to either produce the flash effect or to definitely determine that the effect cannot be induced with the short pulses from the ruby laser. First, the number of pulses should be increased such that a train of pulses can be directed into the fovea. The possibility is high that the effect can be produced and potentiated by more than one pulse in a 250 ns time period.

The second recommendation is to change wavelengths. The sensitivity of the visual system has been shown to increase as the wavelength is decreased from red (6943 μm) to green or blue (argon or frequency-doubled neodymium wavelengths).

The third approach is to maximize the sensitivity of the VECP such that the acuity requirements analogous to the 2.6 cycles per degree condition is increased from 20/200 to approximately 20/20 (approximately 30 cycles per degree).

The fourth recommendation is to examine the VECP response to a stimulus that varies in luminance. To date the grating VECP has measured the response of the visual system to changes in the edges or structure of the stimulus. It is possible that the flash effects would become more apparent when the luminous efficiency of the retina was examined.

PUBLICATIONS

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Physiologic Basis of Laser Effects (Cont)

2. RANDOLPH, D.I., B.E. STUCK, S. WIERZBA, and M.F. SHEA, A technique in evaluating thermal sensitivity at the Rhesus monkey eye and surrounding tissues. Institute Report No. 69. Presidio of San Francisco, California: Letterman Army Institute of Research, February 1981

3. RANDOLPH, D.I., and B.E. STUCK, Sensitivity of the Rhesus monkey cornea and surrounding tissues to CO_2 laser radiation, Aerospace Medical Assoc. Reprints, May 1981

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6100	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	246 APC HL10
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code): (U) Effect of Blood - Oxygen Affinity During Experimental Hemorrhagic Shock and Hypoxemia

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine; 012900 Physiology; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 07	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	1.9	105
	CURRENT 82	1.0	71

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME:
NAME: POC: DA

21. GENERAL USE
Foreign Literature Reviewed

22. KEYWORDS (Precede EACH with Security Classification Code)
(U)Resuscitation Solutions; (U)Experimental Hemorrhagic Shock; (U)Trauma; (U)Blood-gas Transport; (U)Acetylcholinesterase; (U)Lab Animal

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To evaluate relationships between gas transport functions of blood and compensatory physiologic responses to combat trauma, particularly that producing blood loss and cyanosis. To determine safe, effective and practical means of manipulating hemoglobin-oxygen affinity in cases where this would benefit the natural defenses of combat casualties against traumatic injury, chemical agents and disease.

24. (U) Animal models are used to simulate injury encountered in combat casualties, including hemorrhage, burns, fractures, blunt trauma, acetylcholinesterase inhibitors and other noxious agents. Physiologic parameters (heart rate, cardiac output, ventilation, oxygen consumption, regional blood flow, blood and tissue gases, pH, hemoglobin-oxygen affinity, hematocrit, viscosity, oncotic pressure and other pertinent variables) are measured and the status of oxygen transport to tissues evaluated. Hemoglobin-oxygen affinity is manipulated (administration of stored blood and blood substitutes, sodium cyanate, sodium bicarbonate, etc.) to observe the effects of such manipulation on morbidity and mortality.

25. (U) 80 10 - 81 09 When given 2-PAM chloride 30 minutes after challenge, 65 percent of rats with high hemoglobin-oxygen affinity (HOA) survived (24 hours) a lethal dose (4 mg/kg) of diisopropylperfluorophosphate (DFP); with similar treatment, 33 percent of rats with normal HOA survived. High HOA alone significantly increases tolerance to DFP in rats (see LAIR Annual Report, 1980) and the present preliminary results with 2-PAM chloride suggest that substantial protection against DFP may be obtained without use of atropine.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M161102BS10 — Research on Military Disease, Injury and Health Hazards

WORK UNIT NO. 246 — Effect of Blood-Oxygen Affinity During Experimental Hemorrhagic Shock and Hypoxemia

Previous experimental results have indicated that increased hemoglobin-oxygen affinity (HOA) compensates in part for reduced ventilation after cholinesterase inhibition with di-isopropylperfluorophosphate (DFP) and significantly improves survival from doses of DFP that are 100 percent lethal in rats with normal HOA. These results have been extended and confirmed. Additional work has been performed to determine whether 2-PAM chloride would enhance the protective action of increased HOA in rats challenged with lethal doses of DFP. The 2-PAM chloride improved survival at 24 hours after lethal doses of DFP in rats with both normal and increased HOA. The improvement was most pronounced with the increased HOA group, 65 percent of whom survived a lethal challenge of DFP at 24 hours, compared to 25 percent without 2-PAM chloride. The results are preliminary and require additional testing for confirmation.

BODY OF REPORT

WORK UNIT NO. 246

Effect of Blood-Oxygen Affinity During Experimental Hemorrhagic Shock and Hypoxemia

PROBLEM

Future armed conflicts pose the threat of widespread use of anti-cholinesterase poisons, exposure to which can cause respiratory failure and interrupt normal oxygen transport to tissues, producing a fulminating asphyxia and death. Optimum current therapy for this form of poisoning requires the application of assisted breathing and the use of supplemental oxygen as well as alleviation of persistent cholinergic hyperactivity. The latter is accomplished by the injection of atropine and oximes (2-pyridene aldoxime methyl chloride or 2 PAM-chloride). In a combat environment, particularly when confronted with mass casualties, the use of assisted breathing and supplemental oxygen presents obvious and formidable difficulties. Current plans for the medical management of exposure to organophosphorus poison rely upon the distribution of kits for self-injecting the antidote combination of atropine and 2-PAM-chloride. Judging by controlled laboratory experiments with animals, this antidote is expected to reduce the lethal consequences of organophosphorus exposure. However, for obvious reasons, there is no widely published practical experience relating to the efficacy and safety of this drug combination when self-administered by troops in combat. Furthermore, known side-effects, such as mydriasis, could be extremely incapacitating in a combat setting even without organophosphorus exposure. Given the requirement for rapid, self-administration of the antidote, the probability of accidental self-incapacitation is not entirely remote. The present study seeks to provide a better understanding of the pathophysiology of organophosphorus poisoning and to improve upon the medical management of this chemical hazard under combat conditions.

RESULTS AND DISCUSSION OF RESULTS

In the current reporting period, an expanded data base has been developed which conclusively indicates that increased hemoglobin-oxygen affinity (HOA) in rats provides increased tolerance to diisopropylperfluorophosphate (DFP). This result is apparently obtained because of the improved likelihood of saturating hemoglobin at the low alveolar oxygen tensions that are produced by DFP and other organophosphorus poisons. Cyanosis is therefore diminished and tissue oxygenation improved. The effect is generally the same as that produced by assisted ventilation and supplemental oxygen except that it has been accomplished at a different level of the oxygen transport process. To determine to what extent protection could be improved without the use of atropine, 2 PAM-chloride was administered 30 minutes after a lethal DFP challenge of rats having both normal and increased

Effect of Blood-Oxygen Affinity During Experimental Hemorrhagic Shock and Hypoxemia (Cont)

HOA. Both groups demonstrated improved survival at 24 hours compared to untreated controls: 33 percent of rats with normal HOA survived; 65 percent of rats with increased HOA survived (survivals without 2-PAM chloride were 0 percent with normal HOA and 25 percent with increased HOA). These results are preliminary and require additional testing for confirmation. The degree of protection afforded by the relatively late administration of 2-PAM chloride without atropine, however, is of considerable practical interest. Future experiments will address the question of the timing of the 2-PAM chloride injections with regard to tolerance for DFP in rats with increased HOA. These results (and those of others) also imply that cellular energy levels may be a potent factor in modifying an organism's natural ability to combat organophosphorus poisoning. These implications will also be tested in future experiments.

RECOMMENDATIONS

None at the present time.

PUBLICATIONS

None. A manuscript describing these results is currently being prepared.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DAOE 6302	2. DATE OF SUMMARY* 81 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 80 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8a. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	247 APC HL1C
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)*
(U) Response of Muscle to Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine; 002300 Biochemistry; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	0.9	49
	CURRENT 82	1.3	73

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign Intelligence Not Applicable

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Skeletal Muscle; (U) Myoglobin; (U) Metmyoglobin Reductase; (U) Heatstroke; (U) Muscle Injury; (U) Oxygen Util. by Muscle;

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The acutely injured soldier develops negative nitrogen balance and loses muscle mass through unknown mechanisms. One factor that may be involved is myoglobin, a heme-protein that transports oxygen within muscle cells. Myoglobin and its overall metabolic relationships within the muscle cell serve as useful markers in the study of muscle injury. Injured muscle loses myoglobin into the peripheral circulation where it may cause secondary renal damage for unknown reasons. Failure of myoglobin to maintain sufficient intracellular oxygen supply may lead to decreased energy production, weakness, and failure of mechanisms upon which recovery from injury depends.
24. (U) Selected aspects of the effects of injury on muscle will be evaluated. Strategies designed to minimize and/or reverse the detrimental effects of injury on muscle will be determined. The effects of muscle injury on other body systems, including the kidney, will be studied; the effects of various heme-proteins on the kidney will be evaluated. The relationship between myoglobin (and its associated reactions in the muscle cell) and immobilization-induced muscle atrophy, exercise-induced muscle hypertrophy, and recovery from injury will be studied. The effects of cobalt on heme-protein reduction will be investigated.
25. (U) 80 10 - 81 09 The combined influences of dietary iron deficiency and endurance exercise training were evaluated in rapidly growing rats. There were various effects related to iron deficiency and to the training regimen. The influence of iron deficiency appeared predominant in the changes that were seen. Cobalt acetate was found to inhibit both methemoglobin and metmyoglobin reductase activities. This inhibition may be related to the therapeutic efficacy of cobalt salts in cyanide poisoning.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO: 3S162772A874 Care of the Combat Casualty

WORK UNIT NO: 247 The Response of Muscle to Injury

The following investigations have been conducted under this work unit:

STUDY NO 1 Studies concerning the mechanism that controls the redox state of myoglobin

STUDY NO 2 The influence of cobalt on the mechanism that controls the redox state of hemoglobin and myoglobin.

STUDY NO. 1. Studies initiated in FY 79, in collaboration with MAJ E. Wayne Askew MS, were completed. In this, the last of these studies, the combined influences of iron deficiency and endurance exercise training were evaluated in rapidly growing rats. Male rats weighing approximately 130 gm (about 6 weeks of age) were randomly divided into a control or iron-deficient group. Each group underwent a training regimen which ranged from sedentary to a maximum work load on the treadmill (120 min/day, 5 days/wk, 8^{o} incline, 29.5 meters/min); thus some underwent physical training during the induction of dietary iron-deficiency. The control diet contained about 50 mg iron/kg diet, and the iron-deficient diet contained about 6 mg iron/kg diet. Sedentary rats were kept in cages for the duration of the experiment. Trained rats underwent progressive running programs for 12 weeks. At the end of the 12 weeks, biochemical measurements were carried out. The iron-deficient animals had decreased serum iron, liver and muscle iron, and a diminished oxygen-carrying capacity despite normal hemoglobin levels. Skeletal muscle mitochondria demonstrated decreased capacity to oxidize fatty acids and carbohydrates. Iron deficiency decreased the activity of succinic dehydrogenase; the training regimen blunted this decrease. Iron deficiency decreased myoglobin levels in the soleus and red quadriceps, but not in the heart or gastrocnemius. Cytochrome c was decreased in the heart and gastrocnemius, in iron deficiency. Methemoglobin reductase activity was decreased in iron deficiency and was unaffected by the training regimen. The iron deficiency state reduced the physical performance capacity of the animals. The study demonstrates the preferential utilization of iron between and within tissues, the stability of certain iron pools and the lability of others, and the complex interactions of iron deficiency and endurance training. In this study, iron deficiency appeared more important than endurance training in the biochemical changes that were found.

STUDY NO. 2. There has been continued inte ɔst in the therapeutic efficacy of cobalt salts in cyanide poisoning since the late 1800's. Despite a long history of use and widespread investigation, the precise antidotal mechanism of cobalt remains uncertain. Nearly 30 years ago

studies demonstrated the formation of methemoglobin in normal human blood incubated in the presence of cobaltous chloride, and it was concluded that cobalt inhibits the intracellular system that maintains hemoglobin iron in the ferrous state. Since that time, definitive studies in defined systems, using purified enzymes, have not been performed. We found that cobalt ions inhibit the enzymatic reduction of both methemoglobin and metmyoglobin in highly defined in vitro systems. Virtually total in vitro inhibition of the activity of purified methemoglobin and metmyoglobin reductase occurred with the addition of 2.5 mM cobalt acetate to the assay system. Both enzymes were inhibited by lower levels of cobalt in a dose-dependent manner. The similarity in susceptibility to cobalt inhibition is further evidence that the enzymes that reduce methemoglobin and metmyoglobin are functionally similar. The inhibition of methemoglobin reductase may be, in part, responsible for the therapeutic effectiveness of cobalt salts and chelates in cyanide poisoning.

BODY OF REPORT

WORK UNIT NO: 020 — The Response of Muscle to Injury

STUDY NO: 1 — Studies concerning the mechanism that controls the redox state of myoglobin

PROBLEM

Muscle function is often impaired in injured soldiers either directly by the injury or indirectly by immobilization. To facilitate healing and reverse atrophy of muscle, it is necessary to understand the mechanisms involved in exercise-induced hypertrophy and immobilization-induced atrophy of muscle. Muscle is the only tissue that contains myoglobin, the presence of which subserves functions--the precise nature of which remain uncertain. Because myoglobin is a heme-protein, it is presumed that its function, in part, is related to oxygen transport/storage in the muscle cell. It is postulated that myoglobin may be centrally involved in the energy-dependent process of muscle via this function as an intracellular carrier of oxygen.

Myoglobin, like hemoglobin, undergoes freely reversible oxygenation in order to carry out its oxygen transport function. Myoglobin is nearly 20 times more easily oxidized than hemoglobin. The oxidized forms of nemoglobin and myoglobin (methemoglobin and metmyoglobin, respectively) are incapable of carrying the oxygen. The red blood cell possesses several enzymatic mechanisms which maintain hemoglobin in the functional reduced state. We have isolated, purified, and characterized an enzyme (NADH - metmyoglobin reductase) which actively reduces metmyoglobin in vitro.

In a previous study we found that growing rats that had undergone a strenuous 12-week training regimen had increased myoglobin and metmyoglobin reductase activity in selected muscles and a slight, but statistically significant, decrease in hemoglobin levels when compared to sedentary, pair-fed controls. These results indicated that endurance training may influence the patterns of iron utilization, leading to its preferential incorporation into muscle at the expense of the red cell mass. The purpose of the study reported herein was to more thoroughly evaluate the patterns of iron utilization during endurance training. The specific question addressed in the study was whether or not the exercise-induced increase in muscle myoglobin could be diminished or abolished by iron deficiency. These studies were initiated in 1979 in collaboration with MAJ E. Wayne Askew, MS, and the last of the analytical procedures completed during the FY covered by this report.

RESULTS AND DISCUSSION OF RESULTS

Male rats weighing approximately 130 gm (about 6 weeks of age) were randomly assigned to either a control, moderate, or severe iron deficiency diet group. This assignment determined which diet they would receive for the remainder of the study. At the time of assignment to one of the dietary groups, the physical training regimen was also initiated. The animals thus underwent a training regimen during the dietary induction of iron deficiency. The control diet contained about 50 mg of iron/kg diet and the iron deficient about 6 mg of iron/kg diet. The levels of iron intake chosen for this study were based on published estimated requirements for growing rats.

Untrained rats were maintained in a sedentary state in stainless steel cages for the duration of the 12-week experiment. Trained rats underwent a progressive treadmill running program 5 days a week for 12 weeks, at which time the control rats were running 120 min/day at 29.5 meters/min. This training regimen is similar to those that produced certain defined biochemical adaptations to exercise. In addition to pair feeding, the animals were pair exercised to allow the necessary testing of dietary and training effects within dietary groups. Rats were killed after 12 weeks of training. Blood samples were collected, heart, quadriceps, soleus, and gastrocnemius groups were removed, and the following determinations were performed: hemoglobin, hematocrit, blood oxygen affinity, myoglobin, cytochrome c, tissue and serum iron, serum total iron-binding capacity, metmyoglobin reductase, and mitochondrial respiration and P/O ratios with pyruvate-malate as substrates. In addition, several non-heme muscle enzymes were assayed to dissociate possible effects of iron deficiency on general protein synthesis.

There was a major problem with the experimental design of this study which became apparent only after the study was completed. Rats that are about 6 weeks old have accumulated substantial body stores of iron. It is difficult to disturb their iron metabolism despite the imposition of severe dietary iron deficiency. To further complicate the picture, their iron requirements continually fall as they reach maturity. The level of dietary iron intake may have been appropriate at the beginning of the study (i.e., iron intakes were correctly provided to create moderate and severe deficiency), but became increasingly more adequate as iron requirements decreased with age. Despite these confounding problems, enough data were gathered to clearly distinguish the effects of iron deficiency versus those of endurance training.

The iron deficient animals had significantly decreased levels of serum iron, liver iron, and muscle iron. Hemoglobin values and TIBC were not different in the control and iron deficient groups. Despite normal hemoglobin levels, oxygen-carrying capacity was significantly reduced

by the iron deficiency. Iron deficiency decreased myoglobin in the soleus and red quadriceps, but not in the heart or gastrocnemius. Cytochrome c was decreased in the heart and gastrocnemius in the iron deficient animals. Neither myoglobin nor cytochrome c demonstrated changes that could be attributed to the training regimen. In agreement with other studies, methemoglobin reductase activity was increased by iron deficiency.

Skeletal muscle mitochondria demonstrated both decreased fatty acid and carbohydrate oxidation in iron deficiency; however, the decrease was blunted significantly by the training regimen. Dietary iron deficiency severely curtailed the endurance performance of the experimental animals under the conditions of this study.

CONCLUSIONS

There are complex relationships between the metabolism of iron and the age of the animal in which the study occurs. Iron metabolism in the weanling rat is different from iron metabolism in either the growing or adult rat. Such differences were not totally overcome under the conditions of this study and represent major obstacles in any effort to unravel the obscure aspects of iron metabolism. In general, the study demonstrated that iron deficiency and exercise training had variable effects on iron metabolism which underscored the specific hierarchical pattern of iron utilization in various organs and tissues. In general, training effects seemed to be less important than dietary iron deficiency as an explanation for the range of biochemical changes seen. The study clearly demonstrates decreased work performance in iron deficient animals despite the presence of normal hemoglobin values.

RECOMMENDATIONS

This study, initiated in 1979, concludes the nutritional research initiated with the Division of Nutrition Technology. No further investigations along these lines have been initiated. If nutritional research becomes a future concern for the military, studies of iron metabolism versus endurance exercise tolerance may be warranted.

PUBLICATIONS

1. HAGLER, L., R.I. COPPES, Jr., E.W. ASKEW, A.L. HECKER, and R.H. HERMAN. The influence of exercise and diet on myoglobin and metmyoglobin reductase in the rat. J Lab Clin Med 95:222-230, 1980

2. HAGLER, L , E.W. ASKEW, J.R. NEVILLE, P.W. MELLICK, R.I. COPPES, JR., and J.F. LOWDER. Influence of dietary iron deficiency on hemoglobin, myoglobin, their respective reductases, and skeletal muscle mitochondrial respiration. Am J Clin Nutr 34:2169-2177, 1981

3. ASKEW, E.W., L. HAGLER, S. EFSEAFF, J.R. NEVILLE, L.J. ADAMS, and R.I. COPPES, Jr. Dietary iron deficiency and energy metabolism in exercising rats. Fed Proc 40:880, 1981

STUDY NO. 2

The influence of cobalt on the mechanism that controls the redox state of hemoglobin and myoglobin

PROBLEM

There has been continued interest in the therapeutic efficacy of cobalt salts in cyanide poisoning since 1894. Despite a long history of use and widespread investigation, the precise antidotal mechanism of cobalt remains uncertain. In 1954, Shen and co-workers (J Clin Invest 33:560, 1954) demonstrated the formation of methemoglobin in normal human blood incubated in the presence of cobaltous chloride, and concluded that cobalt inhibits the intracellular system that maintains hemoglobin iron in the ferrous state. These studies were performed before any of the putative methemoglobin reductases had been described. In more recent studies of suspensions of intact red blood cells, there was no evidence to suggest that cobalt is a specific inhibitor of methemoglobin reductase; however, specific assays of methemoglobin reductase activity were not performed. Until they are performed, any action or lack of action on methemoglobin reductase activity that is ascribed to cobalt must remain speculative, and it must be clearly distinguished from the evaluation of methemoglobin formation and reduction which occurs in intact red blood cells. Hegesh and Avron (Biochem Biophys Acta 146:91,397, 1967) described an active methemoglobin reductase in the erythrocyte which requires ferrocyanide for in vitro study. We have isolated from muscle a metmyoglobin reductase that actively reduces metmyoglobin in vitro. It is similar to the enzyme described by Hegesh and Avron in that it requires ferrocyanide for in vitro activation, but otherwise appears to be a distinct entity. The isolation of these two met-heme protein-reducing enzymes allows an evaluation of the effect of cobalt on both methemoglobin and metmyoglobin reductase activities. Therefore, we examined the effect of cobalt on the ferrocyanide activated methemoglobin and metmyoglobin reductases and found that cobalt inhibits the activities of these two enzymes in vitro.

RESULTS AND DISCUSSION OF RESULTS

Bovine methemoglobin and metmyoglobin substrates were prepared by methods routinely utilized in this laboratory. Bovine methemoglobin reductase and metmyoglobin reductase were each partially purified (approximately 10-fold) from red blood cells and cardiac muscle, respectively. A 0.1 M solution of cobalt acetate (pH 7.34) was used as the source of the cobalt ion. The enzymatically mediated reduction of methemoglobin and metmyoglobin was measured spectrophotometrically in a

well-defined in vitro system. The addition of cobalt acetate to the assay system decreased the activity of both methemoglobin and metmyoglobin reductase in a dose-dependent manner. Irrespective of the substrate, inhibition was apparent over a narrow range of cobalt concentrations, and was virtually complete when the concentration of cobalt reached 2.5 mM. Because of the small number of experimental observations, the statistical significance of the differences in the degree of inhibition of each of the enzymes against heme protein substrate could not be ascertained. Nevertheless, the responses of the enzymes to the inhibitory effects of cobalt were similar irrespective of whether methemoglobin or metmyoglobin was used as substrate.

Whatever other effects cobalt might exert, it is clear from the results that it does inhibit the enzymatic reduction of methemoglobin in vitro. Cobalt salts and chelates do ionize to variable degrees, and cobalt binds to red cell membranes and penetrates the red cell to bind to hemoglobin. Our data confirm the results of Shen et al demonstrating cobalt inhibition of methemoglobin reduction. It is possible, although unproven, that a level of cobalt sufficient to inhibit methemoglobin reductase activity in intact red cells might result from conventional therapeutic doses.

The physiology and biochemistry of cobalt have been the subjects of extensive investigation. The current understanding of the effects of cobalt includes effects on a number of enzyme systems as measured in vitro. The significance of these in vitro effects in intact organisms are not known, and are currently under investigation. While the significance of cobalt inhibition of methemoglobin and metmyoglobin reductase activity remains unknown, particularly in regard to cyanide poisoning, such inhibition should be added to the known in vitro effects of cobalt.

CONCLUSIONS

Because cobalt compounds tend to form stable complexes, there has been continued interest in the use of the salts and chelates of cobalt in cyanide poisoning, and continued uncertainty about the precise nature of their protective effects. We have found that cobalt ions inhibit the enzymatic reduction of both methemoglobin and metmyoglobin. Virtually total inhibition of methemoglobin and metmyoglobin reductase activity occurred with the addition of 2.5 mM cobalt acetate to the assay system. Both enzymes were inhibited by lower levels of cobalt in a dose-dependent manner. The similarity in susceptibility to cobalt inhibition is further evidence that the enzymes that reduce methemoglobin and metmyoglobin are functionally comparable. The inhibition of methemoglobin reductase may be, in part, responsible for the therapeutic effectiveness of cobalt salts and chelates in cyanide poisoning.

RECOMMENDATIONS

The potentially beneficial effects of cobalt and other cyanide binding materials should continue to be evaluated. Ideally, the development of an agent that could be used prophylactically to prevent cyanide poisoning should be pursued.

PUBLICATIONS

1. HAGLER, L. and R.I. COPPES, Jr. The inhibition of methemoglobin and metmyoglobin reductase by cobalt. Biochem Pharmacol, in press

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 2389	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	248 APC HL07
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Investigating a Circulating Shock Factor of Pancreatic Origin

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002300 Biochemistry; 012600 Pharmacology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	8.0	327
	CURRENT 82	8.2	224

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: POC: DA

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Pancreas; (U) Vascular Monitoring; (U) Kinin; (U) Shock; (U) Pancreatic Shock Factor; (U) Laboratory Animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The pancreas contains hypotensive agents that promote shock in the late stages of low tissue blood flow. The objective was to hemodynamically compare histamine, trypsin, prostacyclin, and glandular kallikrein to a previously isolated and hemodynamically characterized pancreatic shock factor (PSF). Also, enzyme inhibitors were tested for blockade of the PSF-induced shock reaction.

24. (U) The vascular reaction to histamine, trypsin, prostacyclin, and glandular kallikrein will be monitored in a pig and compared to the response of pig PSF. The enzyme inhibitors aprotinin and FOY were used to block PSF either from the pig, dog, and monkey when injected into its own or the other two species.

25. (U) 80 10 - 81 09 Trypsin and histamine do not produce vascular reactions similar to PSF. Glandular kallikrein and prostacyclin have vascular reactions similar to PSF but prostacyclin is not blocked by enzyme inhibitors. Kallikrein is confirmed as a candidate for PSF. PSF exhibits species variability in its inhibition by FOY or aprotinin. Aprotinin is a better inhibitor than FOY but neither can prevent the monkey PSF reaction in the monkey but can block pig or dog PSF in the monkey. These data suggest that an enzme inhibitor that will block monkey PSF in the monkey will be more applicable in human shock, such as in the combat-injured soldier.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO: 3M161102BS10 Research on Military Disease, Injury and Health Hazards

WORK UNIT NO: 248 Investigating a Circulating Shock Factor of Pancreatic Origin

The following investigations have been conducted under this work unit:

STUDY NO. 1 Hemodynamic characterization of a pancreatic shock factor (PSF)

STUDY NO. 2 Isolation and purification of PSF

STUDY NO. 1. The pancreas contains vasoactive substances that exacerbate hypotension after long periods of shock. A pancreatic shock factor (PSF) from the canine pancreas has previously been isolated and shown to be a potent vasodilator and probably a glandular kallikrein. Four vasodilating agents were hemodynamically compared with the vascular reaction of PSF. Glandular kallikrein and prostacyclin produced a vascular reaction similar to PSF but the reactions from trypsin and histamine were not similar. The blocking agents aprotinin and FOY were ineffective in blocking the vascular reaction to monkey or dog PSF when injected into the same species; however, these enzyme inhibitors were effective (aprotinin better than FOY) when crossing species lines (i.e., monkey PSF into the dog). The species variability to enzyme inhibitor blockade indicates that an effective blocker in the monkey against monkey PSF might have therapeutic possibilities in human shock.

STUDY NO. 2. The availability of canine pancreatic tissue was a limiting factor in this column chromatography study and we began using commercially available porcine pancreata. Kilogram quantities of porcine pancreatic tissue were processed and passed through filters, ultracentrifuged, and column chromatographed. Porcine PSF also proved to be a macromolecule. The data are still undergoing tabulation and analysis similar to canine PSF.

BODY OF REPORT

WORK UNIT NO. 248 — Investigating a Circulating Shock Factor of Pancreatic Origin

STUDY NO. 1 — Hemodynamic characterization of a pancreatic shock factor (PSF)

PROBLEM

The pancreas contains vasoactive substances that can exacerbate shock after prolonged low blood flow to the tissues. When resuscitation is delayed in a combat injured soldier, these pancreatic shock factors may prevent salvage of the patient. As described in a previous progress report, we found that the pancreatic shock factor (PSF) was probably an activated glandular kallikrein. PSF acted primarily as a vasodilating substance without myocardial depression. The hypotension associated with the marked vasodilatation was prevented by aprotinin (a kallikrein-binding agent) in the pig, but not in the dog or monkey. Our objective in further studies was to determine if PSF from one animal species would be blocked with antienzyme compounds (aprotinin, FOY) in another animal species. If, for instance, monkey PSF could not be blocked with aprotinin in the monkey, could it be blocked in a pig or dog? The species variability of the blocker in various animals could have important identification, as well as therapeutic, implications. A further objective was to hemodynamically characterize known vasodilating agents and compare them to PSF.

RESULTS AND DISCUSSION OF RESULTS

Aprotinin proved to be a blocker of the PSF shock reaction when pig PSF was injected into the pig, dog, or monkey; when dog PSF was injected into the pig or monkey but not the dog, and when monkey PSF was injected into the pig or dog but not the monkey. These interesting results indicated that other proteins present in dog or monkey serum have a higher affinity for the aprotinin than dog or monkey PSF, respectively. The PSF is liberated from its enzyme-inhibitor complex and is free to be vascularly active.

FOY [ethyl-4-(6-guanidino-hexanoyloxy)-benzoate methanesulfonate], a known inhibitor of kallikrein, was an effective blocker of dog and pig PSF in the monkey only, but was ineffective in blocking monkey PSF in any animal. FOY will prove less useful in the primate model to prevent the shock reaction.

Glandular kallikrein (porcine), trypsin, prostacyclin (PGI_2), and histamine were hemodynamically characterized. Glandular kallikrein and PGI_2 produced vascular reactions similar to PSF. Trypsin and histamine were dissimilar to PSF as they depressed cardiac output. Histamine also exhibited acute tolerance (tachyphylaxis).

Investigating a Circulating Shock Factor of Pancreatic Origin (Cont)

CONCLUSIONS

The pancreas contains a pancreatic shock factor (PSF) which is a glandular kallikrein that lowers systemic resistance in pigs, dogs, and monkeys. The blockade of this vascular reaction with enzyme inhibitors exhibits a species variability not therapeutically conducive in the monkey with aprotinin or FOY. The vascular reactions to trypsin and histamine are not like those produced by PSF. Glandular kallikrein and PGI_2 do have similar reactions, but only kallikrein is known to be blocked by aprotinin.

RECOMMENDATIONS

An enzyme inhibitor should be found that blocks the vascular reaction of monkey PSF in the monkey. Therapeutic intervention in human shock with this inhibitor would then be logical. Other shock factors of pancreatic origin should be tested (hemorrhagic pancreatitis ascites fluid) and compared to PSF and other known hypotensive agents.

PUBLICATIONS

1. TRAVERSO, L.W., and R.R. GOMEZ. Hemodynamic characterization of a canine pancreatic shock factor. Proc Soc Exp Biol Med 168:245-253, 1981

2. GOMEZ, R.R., and L.W. TRAVERSO. Species specificity of Trasylol. (Abstract) Clin Res 29:306A, 1981

STUDY NO. 2 Isolation and purification of PSF

PROBLEM

A canine pancreatic shock factor (PSF) is present in the supernatant of collagenase (bacterial enzyme) digestion of minced pancreatic tissue. The objective of this study is to isolate, purify, and characterize porcine PSF because a commercial porcine source will provide larger quantitites of pancreas than possible with canine sources.

RESULTS AND DISCUSSION OF RESULTS

Porcine PSF was similar to canine PSF. Both canine and porcine PSF are hemodynamically active and are macromolecules, probably proteins. The range of porcine PSF size is between 20,000 and 40,000 Daltons. The agent is present in the supernatant of the homogenate as well as collagenase digested tissue. The enzymatic and physical properties indicated the proteinaceous nature.

Investigating a Circulating Shock Factor of Pancreatic Origin (Cont)

CONCLUSIONS

The pig and dog pancreatic shock factor is a protein between 20,000 and 40,000 Daltons.

RECOMMENDATIONS

The use of antiproteolytic inhibitors of pancreatic acinar cellular enzymes to block shock is indicated.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 2382	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	6.102A	3M161102BS10	EH	249 APC FL08
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:1		

11. TITLE (Precede with Security Classification Code)*
(U) Physiology of Dermal Penetration

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003200 CBR Warfare; 012600 Pharmacology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	4.8	211
FISCAL YEAR CURRENT 82	6.1	231

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
Foreign Intelligence Not Applicable

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NAME: White, Charles T., CPT, MSC, POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Chemical Defense; (U) Skin; (U) Permeability; (U) Physiology; (U) Biochemistry; (U) Pharmacology; (U) Penetration; (U) Laboratory Animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Better understanding of metabolic events in skin before and after injury is necessary for development of safe, effective and rational measures to protect soldiers against environmental hazards and for development of decontamination procedures for casualties incurred in a chemical warfare (CW) environment. The objectives of this line of research are: (1) to determine the mechanisms by which various chemical agents produce aberrations and subsequent tissue damage, and the mechanisms of action of drugs, hormones and other metabolites that may prevent injury, counteract toxic substances, or promote healing; (2) to determine the effects on penetration rates of the physical and chemical properties of the substance, its vehicle, and the skin; and, (3) to determine the events occurring in skin during and subsequent to decontamination.

24. (U) Skin structure and physiology will be correlated with physiology and mechanisms of skin damage and repair. The mechanisms by which nerve agents and vesicants produce physiologic aberration and tissue damage will be investigated, and the mechanisms of action of therapeutic agents, decontaminants and prophylactic substances on skin will be determined.

25. (U) 80 10 - 81 09. Diisopropylfluorophosphate, an organophosphate, enhances steroid and lipid biosynthesis in the skin and other tissues. In contrast, the compound reduces biosynthesis of hepatic and muscle glycogen and of muscle proteins. Paraoxonase activity in the skin is low. The enzyme has four different pH optima in the serum.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO.	3M161102BS10	Research on Military Disease, Injury and Health Hazards
WORK UNIT NO.	249	Physiology of Dermal Penetration

The following investigations have been conducted under this work unit:

STUDY NO. 1 Effects of organophosphate compounds on energy supply systems in the rat

STUDY NO. 2 Effects of hormones on levels of acetylcholinesterase in the skin

STUDY NO. 3 Skin permeability based on chemical structure

STUDY NO. 4 Miliaria and Hypohidrosis

STUDY NO. 5 Effect of organophosphates on paraoxonase in the pig

Studies were conducted to evaluate selective aspects of diisopropylfluorophosphate (DFP), an organophosphate compound, on intermediary metabolism in the rat. DFP administration enhances biosynthesis of steroids and of some lipid components in the skin and other tissues, and reduces liver and muscle glycogen synthesis. In addition, DFP enhances oxidation and incorporation of lysine into muscle proteins. These changes in protein metabolism may be associated with changes in metabolic pools of tissue free lysine.

Brain acetylcholinesterase activity is stimulated by chronic administration of insulin. Insulin has no effect on enzymes in the skin, liver, muscle or in the serum. Glucagon, epinephrine or cortisone did not affect acetylcholinesterase activity.

The enzymatic hydrolysis of paraoxon, an inhibitor of acetylcholinesterase, has been studied in the pig skin and serum. Enzymatic activity in the skin is relatively low. In contrast, the serum enzyme has four different pH optima, ranging from 6.5 to 10.5 and suggesting that perhaps four different enzymes are capable of hydrolyzing organophosphates.

BODY OF REPORT

WORK UNIT NO.	249	Physiology of Dermal Penetration
STUDY NO.	1	Effects of organophosphate compounds on energy supply systems in the rat

PROBLEM

The nicotinic and muscarinic effects of organophosphate toxicity have been adequately described. However, metabolic alterations underlying the signs and symptoms of organophosphate poisoning are sketchy and remain to be defined. A perusal of the literature indicates that organophosphates bind with high affinity to liver microsomes and inhibit hepatic testosterone hydroxylases. These observations suggest that organophosphates are potential inhibitors of steroid metabolism in mammals. In cultured neuroblasts, organophosphate compounds depress the rate of protein synthesis that may be responsible for the degenerative syndrome. After administration of organophosphates, an increase in the activity of plasma beta-glucuronidase has been observed. The increase appears to be liver-dependent. It is evident from the foregoing observations that metabolic changes induced by organophosphates are not fully understood. There is a compelling need for more complete metabolic data relevant to the toxic effects of organophosphates as well as the metabolic factors involved in the protective reactions against such substances. A better understanding of metabolic derangements resulting from organophosphate toxicity will lead to the development of rational and effective protective measures against injurious effects of these compounds. Consequently, this study is concerned with the effects of diisopropylfluorophosphate (an organophosphate) on protein, lipid and carbohydrate metabolism in the rat.

RESULTS AND DICUSSION OF RESULTS

Male rats weighing approximately 200 gm were used in all experiments. The rats were injected intraperitoneally with a solution of DFP (2mg/kg), followed by an injection of one of the metabolic precursors (C^{14}-acetate, C^{14}-glucose, or ^{14}C-lysine). In some experiments, DFP solution was applied topically (7.5 mg/cm^2 skin). Both in vivo and in vitro oxidation rates and incorporation of the various precursors into tissue components were determined.

Compared to the controls, DFP enhanced incorporation of acetate into skin, adipose and hepatic steroids and fatty acids. Oxidation of acetate or incorporation into glycerol was not affected. DFP markedly decreased glucose incorporation into hepatic and muscle glycogen and stimulated lipid synthesis by hepatic and adipose tissue. DFP increased lysine oxidation and lysine incorporation into liver, kidney,

AD-A123 769 LETTERMAN ARMY INSTITUTE OF RESEARCH ANNUAL RESEARCH PROGRESS REPORT FY 1981(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA J D MARSHALL 2/4

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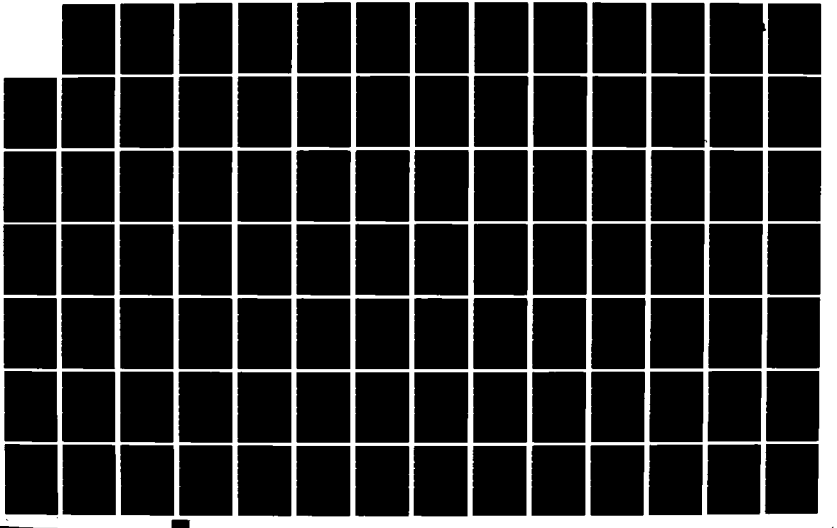

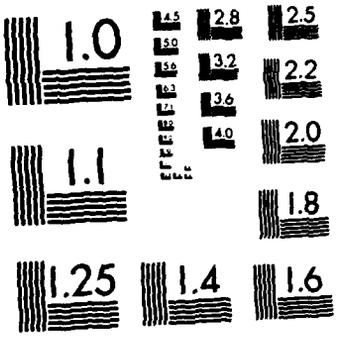

heart and diaphragm proteins. In contrast, DFP decreased lysine incorporation into skeletal muscle proteins and had no effect on protein synthesis in the skin, spleen, brain, and adrenals.

CONCLUSIONS

The data indicate that organophosphates, in addition to their known effects on acetylcholinesterase, induce marked alterations in intermediary metabolism. The results suggest that tissue glycogen is utilized to support accelerated oxidative processes in the muscle during the acute stages of poisoning. It also appears that amino acid oxidation in the muscle is enhanced in support of increased energy needs. Consequently, a decrease in the pool size of free amino acids results in a reduction in tissue, and in particular in muscle protein synthesis.

RECOMMENDATIONS

The mechanisms that underly these metabolic changes, and the effects of various organophosphate antidotes on these processes should be sought.

STUDY NO. 2 Effects of hormones on levels of acetylcholinesterase in the skin

PROBLEM

The toxic action of organophosphates is due to the inhibition of acetylcholinesterase, an enzyme vital for nerve function. Organophosphates react rapidly and covalently with the enzyme to produce an inactive enzyme. Reactivation of the inhibited enzyme proceeds very slowly. An increase in the enzyme activity in animals surviving from organophosphate poisoning has been largely attributed to increased rate of enzyme synthesis and new enzyme molecules. If synthesis of acetylcholinesterase could be stimulated, recovery from organophosphate toxicity should be faster or the toxicity should be alleviated. Since acetylcholinesterase is an allosteric protein, it is subject to metabolic control. Ultimately, an understanding of the mechanisms of acetylcholinesterase control would provide a rational guide for applied research in selecting specific antidotes or in developing preventive measures against the toxic effects of organophosphates. Since numerous metabolic events are regulated by hormones, this study was concerned with the effect of selected hormones on the activity of acetylcholinesterase in the skin and in other tissues.

RESULTS AND DISCUSSION OF RESULTS

Male rats weighing approximately 200 gm were used in all experiments. They were injected subcutaneously every 12 hours for 4 days with each of the following hormones: glucagon, epinephrine, insulin, or cortisone.

Control rats received injections of either the vehicle or saline. Twelve hours after the last injection, acetylcholinesterase activity was determined in the skin, brain, liver, muscle, and serum. Compared to the controls, insulin increased the enzyme activity in the brain. No insulin effect in the other tissues was observed. Glucagon, epinephrine, or cortisone did not alter acetylcholinesterase activity.

CONCLUSIONS

The results indicate the complex nature of acetylcholinesterase and suggest that the brain enzyme may differ from the enzyme in other tissues. Hormone-induced conformational changes in acetylcholinesterase, variations in the isoenzyme components, or the catalytic sites may account for the differential response of acetylcholinesterase to insulin.

RECOMMENDATIONS

Further studies should be conducted to determine the metabolic controls of acetylcholinesterase.

PUBLICATIONS

None

STUDY NO. 3 Skin permeability based on chemical structure

Study has been approved but not initiated.

STUDY NO. 4 Miliaria and Hypohidrosis

Study has been approved and transferred to Work Unit 255, Agency Accession No. DAOG 8396 - Miliaria and Hypohidrosis Prevention.

STUDY NO. 5 Effect of organophosphates on paraoxonase in the pig

PROBLEM

The ultimate purpose of this work is to develop more powerful and safe decontamination and preventative strategies for human skin against the effects of nerve gases and mustards. This requires a new predictive tool and a new animal model that do not depend on crude measurements such as LD50. To develop such a new decontamination system two aspects need to be considered: (1) a representative animal model must be used since initial tests cannot be made on human volunteers; (2) tests should be done with chemical warfare agents or realistic simulants that have similar biological and physical properties as chemical warfare

agents. Current decontamination systems such as the DS 2 or 258 kit or proposed systems such as the NBO/CTAC all operate at unphysiologic pH of about 10 or higher. Current systems also contain organic molecules such as methoxyethanol, phenol, or detergents, all of which damage the skin and/or facilitate penetration of chemical warfare agents. These organic molecules may also alter the natural defense mechanism by activating or deactivating enzymes in the skin that destroy chemical warfare agents. The knowledge of enzymatic activity in skin, vis a vis chemical warfare agents, is therefore important in the development of new decontamination strategies, but that information is not available.

In the past, much work on evaluation of the effects of chemical warfare agents was done with rats and rabbits. However, these two species have a very high density of hair compared to man. It can be calculated that in rats the area of the epidermis within the hair follicles actually exceeds the area of the exposed surface. Because of the protection by hair, rabbits and rats also have a very thin epidermis and stratum corneum compared to man. Because of the hair follicles, the dominant site of entry of the chemical warfare agent will be these hair follicles. For these and other reasons a more representative model is being developed. Despite many dissimilarities, the skin of domestic pigs has remarkable similarities to that of man. It is believed that the thickness of the epidermis of the pig is similar to man although very few measurements are available and the thickness of pig skin is clearly not uniform. Furthermore, in some areas of the pig skin there are serous glands which have cholinesterase-reactive nerve endings similar to ecrine glands of man, but no quantitative data are available. In other areas of the surface of the pig skin many short hairs and sebaceous glands are present and it is believed that these structures contain nonspecific esterases, acetylcholinesterase and other hydrolytic enzymes. Thus, some skin areas of the pig may be useful whereas other areas may be unsuitable for testing decontamination systems or barrier creams, however, the information is not available.

At this time, LAIR is not authorized to use chemical warfare agents. For this reason decontamination work needs to be done with biologically realistic simulants for chemical warfare agents. Besides chemical warfare agents, many pesticides and organophosphates such as paraoxon and DFP are hydrolized by poorly defined enzymes such as phosphatases (tabun), DFPase (DFP) or paraoxonase (paraoxon). Paraoxon appears to be a particularly useful simulant since the enzyme(s) that hydrolizes paraoxon also acts on DFP, tabun and sarin.

In this study paraoxon was used to measure the enzymatic activity of pig serum, human serum, and various skin regions. We also examined the morphologic structure and thickness of those skin areas. This is important since morphology and thickness have been shown to affect penetration of organophosphate pesticides.

RESULTS AND DISCUSSION OF RESULTS

Calibration of equipment and procedures have been completed. Our review of the literature indicates that virtually no information has been published on phosphatases, in pig skin or other pig tissues. In contrast, there are a number of reports on the phosphatase activity in serum of various species and man, but not the pig. For this reason a spectrophotometric assay for the enzymatic hydrolysis of paraoxon has been developed and shows good accuracy and good day-to-day reproducibility with human and pig serum. Enzymatic activity in homogenates from pig skin so far is relatively low. It appears that the activity depends on many factors including the concentration of tissue. It appears that both enzyme activators as well as inhibitors are present and, depending on tissue concentration, may affect the enzymatic rate. Because of this, the activity of paroxonase was investigated in serum of pigs and humans in more detail. Since pH in relation to decontamination is important and since the literature suggested at least two enzymes in serum with optimal velocity at pH 7 and 10, we investigated the pH dependence of the hydrolysis of paraoxon in human and pig serum. The data suggest that phosphatase activity in human and pig serum is similar. Our results suggest, however, four different pH optima in the range from pH 6.5 to 10.5. This suggests that perhaps four different enzymes are capable of hydrolyzing organophosphates. The enzyme with the highest pH optimum has the highest rate of inactivating the organophosphate. At pH 7.5 the enzyme is significantly activated by phosphate ions and inhibited by Tris buffer and perhaps by diethyl malonic acid buffer. This suggests that specific ion effects may have significant effect on the hydrolysis of organophosphates. The enzyme activity varies widely from person to person but the activity level remains relatively stable for any particular person over a period of several weeks. In contrast, the enzyme activity in pigs does not seem to vary greatly from animal to animal. The enzyme activity in pig serum is also stable over a period of weeks. Detergents of various types, including bile salts and nondenaturing zwitterionic detergents, significantly decrease the activity of hydrolysis of the organophosphate both at pH 7.5 and 10.5. Since we are interested in comparing the enzymatic activity in various types of pig skin of various thicknesses and containing various appendages we excised 12 samples from each pig, i.e., three from the head region, five from the trunk (i.e., ventral side of the thorax and cervical region, dorsal thorax) three from the forelimb (axillary region, etc.) and one from the hind limb. Tissue samples from two pigs have been fixed, imbedded in paraffin and sections prepared. Photographs of the sections have been prepared and measurements are being taken of the thickness of the epidermis, hair follicles, and sebaceous glands. A method has been developed that allows for simple and controlled freezing and cryopreservation of the pig skin with apparently little loss in enzymatic activity. This is important in reducing animal numbers and costs, as well as simplifying logistics of experiments.

CONCLUSION

It is already apparent in developing new decontamination strategies and barrier protection systems for human skin that these formulations may alter the natural defense mechanism of the skin. Perhaps by changing the pH, the skin phosphatases may become more active towards hydrolyzing organophosphates. Conversely, organic molecules, such as detergents, may inactivate one or several of the natural phosphatases of the skin. Because of such factors, permeability as well as natural defense of skin may be significantly but unpredictably altered.

RECOMMENDATION

This study should be continued in order to identify and characterize the level of skin phosphatases in various skin areas of the pig so that a realistic animal model for testing decontamination strategies can be developed and used. The information may also be needed in testing barrier creams.

PUBLICATIONS

KLAIN, G.J., J.D. TURNBULL, and S.T. OMAYE. Oxidation of 1-^{14}C-ascorbic acid in the guinea pig: Effect of the route of administration. Int J Vit Nutr Res 51:39-46, 1981

KLAIN, G.J. and W.G. BELL. Differential effects of glucagon, insulin and epinephrine on *in vivo* glucose oxidation in the rat. Metabolism (submitted)

SCHMID, P. and J. JAEGER. Role of appendages in the metabolism of organophosphates in pig skin (Abstract) Ann Meeting Am Acad Derm, 1981.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 6202	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AT	250 APC FL09
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:2		

11. TITLE (Precede with Security Classification Code)

(U) Repellent Science Base

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology; 012600 Pharmacology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	1.5	110
	CURRENT 82	2.3	116

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: Reifenrath, William G., DAC, Ph.D.

NAME: Rutledge, Louis C., M.S., DAC, POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Repellent; (U) Topical; (U) Laboratory Animal; (U) Skin; (U) Formulation; (U) Persistence; (U) Penetration; (U) Evaporation

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) An understanding of the physical, chemical and biological processes that determine the durability and efficacy of repellent formulations will provide a firmer scientific basis for the repellent program and allow a more rational approach to selection of active ingredients and formulation additives. The objectives of this program are to determine the relative contributions of physical, chemical, and biological factors to promoting high intrinsic repellency, long persistence on skin and a pleasant feeling on the skin. Additionally, investigations will be made of effects on repellent efficacy and skin functions arising from interactions with other chemicals (e.g., chemical warfare agents, detoxicants, decontaminants) that contact a soldier's skin in a combat environment.

24. (U) An in vitro evaporation/penetration apparatus will be used with skin samples to measure rates of evaporation and penetration of labeled repellent compounds dissolved in volatile solvents or formulated with controlled-release substances. These compounds will also be evaluated in combination with other labeled substances (like chemical warfare agents, decontaminants, etc.) that have been applied before, with, or after them to determine whether penetration or skin reactions are affected by them or have effect on them. The physiologic basis of repellency in various arthropod species will be explored to the extent permitted by available personnel, knowledge and technology.

25. (U) 80 10 - 81 09. The in vitro evaporation/penetration apparatus was significantly upgraded. Using the model with pig skin, the effect of temperature on the disposition of topically applied m-deet was determined. The percutaneous penetration of m-deet in the grafted athymic nude mouse system and the weanling pig was determined and compared to values reported in other animal models and in man.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M161102BS10 Research on Military Disease, Injury and Health Hazards

WORK UNIT NO. 250 Repellent Science Base

The following investigations were conducted under this work unit.

STUDY NO. 1 Formulation and evaluation of repellents

STUDY NO. 2 Determination of the attractancy properties of seven standard repellents for Anopheles albimanus and Aedes aegypti

STUDY NOS. 1 and 2. The purpose of the research conducted under this work unit is to explore those areas of repellent science that may lead relatively quickly to significant advances in the technology of repellents and the ways in which they can best be exploited. The current effort is directed toward investigating evaporation and penetration processes on skin samples treated with candidate repellents and trial controlled-release formulations. The in vitro apparatus for measurement of repellent evaporation and percutaneous penetration was significantly upgraded during the report period, and the effect of temperature on the disposition of topically applied diethyl toluamide was determined. The percutaneous penetration of diethyl toluamide in the grafted athymic nude mouse system and the weanling pig was also determined. A study was initiated to verify or disprove reports that certain repellents, including diethyl toluamide, become attractive to mosquitoes at low release rates such as those that are presumed to occur on the skin when the material initially applied has dissipated.

BODY OF REPORT

WORK UNIT NO. 250 Repellent Science Base

STUDY NO. 1 Formulation and Evaluation of Repellents

PROBLEM

The mosquito repellent most widely used by the U.S. military in the field is an ethanolic solution containing 75% by weight of N,N-diethyl-m-toluamide (m-deet), the active ingredient. In laboratory tests this repellent can provide 5-10 hours of protection when applied to the dry skin of resting individuals. However, m-deet protects sweating subjects only 2 hours, even at larger doses. M-deet's effectiveness is further reduced by water immersion, abrasion, evaporation, and absorption through the skin. Elevated environmental temperatures and increased wind velocity rapidly reduce the protection time of a repellent.

Various approaches are being taken to develop better mosquito repellents. Chemical synthesis done elsewhere continues to search for compounds with higher intrinsic repellency. A second approach involves development of new concepts of repellency by investigating the mechanisms by which repellents work. These concepts could then be exploited to develop a better repellent. A third approach involves reformulation of m-deet to maintain repellent activity on the skin surface for longer periods. This approach requires knowledge of the ability of the outer layer of the skin to retain chemicals. Model systems are needed to measure loss of repellent from the skin surface by various modes (excessive evaporation, percutaneous penetration, abrasion, and removal by water).

RESULTS AND DISCUSSION OF RESULTS

During FY 81, effort centered on the development and improvement of model systems for determining loss of m-deet from the skin surface by evaporation and/or percutaneous penetration. The in vitro evaporation-penetration model has been improved to make it mechanically more reliable (see Work Unit FL OA). In addition, pig skin was employed in the model to insure a dependable supply of skin.

With this animal model, the percutaneous penetration of m-deet at a dose of 0.32 mg/cm^2 was 18%, and 41% was found to evaporate. In studies with man, it has been reported that 50% of the applied dose of m-deet evaporates from the skin, in close agreement with findings. The percutaneous penetration is comparable to the value (8% at a skin dose of 0.32 mg/cm^2) we reported in the hairless dog, which we have previously found to be a good animal model for prediction of repellent

efficacy in man. Using this model, the effect of elevated air temperature on the disposition of m-deet (at a dose of 0.32mg/cm^2) was determined. As the air temperature was increased from 24C to 32C, percutaneous penetration increased from 18% to 57% while evaporation actually decreased (from 41% to 23%). At the 24C temperature, 19% of the applied dose was found as a residue in the skin, while at the higher temperature, almost no residue was found in the skin.

The percutaneous penetration of m-deet has been determined in several new in vivo models (weanling pig and grafted athymic nude mouse) being developed in Work Unit FL OA. The compound was tested at a dose of 4 ug/cm^2 to allow comparison with human data at that dose. Table 1 summarizes these results and compares then to values previously

Table 1. Percutaneous penetration of radiolabeled N,N-diethyl-m-toluamide (m-deet) at a dose of 4 ug/cm^2

Mean Percent Penetration[1]					
athymic nude mouse					
ungrafted	pig graft	human graft	pig	dog	man
38	31	31	9.5	13	16[2]

[1]Mean percent penetration. Values are the means of three replicates and are corrected for incompleteness of urinary excretion

[2]Data from Feldmann and Maibach

reported for the hairless dog and man. Values found in the nude mouse (ungrafted or with pig or human skin grafts) were considerably higher than those found in the pig, dog, and man. When m-deet (at a dose of 0.32 mg/cm^2) was tested on excised ungrafted athymic nude mouse skin with the in vitro evaporation-penetration model, 74% of the applied dose to penetrated while only 3% evaporated.

CONCLUSIONS

The in vitro skin evaporation-penetration model has been significantly

upgraded in FY 81, so that skin disposition studies of repellents and formulations can proceed more expeditiously. Using this model, the percutaneous penetration of m-deet was found to almost double when the air temperature was increased from 24C to 32C. The percutaneous penetration was assessed in vivo in the weanling pig and grafted athymic nude mouse system for the first time. Penetration values found in the pig were comparable to those previously reported in the hairless dog and man, while the values found with grafted or ungrafted athymic nude mice were considerably higher.

RECOMMENDATIONS

The in vitro evaporation-penetration model should be used for assessment of the safety and efficacy of new formulations of m-deet being developed.

PUBLICATIONS

REIFENRATH, W.G., R.B. ROBINSON, V.D. BOLTON, and R.E. ALIFF. Percutaneous penetration of mosquito repellents in the hairless dog: Effect of dose on percentage penetration. Food Cosmet Toxicol 19:195-199, 1981

REIFENRATH, W.G. and L.C. RUTLEDGE. Evaluation of mosquito repellent formulations. J Pharm Sci (in press)

STUDY NO. 2 — Determination of the attractancy properties of seven standard repellents for Anopheles albimanus and Aedes aegypti

PROBLEM

Several poorly substantiated reports have been published to the effect that certain insect repellents, including diethyl toluamide, became attractive to mosquitoes at low release rates such as those presumed to occur on the skin when the material applied by the user has dissipated. This phenomenon, if true, would be additional reason to re-apply the repellent frequently and would help to explain apparent cases of repellent failure in the past.

RESULTS AND DISCUSSION OF RESULTS

A study to confirm or disprove the attractancy of low doses of seven

standard repellents to Anopheles albimanus and Aedes aegypti was initiated during the report period. Substantive data have not yet been obtained in the study.

CONCLUSIONS

This study is still in its initial phases, and no conclusions can be drawn at this time.

RECOMMENTATIONS

None

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 6203	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	100 APC HL20
b. CONTRIBUTING	61102A	3M161102BS10	BA	251
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)

(U) Strategies for the Prevention and Treatment of Shock after Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016200 Stree Physiology; 002300 Biochemistry; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	2.3	85
	CURRENT 82	6.3	191

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Brown, Danley F., CPT, MS

NAME: POC: PA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Shock; (U) Trauma; (U) Metabolic Changes; (U) Hormones; (U) Prevention; (U) Treatment

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Hemorrhagic shock and trauma are important military medical problems. In future warfare, it may not be possible to evacuate casualties rapidly, so the wounded soldier may have to be treated on the battlefield. Whole blood, large volumes of fluid, and definitive surgical treatment may not be available. We hope to find strategies for troop preparation that can reduce the risk of developing shock after an injury, and to define treatments that can be applied under field conditions that can ameliorate shock if it develops after injury.

24. (U) We will conduct investigations in three areas: 1) the role that preinjury metabolic factors play in inducing resistance to hemorrhagic shock, looking particularly at the importance of intracellular glycogen as a fuel source during low perfusion states; 2) determining the preferred cellular substrates in low flow states and the points where metabolic compensation for low flow fails in order that optimum metabolic interventions can be designed. We will also test the efficacy of ATP, fructose diphosphate, dihydroxyacetone phosphate, and phosphoenolpyruvate as cellular energy sources in low flow states; and 3) methods for reducing cellular energy demand during low flow states.

25. (U) 8010-8109 In the past year, immunoassay techniques for vasopressin, norepinephrine, epinephrine, dopamine, angiotensin I, prostaglandin E, prostaglandin F_{1a}', insulin, and gastrin have been established. These assays, as well as a few others, are essential to conduct the studies defined under 24 above. Study protocols have been prepared and are undergoing division review.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO: 3S162772A874 Care of the Combat Casualty

WORK UNIT NO: 100 Strategies for the Prevention and Treatment of Shock after Injury

To answer a recognized need in the Division of Combat Casualty Care for a laboratory to provide metabolic research expertise, a laboratory facility is being organized; the organization will be completed in FY 82.

BODY OF REPORT

WORK UNIT NO. 100 — Strategies for the Prevention and Treatment of Shock After Injury

PROBLEM

Confronted by large numbers of casualties and unable to provide timely evacuation or obtain adequate supplies of medical materiel needed for the conventional hospital management of shock, front line medical units need new approaches to prevent the increase in mortality and morbidity among casualties that would be expected under those conditions. Since the principal mechanisms of disease that produce death and disability after injury appear to involve metabolic deterioration of critical organs (e.g., myocardium, liver), it seems likely that new approaches to shock management are to be found in methods that can prevent, delay, or reverse the metabolic deterioration. Therefore, a need exists for a laboratory facility to investigate the metabolic events that surround injury, the metabolic effects of "non-metabolic" therapies, and to develop new therapies aimed at correcting metabolic problems.

RESULTS AND DISCUSSION OF RESULTS

In FY 81, the Division of Combat Casualty Care began to organize a laboratory to provide expertise in metabolic research for all relevant division protocols, as well as to originate its own investigations.

CONCLUSIONS

None

RECOMMENDATIONS

The organization of the laboratory was not completed by the close of FY 81. Work should continue to ensure the completion in FY 82.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 6783	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 03 13	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	252 APC HL24
b. CONTRIBUTING				
c. ~~CONTRIBUTING~~	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)*

(U) Isolated Heart Model Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012900 Physiology; 008800 Life Support; 006400 Fluid Mechanics

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AM't.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	0.4	25
FISCAL YEAR CURRENT 82	0.8	54

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Rescuscitative Solutions; (U) Viscosity; (U) Combat Casualty Care; (U) Circulation; (U) Laboratory Animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The immediate resuscitation of the combat casualty requires administration of intravenous fluids. The viscosity of a resuscitative fluid can be critical to the successful outcome of transfusion therapy. Since in vivo viscosity may differ widely from that measured in vitro, the objective of this study is to measure the effective biologic viscosity of currently utilized and projected resuscitative solutions.

24. (U) The isolated coronary vascular bed of the pig will be used as a model of the vascular bed. Pressure-flow relations will be obtained under conditions of maximum vasodilatation.

25. (U) 81 05 - 81 09 Coronary vascular hemodynamics have been characterized and oxygen extraction measured during various flow regimens. Data have been processed for several animals. Stroma-free hemoglobin and perfluorocarbon emulsion have been utilized in three by-passed hearts.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M161102BS10 Research on Military Disease, Injury and Health Hazards

WORK UNIT NO. 252 Isolated Heart Model Development

The following investigations have been conducted under this work unit:

STUDY NO. 1 Biological viscometry of resuscitative solutions

STUDY NO. 1. A procedure for evaluating in vivo relative flow characteristics has been developed using the coronary circulation of the anesthetized pig. Continuous and step function inflow changes were employed utilizing cannulation flow probes and in-line pressure measurements. The resultant pressure-flow relations (PQRs) at the left coronary ostium were found to include a rectilinear segment above a flow of 80 ml min^{-1}. Oxygenated warmed (37C) blood of hematocrits (Hct) 4-60% (hemodilutions and concentrations in isologous plasma) was infused as well as several candidate resuscitative solutions including stroma-free hemoglobin (7.0 gm % SFH). The inverse slope of the PQR provided an index of relative viscosities. For SFH a slope of 4.6 ml min^{-1} $mmHg^{-1}$ was found to be 1.9 times that of blood at Hct 20%. In vitro viscometric data for the solutions was obtained using cone plate geometry. Viscosity of SFH solution demonstrated a slight shear dependence being 1.58 centipoise (versus 2.01 centipoise for Hct 20%) at 450 sec^{-1}, increasing somewhat at lower rates. Comparison of PQR data with those obtained for blood provides a method for evaluating blood substitutes in low flow states such as would be encountered in clinical cardioplegia or hemorrhagic shock. The in vivo data describe flow characteristics that cannot be predicted by the shear rate relation seen in rotational viscometry.

BODY OF REPORT

WORK UNIT NO. 252 Isolated Heart Model Development

STUDY NO. 1 Biological viscometry of resuscitative solutions

PROBLEM

In the treatment of combat casualties involving massive hemorrhage, compatible whole blood is often unavailable for transfusion therapy. For this reason the efficacy of blood substitutes, such as stroma-free hemoglobin or perfluorocarbons, has been investigated. The purpose of this protocol is to improve combat casualty care by providing information for the optimization of the flow properties of resuscitative solutions. A complete evaluation of any blood substitute centers on its ability to carry oxygen to the tissues, and this depends in part on its rheological properties; principal among these is the viscosity of the fluid. Consideration of the viscosity of blood substitutes has thus far been limited to in vitro measurements. As a result, knowledge of the in vivo flow properties of hemoglobin solutions and synthetic blood substitutes is lacking. This protocol represents an attempt to rectify this deficiency by developing a model to measure the biologic viscosity of resuscitative solutions. To this end, an in vivo (η_o) and in vitro (η_i) measurement system has been developed. One of the major obstacles encountered in shock or ischemia is the increased viscosity resulting from reduced flow and decreased plasma volume. The advocacy of stroma-free hemoglobin as a blood substitute in treating shock is based in part on the fact that hemoglobin solutions show a viscosity considerably lower than that of whole blood when measured in vitro. Also, hemoglobin solutions show no shear rate dependence, which by itself may alleviate some of the complicating factors in overcoming low-flow states. Conversely, reduced in vitro viscosity by stroma-free hemoglobin may be offset by diminution of the Fåhraes-Lindqvist effect in vivo. This reasoning is based on the fact that as hematocrit is reduced by addition of stroma-free hemoglobin solution, the pressure required to force solution through the microvasculature may increase to that predicted by the Poiseuille equation. The wide descrepancy between in vitro and in vivo values seen for blood may not be evident with hemoglobin solutions. The advantageous reduction in viscosity provided by hemodilution may thus theoretically be vitiated by the disappearance of the Fåhraes-Lindqvist effect. Only by in vivo viscometry can this possibility be examined.

RESULTS AND DISCUSSION OF RESULTS

A perfusion system has been set up for the in situ measurement of pressure and flow in the porcine coronary bed. Known changes in flow were introduced through this system and the corresponding pressures

measured. One or two intramyocardial pressures were also measured to determine the contractile state and its distributive effect on closing pressures. The surgical model is well-developed and has given much data for analysis. After induction, the animal was anesthetized with halothane and a femoral artery and vein were cannulated. This allowed determination of blood gases, hematocrit, hemoglobin content, oxygen saturation, and fluid administration (Ringer's lactate), if necessary. Halothane was used exclusively for general anesthesia with small amounts of Anectine added to alleviate muscle contraction during medial sternotomy. After resecting the pericardium, the animal was heparinized, bilateral atriotomy was performed, an overdose of halothane administered, and the respirator turned off. Then the left coronary ostium was rapidly cannulated and total by-pass initiated, using a Shiley pediatric oxygenator primed with autologous warmed blood. In some experiments, both coronary ostia were perfused and the coronary sinus isolated via a cannulation flow probe. These cases have demonstrated marked arteriovenous oxygen-extraction capabilities of the empty myocardium for several hours.

When the electrophysiologic, pharmacological, and oxygen-extraction conditions were optimal, a test substance was administered via controlled infusion which generated one or more flow and pressure signals. These signals generated determinations of the η_o of the perfusates. At the end of every experiment, all systems were recalibrated in situ and the data stored for analysis. Several successful experiments have been conducted, but all experimental design criteria have not yet been met, making statistically significant conclusions impossible at this point. The first few animals utilized in this study have, however, confirmed the utility of the porcine myocardium as an experimental model of the vascular bed. When empty and arrested, the heart performs as a biological viscometer for perfusates, while continuing to have an oxygen-extracting capability. Data concerning gases, pH, hematocrits, hemoglobin, total oxygen content, and in vitro viscosities (η_i) are being analyzed.

Also being analyzed are pressure-flow data in response to continuous and step-function changes in flow velocity. Linear and volume flow calibrations are reliably correlated with inertial pressure drops outside the heart, intramyocardial pressures and contractile activities (both direct ventricular and cove-induced). Two batches of stroma-free hemoglobin have been used; both function curves and in vitro viscometry have been tabulated.

Since only small amounts of hemoglobin have thus far been available, only low flow rates have been used in developing these measurements. One batch of fortuitously available perflourocarbon emulsion has been prepared and used in a double oxygenator experiment. It supported the myocardium temporarily, but temperature control and other technical

Isolated Heart Model Development (Cont)

details were not optimal. Subjectively, η_o was adequate and η_i was measured before and after perfusion.

CONCLUSIONS

1. The flow properties of resuscitative solutions are determinable with the present in situ system and can be compared with in vitro viscometry.

2. η_o and η_i show expected deviation from Poiseuille behavior at various hematocrits.

3. Coronary hemodynamic data confirm the well-ordered system of control by vascular compression within the myocardium and preclude, for the present, the utility of returning to the hindlimb model.

4. Perfluorocarbon emulsion has been shown to perfuse the heart adequately in one experiment, but it is difficult to prepare and may not be blood compatible at higher hematocrits or longer perfusion times.

RECOMMENDATIONS

The utility of this approach can be achieved if more batches are bench tested and perfused in situ. The present candidates for emergency resuscitation all have viscometric advantages and drawbacks. Larger volumes of stroma-free hemoglobin at 7.5 and 14 g/dl should be obtained and tested so conclusions can be made at low flow states. Further work on perfluorocarbon emulsions should also be undertaken in a later study.

The present study should be continued using both stroma-free hemoglobin and hemoconcentrated (hematocrit 60) perfusates for comparison of biological viscosities. Automatic data processing should be added to support this protocol so that analysis of data can proceed apace with measurements before solutions expire.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 6204	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	H.TERMINATION	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	CA	202 APC TL01
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:2		

11. TITLE (Precede with Security Classification Code)
Toxicology Support

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
016800 Toxicology; 012900 Physiology; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	81 09	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	3.1	04
FISCAL YEAR CURRENT 82	0.0	00

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21. GENERAL USE
Foreign Intelligence Not Applicable n

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Insect repellents; (U) Toxicology; (U) Skin; (U) Toxic substance; (U) Toxicology testing

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To assure the safety of repellents and repellent formulations developed to protect military personnel from medically important arthropods, tests will be conducted in compliance with the Food and Drug Administration's and the Environmental Protection Agency's Good Laboratory Practice Regulations.

24. (U) Initially, testing will be directed toward acute toxicity of candidate materials to permit testing compounds on human volunteers. These tests will include the Ames Assay, primary eye and dermal irritation, acute oral and dermal toxicity, dermal sensitization, and Drosophila sex-linked recessive lethal test. Long-term testing will be conducted on the most promising compounds.

25. (U) 8010-8109. A total of 33 short-term toxicity tests were conducted on 14 different compounds and formulation ingredients. Tests included the Ames Assay, oral LD_{50} determination, primary dermal and ocular irritating Drosophila sex-linked recessive lethal and acute dermal toxicity. This work unit will be done under the research work units supported.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3M162770A871	Prevention of Military Disease Hazards
WORK UNIT NO.	202	Toxicology Support

One of 12 compounds was mutagenic in the Ames assay. Three of five compounds tested for dermal sensitivity potential were clearly negative. LD_{50} (median lethal dose) values were established for four repellents. Primary dermal irritation potential has been determined for six compound formulations being classed as mild irritants. Both compounds tested for ocular irritancy were mild irritants. Both compounds tested, using the Drosophila sex-linked recessive lethal test, were mutagenic. Two compounds tested for dermal toxicity showed no toxic effects at 2 g/kg body weight. Testing and data analysis will be conducted under the research work units supported.

BODY OF REPORT

WORK UNIT NO.	202	Toxicology Support
STUDY NO.	1	GLP Toxicology Studies of Insect Repellents

PROBLEM

The insect repellent program is directed to the development of better insect repellents for protecting soldiers from insects and insect-borne diseases in the field. In the last several years, the Letterman Army Institute of Research (LAIR), Division of Cutaneous Hazards, has tested a large number of chemical compounds submitted by SRI International, the U.S. Department of Agriculture (USDA), and private industry, against a variety of mosquitoes, sand flies, fleas, bugs, ticks, and mites in animals and in vitro test systems. Several of these materials have shown sufficient repellent activity and persistence on the skin of animals to warrant consideration for use in lieu of, or in conjunction with, the current troop-issue insect repellent, 71.25% N,N-diethyl-m-toluamide (m-DEET) in ethanol. The Division of Cutaneous Hazards also evaluated a number of new formulations of m-DEET prepared at LAIR or submitted by private industry. Several of these new formulations have been more persistent than the current troop-issue repellent in tests on animals.

It is now planned to test the best of the new compounds and formulations on human volunteers to confirm the results obtained in vitro and in animal tests, and to evaluate their performance under conditions of actual use. Before this can be done, it is necessary to obtain certain toxicity data on each compound or formulation to ensure its safety for application to the skin. The toxicity tests required for registering a new insect repellent are prescribed by the Environmental Protection Agency (EPA). The basic toxicity tests required for experimental use of the new compounds and formulations on human volunteers are prescribed by the LAIR and USAMRDC Human Use Committees. If adverse toxicity data are obtained in these tests, the respective material(s) will be eliminated from consideration, and the prospective tests on human volunteers will not be carried out. The toxicity testing program thereby serves as both a safety factor and secondary screen in the development scheme of repellents.

RESULTS AND DISCUSSION OF RESULTS

The following compounds, with their codes, were tested:

Compound	Code
N,N-diethyl-m-toluamide	DEET
N(n-hexyl)-2-oxazolidine	CHR1
N(n-octyl) glutarimide	CHR2
N(n-hexyl) glutarimide	CHR3
(E)-1,2,3,4-tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline	CHR5
(E)-1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl) quinoline	CHR6
3-(N-n-butyl-N-octyl) aminopropionic acid-ethyl-ester	CHR7
Proprietary Compound RH-398	CHR8
Triethylene glycol monohexyl ether	CHR9
N,N-dipropylcyclohexanecarboxamide	CHR10
1-(3-cyclohexene-1-yl-carbonyl) piperidine	CHR11
Formulation of 50% DEET, 25% Isopropanol 25% Dow Corning 200 Fluid	CHF1
Troop Issue DEET, 71.25% DEET, 3.75% other diethyl toluamide and 25% ethanol	TID
Dow Corning 200 Fluid	DC200
Isopropanol	ISO

The Ames assay was conductd on CHR1, CHR2, CHR3, CHR5, CHR6, CHR7, CHR8, CHR9, CHR10, CHR11, CHF1, and DEET. CHR1 was found to be a weak mutagen.

Toxicology Support (Continued)

The oral LD_{50} for four compounds can be found in Table 1.

TABLE 1

Oral LD_{50} in rats for candidate insect repellents (mg/kg body weight).

	Males		Females	
Compound	LD_{50}	95% CI**	LD_{50}	95% CI
CHF1	4362	3374, 6359	2495	1905-3268
CHR1	2558	1940, 3373	1383*	595-3209
CHR2	***		6491	5022-8389
CHR8	4737	4226, 5309	3101	2654-3622

*Approximate lethal dose; **Testing discontinued - compound showed indication of neurotoxicity; ***CI - confidence interval.

The dermal sensitization potential was assessed for CHF1, CHR2, CHR3, CHR5, and CHR6. Compounds CHF1, CHR2, and CHR3 were clearly non-sensitizing. Results for CHR5 and CHR6 were inconclusive and will be repeated.

CHF1 and CHR2 were not acutely toxic at 2 g/kg when applied to the skin of rabbits. No further testing is required if 2 g/kg is not toxic.

Compounds CHF1, CHR2, DEET, and TID were found to be mild primary dermal irritants. ISO and CHF1 formulation carrier liquids were non-irritating.

DEET and CHF1 were found to be primary ocular irritants. Flooding the eye with water after exposure reduced the ocular irritation. CHR2 and CHR8 were found to be mutagenic by the Drosophila sex-linked recessive lethal (SLRL) test.

CONCLUSIONS

It is premature to make definite conclusions based on the limited number of tests many of these compounds have been subjected to. However, it is felt that CHR1, which showed signs of mutagenic potential and also neurotoxicity should be eliminated from the program. Consequently, CHR1 was removed from the list of candidate insect repellents. It is also apparent that some degree of ocular and dermal irritation must be accepted in this program.

RECOMMENDATIONS

It is recommended that testing be continued in support of the Insect Repellent Program.

PUBLICATIONS

1. HANES, M.A., and J.T. FRUIN. Acute lethal dose (LD_{50}) in male and female rats for CHR8. Toxicology Series 25. Technical Note. San Francisco, California: Letterman Army Institute of Research (submitted for publication)

2. POWERS, N.R., R.A. WIRTZ, and J.T. FRUIN. Mutagenic potential of N-(n-octyl)-glutarimide and proprietary compound CHR-8 using the Drosophila melanogaster sex-linked recessive lethal test. Toxicology Series 26) Institute Report 118. San Francisco, California: Letterman Army Institute of Research (submitted for publication)

3. LEWIS C.M., M.A. HANES, and W. REIFENRATH. Acute oral toxicity (LD_{50}) of CHF1 in rats. Toxicology Series 24. Institute Report 119. San Francisco, California: Letterman Army Institute of Research (submitted for publication)

4. FRUIN, J.T., and M.J. LANGFORD. Primary Dermal irritation potential of existing and candidate insect repellents and formulation products for insect repellents. Toxicology Series 22. Technical Note. San Francisco, California: Letterman Army Institute of Research (submitted for publication)

5. FRUIN, J.T., M.A. HANES, and L.C. RUTLEDGE. Primary dermal irritation potential of the insect repellent CHF1 and its components. Toxicology Series 7. Technical Note 81-17TN. San Francisco, California: Letterman Army Institute of Research, September 1981

6. KELLNER, T.P., M.A. HANES, and J.T. FRUIN. The primary eye irritation potential of the insect repellent CHF1 and M-DEET. Toxicology Series 10. Technical Note 81-23TN. San Francisco, California: Letterman Army Institute of Research, September 1981

7. SAUERS, L.J., F.R. PULLIAM, and J.T. FRUIN. The mutagenic potential of n-(n-octyl)-glutarimide. Toxicology Series 1. Institute Report No. 97. San Francisco, California: Letterman Army Institute of Research, July 1981

8. SAUERS, L.J., F.R. PULLIAM, and J.T. FRUIN. The mutagenic potential of: n-hexyl-2-oxazolidone. Toxicology Series 2. Institute Report No. 98. San Francisco, California: Letterman Army Institute of Research, July 1981

9. SAUERS, L.J., F.R. PULLIAM, and J.T. FRUIN. The mutagenic potential of: triethylene glycol monohexyl ether, 3-(N-n-butyl-N-acetyl) aminopropionic acid ethyl ester, proprietary compound RH-398, N,N-diethyl-m-toluamide, N(n-hexyl)glutarimide. Toxicology Series 5. Institute Report No. 107. San Francisco, California: Letterman Army Institute of Research, September 1981.

10. FRUIN, J.T., M.A. HANES, and K. BLACK. The dermal sensitization potential of candidate insect repellents: LAIR formulation CHF1, N(n-octyl) glutaramide, N(n-hexyl) glutarimide, 1,2,3,4- tetrahydro-6-methyl-1-(2-methyl-1-oxo-2-butenyl) quinoline, and 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-1-butenyl) quinolone. Toxicology Series 12. Technical Note. San Francisco, California: Letterman Army Institute of Research (submitted for publication)

11. SAUERS, L.J., F.R. PULLIAM, W. JEDEBERG, and J.T. FRUIN. Ames assay. The mutagenic potential of (E) 1,2,3,4-tetrahydro-6-methyl-1 (2-methyl-1-oxo-2-butenyl) quinoline, 1,2,3,4-tetrahydro-6-methyl-1-(3-methyl-1-oxo-2-butenyl)quinoline, 50% DEET, 25% Dow Corning 200

Fluid, in isopraponyl. Toxicology Series 20. Institute Report 109. San Francisco, California: Letterman Army Institute of Research, September 1981

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE 6316	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	H.Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	6277OA	3M16770A871	CA	203 APC TL08
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:2		

11. TITLE (Precede with Security Classification Code)* (U) Toxicological Screening of Potentially Hazardous Substances Using Drosophila Melanogaster

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
002600 Biology; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 02	81 09	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	1.5	17
	CURRENT 82	0.0	00

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21. GENERAL USE
Foreign Intelligence Not Applicable

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NAME: Rutledge, L.D., DAC POC:DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Toxicology; (U) Mutagenicity; (U) Drosophila melanogaster; (U) Sex-linked recessive lethal test

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The purpose of this work unit is to establish an in-house capability for the toxicologic screening of potentially hazardous substances using the Drosophila melanogaster sex-linked recessive lethal (SLRL) test. This test will be used to assess the mutagenic potential of newly developed compounds for use as a defense against chemical warfare agents and repellents for use against disease-carrying arthropods.
24. (U) A D. melanogaster insectary capable of supporting a SLRL testing program has been established and personnel trained in rearing and testing procedures. Exposure methodology and computer programs for labeling of test insects, data storage, and analysis have been developed. Standard Operating Procedures (SOP) have been written to ensure compliance with Good Laboratory Practices (GLP) Regulations. Pilot studies and testing of experimental compounds have been initiated.
25. (U) 8009-8109. Procedures to prepare water-insoluble compounds for adult exposure have been developed for the test system. Injection procedures and pilot dosimetry studies have been accomplished and further refinements are currently underway. Microbial contamination of the rearing medium has been solved. Additional SOPs have been written to comply with GLP requirements. A pilot study, testing 2-ethyl-1,4-benzoquinone and 2-methyl-1,4-benzoquinone, has been completed. Investigation of the use of plastic in place of glass vials is currently underway. The testing of N,N-bis-2-methallyl-N'-butyl urea and N-(n-octyl)-glutaride has been completed. Pilot studies of methodology and formulation of the P-nitrophenyls have been established. SLRL testing is currently underway for 4-nitrophenyl diphenyl phosphinate and pilot studies have begun for 4-nitrophenyl methyl phosphinate. Further toxicological screening will be conducted under specific program work units.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3m16770A871	Prevention of Military Disease Hazards
WORK UNIT NO.	203	Toxicological Screening of Potentially Hazardous Substances Using Drosophila melanogaster

The following investigations have been conducted under this work unit:

STUDY NO. 1 Mutagenicity testing using the Drosophila melanogaster sex-linked recessive lethal assay

The Armed Forces are often confronted with various toxicology problems associated with requirements for mission completion. Federal requirements must be met concerning human and environmental exposure to potentially hazardous substances. The Department of Defense does not possess in-house capability for compounds that must undergo toxicologic testing to meet federal legal requirements. Establishing an in-house capability for the toxicologic screening of potentially hazardous substances by using Drosophila melanogaster sex-linked recessive lethal test is part of the LAIR toxicology program designed to help meet these requirements. All testing will meet the Food and Drug Administration Good Laboratory Practices Regulation.

STUDY NO. 2 Mutagenicity of tenebrionidae flour beetle secretions using Drosophila melanogaster sex-linked recessive lethal test

The 2-ethyl-1,4-benzoquinone and 2-methyl-1,4-benzoquinone are major secretory products of the Tribolium spp. beetle which commonly infest flours and other stored products. Both compounds were tested for mutagenic activity using the Drosophila melanogaster sex-linked recessive lethal assay and were found to be mutagenic after 72-hour feeding exposures of 1-2 mM.

STUDY NO.3 Analysis of the sex-linked recessive mutation frequency of Drosophila melanogaster when reared in glass vs. plastic vials

Due to cost analysis, plastic vials were considered a potential substitute for glass vials in the sex-linked recessive lethal testing program. Both glass and plastic vials were used concurrently to see what effect they would have upon this test system. The use of plastic vials resulted in a greater mutation frequency of D. melanogaster than the use of glass vials.

BODY OF REPORT

WORK UNIT NO.	203	Toxicological Screening of Potentially Hazardous Substances Using Drosophila melanogaster
STUDY NO.	1	Mutagenicity testing using the Drosophila melanogster sex-linked recessive lethal assay

PROBLEM

The Drosophila mutagenicity test is performed to detect substances that may cause genetic disorders. This test and other required tests are conducted so that such substances may be removed from consideration for human use. This work unit was initiated to determine the feasibility of establishing and maintaining an in-house capability for performing the sex-linked recessive lethal test using D. melanogaster to support Army requirements for toxicologic testing.

RESULTS AND DISCUSSION OF RESULTS

The major steps in establishing the sex-linked recessive D. melanogaster test system have been completed and are now in operation.

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

1. WIRTZ, R.A. and H.G. SEMEY. The Drosophila kitchen-equipment, media preparation and supply. Drosophila Information Bulletin (submitted for publication).

2. WIRTZ, R.A. Handling and containment procedures for use with Drosophila. Drosophila Information Bulletin (submitted for publication)

Toxicological Screening of Potentially Hazardous Substances Using *Drosophila melanogaster* (Continued)

3. WIRTZ, R.A., N.R. POWERS, and J.T. FRUIN. Standard Operating Procedure for: Mutagenicity testing using the *Drosophila melanogaster* sex-linked recessive lethal assay. Institute Report No. 112. San Francisco, California: Letterman Army Institute of Research, January 1982 publication)

4. JEDERBERG, W., R.A. WIRTZ, and N.R. POWERS. Computer-assisted labelling in mutagenicity testing. I. The *Drosophila melanogaster* sex-linked recessive lethal assay. Technical Note No. 82-32TN. San Francisco, California: Letterman Army Institute of Research, March 1982

BODY OF REPORT

WORK UNIT NO. 203 — Toxicological Screening of Potentially Hazardous Substances Using Drosophila melanogaster

STUDY NO. 2 — Mutagenicity of Tenebrionid flour beetle secretions using the Drosophila melanogaster sex-linked recessive lethal test

PROBLEM

There is concern that the secretory and excretory products released by insects into infested food products might be toxic. The Tenebrionid beetles (Tribolium spp.) are the most common insects to infest flours and other stored products. These insects possess glands that secrete a mixture of substituted p-benzoquinones and short chain hydrocarbons (2-ethyl-1,4-benzoquinone (EBQ), and 2-methyl-1,4-benzoquinone (MBQ)). These two compounds are highly reactive, acutely toxic, and perhaps carcinogenic to laboratory animals. This study was performed with two purposes: (1) to examine the mutagenicity of EBQ and MBQ using the Drosophila melanogaster sex-linked recessive lethal (SLRL) assay, and (2) to serve as a pilot study to insure that the in-house capability for toxicologic screening of potential mutagens is functioning.

RESULTS AND DISCUSSION OF RESULTS

The feeding concentrations of 1 mM EBQ and 2 mM MBQ in 1% sucrose fed to males (Canton S strain) resulted in 72-hour mortalities of 25.3% for EBQ and 38.5% for MBQ. The mortality for negative control males (Canton-S) fed on 1% sucrose was 2.5%, while that for positive control males (Canton-S) males fed 1 mM ethyl methane sulfonate in 1% sucrose was 2.3%. The mutation frequency of 1 mM EBQ was 0.15% (n = 100, 10 lethals in 6759 total tests; x-chromosomes),and for 2 mM MBQ was 0.13% (n = 100, 8 lethals in 6255 total tests; x-chromosomes). The concurrent negative control showed a spontaneous mutation frequency of 0.03% (n = 100, 4 lethals in 13,068 tests; x-chromosomes). All males fed EMS developed mutations during the test. The mutation frequencies for

flour-fed EBQ and MBQ were significantly greater ($P = 0.005$ and 0.015, respectively) than the spontaneous rate when analyzed by Fisher's exact test.

The mutation frequencies associated with a particular mating group (age of treated male after exposure) are related to the mutagen activity directly or indirectly upon the germ cells. Both EBQ and MBQ appear to be indirect mutagens.

CONCLUSION

Based on this study, both chemical secretions from Tenebrionid beetles are capable of inducing mutations when tested using D. melanogaster sex-linked recessive lethal assay. Because of the high level of food infestation attributed to the Tribolium spp. flour beetles, the secretion of potentially toxic, mutagenic, and carcinogenic quinones by these insects could represent a considerable toxicologic hazard.

RECOMMENDATION

Further analysis of this potential toxicologic hazard should be conducted using complementary toxicologic tests.

PUBLICATIONS

WIRTZ, R.A. and J.T. FRUIN. Mutagenicity of Tenebrionid flour beetle secretions using the Drosophila melanogaster sex-linked recessive lethal test. (submitted for publication)

BODY OF REPORT

WORK UNIT NO. 203 — Toxicological screening of potentially hazardous substances using Drosophila melanogaster

STUDY NO. 3 — Analysis of sex-linked recessive mutation frequency of Drosophila melanogaster when reared in glass vs. plastic vials

PROBLEM

Among the materials required in this test system are non-recyclable, disposable glass vials (9.5 cm in length, 2.4 cm in diameter). Due to the necessity for their use, but the increasing cost (23 cents per vial) and breakage problems, alternative vials were investigated. Plastic vials (6.5 cm in length, 2.2 cm in diameter) were disposable, cost three cents per vial, and were non-breakable. Before changes could be made in materials used in this test system, the sex-linked recessive lethal testing was conducted and the mutation frequency was compared between the insects reared in glass vials and those reared in plastic vials.

RESULTS AND DISCUSSION OF RESULTS

Each male was crossed with 3 virgin Basc females and the progeny were reared in glass vials while another group of males, each crossed with three virgin Basc females, and their progeny were reared in plastic vials. The frequency of sex-linked recessive lethal mutations were estimated by the Basc method. The mutation frequency of those reared in glass was 0.086% (n = 96, 8 lethals, 9221 total tests, x-chromosomes), while those reared in the concurrently tested plastic had a mutation frequency of 0.202% (n = 96, 18 lethals, 8860 total tests, x-chromosomes). Mutation frequencies of flies reared in plastic vials were significantly greater ($P = 0.0194$) than those reared in glass vials when analyzed by the Fisher's exact test. In concurrent positive controls, all males fed ethyl-methane sulfonate and reared in glass vials demonstrated their capability to produce mutations during the test.

Toxicological Screening of Potentially Hazardous Substances Using Drosophila melanogaster (Continued)

CONCLUSIONS

It is believed the plastic affects the larvae in the vials and, thus, causes a significant increase in the mutation frequency of the offspring.

RECOMMENDATIONS

Further testing with vials made of other types of plastic is being planned.

PUBLICATIONS

WIRTZ, R.A. and N.R. POWERS. Analysis of the sex-linked recessive mutation frequency of Drosophila melanogaster when reared in glass vs. plastic vials. Institute Report. San Francisco, California: Letterman Army Institute of Research (submitted for publication).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 2373	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D.CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	CA	201 APC FL07
b. CONTRIBUTING				
c. ~~CONTRIBUTING~~	STOG	80-7.2:2		

11. TITLE (Precede with Security Classification Code)*

(U) Development of Repellents Against Medically Important Arthropods

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 002300 Biochemistry; 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	84 06	LA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	7.1	323
FISCAL YEAR CURRENT 82	8.0	323

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Rutledge, L.C., M.S., DAC

NAME: Buescher, M.D., CPT, MS, POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Formulations; (U) Laboratory Animals; (U) Human Volunteer; (U) Insects; (U) Arthropods; (U) Vectors; (U) Repellents

23. TECHNICAL OBJECTIVE.* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Arthropods and the diseases transmitted by them can have a major impact on the will and ability of soldiers to fight. Use of repellents is the most cost-effective means available to reduce biting, thus preventing arthropod-borne diseases and maintaining morale and the ability to fight. The repellent now issued is ineffective against several major disease-carrying arthropods. Troops don't like to use it, and it damages plastic and rubber items and synthetic fabrics. With present knowledge and technology we will develop a more effective, longer lasting and pleasant repellent within 5 years.

24. (U) The current Army repellent (diethyl toluamide) will be re-formulated to produce an end-product tailored to military requirements. Other commercial and experimental repellents will be evaluated for use in conjunction with diethyl toluamide to provide a broader spectrum of efficacy of the end-product.

25. (U) 80 10 - 81 09. Certain polymeric additives and microencapsulation techniques have been demonstrated to extend the period of effectiveness of diethyl toluamide on the skin. Several commercial and experimental repellents have been identified which can be used in conjunction with diethyl toluamide to extend its spectrum of efficacy if toxicological standards can be met. A draft Letter of Agreement providing for the formulation studies, performance and safety testing and other activities needed in the advanced development of the improved repellent was initiated in cooperation with the Material Division, U.S. Army Academy of Health Sciences.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3M162770A871	Prevention of Military Disease Hazards.
WORK UNIT NO.	201	Development of Repellents Against Medically Important Arthropods

Research under this work unit is directed toward the development of an improved arthropod and leech repellent for protecting troops in the field from biting insects, mites, ticks, and leeches and the diseases they transmit. The approach employed is to exploit the best available repellent compounds in conjunction with modern methods of controlled-release formulation to develop an end-product tailored to military requirements. Two methods were found to extend the period of effectiveness of the current Army repellent (diethyl toluamide) on the skin. Our method (reported last year) employs polymeric formulation additives, and the other is a microencapsulation process. Several commercial and experimental repellents have been identified which can be used in conjunction with diethyl toluamide to extend its spectrum of efficacy if toxicologic standards can be met. A draft Letter of Agreement (LOA) providing for the formulation studies, performance and safety testing, and other RDT&E activities needed in the advanced development of the improved repellent, was initiated in cooperation with the Materiel Division, Academy of Health Sciences, acting as Combat Developer. This draft LOA is currently being staffed with other interested agencies. Successful conclusion of the LOA effort will represent agreement that technical potential currently exists for advanced development of an improved topical arthropod and leech repellent.

BODY OF REPORT

WORK UNIT NO. 201 Development of Repellents Against Medically Important Arthropods.

PROBLEM

Repellents supplement pesticides, vaccines, and drugs in limiting the impact of biting arthropods and the diseases they transmit on the ability of soldiers to function in the combat environment. However, the repellents currently issued by the Army (diethyl toluamide and ethyl hexanediol) are not effective against all species of insects and must be frequently re-applied in severe weather and extreme climates. Troop acceptance of the Army repellent is poor, and usage in Vietnam was not sufficient to prevent heavy losses from vector-borne diseases. Research under this work unit is directed toward developing improved formulations of diethyl toluamide to provide better performance and increased troop acceptance and toward the identification of other materials which can be used in conjunction with or in lieu of diethyl toluamide to meet the unique requirements of the Armed Forces.

RESULTS AND DISCUSSION

Three microcapsular formulations of diethyl toluamide submitted by Bend Research Inc. and one microcapsular formulation of diethyl toluamide submitted by Eurand America Inc. were tested on white rabbits against the yellow fever mosquito, Aedes aegypti, and the malaria mosquito, Anopheles albimanus. The formulations submitted by Bend Research Inc. were more persistent than the unformulated control in every case. The formulation submitted by Eurand America Inc. was less persistent than its control. These tests demonstrate that microencapsulation is a valid approach to the problem of formulating repellents for improved persistence. The efficacy of certain film-forming polymers as additives to improve persistence was reported in last year's report. (Annual Research Progress Report, FY 80).

Two new repellent compounds submitted by Dr. N.R. Hansl of Creighton University were tested in vitro and on white mice against Aedes aegypti. Both materials were highly repellent to Aedes aegypti but less persistent than diethyl toluamide.

Two new repellents submitted by Cutter Laboratories Inc. (Rohm & Haas 398 and Merck 3535) were tested against nine species of mosqitoes, sand flies, fleas, bugs, ticks and chigger mites. Rohm and Haas 398 was more effective than diethyl toluamide for most of the species tested. Merck 3535 was approximately equivalent to diethyl toluamide overall, but it was ineffective against the bug Rhodnius prolixus. Both materials were as persistent as diethyl toluamide on the skin of test animals.

Development of Repellents Against Medically Important Arthropods (continued)

Five new repellents submitted by the Stanford Research Institute (SRI 434-58, SRI 835-19C and SRI 835-23A) and the U.S. Department of Agriculture (USDA AI3-36166 and USDA AI3-36178) were singled out as "promising" in last year's report, on the basis of tests against five species of mosquitoes. These compounds were tested against sand flies (Lutzomyia longipalpis), fleas (Xenopsylla cheopis) and bugs (Rhodnius prolixus) in FY 81. All were more effective than diethyl toluamide against the sand fly, and two (SRI 835-19C and SRI 835-23A) were more effective than diethyl toluamide against the flea. None were effective repellents against the bug.

Two new repellents currently being advanced by the U.S. Department of Agriculture (USDA AI3-35765 and USDA AI3-36326) were obtained on 18 August 1981, for testing and tests against mosquitoes, sand flies, fleas, bugs and chigger mites are now in progress. These two compounds are currently undergoing advanced toxicity testing at the Army Environmental Hygiene Agency by request of the Department of Agriculture.

The results of toxicity tests conducted at LAIR are given elsewhere in the Annual Research Progress Report, but it may be noted here that both Rohm & Haas 398 and SRI 835-23A have been found to be mutagenic in the Drosophila melanogaster sex-linked recessive lethal test. The significance of the findings, with regard to the possible use of these materials as insect repellents by the Army, has not yet been determined. In this connection, LAIR currently has an urgent requirement for bulk quantities of SRI 434-58, SRI 835-19C, USDA AI3-36166 and USDA AI3-36178 (approximately one liter each) for use in the toxicity testing program. HQ USAMRDC has been requested to arrange for synthesis of these materials through the existing drug synthesis contracts of WRAIR.

A Letter of Agreement (LOA) for advanced development of an improved topical arthropod and leech repellent was initiated in January 1981 in cooperation with the Materiel Division, Academy of Health Sciences, acting as the Combat Developer. This LOA is currently being staffed through other interested agencies by the Academy. It provides for the formulation studies and the performance and safety testing needed developing an improved repellent and for a number of additional RDT& activities that will be needed to produce an end-product suitable for military use. These additional activities include climatic tests, package development, odor testing, infrared spectral analysis, troop acceptance testing and tests for compatibility of the product with rubber and plastic items, uniform fabrics and finishes, topical

decontaminants, face paints, etc.

Several advances and improvements in repellent testing capabilities and methods were achieved during the year: (1) The first tests of repellents against Rhodnius prolixus and Leptotrombidium fletcheri were accomplished at LAIR in FY 81. These species are important vectors of Chagas' disease and scrub typhus, respectively. (2) It was demonstrated that the yellow fever mosquito, Aedes aegypti, is an exceptionally poor predictor for the responses of two important vectors of malaria (Anopheles stephensi and Anopheles albimanus) to repellents. Aedes aegypti has been the traditional standard test species in repellent screening programs. (3) The parallel-line bioassay technique was applied to tests of insect repellents for the first time. This type of test is much used in pharmacology, toxicology, and related fields. (4) An improved version of the arm test for mosquito repellents (Annual Research Progress Report, FY 79) was drafted on the basis of experience accumulated since its initial development. This method has been approved by the LAIR Protocol Review and Human Use Committees and is currently being considered by the USAMRDC Human Use Committee as a type protocol. The refined version has also been submitted to the American Society for Testing and Materials where it is being considered as an ASTM Standard. (5) An in vitro test based on dose-response principles was developed for testing repellents against chigger mites. (6) Techniques for using white rabbits in testing repellents (Annual Research Progress Report, FY 80) were improved and elaborated. These new techniques substantially reduce the need for tests on human subjects in the program.

CONCLUSIONS

It has been demonstrated that the period of effectiveness of diethyl toluamide on the skin can be extended by both the microencapsulation technique (Bend Research Inc. formulations) and the polymer additive technique (LAIR formulations). It is reasonable to expect that other current problems with this material (user resistance, damage to rubber and plastic items, incompatibility with face paints, etc.) can be ameliorated or overcome by similar methods. This approach is likely to be more productive and less costly than that of screening new compounds for a material having ideal characteristics.

The problem of extending the spectrum of efficacy of the Army repellent can be approached by adding a second and, if needed, a third active ingredient to the formulation. This approach was shown to be valid during World War II. Both commercial repellents (Annual Research Progress Report, FY 80) and experimental repellents ("Results and

Discussion" above) can be considered for this role. Although most other repellents are not as persistent as diethyl toluamide, their period of effectiveness on the skin can be extended by formulation techniques or by including higher concentrations of the less persistent materials in the final product.

RECOMMENDATIONS

It is recommended that the draft LOA for an Improved Topical Arthropod and Leech Repellent be finalized and approved and that specific responsibilities for each required aspect of the RDT&E effort be assigned and the resources necessary for their completion be allocated.

Recommend that the thrust of the exploratory and advanced development programs should be toward re-formulation of diethyl toluamide to satisfy the "System Description" section of the LOA. The new formulation should if possible include one or more additional repellents to extend the spectrum of efficacy of the main ingredient.

Recommend that testing of new compounds for possible use in conjunction with or in lieu of diethyl toluamide be continued as a long-range development strategy.

Recommend command action to obtain adequate test quantities of SRI compounds 434-58 and 835-19C and USDA compounds AI3-36166 and AI3-36178.

PUBLICATIONS

REIFENRATH, W.G., and W.A. AKERS. Field testing of repellents against anopheline mosquitoes. Mosquito News 41:276-280, 1981

WIRTZ, R. A., J.D. TURRENTINE, and R.C. FOX. Area repellents for mosquitoes (Diptera: Culicidae): Identification of the active ingredients in a petroleum oil fraction. J Med Entomol 18:126-128, 1981

RUTLEDGE, L.C., M.A. LAWSON, L.L. YOUNG, and M.A. MOUSSA. Non-Correlation of insecticide and repellent tolerances in representative species and strains of mosquitoes. Mosquito News 41:684-688, 1981

RUTLEDGE, L.C., M.A. LAWSON, and L.L. YOUNG. Tests of repellents against Diamanus montanus (Siphonaptera: Ceratophyllidae). J Med Entomol (in press)

BUESCHER, M.D., L.C. RUTLEDGE, R.A. WIRTZ, K.B. GLACKIN, and M.A. MOUSSA. Laboratory tests of repellents against Lutzomyia longipalpis (Diptera: Psychodidae). J Med Entomol (in press)

HOOPER, R.L., and R.A. WIRTZ. Insect repellent used by troops in the field: Results of a questionnaire, Military Med (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE 6087	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	H. Term	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AD	084 JL03
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7,2;5		

11. TITLE (Precede with Security Classification Code)*

(U) CPDA-2 Clinical Trials

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 01	81 09	DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING		
FISCAL YEAR CURRENT		

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research

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RESPONSIBLE INDIVIDUAL

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21. GENERAL USE

Foreign Intelligence Not Applicable

20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Moore, Gerald L., Ph.D., DAC

NAME: Bolin, Robert B., LTC, MC

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Blood Storage; (U) Military Blood Banking; (U) Red Cell Survivals; (U) Adenine

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The final objective of this study, clinicals trials of an improved anti-coagulant is Food and Drug Administration's licensure, which would permit clinical use of red cells after prolonged liquid storage. Shipment of blood into combat areas necessitates delays between drawing and infusion; the impact of these dalays on the quantity of red cells infused will be minimized through use of an improved anti-coagulant-preservative solution.

24. (U) Currently, red cell liquid storage in citrate-phosphate-dextrose-adenine-1 (CPDA-1) anticoagulant-preservative is limited to 35 days. Survivability of packed red cells (PC) stored in CPDA-1 for 35 days is marginally acceptable. In vitro studies of metabolism in red cells and platelets stored in modified CPD-adenine suggest that increased adenine and glucose in the preservative will improve survivability. Such improvements may allow extension of red cell storage time to 42 days or beyond. The Division of Blood Research, LAIR, is coordinating efforts with civilian and container-solution manufacturers in the execution of clinical trials of prom g improved CPD-adenine formulations and CPDA-2.

25. (U) 8010-8109 Human in vivo red cell survival studies have been completed in an effort to extend blood storage to 42-56 days. Completed studies indicate that the survivability of packed red cells and whole blood stored in CPDA-2 for 35 days in significantly superior to that of blood stored in CPDA-1. Furthermore, erythrocyte viability is well preserved after 42 days of storage. Preliminary results of studies performed at 49 days suggest that viability may be preserved in CPDA-2 for prolonged periods of storage. After federal approval of CPDA-2 as a new preservative, this project should be terminated.

* Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 084 CPDA-2 Clinical Trials

The following investigations have been conducted under this work unit:

STUDY NO. 1 Platelet studies - CPDA-2

STUDY NO. 2 Red cell studies - CPDA-2

STUDY NOS. 1 and 2. Recent collaborative efforts with several laboratories, initiated and guided by the Division of Blood Research, LAIR, culminated in the development of a new blood preservative which was approved by the FDA. The new preservative, Citrate-Phosphate-Dextrose-Adenine-1 (CPDA-1) is a marked improvement over previously used preservatives since it extended the shelf life of blood by 67% (i.e., up to 35 days) and improved the quality of the preserved red blood cells. The CPDA-1 preservative is not optimal, and in vitro studies suggested that improved formulations might extend storage beyond 35 days even for packed red blood cells. Such an improved formulation with modified amounts of dextrose and adenine is now undergoing clinical trials to establish human utility and to comply with the requirements for approval. Clinical trials for both red blood cells and platelets have been performed. In vivo red cell survival studies showed that CPDA-2 is far superior to CPDA-1 at day 35 for preservation of whole blood and packed red blood cells (at hematocrits below and above 80%). These studies further demonstrated that CPDA-2 fulfilled all FDA criteria for red blood cell storage to 42 and, possibly, to 49 days. Further studies with CPDA-2 revealed that is has no adverse effects on platelets under storage conditions, and no adverse effects on plasma proteins. This approach has great importance for meeting military needs, since prolonging the storage life of blood will ease the logistical problems related to the provisions of blood for a combat zone. The better the preservative, the more universal will be its acceptance. The use of a militarily oriented blood preservative by the national civilian blood banking community will ensure that future military needs can be met from existing blood supplies.

BODY OF REPORT

WORK UNIT NO. 084 CPDA-2 Clinical Trials

STUDY NO. 1 Platelet studies - CPDA-2

PROBLEM

Before FDA approval of a new preservative, it must be documented that the solution will not adversely affect any usable component of blood, such as plasma proteins and platelets. Approval of CPDA-1 was delayed due to the lack of data concerning the effect of the preservative on platelets. CPDA-2, the new preservative, developed in part by the Division of Blood Research to optimize the concentrations of adenine and glucose for red cell storage, is now ready for clinical use. Concurrent with red cell storage, platelet studies should be performed to insure that the preservative is not injurious to this blood component.

RESULTS AND DISCUSSION OF RESULTS

Twelve normal volunteers were used to obtain in vivo data about platelets prepared and stored in CPDA-2 (treatment group, N=6) and CPD (control group, N=6). Whole blood collected into each preservative was held at ambient temperature for eight hours before processing platelet concentrates. These concentrates were stored in a conventional manner for 72 hr, then labeled with ^{51}Cr and transfused into the original volunteer. Platelet recovery and survival were then determined. CPDA-2 platelets had a mean recovery of 32.5% and survival of 6.1 days, whereas CPD platelet had a mean recovery of 39% and survival of 6.7 days. The differences between CPDA-2 and CPD were not statistically different.

CONCLUSION

Platelet preservation is no different for CPDA-2 collected platelets compared to CPD collected platelets.

RECOMMENDATIONS

These data should be used to support efforts to get Food and Drug Administration (FDA) approval for general blood banking use in the U.S. for CPDA-2.

PUBLICATIONS

One manuscript has been submitted. These data have also been submitted by Fenwal Laboratories to the Bureau of Biologics, FDA, for approval of CPDA-2 as a new preservative.

CPDA-2 Clinical Trials (continued)

STUDY NO. 2 Red cell studies - CPDA-2

PROBLEM

Recent collaborative efforts by several laboratories, initiated and guided by the Division of Blood Research, LAIR, culminated in the development and FDA approval of a new preservative, CPDA-1. This preservative was a marked improvement over CPD and ACD. It extended shelf life from 21 to 35 days and improved the quality of red cells. CPDA-1 is not an optimal preservative, particularly for packed cells stored for 35 days. Studies in this laboratory suggest that adjustments in the concentration of adenine and additional glucose could result in greater than 35-day storage and an improvment in the quality of stored packed cells. This approach has marked impact for military needs since prolonging the shelf life of blood will improve logistic support for combat zone needs. The better the preservative, the more universal its acceptance. The use of a military-oriented preservative by civilian blood banks will ensure military needs are met from existing blood supplies.

RESULTS AND DISCUSSION OF RESULTS

Intramural studies to evaluate red cell survival rates in whole blood and packed red cell units (each at 35, 42, 49, and 56 days of storage) and to determine the maximum acceptable length of storage have been completed. The results of studies performed at 35, 42, and 49 days are summarized below:

CPDA-2 RED CELL SURVIVAL

(24-Hour Post-Transfusion Survival)

Days of Storage	Whole Blood (%)	Packed Red Cells (%)
35	84.4±5.58 (N=5)	91.5±5.9 (N=4)
42	74.2±10 (N=5)	74.0±7.0 (N=16)
49	73.6±5.0 (N=4)	70.2±4.6 (N=5)

Four units of blood stored for 56 days had a mean survival of 66% (range 53-74%). In vitro biochemical studies performed on these units indicated changes associated with blood storage that are comparable with CPD and CPDA-1. Red cell samples from these studies were supplied to investigators at Stanford and UCSF medical centers to measure endocytosis and deformability (by ectocytometry),

respectively. No correlation was found between red cell survival and endocytosis; however, the correlation between survival and deformability was R=0.94 (N=12), and between survival and end % ATP, R=0.73 for N=42. The survival studies at 42 days of storage have been corroborated by extramural studies performed by Dr. E. Beutler, La Jolla, CA.

CONCLUSIONS

These studies have established that CPDA-2 is superior to CPDA-1 and is capable of successfully preserving whole blood or packed red cells for at least 42 days. Preliminary results (N=9) suggest that the maximum storage capacity may be extended to 49 days.

RECOMMENDATIONS

Fenwal Laboratories should be supported in their effort to get FDA listing of CPDA-2 to replace CPDA-1 and allow for 42 days of blood storage. A special military exclusion to use CPDA-2 for 49 days should be considered.

PUBLICATIONS

Four manuscripts have been submitted. These data have also been submitted by Fenwal Laboratories to the Bureau of Biologics, Food and Drug Administration (FDA) for approval of CPDA-2 as a new preservative.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE 6108	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	H.Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	087 APC HL11
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Animal Models for Surgical Repair of Musculoskeletal Structures

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 003500 Clinical Medicine; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	0.1	6
	CURRENT 82	0.0	0

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Cabaud, H.E., LTC, MC, USAR

NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Surgical Repair; (U) Combat Injuries; (U) Trauma; (U) Nerve Graft; (U) Microsurgical Technique; (U) Laboratory Animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Lost duty days, permanent disability, and expenditure of medical resources are the extremely costly results of extremity nerve injuries in military personnel. To return personnel to duty with maximum function in minimum time, improved surgical and therapeutic techniques are needed. Studies completed under this work unit suggest that the method of nerve repair might not be the primary factor in ultimate return of function; rather, some biochemical or immunological phenomenon might prevail. Future studies will concentrate on these aspects.

24. (U) Further evaluation was continued on specimens from the 16 cynomolgus monkeys in which segmental nerve defects simulating combat injuries of 0, 1, 2, or 3 cm had been produced. The digital nerves, far distal to the neurorrhaphy site in the parent (ulnar) nerves, were studied extensively for axon growth and evidence of fibrosis and scarring.

25. (U) 8010-8109 Although there were no statistical differences between the two repair techniques, the digital nerves (end organs) were found to have a markedly decreased percentage of axon regrowth and much more fibrosis than the ulnar nerves just distal to the repair site. These findings suggest that methods must be found which will minimize fibrosis and scar formation so regenerating axons can successfully reach their end organs. This work unit will be combined with another work unit, Agency Accession DAOE 6309, Studies in Combat Injuries to the Extremities.

* Applic to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 087 Animal Models for Surgical Repair of Musculoskeletal Structures

The following investigation has been conducted under this work unit:

Study No. 1 Nerve repair in monkeys

The ulnar nerve of the cynomolgus monkey was used as a model for repair of lacerated peripheral nerves. Sixteen monkeys underwent bilateral ulnar nerve transection and resection of 0, 1, 2, or 3 cm of the ulnar nerve in the mid-forearm to simulate combat-type segmental nerve defects. By using microsurgical techniques, standardized at LAIR, one side was repaired by an epineurial technique under tension and the contralateral side was repaired by using multiple interfascicular sural nerve grafts. Five months after the neurorrhaphies, the animals were evaluated for return of function. Evaluation included axon counts, proximal and distal to the neurorrhaphies, as well as in the mid-graft segment on the grafted side and in the appropriate digital nerves in the hand. Additional evaluation included the weights of the reinnervated intrinsic muscles of the hand and histologic evaluations of the neuromas and reinnervated muscles. Clinically, all neurorrhaphies healed and there was evidence of reinnervation of the intrinsic muscles of the hand. The results indicate the amount of nerve tissue lost during the initial injury is more important than the type of repair in determining the end result after neurorrhaphy. Furthermore, the digital nerves were found to have a markedly decreased percentage of axon regrowth and much more fibrosis than the ulnar nerves just distal to the repair site. These findings suggest that methods must be found that will minimize fibrosis and scar formation so regenerating axons can successfully reach their end organs.

BODY OF REPORT

WORK UNIT NO: 087 Animal Models for Surgical Repair of Musculoskeletal Structures

STUDY NO: 1 Nerve repair in monkeys

PROBLEM

Peripheral nerve injuries are common in both combat and noncombat military accidents. Many war injuries from the Vietnam conflict included severe damage to the peripheral nerves of the upper and lower extremities. During a 24-month period, 54% of all casualties in military hospitals had such injuries. Although our technical capabilities in the surgical repair of peripheral nerves have progressed greatly during the last several years, we still do not have a good method of managing segmental nerve defects. Tension at the repair site has been considered detrimental to nerve regeneration and healing. Consequently, the use of a multiple nerve graft has been advocated. Problems of repairing a nerve under tension (where joints must be flexed, nerves must be mobilized, and vascularity is diminished) are not completely overcome by the use of multiple nerve grafting procedures (in which an avascular unmatched segment is used to bridge the defect and relieve tension). Intrafascicular grafting not only results in the interposition of an avascular segment which loses all endoneurial elements and structure, but this technique also requires two separate neurorrhaphies which regenerating neurites must cross. This study objectively compares epineurial end-to-end repairs with tension to interfascicular grafts without tension following loss of a nerve segment. The injuries produced simulate combat-type segmental nerve defects.

RESULTS AND DISCUSSION OF RESULTS

These results represent data from the continued analysis of specimens obtained from a study for peripheral nerve repair first reported in the previous progress report. Using the model we developed and have previously described, 16 cynomolgus monkeys underwent resections of 0, 1, 2, or 3 cm of both ulnar nerves in the mid-forearm. On one side, a repair was accomplished (using 8-0 nylon) by standard epineurial technique under varying amounts of tension as determined by the amount of defect. The contralateral nerve was repaired by using autogenous cutaneous sural nerve grafts which eliminated all tension at both suture lines; size 10-0 nylon was used to suture the grafts. A microsurgical technique, using appropriate magnification, was used for all nerve repairs. Five months after the nerve sutures, the monkeys were evaluated for return of function. Subjective evaluation included inspection of the neuromas and stimulation of the ulnar nerves proximal to the neurorrhaphies with evaluation of the amount of

contraction in the intrinsic muscles of the hand. Objective evaluation included weights of the ulnar innervated hypothenar intrinsic muscles in the hands, as well as the axon counts of myelinated nerve fibers proximal and distal to the neurorrhaphies and in the reinnervated digital nerves in the fourth and fifth fingers.

Objective evaluations have been completed, and no statistical differences were found between the two techniques. Extensive evaluation of the digital nerves, taken distal to the neurorrhaphy site in the ulnar neves, revealed a markedly decreased percentage of axon regrowth and much more fibrosis than that found just distal to the ulnar repair. Regardless of the repair technique, these adverse findings were consistent, and some digital nerve had as little as 50% axon regrowth and up to twice the amount of neural fibrosis when compared to their parent (ulnar) nerves. This phenomenon obviously leads to reduced return of function.

CONCLUSIONS

We do not have a satisfactory answer to the management of segmental defects of peripheral nerves. Based on a comparison of this and earlier studies, nerves repaired without tension, compared to those with segmental defects, have a greater return of function. Consequently, the most important factor influencing the end result is not the surgical technique, but more likely the amount of nerve tissue lost at the time of injury. From this study it appears that other factors may be important in determining return of function. Certainly, these findings dictate that methods must be found that will minimize fibrosis and scar formation in the nerves at the level of the end organs.

RECOMMENDATIONS

From these studies it is apparent that other considerations such as biochemical and immunologic responses, the role of nerve growth factor, and further work on surgical technique, must be studied to gain more knowledge about the management of peripheral nerve injuries and to maximize ultimate return of function. This work unit will be combined with another work unit, Agency Accession DAOE 6309, "Studies in Combat Injuries to the Extremities."

PUBLICATIONS

1. RODKEY, W.G., H.E. CABAUD, and H.R. McCARROLL. Neurorrhaphy after loss of nerve segment: Comparison of epineurial suture under tension versus multiple nerve grafts. J Hand Surg 5:366-376, 1980

2. RODKEY, W.G., and H.E. CABAUD. Peripheral nerve injury and repair. IN: Current Techniques in Small Animal Surgery, 2nd Edition, edited by J. Bojrab. Philadelphia: Lea and Febiger, in press

3. CABAUD, H.E., W.G. RODKEY, and T.J. NEMETH. Progressive ultrastructural changes following peripheral nerve transection and repair. J Hand Surg, in press

4. HARRIS, H.G., H.E. CABAUD, H.R. McCARROLL, and W.G. RODKEY. Neurorrhaphy after loss of a nerve segment: Experimental studies in primates comparing epineurial suture under tension versus multiple nerve grafts. (Abstract) Ortho Trans 5:100, 1981

5. HARRIS, H.G., H.E. CABAUD, H.R. McCARROLL, and W.G. RODKEY. Neurorrhaphy after loss of a nerve segment: Experimental studies in primates comparing epineurial suture under tension versus multiple nerve grafts. (Abstract) J Hand Sug 6:288, 1981

6. CABUD, H.E., W.G. RODKEY, and T.J. NEMETH. Progressive ultrastructural changes following peripheral nerve transection and repair. (Abstract) Ortho Trans 5:102, 1981

7. CABAUD, H.E., W.G. RODKEY, and T.J. NEMETH. Progressive ultrastructural changes following peripheral nerve transection and repair. (Abstract) J Hand Surg 6:290, 1981

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE 6090	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	H. TERM	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AC	090 JL05
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)*

(U) Investigation of Cell-Free Resuscitating Solutions

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

008800 Life Support; 003500 Clinical Medicine; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 03	81 09	DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	6.6	460
FISCAL YEAR CURRENT 82	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Acute Resuscitation; (U) Stroma-Free Hemoglobin; (U) Blood Substitute Solutions; (U) Hemorrhagic Shock

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Hemoglobin, free of cell constituents, can provide the basis for an ideal resuscitating fluid for the severely wounded soldier. It has several advantages as compared to other blood substitutes or plasma expanders. It is capable of in vivo on-loading and off-loading oxygen with sufficient efficiency to maintain oxygen consumption in experimental animals rendered virtually free of circulating red cells. Hemoglobin can be stored for extended time thus alleviating logistic problems in fluid therapy of mass casualties situations. The object of these studies is to evaluate the effectiveness of the hemoglobin solution as a resuscitating fluid for military use.

24. (U) Hemoglobin, prepared by crystallization from outdated human red cells has been evaluated as a cell-free resuscitation solution in animal models for its effect on critical organ function and maintenance of morphological integrity. Formulations of solutions optimal with regard to concentration and physical configuration of the hemoglobin molecules have been investigated.

25 (U) 8010-8109 The initial objectives of this work unit have been fulfilled. The development of hemoglobin solution as a resuscitation fluid and its evaluation in vivo in small animals have been met and the results have been reported in scietific journals. The data acquired have produced a clear picture of the potential use of hemoglobin solution as a blood substitute. Therefore, this work unit has been terminated and new objectives to be pursued as an evolution of these completed studies have been incorporated in two protocols entitled "Efficacy aspects and clinical evaluation of hemoglobin solutions as resuscitation fluids" and "Chemical modifications of hemoglobin for improved efficacy as a cell-free resuscitating solution".

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 090 Investigation of Cell-Free Resuscitating Solutions

The following investigations have been conducted under this work unit:

STUDY NOS. 1,2,4,5,6 Preparation of hemoglobin, in vivo evaluation, pharmacokinetics, and effects of hemoglobin on organs

STUDY NOS. 1,2,4,5 and 6. The development and evaluation of hemoglobin solution as a resuscitation fluid for combat casualties was initiated in the Division of Blood Research at LAIR in 1975. Since that time the objectives of this work unit have been fulfilled. Many experiments in vitro and in vivo, using small animals, have shown that hemoglobin solution can be prepared in the large quantities needed for massive fluid replacement therapy and can be effective in restoring and maintaining vital signs when used as a resuscitation solution. From these studies new objectives have evolved and these objectives have been incorporated into two more comprehensive and pertinent protocols.

BODY OF REPORT

WORK UNIT NO.	090	Investigation of Cell-Free Resuscitating Solutions
STUDY NOS.	1,2,4,5,6	Preparation of hemoglobin, in vivo evaluation. pharamcokinetics, and effects of hemoglobin on organs

PROBLEM

It has long been evident that significant advantages can be gained by developing a resuscitating solution capable of transporting oxygen, maintaining oncotic pressure, and being readily available when massive clinical transfusions are required. Stringent requirements must be met by resuscitating solition. As a blood substitute, it must be capable of restoring vital functions, but not elicit permanent adverse effects when administered to mass casualty victims. Furthermore, it must be uniquely suited to fulfill the supply, storage, and transportation requirements for field use in combat situations. Plasma, dextran, albumin, and other preparations have been used, and although they appear to be effective as plasma expanders, they donot transport oxygen. As a resuscitating fluid, blood has a limited storage life, must be stored in bulky energy-requiring refrigerators, and requires typing and cross-matching before use.

In most civilian settings in this country, transfusion requirements associated with massive trauma can be met with conventionally stored blood and its components. However, military field requirements frequently demand massive fluid support in areas isolated from supply sources. The inability to predict when modest transfusion requirements may suddenly increase complicates fluid therapy logistics. The ability to stockpile a stable protein solution, capable of carrying and exchanging oxygen, would minimize many of these difficulties.

Hemoglobin is a protein that has such potential. A hemoglobin solution presents numerous advantages compared to other blood substitutes or plasma expanders. Hemoglobin is a component of normal blood, can be prepared from outdated human erythrocytes, does not require typing or cross-matching before use, is capable of transporting oxygen to the tissues, has oncotic activity, has low viscosity, does not cause microaggregates, and may not induce significant immunologic reaction. Furthermore, hemoglobin is highly soluble in physiologic solutions and can be stored for extended periods of time.

The potential value of hemoglobin solution as an oxygen-carrying blood substitute has been recognized also in some special situations: (1) This solution could be used in the treatment of hemorrhagic shock when compatible blood is not available or when constriction of the capillary vessels in the microcirculation would dictate using a fluid with lower viscosity than blood for normovolemic hemodilution. (2) It could be helpful in the military field operating room when prolonged blood loss occurs, thus saving a large volume of donor blood which could be used later. (3) In open heart surgery, hemoglobin solution could be of great advantage in priming the pump and/or maintaining circulation during surgery, again saving the patient's blood intact, without mechanical stress, for better use at the end of surgery. (4) Hemoglobin solution can be used as a perfusate to preserve various organs for long periods of time in a normothermic environment, maintaining the normal oxygen tension and oncotic pressure necessary during preservation. (5) In metabolic studies, solutions of hemoglobin can be formulated with the required components and used in organ perfusion, allowing results that are unaffected by the background compounds present when blood is used.

However, if hemoglobin is used as a blood substitute, it is imperative that it be free of any stromal particles, stromal lipid, or other soluble and insoluble cell components which have been implicated in adverse effects on kidney function and coagulation factors. Hemoglobin has the potential of becoming an important blood substitute and providing the basis for an ideal resuscitating solution for the severely wounded soldier. Developing an effective blood substitute is pertinent not only to military combat casualties but also to civilian casualties.

RESULTS AND DISCUSSION OF RESULTS

This work unit has been terminated because the initial objectives have been fulfilled. The development of hemoglobin solution as a resuscitation fluid and its evaluation in vivo in small animals have been met. A simple, reproducible method for preparing hemoglobin from outdated human red cells has been established. The in vitro characteristics of the hemoglobin solutions, thus prepared, have been studied and reported. Long-term storage conditions, with specific emphasis on non-refrigerated, non-liquid storage have also been developed and reported in scientific publications. In vivo evaluation of the hemoglobin solution, as prepared in our laboratory, has been pursued in small animal models, exchange-transfused with hemoglobin solution to different levels of blood replacement. Survival of animals, in vivo oxygen capacity, oncotic pressure, disposition and organ distribution of hemoglobin, oxygen transport and viscosity at different hemodilutions,

morphologic effects on liver and kidney cells after massive transfusions with hemoglobin solution and several other physiologic, hematologic and biochemical aspects have been investigated. From these studies new insights have developed and further investigations have been directed at systematic improvement of the present product and evaluation efficacy and clinical use of hemoglobin solutions as resuscitation fluids. These new objectives have been incorporated in new protocols.

CONCLUSIONS

The development and evaluation of hemoglobin solution as a resuscitation fluid for combat casualties was initiated in the Division of Blood Research at LAIR in 1975. Since that time the objectives of this work unit have been fulfilled. Many experiments in vitro and in vivo, using small animals, have demonstrated that hemoglobin solution can be prepared in the large quantities needed for massive fluid replacement therapy and can be effective in restoring and maintaining vital signs when used as a resuscitation solution. From these studies new objectives have evolved and these objectives have been incorporated into two more comprehensive and pertinent protocols entitled "Efficacy aspects and clinical evaluation of hemoglobin solutions as resuscitation fluids" and "Chemical modifications of hemoglobin for improved efficacy as cell-free resuscitating solution".

RECOMMENDATIONS

Study on the efficacy and clinical evaluation of hemoglobin solution as a resuscitation fluid should be continued due to its potential application in humans. It is recommended that for these studies higher animals be used (pigs, primates) since they permit better monitoring of physiologic parameters.

PUBLICATIONS

1. DEVENUTO, F., H.I. FRIEDMAN, and P.W. MELLICK. Massive exchange transfusions with crystalline hemoglobin solution and subsequent replacement of hemoglobin and blood volume. Surg Gynecol Obstet 151:361-365, 1980

2. DEVENUTO, F., A.I. ZEGNA, K.R. BUSSE, and C.C. PECK. Evaluation of a reverse osmosis apparatus for field production of USP grade injectable water from sea water, pond water and human urine. Letterman Army Institute of Research Report No. 85 Presidio of San Francisco, CA: Military Med 145:831-835, 1980

Investigation of Cell-Free Resuscitation Solutions (continued)

3. MOORES, W., F. DEVENUTO, W.H. HEYDORN, R.B. WEISKOPF, B.S. BAYSINGER, A.F. GREENBURG, and J.R. UTLEY. Extending the limits of hemodilution on cardiopulmonary bypass using stroma-free hemoglobin solution. J Thorac Cardiovasc Surg 81:155-162, 1981

4. DEVENUTO, F., K.R. BUSSE, and A.I. ZEGNA. Oxygen transport by human blood hemodiluted with crystalline hemoglobin solution. Surg Gynecol Obstet 153:332-336, 1981

5. DEVENUTO, F., and A.I. ZEGNA. Transfusion with pyridoxalated-polymerized hemoglobin solution. Transfusion 21:599, 1981

6. DEVENUTO, F., K.R. BUSSE, and A.I. ZEGNA. Viscosity of human blood hemodiluted with crystalline hemoglobin solution. Transfusion 21:752-756, 1981

7. DEVENUTO, F. Hemoglobin solution: A potential oxygen-transporting resuscitation solution. La Trasfusione Del Sangue, 26:163-177, 1981

8. DEVENUTO, F. Hemoglobin solutions as oxygen-delivering resuscitating fluids. Crit Care med 10:238-245 (in press)

9. MOORES, W.Y., F. DEVENUTO, W.H. HEYDORN, A.G. GREENBURG, and J.R. UTLEY. Effectiveness of hemoglobin solution as seen in a right heart bypass swine model. Crit Care Med 10:279-282 (in press)

10. SCUSCHEREBA, S.T., H.I. FRIEDMAN, F. DEVENUTO, and B.S. BEATRICE. The morphological effects on the retina of massive exchange transfusion with stroma-free hemoglobin. Lab Invest (in press)

11. MOORES, W.Y., F. DEVENUTO, W.H. HEYDORN, and A.F. GREENBURG. Hemoglobin solutions and hemodynamics in a non-shock model. Proc Cur Concepts Casualties (in press)

12. DEVENUTO, F. Acellular oxygen-delivering resuscitation fluids: Hemoglobin solutions. Proc Curr Concepts Combat Casualties (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 3372	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	K. Completion	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	093 APC HL04
b. CONTRIBUTING				
c. CONTRIBUTING	S10G	80-7.2:5		

11. TITLE (Precede with Security Classification Code)

(U) Laser Acceleration of Soft Tissue Wound Healing

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 008800 Life Support; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 01	81 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	1.0	63
FISCAL YEAR CURRENT 82	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Surinchak, John S., SFC

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Wound Healing; (U) Military Trauma; (U) Animal Model; (U) Laser

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Experimental data exist suggesting that laser irradiation of full-thickness skin defects accelerates wound healing. It is necessary to confirm these findings and to determine whether or not the extent to which wounds heal faster is clinically significant. Large wounds, such as those received from mines, shrapnel, or bullets, require debridement and a long recovery. The ability to significantly accelerate healing would save thousands of man-days during the convalescence of soldiers wounded in combat.

24. (U) Full-thickness skin defects of a standard size will be created in rabbits. One group will be treated by daily dressing changes, while a second group will also be irradiated with a helium neon laser at various dosages and treatment times. Wound surface area will be measured every day and statistical comparison made between the control and treated groups. Further studies will include measurement of breaking strength in excised-sutured wounds with and without laser exposure.

25. (U) 80 10 - 81 09 Laser irradiation of full-thickness skin defects does not alter the rate at which full-thickness skin defects close. We have demonstrated an increase in breaking strength of sutured incisions 14 days postoperatively but feel this is not clinically significant enough to be recommended for use in the hospital setting. Experience gained during this research enabled us to develop an animal model for use in other wound healing experiments.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO: 3S162772A874 Care of the Combat Casualty

WORK UNIT NO: 093 Laser Acceleration of Soft Tissue Wound Healing

According to reports in the European literature, laser radiation accelerates the healing of soft tissue wounds. A rabbit model was developed at LAIR to investigate the effects of helium-neon laser radiation on healing. Circular full-thickness skin defects were exposed to the laser radiation at various energy levels and treatment periods. No difference was demonstrated in absolute wound area or rate of healing between the experimental and the control groups. Conversely, the breaking strength of sutured incisions in rats increased by 55% at 14 days postoperatively after having received 2.2 J/cm^2 twice daily. Nonsignificant increases were observed at 28 days postoperatively.

BODY OF REPORT

WORK UNIT NO. 093 Laser Acceleration of Soft Tissue Wound Healing

PROBLEM

The Hungarian surgeon Janos Meister has reported that laser irradiation accelerates healing in a variety of wounds, including chronic decubiti in humans and freshly sutured incisions in rats (Panminerva Medica 17:229, 1975; Experientia 30:1296, 1974). The mechanisms by which the laser affects healing remains uncertain but may involve accelerated formation of cross-links between collagen fibrils secondary to increased concentration of superoxide radicals in the irradiated tissue. Finding ways of increasing the rate at which full-thickness tissue defects heal has military relevance because such wounds are common following debridement of high velocity through-and-through gunshot wounds. We attempted to reproduce certain portions of Dr. Meister's work. Full-thickness skin defects on the backs of rabbits were irradiated with a helium-neon laser at various treatment times and energy levels. Wound surface area was measured daily and compared with an untreated control. After healing, wounds were excised and the force required to cause disruption was measured. Wound breaking strength has also been measured in sutured incisions in rats, one group serving as a control and a second group being irradiated with a laser.

RESULTS AND DISCUSSION OF RESULTS

This study examined the effects of low level helium-neon laser radiation on: 1) wounds that closed primarily by contraction, and 2) the breaking strength of straight line incisions. Circular full-thickness skin defects in rabbits received dosages of 1.1 J/cm^2 during a 30-minute exposure period every 3 days and 2.2 J/cm^2 during a 3-minute exposure period twice daily. Both groups of animals were irradiated until wound closure. No significant differences in healing were observed between laser-treated wounds and untreated controls. Conversely, rat skin incisions that received 2.2 J/cm^2 during 3-minute exposure periods twice daily for 14 days demonstrated a significant increase in breaking strength of 55% over the controls. At 28 days postoperative this difference in breaking strength diminished to a nonsignificant increase of 16% over the controls. Increasing the dosage to 4.5 J/cm^2 yielded a nonsignificant increase of 17% over the controls at 14 days postoperative. These increases, although initially impressive, may not be clinically significant as the wound has regained only 8% of its original strength at 14 days and about 38% at 28 days. The laser radiation would therefore increase these breaking strengths to only 12% and 44%, respectively. Laser treatment of incisions may be

neither clinically nor economically beneficial when equipment cost, treatment time, and personnel costs are considered.

CONCLUSIONS

We have been unable to show that laser irradiation of soft tissue wounds accelerates wound healing. Although it is not significant enough to apply at the clinical level, increases in wound breaking strength were demonstrated.

RECOMMENDATIONS

Recommend termination of research due to lack of significant results.

PUBLICATIONS

1. SURINCHAK, J.S., M.L. ALAGO, R.F. BELLAMY, B.E. STUCK, and M. BELKIN. The effects of low level energy lasers on the healing of full-thickness skin defects (submitted for publication)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 8393	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. NEW	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AC	099 JL08
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80.7.2:5		

11. TITLE (Precede with Security Classification Code): Chemical Modifications of Hemoglobin for Improved Efficacy as a Cell-Free Resuscitating Solution

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 008800 Life Support; 003500 Clinical Medicine; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 07	CONT	DA	C. IN-HOUSE

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 81	0.0	00
CURRENT FISCAL YEAR 82	3.6	158

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
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RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
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TELEPHONE: (415) 561-5875
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: POC:DA

21. GENERAL USE
Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Acute Resuscitation; (U) Stroma-Free Hemoglobin; (U) Blood Substitute Solutions; (U) Intramolecular Cross Link Reagent

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective of these studies is to develop and evaluate an effective hemoglobin solution (cell free) as a blood (red cell) substitute, which can be stockpiled (long shelf life) and utilized in forward combat areas for the resuscitation of casualties (used by paramedics without need for laboratory or diagnostic support). Hemoglobin solutions have a significant advantage over plasma expanders because of the additional capacity to transport oxygen to the peripheral tissue, and would be more stable, economical and available for emergency usage than packed red blood cells.

24. (U) Formulations of hemoglobin solutions that do not cause adverse effects when administered intravenously in animal models are under current investigation. Two limitations of stroma-free hemoglobin, namely: 1) high oxygen affinity, and 2) short intravascular retention time, require additional molecular changes to achieve a useful resuscitation product. Current intramolecular modifications of human hemoglobin A with 1) phosphorylated sugars, 2) diasprin esters, and 3) pyridoxal phosphate are in progress to change the physical chemical properties into the desired physiological range.

25. (U) New work unit.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3S162772A874	Care of the Combat Casualty
WORK UNIT NO.	099	Chemical Modifications of Hemoglobin for Improved Efficacy as a Cell-Free Resuscitating Solution

Modification of stroma-free hemoglobin at the cleft with intramolecular cross-linking agents has been attempted in order to prepare a suitable resuscitation fluid. Two chemical reactions are being investigated and show promise. Most of the tangible progress was made in procurement of improved unmodified stroma-free hemoglobin, preparation of cross-linking reagent, and in analytical systems for the modified hemoglobin product. Preliminary data suggest that the intramolecular cross-linked hemoglobin produced by modification with 3,5-dibromo-salicyl-bis-fumarate has the required properties for a resuscitation fluid.

BODY OF REPORT

WORK UNIT NO. 099 Chemical modifications of hemoglobin for improved efficacy as a cell-free resuscitating solution

PROBLEM

Two intrinsic characteristics of unmodified hemoglobin solution, namely its increased oxygen affinity and rapid plasma clearance, impose distinct limitations on combat field use in the massively transfused soldier by requiring repeated infusions of a solution that has decreased oxygen delivery properties. The goals of molecular modification of hemoglobin have been to modify these characteristics to improve the solution for resuscitative purposes. Specific endpoints desired of modified hemoglobin are defined as a P_{50} between 25 and 40 torr (unmodified hemoglobin P_{50} = 13-17 torr) and a plasma retention time of 12 to 24 hours (unmodified hemoglobin plasma retention time = 2-4 hours).

RESULTS AND DISCUSSION OF RESULTS

Most of the work in this laboratory has addressed the chemical modification of the "β cleft" on the hemoglobin molecule with bifunctional reagents of effectively "staple" the molecule together. This particular area of the molecule has reactive, symmetrically placed amino acid side chains available for chemical modification. The "β cleft" also contains the 2,3-Diphosphoglycerate (DPG) binding site, so that chemical modifiers related in size and charge to DPG will have an increased affinity for this area and enhance the specificity of the chemical modification.

By logical extension of the glycosylation of hemoglobin with glucose to yield hemoglobin A_{1c} derivatives, now used clinically to follow long-term (week-to-week) diabetic control of blood glucose, other phosphorylated mono- and disaccharides were investigated to modify stroma-free hemoglobin. Investigation of different pentose and hexose phosphates under varying reaction conditions of pH, ionic strength, buffer species, temperature, concentration of sugar phosphate, concentration of hemoglobin, time of reaction and oxygen saturation were conducted. No reaction yielded a product with a yield of 25% or greater as judged by High Performance Liquid Chromatography (HPLC). Furthermore, the oxygen affinity of the product mixture was always less than the control stroma-free hemoglobin.

After a review of the proposed reaction intermediates most likely to have been formed, our results confirmed the theory that the transition state energy was too large for the reaction to occur

Chemical Modifications of Hemoglobin for Improved Efficacy as a Cell-Free Resuscitating Solution (continued)

rapidly and the change in free energy too low occur with a good yield.

Efforts were then directed to capitalize on the affinity of the sugar phosphates using a more reactive compound, energetically more favorable to increase reaction rate and yield. The reaction chosen was that of an aldehyde with a primary amine to form a reversible covalent bond which can be easily reduced to an irreversible covalent bond with borohydride. The compound selected was the dialdehyde formed from the reaction with sodium periodate and adenosine triphosphate (ATP), previously characterised by King and Carleson, abbreviated oATP.

oATP was chosen because it has a high affinity for the β cleft, the two aldehydes could react with the symmetrical lysines to yield the required molecular cross-link, and the phosphate moeities on the pentose could interact with the penultimate histidine to lower the oxygen affinity. In order to develop this reaction it has been necessary to reproduce the methodology to separate oATP, $IO_4^{=}$ and $IO_3^{=}$, and to analyze each component using thin layer chromatography (TLC) and gel filtration.

Reaction conditions of oATP with stroma-free hemoglobin as function of temperature, pH, ionic strength, buffer composition, length of time, etc., have been monitored by following the oxygen affinity and the amount of dimer formation on sodium dodecyl sulfate (SDS) gel electrophoresis. The data show an increased P_{50} over control stroma-free hemoglobin, but only 10% dimer formation.

A two dimensional gel electrophoresis system is currently under development so that α and β chains can be separated in one dimension and a monomer-dimer separation in the second dimension. This will allow detection of the partial reaction product where only one of the two aldehyde groups underwent reaction. If this occurred it would explain the increased P_{50} data and the low level of cross-link. For example, if the reaction went to completion as anticipated then one would see only two spots on the gel, one for β-dimer and the other for α-monomer. Using ^{14}C ATP, the label would be detected in the β-monomer area if the half reaction occurred.

Solving this chemical problem is important, together with the data already obtained, the exercise of model building with deoxy human hemoglobin would allow the design of a compound homologous to oATP which might have a more desirable distance between the two aldehyde groups, thus promoting the reaction probability.

Chemical Modifications of Hemoglobin for Improved Efficacy as a Cell-Free Resuscitating Solution (continued)

Another class of reagents that show experimental promise are the 3,5-dibromo-salicyl-bis-fumarate esters. Work is in progress to synthesize this reagent and to begin its testing as a chemical cross-linking agent.

Significant progress has been made also in the isolation of high quality stroma-free hemoglobin. A method developed by Dr. Condie at the University of Minnesota yields a product with the lowest amount of stromal contamination, and of methemoglobin compared to other methods. It is also very simple and can be changed to industrial scale preparation needs.

CONCLUSIONS

The reaction of affinity compounds in the cleft of stroma-free hemoglobin to increase vascular retention and decrease oxygen affinity continue to progress. Currently the reaction of oATP and 3,5-dibromo-salicyl-bis-fumarate esters are under study. Greater emphasis needs to be and will be placed on model building and X-ray coordinate data from crystallography to guide the selection of potential cross-linking agents.

RECOMMENDATIONS

It is recommended that we 1) continue to study the reactions of both the dibromo aspirins and oATP, and 2) build molecular models conforming to the known crystallographic models to select compounds with the most advantageous size and stereo-centrifugation.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOE 6103	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AE	083 APC EL08
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Diagnosis and Treatment of Acute Laser Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 05	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	3.0	219
FISCAL YEAR CURRENT 82	5.3	211

19. RESPONSIBLE DOD ORGANIZATION

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NAME:

21. GENERAL USE

Foreign Intelligence not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Intraocular Trauma; (U) Laboratory Animals; (U) Laser Bioeffect; (U) Ocular Physiology

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23(U) Develop measures of visual function suitable for detecting changes in human vision associated with low level laser exposure which are compatible with clinical ophthalmologic test conditions. Explore techniques of treating ocular trauma, including reabsorption of vitreal hemorrhage and prevention of vitreal band formation.

24(U) State of the art microcomputer and optical solid state technology have been employed to develop human visual tests of dark adaptation and color vision. More conventional apparatus has been developed to measure contrast sensitivity, dynamic visual acuity and spectral sensitivity. Using a standardized ocular trauma model in the rabbit, treat vitreal hemorrhage using urokinase, steroids, and penicillamine systemically as well as intraocularly.

25(U) 8010-8109. Vitreal hemorrhage occurs in the eyes of soldiers subjected to ocular trauma. Current treatment may involve surgical removal of the vitreous, if vitreal bands form. Newer research involving intravitreal injection of substances to prevent fibrosis may reduce the necessity for surgery. Clinical evaluation of the urokinase-injected traumatized rabbit eyes indicated no effect in the treatment of the reabsorption of vitreal bands. Later treatment involving more than 1,500 Plough units resulted in rapid blood absorption. Intraorbital and systemic use of penicillamine results in reduction of vitreal band formation after ocular trauma. Triamcinolone injected intraocularly does not significantly reduce vitreal fibrosis.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 083 Diagnosis and Treatment of Acute Laser Injury

The following investigation has been conducted under this work unit:

STUDY NO. 1 The role of superoxide in the posterior segment of the rabbit eye model

EX-3 The effect of superoxide inhibitors in preventing vitreal band formation after double penetrating wounds to the posterior segment of the rabbit eye

Traumatic ocular injury frequently results in the formation of vitreal bands as a direct result of a tract in the vitreous, into which blood, fibroblasts, and debris are deposited. This combination of necessary agents results in the fibroblasts initiating the process of procollagen tissues in the tract with subsequent maturation of the collagen. As the collagen matures, contraction of the tissues occurs. This process ultimately results in detachment of the retina.

Double perforating wounds were induced in rabbit eyes. The wound site of the test eye was injected with 5 mg triamcinolone with follow-up injections in a 0.15cc solution of saline at 24 hr intervals for two days. The control eye was injected with saline solution. In a second experiment, 1 mg of penicillamine was injected into the test eye while the animal continued to receive 5 mg penicillamine, intramuscularly for four days after trauma. A semi-quantitative scaling technique was established using indirect ophthalmoscopy (grades 1-4, based upon estimates of vitreal band formation).

There were no differences in vitreal band formation in the eyes treated with triamcinolone as compared to saline injected eyes. Penicillamine injected eyes showed an 80% reduction in vitreal band formation.

BODY OF REPORT

WORK UNIT NO.	083	Diagnosis and Treatment of Acute Laser Injury
STUDY NO.	1	The role of superoxide in the posterior segment of the rabbit eye model
EX-3		The effect of superoxide inhibitors in preventing vitreal band formation after double penetrating wounds to the posterior segment of the rabbit eye

PROBLEM

Ocular trauma presents a difficult clinical problem. In many cases total loss of the eye may result. In other cases vitreal fibrosis and proliferation of collagenous bands obstruct vision and may lead to retinal detachments. The visual treatment of vitreal bands is currently complete vitreous replacement with saline or hyaluronic acid. Vitrectomy is a high-risk procedure and may result in retinal or lenticular detachment at the time of surgery. Such patients might be spared surgical intervention if a technique for preventing bands could be explored and perfected for clinical use.

RESULTS AND DISCUSSION OF RESULTS

A well established method of producing double perforating wounds in the rabbit eye was employed. Immediately after injury the wound site was injected with 5 mg triamcinolone with followup injections in a 0.15cc solution of saline at 24 hr intervals for two days. The control eye was injected with saline solution. In a second experiment, 1 mg of penicillamine was injected into the test eyes while the animal continued to receive 5 mg penicillamine, intramuscularly for four days after trauma. A semi-quantitative scaling technique was established using indirect ophthalmoscopy (grades 1-4, based upon estimates of vitreal band formation).

Each of three independent observers evaluated the eyes. Each medication was given in a "blind" fashion. That is, none of the investigators knew what was being administered.

CONCLUSIONS

There were no differences in vitreal band formation in the eyes treated with triamcinolone as compared to saline injected eyes. Penicillamine injected eyes showed an 80% reduction in vitreal band formation.

Diagnosis and Treatment of Acute Laser Injury (Cont)

RECOMMENDATIONS

Further experiments should be conducted to test the side effects of penicillamine injected into normal rabbit eyes. A precise dose schedule for penicillamine, both intraocular and systemic, should be determined.

PUBLICATIONS

BELKIN M., and J. F. WEISS. Use of triamcinolone after ocular trauma. Letter to the Editor, Archives of Ophthalmology. September 1981

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 083 Diagnosis and Treatment of Acute Laser Injury

The following investigation has been conducted under this work unit:

STUDY NO. 3 Ultrasonographic diagnosis and monitoring of laser-induced retinal edema

Subtle retinal injury, such as that resulting from acute laser exposure, may not be directly evident from a simple non-dilated ophthalmologic examination. The use of ultrasound has been improved by rapid increases in technology, providing the military ophthalmologist with a markedly improved testing technique with resolution on the order of 100-150 microns. With this new technology, the exact location and extent of retinal edema after laser injury may provide a suitable means of detecting laser injury and initiating early therapy.

Multiple single exposures to a Q-switched neodymium (20 ns) laser at 0.5 to 1 mJ were placed in the eyes of Rhesus monkeys. Each exposure produced a large lesion (300 microns) with a swollen border. A & B mode ultrasonography was performed immediately, 1 hour, 24 hours, and 1 week after exposure. Applanation ultrasonography was the only methodology employed.

A and B mode ultrasonography failed to reveal the extent of swelling or retinal involement for any exposure condition.

BODY OF REPORT

WORK UNIT NO.	083	Diagnosis and Treatment of Acute Laser Injury
STUDY NO.	3	Ultrasonographic diagnonis and monitoring of laser-induced retinal edema

PROBLEM

Acute laser injury involving pulsed laser exposures from visible and near-infrared laser sources will be an increasing problem for the near term. The military ophthalmologist can be expected to see a series of combat casualties resulting from accidental or purposeful exposure to laser sources. These exposures are not characteristic of his experience with argon laser photocoagulation, as both the number of lesions and appearance of the retinal pathology may be variable. Subtle retinal alterations may consist of depigmented areas in the fovea and foveocular areas. High resolution ultrasonography may be able to provide clinical interpretation of the extent of retinal damage.

RESULTS AND DISCUSSION OF RESULTS

Multiple single exposures to a Q-switched neodymium (20 ns) laser at 0.5 to 1 mJ were placed in the eyes of Rhesus monkeys. Each exposure produced a large lesion (300 microns) with a swollen border. A & B mode ultrasonography was performed immediately, 1 hour, 24 hours, and 1 week after exposure. Applanation ultrasonography was the only methodology employed.

CONCLUSIONS

A and B mode ultrasonography failed to reveal the extent of swelling or retinal involvement for any exposure condition.

RECOMMENDATIONS

Resolution of A & B mode ultrasonography of the eye is increased by the use of the immediate probe technique. Further experiments at lower laser exposure levels should be tested with a 10 and 5 MHz pulse and the water bath technique.

PUBLICATIONS

None

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 083 Diagnosis and Treatment of Acute Laser Injury

The following investigation has been conducted under this work unit:

STUDY NO. 4 Treatment of corneal, retinal, and vitreal effects of laser injury

EX-1 Use of urokinase in rapid absorption of vitreal hemorrhage

EX-2 Measurement of "acute" phase serum proteins in injured animals

EX-1. Following acute high level exposure to a single ultrashort pulse of neodymium, ruby, or frequency-flashed neodymium laser radiation at intraocular energies that exceed the ED_{50} for vitreal hemorrhage, large quantities of blood appear in the vitreous. This blood not only prevents the injured individual from seeing, but may lead to the development of vitreal bands and ultimately to retinal detachments. Rapid removal of the vitreal hemorrhage at present consists of total removal of the vitreous. Simpler measures developed in this research may be of immediate benefit to the ophthalmologist confronted with many cases of hemorrhage in the combat theater.

Urokinase is an exzyme system capable of breaking down blood (specifically erythrocytes) to permit rapid reabsorption by phagocytes present in the vitreous. Vitreal hemorrhage in rabbit eyes was induced by a Q-switched laser. In each case (a total of 50 eyes) one eye served as a control (saline injection) while the other eye was given 1500 Plough units of urokinase into the vitreous immediately after laser exposure. In these cases the urokinase increased the vitreal fibrosis. In a second experiment, the dose delivery schedule was modified and urokinase was increased to 2000 Plough units in 0.15cc saline solution but administered at 12 hrs post-laser. Treated eyes showed resolution of hemorrhage 16 hrs more rapidly than control eyes.

EX-2. Following a tenet that laser injury to the eye or skin can produce serum protein alterations, a laser dosimeter was tested. Electrophoresis of serum proteins was performed in rats, rabbits, and primates that had received corneal and skin exposures to the carbon dioxide laser and ruby laser to the retina. Preliminary data showed acute-phase protein changes at doses that produced definite corneal

and cutaneous changes in rats after CO_2 laser exposures at 10.6 um. Changes were more pronounced for the cutaneous exposures than for the single exposure to the cornea. Changes after retinal exposures were less definitive. Dose correlation to the acute-phase protein change is required in future experiments.

BODY OF REPORT

WORK UNIT NO.	083	Diagnosis and Treatment of Acute Laser Injury
STUDY NO.	4	Treatment of corneal, retinal, and vitreal effects of laser injury
EX-1		Use of urokinase in rapid absorption of vitreal hemorrhage

PROBLEM

After penetration of the retina has occurred, large quantities of blood exude from the deep choriocapillaris into the vitreal space and into the vitreous itself. Vitreal bands may develop if the blood is not rapidly removed. Theoretically, specific enzyme systems are capable of breaking down blood (specifically erythrocytes) to permit rapid reabsorption by phagocytes present in the vitreous. Urokinase is such an enzyme system.

RESULTS AND DISCUSSION OF RESULTS

Using a Q-switched ruby laser operating on a TEM_{00} mode, rabbit eyes were exposed to single 20-nanosecond pulses of laser radiation at energy levels to 1 millijoule. In each case (a total of 50 eyes) one eye served as a control (saline injection) while the other eye was given 1500 Plough units of urokinase into the vitreous immediately after laser exposure. In these cases the urokinase increased the vitreal fibrosis. In a second experiment, the dose delivery schedule was modified and urokinase was increased to 2000 Plough units in 0.15cc saline solution but administered at 12 hrs post-laser. Treated eyes showed resolution of hemorrhage 16 hrs more rapidly than control eyes.

CONCLUSIONS

Modification of the dose delivery and dose of urokinase results in rapid reabsorption of vitreal blood. Effects of urokinase alone injected into normal eyes must be evaluated to determine the absence of possible "toxic" effects on retinal function.

RECOMMENDATIONS

Further studies on complications of urokinase should be conducted. Techniques involving combination of urokinase and cryoprecipitate macrophages are required to provide necessary factors in vitreal hemorrhage reabsorption.

PUBLICATIONS

None

EX-2 Measurement of "acute" phase serum proteins in injured animals

PROBLEM

The problem is to determine if detectable acute-phase protein change was evident in the serum of animals exposed to laser radiation. The potential exists for using these techniques as a biologic dosimeter. If the acute-phase protein change has a unique signature for laser exposure, this would provide a valuable assessment capability.

In this pilot study, white rats were exposed to CO_2 laser radiation and Rhesus monkeys were exposed to a ruby laser. Blood was taken from the subjects 24 hours before and 1 hour and 24 hours after exposure. Exposure doses were near and above those required to produce a change, e.g., cornea lesion (rat), cutaneous burn (rat), and retinal burn (rat).

RESULTS AND DISCUSSION OF RESULTS

Acute-phase protein changes were observed for the cutaneous exposures in the rat. Corneal exposure produced less change, probably due to involvement of smaller surface area. Retinal lesions produced minimal change which requires further evaluation to assure validity.

CONCLUSIONS

Preliminary data indicate some minimal degree of acute serum protein acuity as a result of CO_2 laser radiation. The data are non-specific, that is there may be a relationship between laser injury and acute protein response. More definitive studies should be explored.

RECOMMENDATIONS

Further detailed experiments should be designed with limited scope to evaluate dose dependence of the acute-phase protein changes to verify preliminary data.

PUBLICATIONS

None

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 083 Diagnosis and Treatment of Laser Injury

The following investigation has been conducted under this work unit:

STUDY NO. 6 Ocular ballistic injury

Ocular trauma due to penetration of the globe by metallic or non-metallic fragments results in an immediate ophthalmologic emergency. Such fragments frequently occur in combat under conditions of "buttoning up" inside a tank or armored personnel carriers. These fragments are generally metallic, traveling at relatively low velocities (100-200 ft/sec) and may produce sufficient tracts and openings in the cornea or sclera to produce ocular hypotony. A method of producing such injuries in vitro would provide location of fragments for removal and treatment by the combat ophthalmologist.

BODY OF REPORT

WORK UNIT NO. 083 Diagnosis and Treatment of Acute Laser Injury

STUDY NO. 6 Ocular ballistic injury

PROBLEM

Those who wear glasses (ammetropes) as well as those who require no corrective eyewear (emmetropes) are exposed to ocular injury from penetration of the eye by a wide variety of combat weapons. When glass lenses are struck by fragments, the wearer may be presented with a complicated pattern of glass and metal fragments in the anterior chamber, posterior compartment, or retina.

RESULTS AND DISCUSSIONS OF RESULTS

In three separate experiments, metallic spheres (0.25 gr.) were projected into freshly enucleated porcine eyes imbedded in 20% gelatin (a simulator of the orbital muscle and tissue wall). Initial data, from velocities of 300-500 ft/sec, produced through penetration of the entire globe. In a second experiment, velocities were reduced to 200 ft/sec but new reproducible terminal velocities were recorded. It was necessary to contract a sealed mini-gun system consisting of a compressed air valve, a magnetic breach, and glass tube barrel to obtain successful velocity and trajectory measures for the in vitro ocular ballistic studies.

CONCLUSIONS

From initial experiments, only eight eyes were exposed in the most successful model weapon. It was possible to penetrate the porcine cornea and ultrasonographically demonstrate fragments in the anterior chamber or retina.

RECOMMENDATIONS

A mini range must be completed in which exact velocity measurements, using a helium neon laser and photodiodes or chronograph, can be tested before further experiments can be conducted.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DA OE 6099	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	085 APC HL05
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)* (U) Development of a Rapid System for Assessing Blood Anticoagulant-Nutrient Preservatives

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clin Medicine; 009800 Medical and Hosp Equip; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 07	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	0.1	28
	CURRENT 82	0.4	17

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
Foreign Literature Reviewed

20. PERFORMING ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME:
NAME: POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Rapid Screening Techniques; (U) Blood Preservation; (U) Temperature; (U) Blood Respiratory Function

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To develop and test a high temperature screening technique for evaluating new or modified anti-coagulant nutrient preservative solutions (ANPS) used for storing blood and blood products administered to combat casualties. The effort seeks to provide a more rapid and efficient means to (a) optimize the composition of ANPS, (b) identify factors responsible for the collection and storage lesions produced with present procedures and (c) better understand differences in individual blood donors with respect to storagability. An additional objective is to determine the optimum temperature for storage of whole blood.
24. (U) Experiments are designed to determine effects of selected-temperatures between 0 and 37C on biochemical and physiochemical changes in harvested human blood during storage in standard ANPS such as ACD, CPD and CPD-adenine. Measurements include adenosine triphosphate, 2,3-diphosphoglycerate, plasma and intraerythrocyte pH, methemoglobin, gas tensions, potassium, red cell deformability, viscosity and oncotic pressure. Rates of change with time in the measured parameters at each temperature are compared using Arrhenius plots and statistical means to assess whether the slow changes at low temperatures are reliably predicted from observing rapid changes at high temperatures. Theoretically, a thirty-fold decrease in the time required for evaluating ANPS can be anticipated working at 37C rather than 4C, the usual storage temperature. If proven feasible, the approach will be applied to systematically explore promising modification of ANPS and storage/collection techniques.
25. (U) 80 10 - 81 09 Progress on this work unit has been detailed in previous Annual Reports. Loss of trained personnel and transfer of the principal investigator has interrupted work on this effort during the current reporting period.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 085 Development of a Rapid System for Assessing Blood Anticoagulant-Nutrient Preservatives

Earlier theoretical and in vitro experimental work has indicated that storage of erythrocytes at a temperature of 1C rather than 4-6C, 6C being the upper limit of the permissible range, would significantly improve the quality of transfused red cells and possibly permit an extension of the storage periods based on enhanced in vivo survivability. Because of the departure of the medical officer collaborating on the in vivo human studies needed to confirm these expectations, however, it has not been possible to complete this phase of the study. It is currently planned to pursue this objective using an animal model being developed by a newly appointed investigator in the Division of Blood Research.

BODY OF REPORT

WORK UNIT NO. 085

Development of a Rapid System for Assessing Blood Anticoagulant-Nutrient Preservatives

PROBLEM

Earlier theoretical and experimental work within this work unit has indicated that the storagability of erythrocytes would be affected to a significant extent by changes of storage temperature in the range of 1-6C. For instance, reduction in ATP concentration during storage at 6C was estimated to proceed twice as rapidly as it does at 1C. ATP has been thought to be related to post-transfusion red cell survivability and considerable experimental effort is devoted to devising chemical and physical preservation schemes to enhance the level of this constituent during storage. A similar effect of temperature was found for DPG; H+ ion, on the other hand, accumulates much more rapidly at 6C than at 1C. Blood is commonly stored at "about" 4C, although a range of 1-6C has been generally accepted as permissible. Few references, however, can be found in the published literature regarding the determination of the optimum red cell storage temperature. This last objective is within the scope of work planned under this work unit and a considerable amount of in vitro work supporting this technical goal has been completed. A decisive phase of the work was planned to include actual in vivo red cell survival studies using humans and transfused blood stored in this acceptable 1 to 6C temperature range. The medical officer who was to collaborate on this phase of the work, however, has separated from the service, and support for the effort has not been available. Current plans call for coordinating and continuing this work with the Division of Blood Research and using an animal model being developed by a newly appointed investigator for the in vivo testing of various blood products.

RESULTS AND DISCUSSION OF RESULTS

None

CONCLUSIONS

None

RECOMMENDATIONS

The optimum temperature for storage of erythrocytes should be determined. The resources required to accomplish this objective are not large and the impact on blood preservation technology could be significant.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOE 6077	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	253 APC HLOJ
b. CONTRIBUTING	62772A	3S162772A874	AB	086
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code): (U) Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 008800 Life Supp; 016200 Stress Physiol; 009800 Medical & Hosp Equipment

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 11	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	0.0	26
FISCAL YEAR CURRENT 82	1.3	50

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
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RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
NAME: Letterman Army Institute of Research
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: POC: DA
NAME:

21. GENERAL USE
Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code): (U)Combat Surgery; (U) Trauma; (U) Combat Anesthesia; (U) Left Ventricular Function; (U) Artificial Blood; (U) Laboratory Animal

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Newly developed artificial blood substitutes and whole blood, stored using new techniques, must be physiologically evaluated to assess their effectiveness in combat resuscitation efforts. The objective of this work unit is to use an appropriate subprimate model which will permit precise measurements of ventricular hemodynamic and metabolic functions. This model has been used to investigate the importance of different anesthetic agents, artificial blood substitutes, various levels of hemodilution and beta endorphin antagonists on left ventricular function during conditions resulting from combat injury.

24. (U) A perfused in situ heart model, using total and right heart bypass with control of heart rate, blood pressure, and left ventricular function, has continued to be employed. Left heart performance and myocardial oxygen transport dynamics are assessed to determine the ability of newly developed resuscitation techniques and agents to support normal tissue function following combat injury.

25. (U) 80 10 - 81 09 A cardiovascular investigational laboratory is functioning for active measurements of stroke volume, dp/dt ejection fraction, myocardial metabolism and coronary flow. Studies during the last year have subjected animals to anemia, perfusion with stroma-free hemoglobin solution, and administration of naloxone (a beta endorphin antagonist). Hemodilution to a hematocrit of 15% appears to be acceptable in this model without a significant decrease in heart performance. If the hemodilution is extended to a hematocrit of 5 or 10%, no myocardial function occurs unless the perfusion is done with stroma-free hemoglobin solution. Current studies are examining the effect of naloxone on heart function and the establishment of a critical hematocrit level during conditions resulting from combat injury, such as hypothermia, hypotension, and hypoxia.

* Complete in accordance with originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M161102BS10 — Research on Military Disease, Injury and Health Hazards

WORK UNIT NO. 253 — Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier

The following investigations have been conducted under this work unit:

STUDY NO. 2 The effect of variation in the oxyhemoglobin dissociation curve on left ventricular function in swine

STUDY NO. 3 Anesthetic agents and their effect on left ventricular function during normoxia and hypoxia

STUDY NO. 4 The effect of stroma-free hemoglobin solution on myocardial function in a nonshock, subtotal exchange model

STUDY NO. 5 The effects of naloxone on myocardial function

STUDY NO. 6 The effects of hemodilution in conjunction with hypotension, hypoxia, or hypothermia on myocardial function

STUDY NO. 2. The relationships between preservation of myocardial performance and the oxyhemoglobin dissociation curve of priming solutions have been investigated in the isolated swine heart preparation described previously. These studies have been designed to determine whether or not the P_{50} of resuscitation fluids, including whole blood, is a significant determinant of recovery from hemorrhagic shock secondary to massive combat wounds. Animal studies have been completed indicating that variations of P_{50} have a significant effect on left ventricular functions at normal oxygen tensions and hemoglobin concentrations. Further studies examining the role of P_{50} variation during anemia have been completed, and analysis of these data shows that anemia does not heighten the effects of a change in P_{50} on left ventricular function, but increased affinity does result in decreased coronary sinus PO_2 values and decreased myocardial tissue PO_2 values. Further work is underway to determine whether or not the oxyhemoglobin dissociation curve has an important part in determining myocardial performance during hypoxia and limited coronary artery blood flow. This additional work is being done under Study No. 6 and all of the animal work and preliminary analysis under Study No. 2 have essentially been completed.

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

STUDY NO. 3. The myocardial effects of the major anesthetic agents have been studied in our swine heart model in an attempt to evaluate these agents under conditions analogous to combat-induced stress. Previous studies completed under this work unit have substantiated that halothane decreases left ventricular function during normoxia and especially during hypoxia, whereas morphine sulfate had a minimal effect. Further analysis of this depression has revealed that the decrease in function was due more to a decrease in myocardial compliance rather than a decrease in contractility. All animal work contemplated in Study No. 3 has been completed and additional work in this area is contemplated through extramural contract studies.

STUDY NO. 4. Work has continued to progress in the evaluation of stroma-free hemoglobin solutions on myocardial performance. In initial studies with the in situ swine heart model, we evaluated a subtotal exchange transfusion comparing stroma-free crystalline hemoglobin solution with a 7% bovine albumin solution to produce a hematocrit level of 5%. These studies show that while myocardial performance is decreased by approximately 50% with stroma-free hemoglobin solution, the animals were able to maintain this level of cardiac performance, whereas animals exchanged with the albumin solution were unable to sustain any degree of myocardial work. Animals with a hematocrit of 10% exchanged with albumin were also unable to sustain significant cardiac work. All animal studies in Study No. 4 have been completed and no further experiments with stroma-free hemoglobin solution are being contemplated for inclusion under Study No. 4. There is, however, a provision for investigation of these solutions under the general topic of Study No. 6.

STUDY NO. 5. The effects of naloxone on myocardial function: The right heart bypass model continues to be used for this study, evaluating the effects of the beta-endorphin antagonist naloxone to favorably affect left ventricular function. Most of the animal experiments in the initial group (n=10) have been completed and a preliminary analysis supports the conclusion that naloxone appears to have no effect in animals stressed with a period of hypotensive cardiopulmonary bypass, but that there may be a modest favorable effect on those animals given naloxone following a normotensive cardiopulmonary bypass stress. We are currently obtaining the beta-endorphin levels on the samples, and we plan to submit an abstract outlining our initial findings. Porcine and human beta-endorphin have been measured in plasma samples with and without intervention. The assay techniques have been refined to give greater than 80% recovery. Interassay variability is a problem, but continuing technical refinements in sample handling are minimizing these sources of error. During total heart bypass, endorphin levels appear to correlate inversely with duration. This may be a result of non-specific protease

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

release. True decrease in secretion of beta-endorphin from pituitary shutdown is another possibility. In cases where naloxone reversibility has been demonstrated, endorphin levels are analyzed concomitantly with cases where no effect of naloxone was seen. Data analysis is in progress. Neuroendocrine response in conscious animals and during anesthesia are being investigated.

STUDY NO. 6. The effects of hemodilution in conjunction with hypotension, hypoxia, or hypothermia on myocardial function: This study has just been initiated, as was the case with Study No. 5, but only two animals have been studied. The right heart bypass swine model has again been used, and it appears that it will be possible to document the effects of hemodilution at three different blood pressures in a single animal. Preliminary analysis of the data from these two animals seems to imply that, even in the range of blood pressure of 45-65 torr, coronary blood flow is quite dependent upon blood pressure.

BODY OF REPORT

WORK UNIT NO. 253 — Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier

STUDY NO. 2 — The effects of variation in the oxyhemoglobin dissociation curve on left ventricular function in swine

PROBLEM

Recently, with the understanding that the oxyhemoglobin dissociation curve is affected by concentrations of 2,3-diphosphoglycerate (2,3-DPG) and that stored blood has a low 2,3-DPG level, there has been concern that massive transfusions with blood stored for prolonged periods may have a detrimental effect on oxygen delivery to critical tissues. Myocardial function is intimately tied to adequate oxygen transport which, if less than optimal, may depress heart performance in the combat-injured soldier. Some studies have suggested that there is a relationship between P_{50} and left ventricular performance. If an adequate P_{50} is crucial to preserving heart performance during periods of combat injury, then aged blood with a low P_{50} and low 2,3-DPG may have limited usefulness, and fresh blood or blood with enriched 2,3-DPG must be made available. If P_{50} is not a major determinant of left ventricular function, aged blood could be employed, especially during combat situations which would require massive transfusions and maximal utilization of blood bank resources.

RESULTS AND DISCUSSION OF RESULTS

Our in situ perfused swine heart model has been used for this study. As previously outlined, left ventricular function and metabolic responses have been directly evaluated.

Currently, in this study, we have evaluated myocardial function following exchange transfusions with blood having various P_{50} characteristics and hematocrit levels. As reported previously, our initial study with this preparation, examining the situation at a normal hematocrit level, showed that left ventricular performance is affected adversely when animals are subjected to blood having a lowered P_{50}. This change in performance was accompanied by documented and statistically significant changes in the P_{50}, n-value, and coronary sinus gas values for the animals. The group of animals subjected to exchange with high P_{50} blood had preservation of myocardial performance but did not show an improved performance compared to that with blood having a normal P_{50} value. A second phase of this study has examined the effect of altered P_{50} on the left ventricular function in an animal exchanged with blood at a lowered hematocrit level. These animal

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

studies have been completed. They reveal that increased oxygen-hemoglobin affinity during anemia does not result in decreased left ventricular function when compared to exchange transfusion of blood having a decreased oxyhemoglobin affinity. Increased affinity did result in a lowered tissue and coronary sinus PO_2 value. This finding indicates a lower level of oxygen availability in the tissues of the working myocardium being perfused with low P_{50} blood.

CONCLUSIONS

Our conclusion is that P_{50} is an important determinant of left ventricular function; the question of its clinical importance remains to be answered. Further studies under Study No. 6 will examine the situation in animals stressed at a lower pressure, temperature, and oxygen tension.

RECOMMENDATIONS

The findings in this study have helped answer the question of how vital a role P_{50}-changes play in myocardial performance. Additional work is needed to weigh adequately the role in a clinical situation analogous to that experienced by soldiers in the combat field. The problem of evaluating the role of P_{50} in myocardial performance is being assessed with an in situ swine model being done at Letterman Army Institute of Research (Study 6) and in other extramural laboratories. These laboratories currently involve facilities at the University of California at San Diego and the VA Medical Center at San Diego, both under the direction of the principal investigator. We recommend continued work examining this question as mentioned under Study No. 6, and with appropriate collaboration of those extramural programs mentioned above.

PUBLICATIONS

1. MOORES, W.Y. Oxygen delivery: hemodilution, oxyhemoglobin dissociation, stroma-free hemoglobin during cardiopulmonary bypass. In: Pathophysiology and Techniques of Cardiopulmonary Bypass. Baltimore: Williams & Wilkins (in press)

2. MOORES, W.Y., D.C. WILLFORD, and J.A. SWAIN. The role of oxygen-hemoglobin affinity in determining postperfusion myocardial performance: a laboratory and clinical corrologic study. Bulletin of Cardiovascular Research Center, Baylor University (in press)

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

STUDY NO. 3 — Anesthetic agents and their effect on left ventricular function during normoxia and hypoxia

PROBLEM

The effects of anesthetic agents on myocardial function have been well worked out for the normal situation encountered in civilian operating room practice where the patient is at an optimum oxygenation level. Unfortunately, during combat situations patients may have to be anesthetized during conditions of decreased oxygen tension. The ultimate survival of these patients is closely connected with their myocardial performance. Safe anesthesia would require optimization of myocardial performance even during conditions of hypoxia. This information becomes crucial if the field anesthesiologist is to select the optimal available anesthetic agent during these combat stress situations. In the past, this particular problem has been addressed in Work Unit 021 (DAOE 6079), "Anesthetic Management and Perioperative Care of the Acutely Wounded Soldier." During this last year, work has been conducted under this work unit and is reported here.

RESULTS AND DISCUSSION OF RESULTS

The perfused swine heart model has been used and animal studies examining the response of halothane, morphine, and infiltration anesthetic regimens have been conducted. The techniques for accurately measuring anesthetic concentrations with a mass spectrometer and for accurately adjusting the animal's oxygen tension to a level of 50 torr have been perfected. With these technical refinements, it has been possible to complete the evaluation of a series of animals at normoxia and at the hypoxic level of 40 torr. The initial results from this study have shown that halothane, as expected, decreased myocardial performance during normoxia. This drop in performance is accompanied by a decrease in myocardial oxygen consumption. The new finding during hypoxia was that halothane anesthesia not only decreases myocardial performance significantly more during hypoxia, but also that this decrease in performance is not accompanied by a corresponding reduction in oxygen consumption. The experiments performed with morphine anesthesia substantiated that, under conditions of normoxia, morphine has no appreciable depressive effects on myocardial performance and that its depressive effects during hypoxia are relatively less than with halothane (approximately 25% versus 66%). This depressed function is not accompanied by an increase in myocardial oxygen consumption. The results from the animals examined under infiltration anesthesia were similar to those with the animals examined under morphine anesthesia. These data have been analyzed to determine the mechanism for the change in myocardial performance seen with halothane.

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

CONCLUSIONS

Our conclusion is that halothane may be an appropriate anesthetic agent to use during normoxic conditions since the depression of myocardial performance is accompanied by a decrease in myocardial oxygen consumption. The amount of oxygen consumed per unit of cardiac work is not increased which prevents ischemic damage to the myocardium. During hypoxia, halothane is not a good anesthetic since the depression of myocardial function is enhanced and this depression is accompanied by an increased oxygen consumption, thereby subjecting the myocardium to a greater risk of ischemic damage.

RECOMMENDATIONS

Additional work is needed to examine the anesthetic agents during periods of hypoxia and other situations of deranged physiology such as hypotension and anemia that are encountered in a combat injury situation. The question of an appropriate choice of an anesthetic agent during situations of combat stress needs to be answered by additional studies examining various anesthetic agents during anemia and hypotension in a controlled swine heart model. The major thrust of studies attempting to answer these important questions is currently being undertaken with an extramural contract grant under the direction of Dr. Richard B. Weiskopf. No further animal studies are presently contemplated in this study; however, the principal investigator continues to perform studies examining this question in the perfused swine heart model at the laboratory facilities at the University of California in San Diego and the VA Medical Center in San Diego.

PUBLICATIONS

1. MOORES, W.Y., R.B. WEISKOPF, M. BAYSINGER, and J.R. UTLEY. Effects of halothane and morphine sulfate on myocardial compliance following total cardiopulmonary bypass. J Thor Cardiovas Surg 81:155-162, 1981

2. SANSONETTI, D., W.Y. MOORES, R. MACK, R. SCHEUSSLER, R. WEISKOPF, and J.R. UTLEY. Common effects of halothane on diastolic heart function in swine on cardiopulmonary bypass. Circulation, 1981 (in press)

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

STUDY NO. 4 — The effect of stroma-free hemoglobin solution on myocardial function in a nonshock, subtotal exchange model

PROBLEM

Resuscitation of the combat injured soldier may require the use of various artificial blood substitutes as well as whole blood. These solutions must be adequately evaluated in terms of their effects on myocardial function. Several studies examining stroma-free hemoglobin solutions have been accomplished in a shock model. However, it is appropriate to examine the effects of these resuscitation techniques in an animal model which allows evaluation of myocardial function in a nonshock situation as might be encountered during recovery and convalescence from combat injury. This study should help determine if casualties should be transfused with hemoglobin solution or an artificial blood substitute that carries oxygen, or if a nonoxygen-carrying blood substitute, such as albumin solution, would be adequate.

RESULTS AND DISCUSSION OF RESULTS

During the last year, the in situ perfused swine heart model has been used to evaluate the effects of an exchange transfusion of stroma-free hemoglobin solution on left ventricular function. The standard parameters of myocardial performance (stroke volume, etc.) have been examined under conditions of controlled pre-load, after-load, and rate and an index of myocardial metabolism and oxygen utilization has been used. These studies have been done with hemoglobin solution that has been exchanged in a pig animal model so that the subsequent hematocrit was 5%. Experiments comparing stroma-free hemoglobin solution with albumin solution to produce a hematocrit level of 5% has revealed that animals transfused with the stroma-free hemoglobin solution were able to maintain a work performance at approximately 50% of their control value and were able to sustain this level of work performance for the standard work trial period. The animals exchanged with the albumin solution to produce a similar hematocrit level were initially able to support the same level of cardiac performance but, within minutes of the work trial period, these animals were no longer able to perform any useful cardiac work. Those animals perfused with stroma-free hemoglobin solution showed signs of inadequate oxygen delivery, such as high lactate levels. However, the hearts were able to work with the stroma-free hemoglobin solution. Albumin exchanged to produce a hematocrit of 10% did not allow myocardial work to be sustained.

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

CONCLUSIONS

Stroma-free hemoglobin solution is promising in terms of supporting useful cardiac work under conditions of severe anemia. Support of cardiac function occurred even though the present form of hemoglobin solution has a depressed P_{50} with a left-shifted oxyhemoglobin dissociation curve.

RECOMMENDATIONS

Additional work is necessary to define the role of stroma-free hemoglobin in those situations where the hematocrit level is not severely depressed. Continued work should be done to improve the solution so that cardiac performance can be maintained without causing anaerobic metabolism. We are evaluating stroma-free hemoglobin solution perfusion at hematocrit levels greater than 5%. We are also evaluating improved solutions which have a more normal oxyhemoglobin dissociation curve and better in vivo retention.

PUBLICATIONS

1. MOORES, W.Y., F. DEVENUTO, W.H. HEYDORN, R.B. WEISKOPF, M. BAYSINGER, and J.P. HANNON. Improved porcine myocardial performance during severe anemia using a stroma-free hemoglobin solution. (Abstract) Fed Proc 39:709, 1980

2. MOORES, W.Y., F. DEVENUTO, W.H. HEYDORN, R.B. WEISKOPF, M. BAYSINGER, and J.R. UTLEY. Extending the limits of hemodilution on cardiopulmonary bypass using stroma-free hemoglobin solution. J Thorac Cardiovasc Surg 81:155-162, 1981

3. GREENBURG, A.G., J. PESKIN, D. HOYT, and W.Y. MOORES. Is it necessary to improve the intravascular retention of hemoglobin solution. Crit Care Med, 1981 (in press)

4. MOORES, W.Y., F. DEVENUTO, W.H. HEYDORN, A.G. GREENBURG, and J.R. UTLEY. Effectiveness of stroma-free hemoglobin solution as seen in a right heart bypass swine model. Crit Care Med, 1981 (in press)

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

STUDY NO. 5 — The effects of naloxone on myocardial function

PROBLEM

There has been heightened interest inspired by the work of Fadden and Holaday about the possibility that naloxone may significantly increase survival in hemorrhagic shock. The basic hypothesis is that beta-endorphins have a disadvantageous effect on myocardial function and may well be the myocardial depressant factor seen in shock-like states. Naloxone is a specific beta-endorphin antagonist and might be expected to be useful in dealing with the myocardial function that follows shock states. The treatment of shock, both hemorrhagic and septic, continues to be a subject of great concern in military medicine. Shock states manifested by hypotension and myocardial depression must be effectively treated if adequate resuscitation is to occur. Previous studies examining the effects of naloxone have not been done in an animal model with carefully controlled pre-load, after-load and heart rate. It would seem appropriate to evaluate this potentially beneficial agent in the swine model.

RESULTS AND DISCUSSION OF RESULTS

Initial protocol attempts to determine the effectiveness of naloxone in treating depression after a shock period have revolved around examination of myocardial function after a period of cardiopulmonary bypass and after administration of naloxone. Two groups of animals have almost been completed, examining first a normotensive period of cardiopulmonary bypass and second a hypotensive period of cardiopulmonary bypass. Beta endorphin levels are to be examined during various periods within each animal experiment. Although some of these samples have been analyzed, the majority are not yet completed and any final conclusions would have to await completion of these determinations. Animals subjected to a normotensive period of cardiopulmonary bypass did have a modest drop in left ventricular function. When naloxone was administered, there was no dramatic change in any of the cardiovascular parameters. However, repeat left ventricular function curve measurements were, on several occasions, consistent with some improvement in left ventricular function. Those animals subjected to a hypotensive period of cardiopulmonary bypass with a mean arterial blood pressure of 40 torr had a much greater depression in their myocardial function, as measured by a controlled left ventricular function curve. Naloxone was given to these animals, and again there was no appreciable beneficial effect seen immediately, nor could any improvement in left ventricular function be discerned through the systemic left ventricular function curve measurement. These results, which must await completion of the beta-endorphin

analyses, may lead us to the conclusion that naloxone may have a role in mild depression of the myocardium following a cardiopulmonary bypass stress, but it does not appear to have a direct role in a major depression situation.

CONCLUSIONS

Our preliminary conclusion at this stage is that naloxone does not appear to be a dramatic positive ionotropic agent capable of providing functional recovery after a major stress, but that it may have a mild ionotropic type of action, possibly mediated through beta-endorphin antagonism, in a mild stress state.

RECOMMENDATIONS

The treatment of shock states is a crucial item for the military and it is recommended that any investigations allowing one to treat these conditions in an effective fashion should continue to be pursued. Our preliminary findings seem to indicate that one may have to use very sensitive indicators to discern an effect from naloxone. It is hoped that continued studies in this area will help elucidate those situations in which beta-endorphin antagonism has real value during a standard postshock resuscitation effort.

PUBLICATIONS

None.

STUDY NO. 6

The effects of hemodilution in conjurction with hypotension, hypoxia, or hypothermia on myocardial function

PROBLEM

Hemodilution is universally employed in almost any resuscitation attempt where a hypovolemic situation is being countered. There currently is a modest amount of literature examining the question of a normal animal's response to hemodilution and decreased availability of oxygen. Unfortunately, most combat resuscitation efforts in the field will be carried out under less than optimum conditions and might well involve the simultaneous treatment of shock, hypoxia, and hypothermia. It becomes imperative, therefore, to investigate the effects of hemodilution not only during normal physiologic situations, but during physiologic situations that are analogous to those encountered in a combat injury situation. This study attempts, therefore, to examine

Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier (Cont)

various levels of hemodilution and to determine which level is appropriate if one must attempt resuscitation under the adverse conditions of hypotension, hypoxia, or hypothermia.

RESULTS AND DISCUSSION OF RESULTS

Only two animals have been studied. As mentioned in the abstract section of this annual report, most of the present investigator's efforts have been spent in Study No. 5, examining the effects of naloxone. The two animals that were studied were helpful in establishing the feasibility of this protocol. Specifically, the animals could be subjected to left ventricular function curves at three separate blood pressures (65 torr, 55 torr, and 45 torr) during a control normal hematocrit perfusion. The animals could subsequently be exchange transfused to a level of hemodilution of approximately 50% and the three left ventricular function curves again repeated. The animal preparation appeared to be stable for the completion of all of these experiments. There are insignificant data for any meaningful discussion; however, examination of the data obtained thus far appears to substantiate the fact that coronary blood flow is dependent upon mean aortic perfusion pressure even when that pressure is varied over a relatively modest range of 20 torr.

CONCLUSIONS

No substantive conclusions can be reached at this time due to the recent initiation of this study and small number of observations.

RECOMMENDATIONS

This study is feasible and will be carried out as specified. We plan to examine the effects of hemodilution on myocardial function during periods of hypotension.

PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 7064	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 06 08	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AB	086 APC HL25
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)

(U) Corticoid Protection of Cerebral Edema Induced by Gold Thioglucose

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology; 016200 Stress Physiology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 06	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	0.1	01
FISCAL YEAR CURRENT 82	0.3	08

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: POC: DA

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Brain Edema; (U) Laboratory Animal; (U) Gold Thioglucose; (U) Corticoids; (U) Hypothalamic Ultrastructure

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Since the initial effect of gold thioglucose is edema, this drug can be used as a model to study cerebral edema incurred from battlefield shock and trauma. In addition, gold thioglucose can serve as an indicater of hypothalamic function in shock and trauma. Since traumatic wounds and various shock syndromes are frequent combat injuries, the potential cerebral damage is of military importance.

24. (U) Previously, mice were injected with hydrocortisone and subsequently challenged with gold thioglucose. The brains were perfused with gluteraldehyde and the fixed ventromedial hypothalamus was removed and prepared for electron microscopic examination. These specimens will be examined using the electron microscope and the effect of hydrocortisone on cerebral edema induced by gold thioglucose will be assessed.

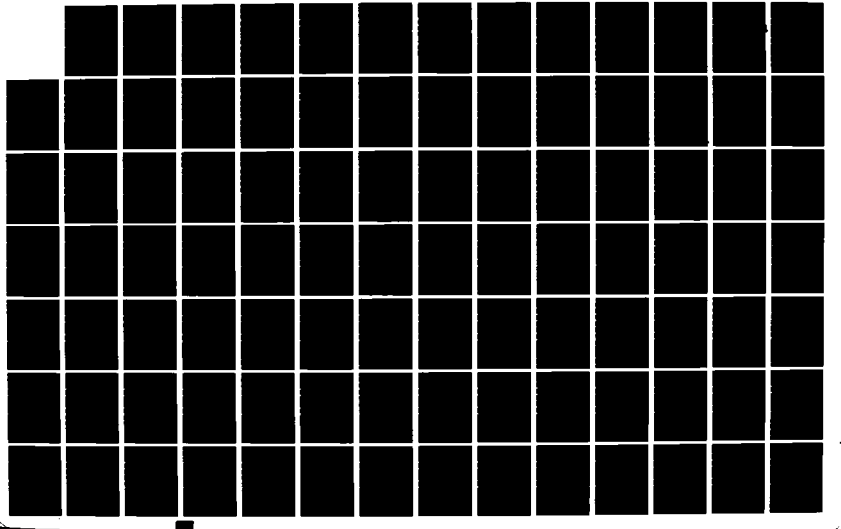
25. (U) 81 06 - 81 09 Hydrocortisone treatment before a gold thioglucose challenge blocks development of gold thioglucose-induced lesions in the ventromedial hypothalamus. Corticoid treatment may prove beneficial for the management of brain edema during shock and trauma. Moreover, these results implicate the possibility of altered hypothalamic function during shock and trauma due to potential glucocorticoid secretion.

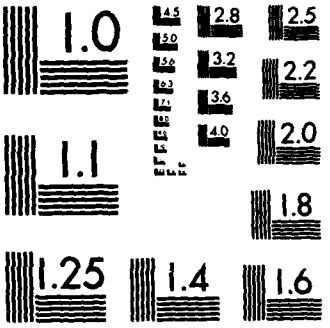
Release to contractors upon originator's approval.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

AD-A123 769 LETTERMAN ARMY INSTITUTE OF RESEARCH ANNUAL RESEARCH PROGRESS REPORT FY 1981(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA J D MARSHALL 3/4

UNCLASSIFIED OCT 81 F/G 6/5 NL





ABSTRACT

PROJECT NO: 3S162772A874 Care of the Combat Casualty

WORK UNIT NO: 086 Corticoid Protection of Cerebral Edema Induced by Gold Thioglucose

Gold thioglucose (GTG), administered intraperitoneally, causes lesions in the ventromedial hypothalamus (VMH). This hypothalamic response to GTG is ameliorated by stress or corticoid injections. Ultrastructural experiments were undertaken to determine if corticoid pretreatment completely abolishes GTG edema and necrosis in the VMH. A hydrocortisone injection and GTG challenge in mice showed no sign of edema or pathology in the VMH. This altered VMH response to GTG in animals with high blood corticoid levels suggests that corticoid protection of the VMH may be important in maintaining hypothalamic function and reducing cerebral edema during stress or shock.

BODY OF REPORT

WORK UNIT NO. 086

Corticoid Protection of Cerebral Edema Induced by Gold Thioglucose

PROBLEM

Since Guilleman and Schally won the Nobel prize for discovering hypothalamic-releasing factors, the hypothalamus can be considered the "master gland" for the control of endocrine homeostasis. The ventromedial hypothalamic area (VMH) is sensitive to the anti-arthritic drug, gold thioglucose (GTG). The lesions produced in the VMH when GTG is injected intraperitoneally in mice are the manifestation of cerebral edema. GTG does not cause the edema or lesions in the VMH by destroying microvasculature; ultrastructural observations have shown that only neural tissue damage is seen initially following the GTG challenge. Recently it has been demonstrated that corticoid pretreatment prevented GTG lesion formation. Food deprivation and cold stress also eliminate GTG necrosis. Increases in blood glucocorticoid levels are a common response to shock or stress. Thus, it appears that the corticoid alteration of the VMH in response to GTG may be a protective mechanism for the hypothalamus during stress or shock. To determine if the corticoid treatment prevented GTG-induced lesion formation in the VMH completely, electron microscopic observations were undertaken.

RESULTS AND DISCUSSION OF RESULTS

Mice were injected with hydrocortisone and subsequently challenged with GTG. The brains were perfused with 5% glutaraldehyde and phosphate buffer. The hypothalamic area was removed from the brain and processed by standard techniques for electron microscopy. Control animals displayed the typical VMH pathology associated with necrosis: dissolution of the neuropil and extensive edema. Conversely, hydrocortisone-treated and GTG-injected animals showed no signs of pathology in the VMH and could not be distinguished from normal untreated animals. This result suggests that conditions that elevate blood corticoid levels, such as stress or shock, alter the normal response of the VMH to a GTG challenge and prevent edema. This corticoid protection of the hypothalamus from GTG edema and necrosis may be important in maintaining body function during stress or shock.

CONCLUSIONS

Corticoid pretreatment protects the hypothalamus against development of edema and GTG lesions. The same result is seen in stressed animals. Because shock is a stress phenomenon and corticoid levels are elevated, the same results would be expected. Perhaps even more significant is the fact that hypothalamic function is altered during corticoid treatment and probably during stress, shock, and trauma.

Corticoid Protection of Cerebral Edema Induced by GTG (Cont)

RECOMMENDATIONS

The effect of shock on hypothalamic function using GTG as a probe should be investigated. In addition, alterations of the hypothalamus before shock or trauma may ensure survivability. GTG can be used as a model compound to produce changes in the hypothalamus.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOE 6309	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	088 APC HL12
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)

(U) Studies in Combat Injuries to the Extremities

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 012600 Pharmacology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 08	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	0.1	11
FISCAL YEAR CURRENT 82	0.6	21

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

POC:DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Nerve; (U) Nerve Graft; (U) Microsurgical Technique; (U) Combat Injuries; (U) Fractures; (U) Ligamentous Injuries; (U) Trauma; (U) Lab Animal

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Bony and ligamentous injuries to the extremities due to combat frequently result in delayed healing and permanent disability. Prolonged hospitalization and multiple surgical procedures delay return to duty, and eventual medical separations are common sequelae to such injuries. Multiple systemic and mechanical factors are known to retard healing of musculoskeletal structures, but considerable controversy still exists about how bone and ligament healing can be accelerated. Biochemical and immunological alterations and various surgical modalities will be investigated. Results will be transferred into management principles and techniques for combat injuries to the extremities.
24. (U) The canine anterior cruciate ligament has been well-established as a model for ligamentous injuries in earlier studies under this work unit. The anterior cruciate ligaments of 12 dogs were severed, repaired in a conventional manner, then reinforced with a completely biodegradable ligament. They were evaluated for function and mechanical strength at 4 mo. Another study currently is in progress to reinforce injured and repaired ligaments with a braided material that is 80% biodegradable and 20% non-absorbable. This material may allow adequate fibrous ingrowth for neoligament formation to occur.
25. (U) 8010-8109. The completely biodegradable ligament is gone 5 wk post surgery, but initial ligament healing is well underway. At 4 mo, all reinforced ligaments had healed and provided functional stability to the joints, and mechanical strength was about 50% of the controls. The ligament that is 80% biodegradable and 20% permanent shows great promise as an internal splint for repaired soft tissue structures of the musculoskeletal system. Another work unit, Agency Accession DAOE 6108, is being combined with this work unit.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO.	3S162772A874	Care of the Combat Casualty
WORK UNIT NO.	088	Studies in Combat Injuries to the Extremities

The following investigations have been conducted under this work unit:

STUDY NO. 3 Evaluation of repair techniques in treating avulsion fractures and injuries of the anterior cruciate ligament

Bony and ligamentous injuries to the extremities due to combat-type trauma result in the expenditure of large amounts of medical resources, and often lead to permanent disability. Many factors can affect bone and ligament healing, and specifically we are attempting to accelerate such healing through various mechanical, biochemical, and immunological alterations. Using the ligamentous injury model previously developed under this work unit, the anterior cruciate ligament (ACL) was transected in one knee joint in each of 12 dogs. The ACLs were repaired in a conventional manner and then reinforced with a completely biodegradable ligament made of braided polyglycolic acid (PGA) suture. Five weeks after repair, initial healing had firmly attached the repaired ACL to the femoral condyle, and the PGA ligament had resorbed completely without inflammatory or fibrotic response. Inspection and testing of the repaired ACLs at 4 months revealed that all had healed and already had attained a strength of up to 50% of the controls. This degree of healing permitted satisfactory return of function in all the operated knees. Another study now in progress to reinforce repaired ligaments with a braided material is 80% biodegradable and 20% nonabsorbable. This material is promising because it may allow adequate fibrous ingrowth for neoligament formation.

BODY OF REPORT

WORK UNIT NO.	088	Studies in Combat Injuries to the Extremities
STUDY NO.	3	Evaluation of repair techniques in treating avulsion fractures and injuries of the anterior cruciate ligament

PROBLEM

Injury to the anterior cruciate ligament (ACL) and the resulting rotatory instability of the knee is a militarily devastating handicap. A significant percentage of soldiers who sustain ACL injuries in training or combat develop knee instability and require medical separation regardless of methods of treatment. Although excellent functional, anatomical, and biomechanical studies of the ACL have been reported, there is still considerable disagreement as to whether or not a ruptured or avulsed ACL should be repaired, discarded, replaced, or ignored. Based on the results of our previous studies under this work unit, this current study evaluated the results of primary repairs of anterior cruciate ligament reinforced with biodegradable synthetic material. Results can be transferred to the management of most ligamentous injuries of the extremities.

RESULTS AND DISCUSSION OF RESULTS

The anterior cruciate ligament was transected at the femoral origin in one knee joint in each of 12 dogs. The ACLs were repaired in a conventional manner and reinforced with a synthetic ligament made of braided polyglycolic acid (PGA) suture. At 2 weeks, the PGA ligament was still providing excellent support for the healing ACL, and there was no synovitis within the knee joint. After 5 weeks, initial healing had firmly attached the repaired ACL to the femoral condyle, and the PGA ligament had resorbed without inflammatory or fibrotic response. All repaired and reinforced ligaments healed clinically and provided functional stability to the knee joints. Biomechanical testing of the repaired ACLs at 4 months produced a maximum strength of 54.18 ± 6.29 kg_f, about 50% of the strength of the controls. At the same time, sulfur^{35} uptake studies revealed viable active collagen-producing cells in the repaired ACLs. Thus, the biodegradable PGA ligament successfully reinforced the repaired ACLs to allow satisfactory functional healing in all the animals in this study.

Because the biodegradable ligament is completely resorbed by 5 weeks, we believe that an effort should be made to prolong its protective effects. Consequently another study, currently in its early stages,

will evaluate as an integrated splint a braided material that is 80% biodegradable and 20% nonabsorbable. Using our experimental model for ligamentous injuries, this material will be used to reinforce and internally splint ACLs repaired in a conventional manner. However, no external immobilization will be used so that the animals may start immediate postoperative weight-bearing. This material is promising because its strength may obviate the need for long-term external casts or splints. The internal splinting might allow the combat injured soldier to return to some degree of function in a short time. Furthermore, we believe that as the biodegradable portion is resorbed, fibrous ingrowth will occur in and around the nonabsorbable portion, thus leading to formation of neoligament. If neoligament does form, this material would be extremely useful in treating combat injuries in which there is segmental loss of ligament or tendon tissue.

Working with the Audio-Visual Production Officer, we have been able to record with high speed photography the mechanism by which the anterior cruciate ligament fails. Film speeds of 1000 frames per second have documented the sequence of events that occur when the ACL is loaded to the point of failure. We now better understand the biomechanics, and this information will aid us in planning future studies.

We have worked in cooperation with the Department of Biology at the University of San Francisco by having one of their undergraduate students spend time with us. This program seems mutually rewarding. The student currently assigned has been helpful in determining the biomechanical properties of the synthetic ligaments with which we are working. We have provided consultation and assisted investigators from Oak Knoll Naval Hospital on a study involving combat fracture healing. The study is still under evaluation and results are not yet available.

We have collaborated with investigators from the School of Medicine, University of San Francisco, on a study to evaluate the effects of preservation on bone-ligament-bone preparations. Fresh specimens were compared with those frozen by different methods. At least one freezing technique has been identified in which the biomechanical properties and cell viability of the preserved specimens were equal to those of fresh specimens. Further investigation might be warranted for preservation of tissue destined for homogenous transfer.

CONCLUSIONS

The polyglycolic acid material appears to be quite satisfactory for use as a biodegradable ligament. The results obtained in this current study are encouraging because the technique is not complicated, and it avoids use of autogenous tissue to achieve healing of repaired

anterior cruciate ligaments. This PGA ligament also has potential use for any situation where temporary internal splinting or reinforcement of repaired ligaments is indicated. We recognize that the main drawback is the rapid rate of resorption and the consequent need for postoperative protection with external splints or casts. Therefore, we are extremely encouraged by our new study with the material, which is 80% biodegradable and 20% nonabsorbable, possibly eliminating the need for postoperative external immobilization.

RECOMMENDATIONS

Further studies are necessary to evaluate internal splints for repaired tendons and ligaments. We should examine additional materials that are partially or completely biodegradable, and we will consider techniques that will lessen the devastating effects of combat-type injuries to ligaments and tendons. Also, consideration should be given to studies that examine the biochemical and immunological factors that might accelerate healing of bony and ligamentous injuries to the extremities from combat trauma. Another work unit, Agency Accession DAOE 6108, "Animal Models for Surgical Repair of Musculoskeletal Structures," will be combined with this work unit.

PUBLICATIONS

1. CABAUD, H.E., W.G. RODKEY, and J.E. FITZWATER. Medial meniscus repairs: An experimental and morphological study. Am J. Sports Med 9:129-134, 1981

2. CABAUD, H.E., J.A. FEAGIN, and W.G. RODKEY. Acute anterior cruciate ligament injury and augmented repair. Experimental Studies. (abstract) Ortho Tans 5:144, 1981

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OE 6317	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NI	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AD	089 JL04
b. CONTRIBUTING				
c. ~~CONTRIBUTING~~	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)* (U) Development of Optimal red Blood Cell Products

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002300 Biochemistry; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 01	82 10	DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	2.7	189
FISCAL YEAR CURRENT 82	4.7	200

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Blood Storage; (U) Adenine; (U) Optional Additive Solutions; (U) 2,3-DPG

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Forward resuscitation of the wounded soldier requires that front line medical units maintain an adequate supply of viable, functional whole blood or packed red cells. This inventory must be available in spite of large fluctuations in usage, and delays limitations, or interruptions in normal supply lines. This dictates that stored blood have the longest possible shelf life and be of the highest quality. The work unit addresses the development of extended liquid storage of blood (42-100 days) as well as the improvement of the oxygen transport function of the stored blood.

24. (U) Chemicals known to improve red cell adenosine triphosphate (ATP) (survival) and 2,3-diphosphoglycerate (2,3-DPG) (function) will be evaluated singly and in combination using modern optimization techniques. Maximally effective formulations of citrate phosphate dextrose (CPD) adenine and optimal additive systems will be developed. The 2,3-PDG maintenance problem will be studied and the membrane integrity limits of long-term liquid storage defined.

25. (U) 8010-8109 Optional additive system (OAS) solutions were evaluated including a saline-adenine-glucose (SAG) solution, and ascorbate-2-phosphate (AsP) added to CPDA-1 and CPD whole blood. Both maintained red cells for 42 days while the latter also maintained elevated P_{50} via 2,3-DPG preservation. Long-term solution stability studies of AsP indicate solution stability for at least 12 months. The OAS containing AsP is being optimized using computer assisted experimental designs. Two commercial experimental SAG solutions ± mannitol have been evaluated.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 089 Development of Optimal Red Blood Cell Products

The following investigation has been conducted under this work unit:

Study No. 1 In vitro development of optimal formulations

Studies have continued in the development of an Optional Additive System (OAS) containing saline, adenine, glucose and ascorbate-2-phosphate (AsP). The 25 C solution stability of this OAS has been confirmed for periods of at least 1 year. Using computer-generated experimental designs, this OAS formulation is now being optimized. Discussions are in progress with Cutter Laboratories for toxicity testing and clinical trials of the optimal OAS which are scheduled to start during FY 82.

Limited studies have been done on ADSOL and saline-adenine-glucose (SAG) systems; these do not contain AsP, but may contain mannitol to retard hemolysis. These in vitro studies indicate that both ADSOL and SAG are similar to CPDA-2 in their ability to preserve red cells and, with mannitol, exhibit reduced hemolysis. Neither solution can maintain red cell function in storage, as can the OAS solution with AsP. ADSOL has excessive amounts of glucose and mannitol which may prove toxic to neonates and diabetic subjects.

BODY OF REPORT

WORK UNIT NO.	089	Development of Optimal Red Blood Cell Products
STUDY NO.	1	In vitro development of optimal formulations

PROBLEM

Military blood banking differs from its civilian counterpart because of unique logistical limitations imposed in combat situations. In a civilian setting blood is drawn, stored under "ideal" conditions, and used in a geographically-contained community at a relatively predictable rate. Under these conditions blood shortages are minimal, and loss due to outdating is less than 10%. The wartime use of blood in the Army may be illustrated by the Vietnam experience, which is probably a best-case example. The blood used in Vietnam was drawn in CONUS and had CPD anticoagulant added; it had a 21-day dating period. The time required to process and ship this blood to field medical units was 7 to 14 days leaving only 7 to 14 days of shelf life. Due to limited shelf life and the fluctuation in casualty rate, outdating was possibly as high as 50%, leaving inventories dangerously low in many instances. These problems could have been aleviated if the shelf life of blood had been 35 to 42 days. In future conflicts, the U.S. may not have air superiority, thus logistical problems will be compounded in all areas of supply, including fresh blood and blood products. To support the wounded soldier with available blood products, it will be imperative to store blood for extended periods of time. In addition, it is essential that stored blood maintain its functional qualities. These ends can be met by the development of new systems for blood storage that extend the shelf life (viability) and improve the oxygen-delivering quality of red cells. A significant step in this direction was taken with the development of CPDA-1 anticoagulant which allows for 35-day storage of whole blood or packed cells of hematocrit not over 80. New efforts are underway to extend blood storage beyond 35 days, and also to improve the quality of long-term stored blood. At this time, specific studies are underway to develop an Optional Additive System (OAS). The development of CPDA-1, while offering a significant improvement in blood storage does not achieve the results in red cell storage that are attainable with a glucose-adenine mixture.

CPDA-2 was shown to be a significantly superior product, based on clinical trials (work unit JL03) and in vitro tests, compared to CPDA-1. Red cells could be stored in CPDA-2 for up to 49 or 56 days. The best approach to extended quality storage of red blood cells is by use of specific solution for addition to packed red cells. This approach is termed an Optional Additive System.

Development of Optimal Red Blood Cell Products (continued)

Solutions are being developed and tested (in vitro) which allow for extended storage of packed red cells, and at the same time improve the functional quality of these cells by maintaining the concentrationn of red cell 2,3-DPG. The development of these systems will provide military blood banking with the capability to a) store red blood cells to extended periods of time beyond 35 days, b) improve the functional qualities of these cells (i.e., their oxygen off-loading characteristics) by maintaining normal P_{50}, and c) make available for separate use, fresh plasma components in maximum quantities, free of adenine or other additives.

RESULTS AND DISCUSSION OF RESULTS

Studies have continued with the development of OAS solution using ascorbate-2-phosphate (AsP) to maintain red cell O_2 delivery function. AsP solutions in saline and SAG were stored as individual samples in 50 ml transfer packs, each sealed and heat processed (to prevent mold) in 1/2 pint canning jars. These solutions are being assayed over a 3-year period. After 12 months, no loss of stability is seen in the AsP at room temperature. Similar studies were being planned to reevaluate the solution stability of DHA, but were cancelled when the company holding the patent on using DHA terminated the manufacture of blood bags. Final studies are in progress to obtain the optimal formulation of an OAS solution containing saline, adenine, glucose and AsP. Those studies were designed with the aid of a computer program entitled "Computer Optimized Experimental Design" (COED). The copyrighted COED program is available on a time-leased basis from CompuServe Corp., Columbus, OH. We evaluated COED, decided it would be a powerful tocl to minimize gathering of experimental data while maximizing the knowledge gained, and leased access to the program through FY 1982. When the COED-generated experiments are completed they will be analyzed and optimized by the companion program to COED entitled "Response Surface Methodology-3".

Studies were also done on two OAS systems containing saline, adenine and glucose $\pm$ mannitol. These solutions were commercially prepared by Fenwal Laboratories (ADSOL) and Cutter Laboratories (SAG), respectively. The purpose of these solutions is to provide a more controlled alternative to long-term packed cell storage than the use of CPDA-2. Our in vitro studies indicate that both solutions are effective in maintaining red cell ATP for 42 days of 4 C storage. Addition of mannitol to either solution retards hemolysis up to 90% and also causes a slight improvement in ATP maintenance. The ADSOL solution contains an excessive amount of glucose (i.e., 600 mg/dl after 42 days of storage) and mannitol which may prove clinically hazardous in certain patients.

Joint studies between our laboratory and Cutter Laboratories are being planned in FY 1982 for clinical trials of the saline, adenine, glucose, AsP solution. Further advances in this project during FY 81 were limited by requirements of the technical personnel to heavily support the hemoglobin solution safety project (JL07).

CONCLUSIONS

The best choice for an OAS solution to both extend red cell storage and improve red cell function appears to be solution of saline, adenine, glucose, and AsP. Modern computer-assisted techniques are being used to optimize this formulation. SAG-type systems are similar in performance to CPDA-2 for extended red cell storage but do not aid in red cell function as so the OAS systems. In SAG is put on the commercial market it will probably be quickly replaced by the OAS system containing AsP.

RECOMMENDATIONS

Studies should be completed to optimize the OAS solution containing AsP. Cooperation should be continued between LAIR and Cutter Labs to do toxicology and Clinical Studies for FDA "approval" of this solution. Continued evaluation of the COED-RSM3 computer package should be done with the idea of expanding its use to a LAIR-wide resource.

PUBLICATIONS

1. PECK, C.C., G.L. MOORE, and R.B. BOLIN. Adenine in blood preservation. CRC Reviews in Clinical Laboratory Science, 13:173-212, 1981

2. MOORE, G.L., C.C. PECK, P.R. SOHMER, and T.F. ZUCK. Some properties of blood stored in anticoagulant CPDA-1 solution. Transfusion, 21:135-140, 1981

3. UNRUH, K., M.E. LEDFORD, A. ZEGNA, and G.L. MOORE. Adaptation of the biotonometry P P-50 technique to the IL Model 213 blood gas analyzer. LAIR Technical Note, No. 80-15TN, Oct 80

4. MOORE, G.L., M.E. LEDFORD, M.R. BRUMMELL, and K.A. UNRUH. Red cell storage for 56 days in modified CPD-adenine: an in vitro evaluation. Transfusion (in press)

5. MOORE, G.L., M.E. LEDFORD, M.R. BRUMMELL. Improved red cell storage using optional additive systems (OAS) containing adenine, glucose, and ascorbate-2-phosphate. Transfusion (in press)

6. MOORE, G.L., M.E. LEDFORD, M.R. BRUMMELL. Red cell ATP and 2,3-DPG concentrations as a function on dihydroxyacetone supplementation of CPD-adenine blood. Vox Sang, 41:11, 1981

7. MOORE, G.L., M.E. LEDFORD, A. MERYDITH. A micro-modification of the Drabkin hemoglobin assay for measuring plasma hemoglobin in the range of 5 to 2000 mg/dl. Biochem Med (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DAOG 3369	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	256 APC HL14
b. CONTRIBUTING	62772A	3S162772A874	AA	091
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) A Porcine Model for Studies in Combat-Related Trauma

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012900 Physiology; 002300 Biochemistry; 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY		16. PERFORMANCE METHOD
79 10	CONT	DA		C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	1.5	120
FISCAL YEAR CURRENT 82	2.5	169

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: POC: DA

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Hypovolemic Shock; (U) Swine; (U) Trauma; (U) Resuscitation; (U) Hemodynamics; (U) Metabolic Function; (U) Laboratory Animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) There is a distinct need for a large, nonprimate, animal model to conduct simulated studies of combat-related trauma, severe blood loss, and consequent shock. From a scientific standpoint, the domestic pig would appear to be an attractive species for meeting this need, much more so than the commonly used mongrel dog. Use of the pig, however, has been hampered by a lack of knowledge about its normal physiologic and biochemical characteristics and the impact thereon of simulated combat injuries. This information is needed to more accurately describe such injuries and to foster rational development of improved treatment modalities.

24. (U) Surgical and technical procedures will be developed to study the physiologic and biochemical characteristics of the conscious, unencumbered animal. The effects of injuries and severe blood loss, such as seen in the combat environment, will be described and the effects of conventional and innovative treatment modalities will be evaluated.

25. (U) 80 10 - 81 09 Conscious domestic pigs, under basal conditions, have higher pH_a, $[HCO_3^-]_a$ and $[BE]_a$ values, lower P_aO_2 and S_aO_2 values, and the same P_aCO_2 values as humans measured under similar conditions. Regulation of F_IO_2 and ventilation in the anesthetized pig can be used to adjust the levels of P_aO_2, P_aCO_2, $[HCO_3^-]_a$, and $[BE]_a$. In conscious, unrestrained pigs 50% hemorrhage led to symptoms and hemodynamic, acid-base, and blood metabolite changes that were nearly identical to those described for similarly hemorrhaged humans. Major effects included transient nausea, vomiting, dizziness, hypotension, hyperglycemia, lactacidosis, elevated arterial magnesium and creatinine levels, but reduced arterial potassium, P_aCO_2, $[HCO_3^-]_a$, and $[BE]_a$ levels. These pigs recovered from severe hemorrhage without medical intervention. Swine, therefore, offer an excellent model to study the role of severe blood loss as it occurs in combat casualties.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO: 3M161102BS10 Research on Military Disease, Injury and Health Hazards

WORK UNTT NO: 256 A Porcine Model for Studies in Combat-Related Trauma

The following investigations were conducted under this work unit:

STUDY NO. 1 Normal physiologic and biochemical values for the domestic pig (Sus scrofa)

STUDY NO. 2 Physiologic aspects of porcine hemorrhage

STUDY NO. 1. During the past year all work on two experiments was completed. These were concerned with determining the feasibility of establishing steady-state ventilatory conditions in the anesthetized domestic pig such that arterial Po_2 and pH approximated values observed in humans and, under such conditions, to determine the porcine population characteristics for arterial electrolytes and the blood gas and acid-base status of both arterial and venous blood. Mean arterial electrolyte values (mEq/l) were: sodium, 139; potassium, 4.8; calcium 4.8; magnesium 1.8; chloride, 100; bicarbonate, 27.7; phosphate, 4.3; albuminate, 7.1; globulinate, 6.2; and lactate 1.8. Mean arterial blood gas and acid-base values were: Po_2 97 torr; S_aO_2, 94%; C_aO_2, 15.4 ml/dl; pH, 7.399; Pco_2, 47 torr; and base excess 2.8 mEq/l. Venous values from seven vascular sites differed significantly from arterial values and from each other. A third, uncompleted study, concerned the arterial blood gas and acid-base status of conscious, unrestrained pigs under near-basal conditions. To date, 40 pigs have been evaluated. These animals had higher pH, bicarbonate, and base excess values but lower Po_2 values than those observed in humans under basal conditions. Pigs, nevertheless, are similar enough to humans to be an attractive animal model when the above variables are to be altered experimentally.

STUDY NO. 2. During the past year all work on three experiments was completed. These concerned the hemodynamic alterations, blood-gas and acid-base status, and plasma metabolite, electrolyte, and enzyme changes wrought by moderate (30%) and severe (50%) blood volume loss in the conscious animal. Severe blood loss led to symptoms similar to those reported for humans, yet all animals spontaneously recovered without blood replacement or other interventions. Arterial pressure (mean, systolic, and diastolic) alterations during and following hemorrhage also were remarkably similar to those reported for comparably hemorrhaged humans. Severe blood loss led to metabolic acidosis that was largely compensated. Immediately after hemorrhage these pigs had a mean arterial pressure of 46 torr, Pco_2 of 28.4 torr, bicarbonate of 21 mEq/l, and base excess of 1.3 mEq/l; all of these

were substantially lower than control values. Arterial pH was lowered only slightly and transiently, while Po_2 increased. Pigs subjected to 30% hemorrhage showed far fewer, and less marked, changes in all of the above variables. Both 30% and 50% blood loss led to substantial fluid shifts from the extra- to the intravascular compartments during spontaneous recovery. A fourth experiment currently being completed showed that conscious animals subjected to 50%, and to a much lesser extent 30%, blood loss exhibited transient hyperglycemia and lactacidosis. They also showed transient increases in plasma creatinine and magnesium, and transient decreases in potassium. Plasma urea concentration increased progressively following hemorrhage, and the concentrations of several plasma enzymes were reduced. Most of the hemodynamic, blood gas, acid-base, metabolite, and electrolyte changes are remarkably similar to those reported for similarly hemorrhaged humans.

BODY OF REPORT

WORK UNIT NO. 091 — A Porcine Model for Studies in Combat-Related Trauma

STUDY NO. 1 — Normal physiological and biochemical values for the domestic pig (Sus scrofa)

PROBLEM

In the past, and at the present time, mongrel dogs have served as the predominant large animal species for medically oriented research on problems of combat-related trauma. Such usage is largely attributable to tradition and to the availability of dogs at local pounds and animal shelters. In recent years, however, the use of dogs in medical research has come under increasing criticism by scientists because they exhibit functional characteristics that are not seen in humans. The domestic pig, consequently, is becoming an attractive alternative to the dog as a large animal model for human-oriented research. Pigs are readily available in all parts of the country and can be acquired in a variety of ages, sizes, and genetic backgrounds. Between-animal functional variances, therefore, are usually far less than those seen in mongrel dogs. But more important than these considerations, available information shows the pig to be far superior to the dog in terms of his physiologic and biochemical similarities to man. In many research situations these similarities should allow substitution of pigs for nonhuman primates, hence conserving an expensive and rapidly diminishing laboratory animal resource. A major impediment to more extensive use of pigs in combat injury and other medical research projects is a lack of detailed knowledge about the population characteristics for certain key aspects of normal porcine physiology and biochemistry. Without this knowledge, rational experimental work involving pigs cannot be designed, nor can meaningful information about the functional changes associated with simulated combat injuries be obtained. It is to these problems that the experiments conducted under this study are directed.

RESULTS AND DISCUSSION OF RESULTS

During the past year all work on two experiments was completed, a third was continued, and a fourth was initiated. The two completed experiments concerned the feasibility of regulating and eventually stabilizing the ventilation of anesthetized pigs such that arterial pH and Po_2 values approximated those seen in humans during thoracic surgical procedures. Fifteen young domestic pigs were thus anesthetized with nitrous oxide, and mechanical ventilation and inspired oxygen tension were regulated to achieve an arterial pH of approximately 7.40 and Po_2 of approximately 100 torr. Once established, it was possible to maintain steady-state values for at

A Porcine Model for Studies in Combat-Related Trauma (Cont)

least a half hour without further adjustments of ventilation or inspired Po_2. Under these stabilized conditions, the following population characteristics for arterial cation concentrations (mean mEq/l ± S.D.) were obtained: sodium, 139 ± 2.40; potassium, 4.8 ± 0.58; calcium, 4.8 ± 0.29; magnesium, 1.8 ± 0.20; and total cations, 150.3 ± 2.71. Anion concentrations (mean mEq/l ± S.D.) were: chloride, 100 ± 2.50; bicarbonate, 27.7 ± 1.93; phosphate, 4.3 ± 1.22; albuminate, 7.1 ± 0.92; globulinate, 6.2 ± 0.41; lactate, 1.8 ± 0.60; and total anions 147.1 ± 6.21. Population characteristics for arterial blood gas and acid-base status were: Po_2, 97 ± 8.6 torr, S_aO_2, 94 ± 1.4%, C_aO_2, 15.4 ± 0.90 ml/dl; pH, 7.399 ± 0.0117; Pco_2, 47 ± 3.5 torr; and base excess 2.8 ± 1.80 mEq/l. Characteristics for pulmonary artery mixed venous blood were: Po_2, 36 ± 4.2 torr, S_aO_2, 51 ± 7.9%; C_aO_2, 8.5 ± 1.46 ml/dl; pH, 7.335 ± 0.0259; Pco_2, 57 ± 5.8 torr; HCO_3^-, 29.6 ± 2.31 mEq/l; and base excess 3.9 ± 2.14 mEq/l. Comparisons of venous values obtained from various vascular sites (pulmonary artery, anterior vena cava, posterior vena cava, internal jugular vein, femoral vein, and coronary sinus) revealed numerous between-vessel differences in blood gas and acid-base status. Anesthesia with mechanical ventilation appeared to produce defects in alveolar-arterial gas exchange similar to those reported for other species.

The third experiment conducted, but not completed, during the past year was concerned with determining the arterial blood gas and acid-base status of young domestic pigs, measured under near-basal conditions. The animals received surgically implanted carotid catheters and blood samples were taken 7-10 days postsurgery, while the animal was in an awake, well-rested, unrestrained, recumbent position subsequent to an overnight fast. To date 40 pigs have been so evaluated, and the following arterial values (mean ± S.D.) were obtained: pH, 7.502 ± 0.0160; Pco_2, 41.0 ± 2.53 torr; Po_2, 79.1 ± 4.05 torr; HCO_3^-, 31.0 ± 2.40 mEq/l; and base excess 8.2 ± 2.21 mEq/l. In addition, a group of 6 similarly treated pigs was evaluated at hourly intervals for six hours. The arterial blood gas and acid-base values obtained from these animals were essentially the same as those indicated above. The only significant diurnal changes were a 6.6 torr decrease in Po_2 and a 0.9 mEq/l decrease in base excess, both occurring midway through the experimental period. Completion of this experiment requires construction of a Siggaard-Anderson nomogram that is applicable to porcine blood. This effort is currently in progress.

The fourth experiment was concerned with setting up procedures for measuring the total erythrocyte and plasma volumes of chronically-catheterized, conscious, unrestrained pigs using ^{51}Cr and ^{125}I-labelled albumin as indicators, and to determine the erythrocyte storage characteristics of the porcine spleen. Six intact and six splenectomized animals have been studied to date, and the data are currently being evaluated.

A Porcine Model for Studies in Combat-Related Trauma (Cont)

CONCLUSIONS

The foregoing experiments have shown that the arterial pH and Po_2 values of anesthetized pigs can be stabilized at values that closely approximate those characteristics of humans. Under such circumstances, however, arterial values for Pco_2, HCO_3^-, phosphate, and base excess values tend to be higher while S_aO_2 and C_aO_2 values tend to be lower than those characteristic of humans. In conscious unrestrained pigs studied under near basal conditions, arterial pH, HCO_3^-, and base excess values are distinctly higher than those of humans measured under similar conditions. The conscious domestic pig, nevertheless, is an attractive large animal model for studies of combat-related injuries.

RECOMMENDATIONS

To properly evaluate the blood gas and acid base status of pigs, a Siggaard-Anderson nomogram applicable to porcine blood needs to be constructed. The erythrocyte storage role of the porcine spleen should be evaluated by studies of splenectomized animals and animals in which splenic discharge of erythrocytes is elicited with epinephrine injections. Studies of arterial oxygen transport, hemodynamic characteristics, and the regional distribution of blood flow should be conducted in conscious unrestrained pigs.

PUBLICATIONS

1. HANNON, J.P. Domestic swine in physiological research. I. A biomedical model. Institute Report No. 91, Presidio of San Francisco: Letterman Army Institute of Research, May 1981

2. HANNON, J.P., J.H. SKALA, and W.Y. MOORES. Domestic swine in physiological research. II. Electrolyte values for arterial serum from young anesthetized pigs maintained under steady-state ventilatory conditions. Institute Report No. 92. Presidio of San Francisco: Letterman Army Institute of Research, May 1981

3. HANNON, J.P. and W.Y. MOORES. Domestic swine in physiological research. III. Blood gas and acid-base values of arterial and venous blood from young anesthetized pigs maintained under steady-state conditions. Institute Report Nol 113. Presidio of San Francisco: Letterman Army Institute of Research (in press)

A Porcine Model for Studies in Combat-Related Trauma (Cont)

STUDY NO. 2 Physiologic aspects of porcine hemorrhage

PROBLEM

Virtually all previous studies of the physiology and biochemistry of hemorrhage and resultant hypovolemic shock have been conducted in anesthetized animals. Rarely does one see investigations utilizing conscious animals. In addition, the majority of large animal studies have been conducted with canine models. These studies, in general, suffer from two major deficiencies: First, combat injuries rarely, if ever, occur in anesthetized soldiers and it is well-established that anesthetic agents seriously modify many of the normal physiologic and biochemical responses to severe injury and blood loss. Second, in terms of many highly pertinent functional variables, the dog is not a good model for characterizing responses to severe hemorrhage so often seen on the battlefield. The applicability of such experimental information to the combat-injured soldier is critical to the rational development of effective medical treatment procedures at front line positions.

The domestic pig, in terms of its known functional characteristics, appears superior to the dog as an animal model for physiologic and biochemical studies which are relevant to humans injured in combat. The pig, furthermore, can be readily studied under conscious unencumbered conditions in the laboratory. But, it is only in recent years that medical researchers have started to use the pig for studies of hemorrhage and shock, and even in these few instances virtually no experimental work has involved conscious animals. The present study, therefore, was designed to develop surgical procedures for monitoring the functional characteristics of conscious pigs over extended periods of time and to collect data on physiologic and biochemical responses to severe blood loss.

RESULTS AND DISCUSSION OF RESULTS

During the past year all work on three experiments was completed and a fourth experiment is currently in the final stages of completion.

In the first experiment, a porcine animal model designed to simulate physiologic characteristics of the combat casualty was used to assess the effects of severe blood loss on heart rate and arterial pressures in the absence of anesthesia or other interventions. Chronic catheters were placed surgically in the aorta, via the carotid artery, of 8 young domestic pigs. Seven to 9 days after surgery each animal was brought into the laboratory and the catheter was connected to a three-way stopcock and a pressure transducer for blood removal and pressure recording. After 30 minutes of unrestrained and uninterrupted supine

rest, control measurements were made. Thereafter, 50% of the estimated blood volume was removed progressively over a one-hour period. No physiologic changes were seen until blood loss exceeded 10%. A transient increase in heart rate occurred at 20% loss, but subsequent rates were no different from control values. Systolic, diastolic, and mean arterial pressures decreased progressively between 20 and 50% blood loss; the respective values at 50% blood loss were 84 $\pm$ 3.2, 31 $\pm$ 3.2, and 49 $\pm$ 2.8 torr. Signs displayed during hemorrhagic hypotension were similar to those reported for similarly hemorrhaged humans; i.e., lethargy, dizziness, nausea, and vomiting. The pigs spontaneously and successfully compensated for 50% blood loss without blood replacement or other interventions, as judged by 24-hour survival beyond the hemorrhage episode.

In the second experiment, young domestic swine, six animals per group, were subjected to 30 and 50% hemorrhage of their estimated blood volume over a one-hour period while in a conscious recumbent state. Before and for five hours after hemorrhage, hemodynamic functions were measured to assess the characteristics of spontaneous recovery from hemorrhagic hypotension. Six additional pigs, treated similarly except for hemorrhage, served as controls. Immediately after 30% hemorrhage, arterial mean, systolic and diastolic blood pressures were 79, 104, and 59 torr, respectively. During the 5-hour recovery period, these pressures reverted to 105, 129, and 81 torr, nearly the same as pre-hemorrhage values. Heart rates were unaltered by hemorrhage but increased slightly during recovery. Pulse pressure was not significantly affected by hemorrhage or recovery, while hematocrits declined during and following blood loss. After 50% hemorrhage, arterial mean, systolic, and diastolic pressures were 46, 79, and 26 torr, respectively. During the recovery period these pressures rose to 81, 104, and 62 torr; all remained significantly below pre-hemorrhage levels. Pulse pressure increased significantly during the recovery period, while hematocrits decreased to an even greater degree than those in the 30% group. Heart rates were not significantly changed after 50% hemorrhage, but rose markedly during the first 4 hours of the recovery period. In both hemorrhage groups, spontaneous recovery was associated with a progressive decrease in hematocrit which reflected a transfer of interstitial fluid to the circulation.

The third experiment was conducted simultaneously with the second. It concerned the blood gas and acid-base changes associated with hemorrhage and subsequent spontaneous recovery in the conscious animal.

This experiment showed that 50% blood loss led to a metabolic acidosis that was largely compensated. Accordingly, the group mean for arterial pH decreased slightly, from 7.500 to 7.464, Pco_2 from 41.0 to 28.4 torr, $[HCO_3^-]$ from 31.0 to 21.0 mEq/l, and base excess from 8.1 to -1.3 mEq/l, while arterial Po_2 rose from 79.7 to 98.8 torr. During the

five-hour period of spontaneous recovery, all the foregoing changes reverted to and eventually exceeded values recorded in the initial control period or in the control group measured at the same time point of recovery. Except for arterial Po_2, which remained at control levels, the acid-base values of pigs subjected to 30% hemorrhage also rose and eventually exceeded control levels as the period of spontaneous recovery progressed. On the basis of linear regression and correlation analysis, it appeared that arterial chemoreceptor drive for ventilation became inoperative during and for 5 hours after hemorrhage. These analyses also indicated that baroreceptor drive of heart rate was eliminated during hemorrhage but returned during spontaneous recovery.

The fourth experiment concerned the metabolic, electrolyte, and enzyme changes in arterial plasma taken from the pigs used in the above two experiments. Fifty percent hemorrhage, and to a much lesser extent 30% hemorrhage, led to prompt hyperglycemia and lactacidosis; mean glucose level following 50% blood loss rose from 4.84 to 9.40 mmoles/liter, lactic acid from 1.13 to 11.36 mmoles/liter. Plasma creatinine and magnesium concentrations also were elevated immediately after 50% (but not 30%) hemorrhage; creatinine increased from 79 to 119 umoles/liter, and magnesium increased from 1.15 to 1.56 mEq/liter. In contrast, 50% blood loss was associated with a decrease in potassium concentration from 4.5 to 3.7 mEq/l. All of the foregoing changes were transient and the concentrations reverted to near normal levels over a 5-hour period of spontaneous recovery. Plasma urea concentration was unaltered immediately after 30 or 50% blood loss. Over the period of spontaneous recovery, however, the values in pigs subjected to 50% hemorrhage increased from 2.64 to 4.79 mmoles/liter. Hemorrhage had no immediate effect on the concentrations of several plasma enzymes, but during the recovery period decreased concentrations of alanine transaminase, lactic dehydrogenase, creatine kinase, and alkaline phosphatase were observed. These effects were similar in both hemorrhage groups and appeared attributable to fluid shifts from the extra- to the intravascular compartments.

CONCLUSIONS

The heart rate and arterial pressure changes associated with severe hemorrhage in conscious swine are remarkably similar to those reported for conscious humans. The pig may tolerate a somewhat greater blood loss than man without fatal consequences, but this has yet to be established.

As evidenced by 24-hour survival, conscious young domestic pigs can successfully compensate for 30 and 50% losses of estimated blood volume. This is attributable to rapid transfer of fluid from the interstitial to the intravascular space. Such transfer replenishes blood volume and returns arterial pressures toward prehemorrhage

values. Estimates of the magnitude of this transfer over a 5-hour posthemorrhage period indicate nearly complete recovery in pigs subjected to 30% blood volume loss and about one-half recovery in pigs subjected to a 50% blood volume loss.

On the basis of results reported in the literature, anesthetic agents seriously modify the physiologic responses to hemorrhage. Equivalent blood loss causes a far greater decrease in arterial pressure in the anesthetized animal as compared to the conscious animal. The conscious pig survives much greater blood losses than the anesthetized pig.

The pig would appear to be superior to the dog for human-oriented studies of physiologic compensations to severe blood loss. The large erythrocyte storage capacity of the canine spleen plays a major compensatory role in the restoration of blood volume following hemorrhage--a role not nearly so important in the human and pig.

In view of the similarities in human and porcine conpensations to hemorrhage, it would appear likely that humans can successfully survive moderately severe blood loss without resuscitative intervention. If this can be firmly established, it would have a major impact on the management of certain combat casualties, i.e., those in which blood loss does not exceed limits compatible with spontaneous recovery.

RECOMMENDATIONS

Spontaneous compensations of the conscious pig to severe hemorrhage should be described in terms of changes in tissue blood flow and the kinetic characteristics of fluid transfer from the interstitial and intracellular space to the vasculature.

Alterations in metabolic status as reflected by oxygen transport characteristics and blood chemical changes should be delineated during and after hemorrhage in the conscious pig.

The critical physiologic and biochemical factors leading to fatalities following massive hemorrhage of the conscious pig should be described.

PUBLICATIONS

1. DIXON, R.S., P.B. JENNINGS, and J.P. HANNON. Physiologic aspects of porcine hemorrhage. I. A vascular catheter for chronic implantation in swine. Institute Report No. 93. Presidio of San Francisco: Letterman Army Institute of Research, July 1981

A Porcine Model for Studies in Combat-Related Trauma (Cont)

2. HANNON, J.P., P.B. JENNINGS, Jr., and R.S. DIXON. Physiologic aspects of porcine hemorrhage. II. Alterations in heart rate and arterial pressure during fifty percent blood volume loss in the conscious animal. Institute Report No. 94. Presidio of San Francisco: Letterman Army Institute of Research, July 1981

3. HANNON, J.P., P.B. JENNINGS, Jr., and R.S. DIXON. Physiologic aspects of porcine hemorrhage. III. Heart rate and arterial pressure changes during spontaneous recovery from 30 and 50 percent blood volume loss in the conscious animal. Institute Report No. 95. Presidio of San Francisco: Letterman Army Institute of Research, July 1981

4. HANNON, J.P. Physiologic characteristics of non-fatal hemorrhage in the conscious pig. (abstract) Circ Shock 8:190, 1981

5. HANNON, J.P., P.B. JENNINGS, Jr., and R.S. DIXON. Physiologic aspects of porcine hemorrhage. IV. Blood gas and acid-base status of the conscious animal following 30 and 50 percent blood loss. Institute Report No. 111. Presidio of San Francisco: Letterman Army Institute of Research (in press)

6. HANNON, J.P., and J.H. SKALA. Physiologic aspects of porcine hemorrhage. V. Metabolite, electrolyte, and enzyme changes in arterial plasma during spontaneous recovery from 30 and 50 percent blood volume loss in the conscious animal. Institute Report. Presidio of San Francisco: Letterman Army Institute of Research (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 2374	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AB	092 APC HL15
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Procede with Security Classification Code)*

(U) Pharmacologic Stabilization of the Combat Casualty

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

008800 Life Support; 012900 Physiology; 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	3.8	197
FISCAL YEAR CURRENT 82	5.1	157

19. RESPONSIBLE DOD ORGANIZATION

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NAME: POC; DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Resuscitation; (U) Laboratory Animal; (U) Irreversibility; (U) Hemorrhagic Shock; (U) Critical Organ Failure

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) For optimal management of combat casualties, a rapid and effective evacuation to treatment facilities that can provide surgical care is necessary. In future wars, battlefield conditions may preclude rapid evacuation of the combat casualty. Therefore, it is necessary to develop nonsurgical therapies which combat medics in the field can use to delay the pathophysiologic consequences of blood loss and neglected wounds.

24. (U) A small animal fixed-volume withdrawal hemorrhagic shock model (50% loss of blood volume over 1 hour) was developed using conscious rats to investigate the effectiveness of potential "antishock drugs." A small animal uncontrolled hemorrhage model was also developed.

25. (U) 80 10 - 81 09 A fixed-volume withdrawal hemorrhagic shock model using conscious rats was used to study possible antishock agents. Twenty percent of the untreated control animals survive beyond 6 hours following the completion of hemorrhage. The following drugs improve survival in the fixed-volume withdrawal model when compared to the untreated controls: naloxone (1 mg/kg, i.v.), captopril (1 mg/kg, i.v.), 7.5% NaCl (volume equal to 10% of shed blood, i.v.), and atropine (0.5 mg/kg, i.p.). The following drugs have been shown not to increase survival in this shock model: fructose-1,6-diphosphate (200 mg, i.v.), imidazole (30 mg/kg, i.v.), morphine (0.7 mg/kg or 0.07 mg/kg, i.v.), diphenhydramine hydrochloride (1 mg/kg, i.v.), indomethacin (1 or 10 mg/kg, i.v.), and verapamil (0.75 mg/kg, i.v.). Work is in progress evaluating different proposed therapeutic agents, such as thyrotropin-releasing hormone and prostacycline. Combinations of the agents that have been shown to improve survival are also being studied.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO: 3S162772A874 Care of the Combat Casualty

WORK UNIT NO: 092 Pharmacologic Stabilization of the Combat Casualty

The following investigations have been conducted under this work unit:

STUDY NO. 1 Antishock drugs

STUDY NO. 2 Drug therapy in exsanguination

STUDY NO. 1. A hemorrhagic shock model using conscious rats was developed to investigate a number of potential antishock drugs. In brief, rats were anesthetized and silastic catheters inserted in the right external jugular vein and carotid artery. The catheters were tunneled dorsally and pocketed subcutaneously between the animal's scapulae. Patency was maintained by filling the catheter with 1:1000 dilution heparin. The following day the catheters were exteriorized and the rats bled 50% of their estimated blood volume for one hour. Survival was assessed at 6 hours. A radioiodinated serum albumin study was performed to test the validity for calculating rat blood volume as a percent of body weight. The rats used in this study (males weighing 325-400 g), were found to have an average of 6.1 ml blood per 100 g body weight.

STUDY NO. 2. This study examined the possibility of using pharmacological agents to increase survival of conscious rats, which were bled to 50% of total blood volume. Twenty-four hours before exsanguination, the rats were anesthetized and catheters placed in the carotid artery and jugular vein. The next day the animals were bled to 50% of their calculated blood volume for one hour. The agents to be tested were administered intravenously starting 15 minutes after the beginning of hemorrhage. Survival was assessed at 6 hours. The following drugs were tested against a series of concurrent controls: diphenhydramine hydrochloride 1 mg/kg (D), fructose-1,6-diphosphate 500 mg/kg (FDP), naloxone 1 or 2 mg/kg (N), indomethacin 1 or 10 mg/kg (I), imidazole 30 mg/kg (IM), captopril 1 mg/kg (C). Ringer's lactate equal to two times the volume of shed blood (RL), and 7.5% NaCl equal to 10% shed blood (NaCl) were also studied:

Pharmacologic Stabilization of the Combat Casualty (Cont)

Proportion of animals surviving six hours (*p<0.05 compared to control)

CONTROL	D	FDP	N*	I	IM	C	RL	NaCl*
% Surviving								
0.20	0.07	0.05	0.41	0.10	0.20	0.38	0.62	0.77
(98)	(15)	(19)	(34)	(23)	(25)	(13)	(21)	(13)

Figures in parentheses are number of animals in each group

Altering histamine and prostaglandin metabolism does not appear to affect survival in the fixed-volume-withdrawal-hemorrhage model. These data suggest that naloxone and 7.5% NaCl may be useful as temporizing measures when standard treatment of hemorrhage is not possible.

BODY OF REPORT

WORK UNIT NO. 092 Pharmacologic Stabilization of the Combat Casualty

STUDY NO. 1 Antishock Drugs

PROBLEM

The most important factor in survival of the combat casualty is rapid evacuation to facilities providing definitive surgical care. The low in-hospital mortality rate in Vietnam (2% in 1969) documents the effectiveness of rapid evacuation of the combat casualty. A delay in evacuation of the wounded would result in an increased death rate for those casualties whose wounds were not quickly treated. Some casualties would experience moderate to severe blood loss, and would be expected to experience prolonged hypotension and inadequate blood flow to the vital organs. It is our intention to develop interventions that can be used by the field medic to prolong survival in the combat casualty.

A rat hemorrhagic shock model has been developed that simulates blood loss due to battlefield trauma. Possible treatment modalities will be tested using this model. Some of the treatment modalities that were tested in this conscious fixed-volume withdrawal model were: 1) benadryl (1 mg/kg i.v.), an antihistamine which increases venous return; 2) Ringer's lactate (2 parts to 1 part shed blood over 1 hour, i.v.), the standard battlefield crystalloid fluid replacement; 3) naloxone (1 and 2 mg/kg, i.v.), a beta-endorphin blocker; and 4) fructose-1,6-diphosphate (200 mg given over 3 hours, i.v.), a glycolytic intermediate.

RESULTS AND DISCUSSION OF RESULTS

Twenty percent of the untreated control animals in the study survived. Benadryl 1 mg/kg decreased survival to 7%. Naloxone 1 and 2 mg/kg increased survival (41%) when compared to untreated controls. Ringer's lactate increased survival (62%) when compared to controls, and fructose-1,6-diphosphate from a U.S. manufacturing source caused adverse effects in the rats; pyrogens are suspected in this batch of FDP.

CONCLUSIONS

In this study we have shown that, given appropriate treatment, the survival rate of rats experiencing hemorrhagic shock can be significantly increased. Naloxone shows promise as an antishock agent. Other proposed therapies should be studied. Study 1 has essentially been completed.

RECOMMENDATIONS

The fructose-1,6-diphosphate study should be repeated using a pure, pyrogen-free preparation. Several additional drugs should be tested, as well as combinations of therapeutic agents with Ringer's lactate as volume replacement. Please refer to Study 2 for a continuation of this work.

STUDY NO. 2 Drug therapy in exsanguination

PROBLEM

For optimal management of combat casualties, a rapid and effective evacuation is necessary to treatment facilities that can provide surgical care. However, rapid evacuation in a future war may not be possible. It is necessary to develop interventions which can be used in the field by a combat medic to improve or prolong survival of the combat casualty. Many publications in recent years have shown that drug therapy increased survival in a variety of animal shock models. Some of these proposed antishock drugs are: naloxone (blockade of beta-endorphin opiate receptors), indomethacin (interference with the formation of vasoactive prostaglandins), captopril (inhibition of angiotensin-converting enzyme), 7.5% sodium chloride (mechanism under study), verapamil (calcium channel blockade), thyrotropin-releasing hormone (mechanism under study), and prostacycline (mechanism under study). These published data often show dramatic improvements in survival rates (for example, survival increases from 0 to 90% when dogs are given 7.5% sodium chloride after having been subjected to hemorrhage). The relevance of many of these shock models to the treatment of the bleeding soldier is questionable. This study is being performed to investigate the effectiveness of various proposed antishock drugs, using a model designed to simulate blood loss due to battlefield trauma.

A fixed-volume withdrawal hemorrhagic shock model has been developed using conscious rats. Catheters are placed in the jugular vein and carotid artery of anesthetized 325-375 g rats. The next day, the conscious instrumented rats are bled 50% of their estimated blood volume for one hour; at 15% loss, the rat is given the "antishock drug" under study. Hematocrit and respiratory rates are measured throughout the study. Survival is assessed at 6 hours and at 6 days.

RESULTS AND DISCUSSION OF RESULTS

Twenty percent of the untreated control animals survived. Diphenhydramine hydrochloride (1 mg/kg), fructose-1,6-diphosphate (200 mg), naloxone (1, 2, and 5 mg/kg), indomethacin (1 and 10 mg/kg),

imidazole (30 mg/kg), captopril (1 mg/kg), morphine (0.7 and 0.07 mg/kg), Ringer's lactate (one part to each part shed blood), 7.5% sodium chloride (equal to 10% shed blood volume), verapamil (0.75 mg/kg), atropine (0.5 mg/kg), and a combination of lactated Ringer's solution and naloxone have been studied. The following agents increase the survival rate when compared to the untreated control group: naloxone (1 mg/kg), captopril (1 mg/kg), 7.5% sodium chloride (volume equal to 10% shed blood volume), and atropine (0.5 mg/kg i.p.). The other drugs tested either failed to significantly increase survival in the fixed-volume withdrawal rat model, or they had a detrimental effect when combined with hemorrhage. The indomethacin data may be incorrect because we were using an impure preparation. The pharmaceutical company has supplied us with a pure sample so we can repeat the experiment.

Sixty-two percent of the exsanguinated rats survived when treated with Ringer's lactate. The combination of Ringer's lactate and 1 mg/kg naloxone did not significantly improve survival when compared to the Ringer's lactate group.

Uncontrolled arterial hemorrhage studies were also performed. Rats pretreated with either naloxone or captopril were allowed to exsanguinate from their carotid arteries. Rats pretreated with naloxone (5 mg/kg) showed a greater rate of bleeding than an untreated control group.

CONCLUSIONS

These data are meaningful in the context of our small-animal shock models. Care must be taken in extrapolating these data to the bleeding soldier. Unless the bleeding can be controlled, pharmacologic agents that have a pressor effect may actually be detrimental to the survival of the combat casualty. It is necessary to repeat studies of the more promising "antishock drugs" such as 7.5% sodium chloride, in a more realistic mammalian model.

RECOMMENDATIONS

Several additional drugs should be investigated. These include thyrotropin-releasing hormone, prostacycline, pure indomethacin, and 2-PAM chloride. In addition, we will be conducting studies using bongkrekic acid in the fixed-volume withdrawal shock model. For the promising therapeutic agents, such as naloxone, 7.5% sodium chloride, and captopril, we recommend studying their actions in a more relevant, realistic large animal model. Combinations of successful agents need to be tested.

Pharmacologic Stabilization of the Combat Casualty (Cont)

PUBLICATIONS

1. MAHONEY, E.M. Drug therapy in fixed-volume exsanguination of unanesthetized rats. (abstract) Circ Shock 8:219, 1981

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 6272	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AD	094 JL06
b. CONTRIBUTING				
c. ~~CONTRIBUTING~~	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Pharmacologic and Metabolic Amplification of Fresh and Stored Blood

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

008800 Life Support; 003500 Clinical Medicine; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	C. IN-HOUSE

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 81	0.1	24
CURRENT FISCAL YEAR 82	6.5	50

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Acute Resuscitation; (U) Sodium Benzoate; (U) Phosphoenolpyruvate; (U) Hemorrhagic Shock; (U) Blood Amplification; (U) Laboratory Animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objectives of these studies are to develop and evaluate solutions that will improve the in vivo red cell function of fresh and stored blood. A cell-free resuscitation fluid, which augments the delivery of oxygen to wounded body tissues and adequately replaces the blood volume lost due to hemorrhage, will facilitate the immediate resuscitation of combat casualties and will diminish the immediate requirement for stored blood transfusions. A liquid preservation additive or rejuvenation solution which extends the shelf life of blood and improves function of the stored cells following transfusion will also decrease the amount required and waste of stored blood supplies.

24. (U) Metabolic and chemical alteration of intraerythrocytic hemoglobin will be performed; these erythrocytes will then be tested to determine the effect of these alterations on tissue oxygen delivery, cell viability and resuscitation from shock. Following demonstration of the efficacy of using of red cells with altered hemoglobin function, solutions will be tested in live animals to identify non-toxic means to alter red cell hemoglobin function in vivo.

25. (U) 8010-8109 Incubation of red cells with phosphoenolpyruvate (PEP) has been found to improve red cell oxygen delivery, as well as to reverse other adverse storage related red cell alterations. Blood treated with PEP maintains its new properties following exchange transfusion in the rat. Shocked rats resuscitated with blood treated with PEP appear to have a lower mortality rate than rats treated with blood stored for less than 24 hours.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3S162772A874 Care of the Combat Casualty

WORK UNIT NO. 094 Pharmacologic and Metabolic Amplification of Stored and Fresh Blood

Experiments were conducted to determine the efficacy of using phosphoenolpyruvate (PEP) for the in vivo and in vitro amplification and rejuvenation of fresh and stored blood. The first experiment determined the ability of PEP to reverse functional defects of blood subjected to long-term storage. The second experiment was done to determine the effect of pre-treating blood with PEP before long-term liquid storage. The third experiment compared the effect of PEP on red blood cells from a number of animal species whose hemoglobins differ in their sensitivity to 2,3-DPG. The fourth experiment tested in vivo efficacy of using high P_{50} red blood cell suspensions to rescue rats from hemorrhagic shock. The results of these studies indicate that: 1) PEP has no direct interaction with hemoglobin, and 2) incubation of stored blood with PEP results in a dramatic rise in 2,3-DPG and ATP, and 3) treated red blood cells maintain their high intracellular-metabolite concentration and high P_{50} at least 24 hours after transfusion.

BODY OF REPORT

WORK UNIT NO. 094 Pharmacologic and Metabolic Amplification of Fresh and Stored Blood

PROBLEM

The basic pathophysiologic defect of hemorrhagic shock is impaired oxygen delivery. Oxygen transport is dependent on hemoglobin concentration and hemoglobin oxygen affinity (conventionally expressed as P_{50}, the tension at which hemoglobin is 50% saturated). The conventional approach to the study of resuscitation from shock has focused on determining the most efficacious means of increasing flow (usually by increased volume) and by increasing hemoglobin concentration by transfusing red blood cells. The third variable that is the P_{50}, has received little attention from investigators interested in shock. Various strategies designed to alter the P_{50} favorably have been explored.

The higher the intraerythrocytic 2,3-DPG level, the higher the P_{50}; functionally, this means greater oxygen-delivering capacity of the blood. Liquid storage causes rapid depletion of 2,3-DPG and a subsequent decrease in the oxygen transport capacity of stored blood. Because of 2,3-DPG cannot cross the red cell membrane, it is not possible to simply provide 2,3-DPG, either as a blood preservative additive or directly to the shocked individual, to raise intraerythrocytic 2,3-DPG levels. We examined phosphoenolpyruvate (PEP) as a metabolic intermediate which raises the intracellular 2,3-DPG to levels previously unattainable. We have also used a small animal shock model to determine the in vivo effect of using these high P_{50} red cells for resuscitation purposes.

RESULTS AND DISCUSSION OF RESULTS

The first experiments were conducted to evaluate the effects of PEP incubation on human blood stored in CPD. In the first experiment, blood was collected and stored at 4 C. At weekly intervals aliquots were removed, and incubated with PEP for up to four hours. In the second experiment, blood was treated with PEP prior to storage at 4 C. Even after 42 days of storage, treatment of blood with PEP resulted in 2,3-DPG levels three times normal, and the ATP levels returned to normal. Morphology was also improved. Blood up to 100 days old has been treated with PEP and 2,3-DPG, and ATP levels were increased significantly. Unlike other techniques that have been used for red blood cell rejuvenation, incubation with PEP results in a dramatic increase of 2,3-DPG without depletion of ATP. This technique may have significant potential as an adjunct to conventional blood preservation systems.

Pharmacologic and Metabolic Amplification of Stored and Fresh Blood (continued)

The third experiment was conducted to identify the laboratory animals whose red cells respond to incubation with PEP in a matter analogous to human cells. Blood from rats, dogs, sheep, cows, monkeys, rabbits, and pigs was tested for its response to incubation with PEP. Rat, rabbit, monkey and dog erythrocytes demonstrate a rise in P_{50} which correlates with the increase of 2,3-DPG. There is no effect on P_{50} in red cells from sheep or cows, species whose hemoglobin is not sensitive to 2,3-DPG. PEP was transported across the membrane of all species examined except the pig, and it appears that rats, dogs, rabbits and monkeys are appropriate models for in vitro and in vivo evaluation of the effects of PEP treatment of red blood in the therapeutic manipulation of the oxygen dissociation curve.

The final experiment was done to evaluate the effect of using PEP-treated red blood cells to resuscitate rats from shock. Rats were bled 50% of their estimated blood volume and reinfused 20 minutes later with normal or PEP-treated (high P_{50}) red blocd cell suspensions. It was shown that the high 2,3-DPG levels and consequent high P_{50} of the treated cells was maintained at least 24 hours after transfusion. Although the rate of survival from shock was apparently improved, due to the design of the experiment, it is not possible to attribute the effect to improved oxygen delivery. In addition, although treatment with PEP improves the morphology of human cells, rat red cells are more sensitive to PEP incubation, and hemolysis became a significant problem. Isolated organ profusion, using human red cells treated with PEP, must be done to determine the the effect of using high P_{50} red cells on oxygen delivery to hypoxic tissues.

CONCLUSIONS

Treatment or pretreatment of stored blood with PEP results in a dramatic increase in both intraerythrocytic 2,3-DPG and P_{50}. This effect is maintained through subsequent storage and blood transfusion. Use of red cells with an abnormally high P_{50} for resuscitation may be useful; it is difficult to examine this use of PEP-treated cells in a small animal shock model.

RECOMMENDATIONS

Alternative methods of raising P_{50} must be found. Use of the isolated perfused organ model for measurement of oxygen consumption is providing the definitive answer to the question of the effect of P_{50} on tissue oxygen extraction from blood.

Pharmacologic and Metabolic Amplification of Stored and Fresh Blood (continued)

PUBLICATIONS

1. SCOTT, R.L., and SOHMER, P.R. Comparative aspects of the effect of phosphoenolpyruvate on mammalian erythrocyte metabolism. Clinical Research 42(A) (abstract), 1981

2. SOHMER, P.R., and SCOTT, R.L. Rejuvenation of CPD stored blood with phosphoenolpyruvate. Clinical Research 42(A) (abstract) 1981

3. SOHMER, P.R., and SCOTT, R.L. Phosphoenolpyruvate (PEP) effects on fresh and stored red blood cells. Proceedings of Society of Experimental Biology and Medicine (in press)

4. SOHMER, P.R., and SCOTT, R.L. Regeneration of red cell 2,3-DPG and ATP with phosphoenolpyruvate. Transfusion (abstract) 1981

5. SCOTT, R.L., and SOHMER, P.R. Comparative effects of phosphoenolpyruvate on selected mammalian erythrocytes. Comparative Biochemistry and Physiology (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 2387	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	095 APC HL16
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2.5		

11. TITLE (Procede with Security Classification Code)
(U) Metabolic Support Following Combat Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine; 008800 Life Support; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	1.1	87
FISCAL YEAR CURRENT 82	3.1	88

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: POC: DA
NAME:

21. GENERAL USE
Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Body Compositional Change; (U) Wound Healing
(U) Military Trauma; (U) Parenteral Nutrition; (U) Animal Model

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The objectives of these studies are 1) to determine if the response to metabolic support following hemorrhage is the same as has been shown for trauma and surgical injury, and 2) to identify hypocaloric metabolic support systems which result in near-normal rates of protein synthesis, erythropoiesis, and reticuloendothelial function.

24. (U) Rats were maintained in either a fed, semi-starved (20% of their daily caloric requirements), or fasting state for 4 days. Shock was induced in half the rats from each group by removing 35% of their estimated blood volume. Fibronectin synthesis and degradation, total plasma protein synthesis, phagocytic capacity, and survival were assessed to determine the relationship of nutritional support and hemorrhagic shock.

25. (U) 80 10 - 81 09 It has been found that simple starvation dramatically reduces circulating fibronectin concentrations, and return to normal is rapid following refeeding. Fibronectin concentration is correlated with survival in casualties of traumatic and hemorrhagic shock and burn injury. Thus, treatments (i.e., pharmacologic or metabolic support) should be designed to maintain or increase fibronectin synthesis. There seems to be no difference in the rate of fibronectin degradation due to nutritional state, implicating synthesis or secretion as the regulatory step in determining total circulating fibronectin levels. Total protein synthesis is retarded by a lack of calories and shock. Mortality due to shock is greater during starvation and in animals fed a l amino acids than we found in normally fed animals or those receiving glucose alone.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO: 3S162772A874 Care of the Combat Casualty

WORK UNIT NO: 095 Metabolic Support Following Combat Injury

The following investigations have been conducted under this work unit:

STUDY NO. 1 Effect of isotonic dextrose and amino acids on body composition and protein synthesis during hemorrhagic shock.

STUDY NO. 2 Effect of metabolic support on reticuloendothelial system (RES) function during shock

STUDY NO. 1. Studies were conducted to investigate the interaction of hemorrhagic shock and nutritional status on survival and plasma protein synthesis. Rats were fed one of four diets for four days. At the end of the feeding period, shock was induced in half the rats from each diet group by removing 35% of their calculated blood volume. Plasma protein synthesis was then estimated by following the incorporation of C^{14} valine into protein. Shocked rats had a lower rate of plasma protein synthesis than controls. There was no discernable effect of dietary pretreatment on control rate of protein synthesis or amino acid oxidation rate.

STUDY NO. 2. Studies were designed to test the effect of metabolic support strategies and shock on fibronectin status. Fibronectin is a circulating plasma protein which correlates closely with both survival and the capability of liver, spleen, and lung to phagocytize foreign circulating material, nonviable erythrocytes, fibrin degradation products, and other waste material. Animals were pretreated for four days with one of four diets, at which time half the animals from each treatment were subjected to hemorrhagic shock. Purified fibronectin, iodinated with I^{125} was injected intravenously, and the clearance from the circulation and uptake by individual organs was measured. In starving animals, shock resulted in a prolonged fibronectin half-life compared with non-shocked animals. There was no difference in the rate of clearance in fed animals, whether in shock or not.

BODY OF REPORT

WORK UNIT NO. 095 — Metabolic Support Following Combat Injury

STUDY NO. 1 — Effect of isotonic dextrose and amino acids on body composition and protein synthesis during hemorrhagic shock

PROBLEM

The effect of hemorrhagic shock on plasma protein synthesis is unknown. Previously it was assumed that the metabolic response to shock was similar to the metabolic response to trauma. Recently it has become evident that many dissimilarities exist. In addition, although the importance of prior nutritional status and intake on the response to injury or surgery seems relatively clear, the interaction between nutrient intake and the response to shock has remained unexplored.

It has been suggested that provision of amino acids in the postinjury period promotes greater protein flux and is responsible for increased rates of synthesis of important plasma proteins. Pretreatment with amino acids has also been shown to cause this effect. Protein synthesis, however, is an energy-consuming process and may, in part, be responsible for the increased post-traumatic metabolic rate. Shock produces a period of decreased oxygen availability and consumption and decreased metabolic rate. Thus, we were interested in the effect that different nutritional measurements might have on plasma protein synthesis following hemorrhagic shock.

RESULTS AND DISCUSSION OF RESULTS

Male Sprague-Dawley rats (350-550 g) were fed one of four diets for four days: 1) glucose plus amino acids, 20 Kcal/100 g body weight/day; 2) amino acids, 4 Kcal/100 g body weight/day; 3) glucose, 4 Kcal/100 g body weight/day, or 4) starvation. On the fourth day, animals to be shocked (n=6/diet) were bled 35% of their calculated blood volume. Control animals (n=6/diet) were cannulated but not bled. A pulse of U-^{14}C-L-valine (10 uCi/rat) was injected via the jugular vein and the appearance of ^{14}C in plasma protein and expired $^{14}CO_2$ was monitored for 10 hours. There was no difference in the rate of $^{14}CO_2$ expiration among any of the treatments. Shocked rats exhibited a significantly lower rate of incorporation of ^{14}C-valine into protein at 1, 3, and 5 hours. These data indicate the effect of uncomplicated hemorrhagic shock on protein synthesis is dissimilar to the effect of trauma, with and without associated hemorrhage. Observations made when both assaults (shock and trauma) occur simultaneously may result from the combined effects of distinctly different mechanisms.

CONCLUSIONS

Although hemorrhagic shock is undeniably a stress characterized by increased catecholamine release and other responses, there appear to be important differences in the animal's response to shock when compared to other forms of stress, such as fear, pain, cold, surgical injury, or trauma. In addition, anesthesia plays a significant role in determining both survival and the metabolic response to shock, particularly in the rat. It is important to identify the effect each of these variables plays in determining the whole animal response to hemorrhagic shock.

RECOMMENDATIONS

Similar experiments should be performed examining the effects of alternate metabolic support strategies on protein synthesis in the post-shock period.

PUBLICATIONS

1. SCOTT, R.L. and J.A. O'CONNOR. Branched chain amino acid metabolism in injured muscle. (abstract) Clin Res 29:60A, 1981

2. BOWERSOX, J.C., J.A. O'CONNOR, and R.L. SCOTT. Effect of diet and hemorrhagic shock on plasma protein synthesis. (abstract) J Parent Ent Nutr (in press)

3. O'CONNOR, J.A., R.L. SCOTT, P.W. MELLICK, and M.D. CALDWELL. Perfused rat hindlimb wound model: lambda-carrageenan induced. Am J Physiol (in press)

STUDY NO. 2 — Effect of metabolic support on reticuloendothelial system (RES) function during shock

PROBLEM

It has been shown that the circulating levels of fibronectin are responsible for modulating activities of the hepatic and splenic clearing mechanisms. RES blockade, which increases susceptibility to sepsis, shock, and trauma, is associated with depletion of this protein. Administering this protein to rats prevented decreased RES function usually found after surgical trauma or hemorrhage. Despite its apparent importance in the response to injury and shock, factors that regulate circulating levels of fibronectin are unknown. Consumptive depletion and simple loss due to hemorrhage are two factors, but possible determinants of the rates of synthesis,

secretion, and degradation require careful and controlled examination to identify.

Strikingly elevated rates of mortality, morbidity, and infection are observed following injury of the chronically or acutely malnourished. In spite of the accumulation of an enormous body of literature describing effects of postinjury metabolic support strategies, there are as yet no acceptable methods for quantitating the efficacy of a specific therapy. The effects of metabolic support regimens on fibronectin, a component of the RES known to correlate with mortality and morbidity, have not been investigated. This protocol was designed and conducted to provide a systematic and careful examination of the effects of shock and resuscitative and metabolic support systems on fibronectin status.

RESULTS AND DISCUSSION OF RESULTS

In these experiments, fibronectin degradation was not influenced by hemorrhagic shock alone. In fact, starvation in conjunction with shock resulted in a prolonged half-life of plasma fibronectin when compared to non-shocked starving animals. We have not been able to complete the measurements of fibronectin synthesis; thus, no statement can be made concerning fibronectin turnover following shock.

We have found that simple starvation results in a highly significant decrease in circulating fibronectin levels in fasting people. The depression is reversed by refeeding, and levels are normal by the fifth postfast day. Studies are in progress to determine if intravenous hyperalimentation following surgery and trauma results in increased circulating fibronectin.

CONCLUSIONS

Starvation has a significant deleterious effect on fibronectin status, both in man and laboratory animals. Although endoxotic, hemorrhagic, and traumatic shock are reported to both reduce circulating levels of fibronectin and depress RES function, there is no difference in the rate of fibronectin degradation after hemorrhagic shock in the anesthetized rat. We are currently examining the interaction of shock and metabolic support on rates of fibronectin synthesis in rats. Current studies are designed to determine the in vivo phagocytic capacity of the animal in shock as influenced by nutritional status.

RECOMMENDATIONS

We will continue to explore the effect of pre- and post-shock metabolic support on fibronectin turnover in rats.

Metabolic Support Following Combat Injury (Cont)

PUBLICATIONS

1. SCOTT, R.L., P.R. SOHMER, M.G. MACDONALD, and R.H. HERMAN. Effect of fasting on fibronectin in humans. (abstract) Clin Res 29(3):663A, 1981

2. SCOTT, R.L., P.R. SOHMER, and M.G. MACDONALD. Effect of starvation on fibronectin and coagulation status in man. (abstract) J Parent Ent Nutr (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 7166	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 06 19	D. CHANGE	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AC	096 JL07
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code): (U) Safety Aspects of Acellular Hemoglobin Solutions as a Resuscitation Fluid

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
008800 Life Support; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 03	CONT	DA	C. IN-HOUSE

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	1.1	85
FISCAL YEAR CURRENT 82	13.6	324

19. RESPONSIBLE DOD ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Bolin, Robert B., LTC, MC
NAME: Boswell, Garry W., CPT, MSC POC:DA

21. GENERAL USE
Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Blood Substitute Solutions; (U) Stroma-Free Hemoglobin; (U) Acute Resuscitation; (U) Organ Function; (U) Human Volunteer

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective of these studies is to evaluate the safety aspects of hemoglobin solutions for their potential use in fluid replacement therapy in forward resuscitation for military combat casualties. These solutions can be maintained for long periods of time and can be stockpiled, thus preventing logistic problems in battlefields. It is necessary to establish that these resuscitation solutions are clinically safe in order to obtain an Investigational New Drug (IND) approval from the Bureau of Biologics, so that clinical studies in humans could be projected for the future.

24. (U) In vitro and in vivo studies are currently being done to investigate the effect of hemoglobin solution on platelet integrity and function, coagulation activity, renal function, and possible saturation and/or blockage of the reticuloendothelial system.

25. (U) 8106-8109 This project was started recently. Hemoglobin solutions are being analyzed for contaminants that could be involved in coagulation and hemostasis, such as phospholipids and fatty acids. Hemostasis tests have been established to quality control present and future blood substitutes. A biologic assay, the Wessler rabbit model, has been been used for determination of possible thrombosis-inducing activity in hemoglobin solutions. With this assay, the hemoglobin solutions tested do not show thrombosis-inducing activity. In vivo experiments have been started in the dog to determine if hemoglobin can activate clotting. The results obtained from the first group of animals are being analyzed.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3S162772A874	Care of the Combat Casualty
WORK UNIT NO.	096	Safety aspects of acellular hemoglobin solution as a resuscitation fluid

The following investigation has been conducted under this work unit:

STUDY NO.	1	Hemostatic aspects of acellular hemoglobin

Investigations on the safety of hemoglobin solutions have been started with the aim of satisfying requirements for an Investigational New Drug (IND) approval so that clinical trials in humans could be projected in the future. Standard operating procedures have been established and quality controls have been implemented to comply with regulations and ensure integrity of data generated in these studies. A simple, fast assessment of possible thrombogenic properties of hemoglobin solutions has been done by using the Wessler rabbit model. The results indicate that several hemoglobin preparations tested do not show thrombogenic activity. Potential procoagulant agents such as lipids and fatty acids are not present in the hemoglobin solutions tested. In vivo experiments in the dog have been started to determine if infusion of hemoglobin solution can activate the clotting mechanism. The results obtained from the first group of animals are being analyzed.

BODY OF REPORT

WORK UNIT NO.	096	Safety aspects of acellular hemoglobin solution as a resuscitation fluid
STUDY NO.	1	Hemostatic aspects of acellular hemoglobin

PROBLEM

Development and evaluation of hemoglobin solution as a resuscitation fluid for combat casualties was initiated in the Division of Blood Research at LAIR in 1975. Considerable progress has been made and many of the initial objectives have been fulfilled. A simple, reproducible method for preparing hemoglobin from outdated human red blood cells has been established. The in vitro characteristics of the hemoglobin solutions, thus prepared, have been studied and reported. Long-term storage conditions, with specific emphasis on non-refrigerated, non-liquid storage, have also been developed and reported in scientific publications. In vivo evaluation of the hemoglobin solution, as prepared in our laboratory has been pursued in various animal models exchange-transfused with hemoglobin solution to different levels of blood replacement. Survival of animals, in vivo oxygen capacity, oncotic pressure, disposition and organ distribution of hemoglobin, oxygen transport and viscosity at different hemodilutions, morphologic effects on liver and kidney cells after massive transfusions with hemoglobin solution and several other physiologic, hematologic, and biochemical aspects have been investigated. The experience acquired during these past years has enabled us and other investigators to study the potential application of hemoglobin solution in a far less ambiguous manner than was previously possible. The studies done in our laboratory have produced a clear picture of the limitations of the current products and an insight into approaches for systematic improvement. Although investigations are being continued on the improvements of the present product, the hemoglobin solution, as presently prepared by the crystallization procedure, could be useful in several applications, provided that its in vivo safety aspects could be demonstrated. Some of the potential applications of the present hemoglobin solutions are: 1) transfusion in patients who cannot receive immediate medical assistance, but could be cared for with blood transfusion after a few hours; 2) transfusion in patients who will receive definitive medical assistance after a prolonged period of time, provided hemoglobin and blood volume losses could be restored by periodic or continuous infusion of hemoglobin solution; 3) open heart surgery; 4) organ perfusion; 5) uncontrolled bleeding; 6) poison clearance (e.g., cyanide removal of methemoglobin).

In most civilian settings in this country, transfusion requirements associated with massive trauma can be met with conventionally stored blood and its components. However, military requirements frequently demand massive fluid support in areas remote from the supply source, presenting uniquely difficult storage and transportation problems. The inability to accurately predict when modest transfusion requirements suddenly become great further complicates fluid therapy logistics. The ability to stockpile a stable protein capable of carrying oxygen avoids many of these difficulties.

A goal of the Division of Blood Research at LAIR is to obtain an Investigational New Drug (IND) approval for the hemoglobin solution so that clinical studies in humans could be projected for the future. Determination of safety features have been stressed in order to obtain the IND approval. The objectives of these studies are to investigate safety aspects of acellular hemoglobin solutions presently available and also of those modified hemoglobin solutions which show promise as resuscitation fluids.

RESULTS AND DISCUSSION OF RESULTS

In order to comply with federal regulations we have established standard operating procedures for every assay, method, and determination used in these investigations. Also we have implemented quality controls, set up specifications, and implemented a system for maintaining the quality and integrity of data generated in these studies.

A study has been performed to establish a simple, fast assessment of thrombogenic properties of hemoglobin solutions. For this purpose, a Wessler rabbit model has been used to determine if a substance is thrombogenic or can amplify thrombosis using xenotypic biologic material. Rabbits were anesthetized and segments of the two jugular veins were exposed. Control solutions (plasma or isotonic saline for negative control, serum for positive control) or hemoglobin solutions were injected into a marginal ear vein. At 10 seconds after injection the segment from each of the two exposed jugular veins were ligated. After 10 minutes the segments from the veins were isolated and opened to see if a clot had formed. Results have shown that a clot forms when serum (positive control) is injected in the ear vein of the rabbits; no clot is observed when plasma or isotonic saline solutions (negative control) are used. When hemoglobin solutions from different preparations were tested, a clot did not form, indicating that these solutions do not possess thrombogenic properties.

Safety Aspects of Acellular Hemoglobin Solutions as a Resuscitation Fluid (continued)

Extensive studies were done to define possible lipid or phospholipid contaminiation of hemoglobin solutions. Three assay systems were developed for this purpose. A four-pass thin layer chromatography system was established which detects all major lipid classes after the first solvent pass, and which classifies all the phospholipids after the fourth pass. Minimum detectability in this system was 0.05-0.20 µg of individual phospholipid. A second, two-dimensional, thin layer procedure was also developed to separate phospholipids into classes to verify the results of the first procedure, and to allow for extraction of the separated phospholipids for subsequent fluorometric quantitation. Analysis of many lots of hemoglobin solution, prepared by crystallization, revealed some phospholipid contamination in several early lots. However, reinforced strict adherence to the preparative standard operating procedure eliminated evidence of phospholipids from all subsequent lots. A trace amount of triglyceride remains in these preparations. Analysis of hemoglobin solutions prepared by Dr. Condie, University of Minnesota, also confirmed the absence of phospholipids but demonstrated the presence of triglycerides.

Investigation into possible contamination of hemoglobin solutions by free fatty acids was undertaken. A gas chromatographic procedure was developed utilizing flame ionization detection and a 12-ft glass column packed with SP2330. Following selective extraction of free fatty acids from hemoglobin solution with a chloroform:heptane: methanol mixture the fatty acids were derivatized with BF_3/methanol, then chromatographed as the methyl esters. The sensitivity of this method for individual fatty acids was approximately 3 µg/ml hemoglobin solution or less, depending on which compound was measured. Identification of the individual fatty acid was made by comparing the unknown peak retention time to that of a known standard. The fatty acids of concern in this study were saturated and unsaturated compounds containing 14 to 24 carbons. Using this procedure, samples of hemoglobin solutions prepared by crystallization and by the Minnesota process were analyzed, and no measurable fatty acids were found in either group of samples.

The hemostatic effects of hemoglobin solution in vivo was evaluated in mongrel dogs. Phospholipid and fatty acid-free hemoglobin solution was bolus infused intravenously at approximately 60 ml/min to a total dose of 15 ml/kg. Blood was drawn before infusion, at 5, 15, 60, and 240 min after infusion. Ten dogs were evaluated with hemoglobin and eight dogs were evaluated with albumin (controls). Hematologic parameters tested were CBC, platelet count, thrombin time (PT), activated partial thromboplastin time (aPTT), prothrombin time (PTT), Kaoline coagulation time (KCT), fibrinogen degradation products (FDP), fibrogen, protamine sulfate precipitation, platelet

aggregation and plasma complement (C_3). No differences were seen in the treatment and control animals for platelet aggregation, plasma complement, PT, CBC, and fibrinogen. The aPTT, PTT, and KCT were slightly prolonged in the hemoglobin dogs compared to controls (142%). Platelet counts were depressed at 5 min (40% of starting values) compared to controls (80% of starting values) suggesting a transient thrombocytopenia.

CONCLUSIONS

Investigation on the safety aspects of hemoglobin solutions have been started recently with the aim of satisfying the requirements for an Investigational New Drug approval so that clinical trials in humans could be projected in the future. Standard operating procedures have been established and quality controls have been implemented in order to comply with regulations and ensure integrity of data generated in these studies. A simple, fast assessment of possible thrombogenic properties of hemoglobin solutions has been done by using the Wessler rabbit model. The results indicated that several hemoglobin preparations tested do not show thrombogenic activity. Analyses of lipid and fatty acid contents have demonstrated that these possible coagulant agents are not present in the hemoglobin solutions tested. In vivo experiments in the dog to determine if infusion of hemoglobin solution can activate clotting mechanisms have been analyzed. The transient effect of hemoglobin on platelet equestration may affect hemostasis in casualty situations.

RECOMMENDATIONS

Significant advances can be gained by the use of resuscitation solution capable of transporting oxygen and being readily available when massive transfusions are required. Stringent requirements must be met by a resuscitation solution in order to be effective. As a blood substitute, this solution not only must be capable of restoring vital functions, but also must not elicit adverse effects when administered to mass casualty victims. It is, therefore, recommended that the safety aspects of hemoglobin solutions be investigated in full, not only in vitro but also in vivo, simulating conditions which occur in battlefield situations. Furthermore, by establishing methodologies and procedures for these investigations it is recommended that they be used not only for hemoglobin solutions presently available but also for any resuscitation solution, including modified hemoglobin solution, which may be developed in the future and which may show a promising potential as a blood substitute. Further evaluation of hemoglobin's effect on hemostasis is needed. Organ sequestration, phenomena in animals other than dog, large dose infusions are now being investigated.

Safety Aspects of Acellular Hemoglobin Solutions as a Resuscitation Fluid (continued)

PUBLICATIONS

1. Bolin, R.B. Coagulation aspects of acellular oxygen-delivering resuscitation fluids. Proc Curr Concepts Combat Casualties (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 6784	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 03 13	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AA	097 APC HL23
b. CONTRIBUTING				
c. ~~CONFIGURING~~	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)

(U) Mechanisms of Wound Healing Enhancement

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012900 Physiology; 003500 Clinical Medicine; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	0.3	09
FISCAL YEAR CURRENT 82	0.5	15

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ASSOCIATE INVESTIGATORS

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NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Laboratory Animal; (U) Wound Healing; (U) Military Trauma; (U) Animal Model; (U) Amnion

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Amnion is widely used as a biologic dressing for thermal injuries. Experimental data suggest the presence of biochemical compounds that suppress bacterial growth and increase vascularization at the wound site. Its use in full-thickness skin wounds, which might result from combat injuries such as mines, shrapnel, or bullets, has not been explored. It is necessary to determine if amnion can significantly increase the rate of healing of wounds of this nature, thereby saving thousands of man-days during the convalescence of soldiers who have received combat injuries.

24. (U) Full-thickness skin defects of a standard size will be created in rabbits. One group will be treated with conventional dressings, while the second group will utilize the amnion dressing. Wound surface areas will be measured every third day during dressing changes, and statistical comparisons will be made between the control and treated groups. Wound sites will be sampled for bacterial growth during dressing changes. Immunologic effects will also be observed. A second study, conducted as above, will determine the effects of amnion on chronic infected wounds.

25. (U) 81 05 - 81 09 Work on this study was recently begun. Preliminary data suggest amnion may significantly increase the rate of healing of full-thickness skin defects. These preliminary data do not support reports that amnion suppresses bacterial populations.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO: 3S162772A874 Care of the Combat Casualty

WORK UNIT NO: 097 Mechanisms of Wound Healing Enhancement

The following investigations have been conducted under this work unit:

STUDY NO. 1 Evaluation of amniotic wound dressings

STUDY NO. 1. Amnion is widely used as a biological dressing for thermal injuries. Its use in full-thickness skin wounds, which might result from combat injuries such as mines, shrapnel, or bullets, has not been explored. Using a wound-healing rabbit model developed at LAIR, we recently started research on its reported ability to accelerate healing and suppress bacterial populations. Initial results indicate that amnion may significantly increase the rate of healing of full-thickness skin defects. These preliminary data do not support results that amnion suppresses bacterial populations.

BODY OF REPORT

WORK UNIT NO. 097 — Mechanisms of Wound Healing Enhancement

STUDY NO. 1 — Evaluation of amniotic wound dressings

PROBLEM

Amnion is widely used as a biological dressing for thermal injuries. Experimental data suggest the presence of biochemical compounds that suppress bacterial growth and increase vascularization at the wound site. Its use in full-thickness skin wounds, which might result from combat injuries such as mines, shrapnel, or bullets, has not been explored. It is necessary to determine if amnion can significantly accelerate healing of wounds of this nature, thereby saving thousands of man-days during the convalescence of soldiers. Using a rabbit wound healing model developed at LAIR, we will create full-thickness skin defects and compare the rate of healing between amnion-treated wounds and those covered with a conventional dressing. Wound sites will be sampled for bacterial growth. Work on this project has just been initiated.

RESULTS AND DISCUSSION OF RESULTS

Initial data suggest amnion may significantly increase the rate of healing of full-thickness skin defects. These preliminary data do not support the reports that amnion suppresses bacterial populations.

CONCLUSIONS

At this early stage of investigation no conclusions can be formulated.

RECOMMENDATIONS

Continuation of this project, with the addition of another group of animals, to compare the healing of a wound covered with an occlusive dressing to amnion and the control is recommended.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 8398	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. NEW	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A875	CE	307 APC TL10
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:1		

11. TITLE (Precede with Security Classification Code)

(U) Toxicologic Assessment of Decontamination Materials

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016800 Toxicology; 003200 CBR Warfare; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 02	81 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	0.4	14
FISCAL YEAR CURRENT 82	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: Hanes, M.A., VC

NAME: Jederberg, W., CPT, MC POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Toxicology; (U) Decontaminants; (U) Decontamination; (U) Skin; (U) Dermal; (U) Chemical warfare

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) If chemical warfare agents are used on the modern battlefield, effective decontaminants and decontamination systems are needed. Decontamination should be harmless, or the extent of danger associated with decontamination should be known. The M258 Decontamination Kit will be evaluated from a safety standpoint using various modifications of the standard dermal irritation test.

24. (U) Studies will be conducted to identify the level of irritation caused by the kit when used as directed, with modified use, and with various modified decontamination materials.

25. (U) New work unit.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3M162734A875	Medical Systems in Chemical Defense
WORK UNIT NO.	307	Toxicologic Assessment of Decontamination Materials

The Following investigation has been conducted under this work unit:

GLP STUDY NOS.	81011, 81018, 81019, 81020, 81021, 81023, 81024, and 81026	Evaluation of the M258A-1 Decontamination Kit for dermal irritation and injury

The modified Draize method was used for the evaluation of the primary dermal irritation potential of the M258A-1 Decontamination Kit. The effect of occlusion, condition of the skin, age of components, and immediate rinsing were explored.

The components present a dermal irritation hazard to the user which is minimized by immediate rinsing.

BODY OF REPORT

WORK UNIT NO.	307	Toxicologic Assessment of Decontamination Materials
STUDY NOS.	81011, 81018, 81019, 81020, 81021, 81023, 81024, and 81206	Evaluation of the M258A-1 Decontamination Kit for dermal irritation and injury

PROBLEM

Insufficient data are available to assess the health hazard of the components of the M258A-1 Decontamination Kit when applied directly to the skin of man.

RESULTS AND DISCUSSION OF RESULTS

Eight studies were conducted in compliance with the Food and Drug Administration Good Laboratory Practices Regulations. These studies consisted of evaluations of the primary dermal irritancy of the components of the M258A-1 Decontamination Kit by modifications of the method of Draize. Evaluation was made of the effect of fresh versus old components, occlusion versus non-occlusion of exposed sites, abraded versus unabraded skin, and the impact of immediate rinsing. The components of the kit were categorized as mild to severe primary dermal irritants under various conditions. The irritancy was reduced by the immediate rinsing of the application site with saline.

CONCLUSIONS

Provisions should be made for the user to rinse away the components of the decontaminaion kit immediately after they are used, as directed. Further evaluations must be made to determine the impact of these substances on the functional integrity of the skin.

PUBLICATIONS

1. FRUIN, J.T. and M.A. HANES. Primary dermal irritation potential of components of the M258A-1 Decontamination Kit (Study 1). Toxicology Series 6. Institute Report No. 101. San Francisco, California: Letterman Army Institute of Research, September 1981

2. FRUIN, J.T. and M.A. HANES. Primary dermal irritation potential of components of the M258A-1 Decontamination Kit (Study 2). Toxicology Series 8. Technical Note No. 81-21TN. San Francisco, California: Letterman Army Institute of Research, September 1981

3. FRUIN, J.T. and M.A. HANES. Primary dermal irritation potential of components of the M258A-1 Decontamination Kit (Study 3). Toxicology Series 9. Technical Note No. 81-22TN. San Francisco, California: Letterman Army Institute of Research, September 1981

4. FRUIN, J.T. and M.A. HANES. Primary dermal irritation potential of components of the M258A-1 Decontamination Kit (Study 4). Toxicology Series 11. Technical Note. No. 81-18TN. San Francisco, California: Letterman Army Institute of Research, September 1981

5. FRUIN, J.T. and M.A. HANES. Primary dermal irritation potential of components of the M258A-1 Decontamination Kit (Study 5). Toxicology Series 13. Technical Note No. 81-24TN. San Francisco, California: Letterman Army Institute of Research, September 1981

6. FRUIN, J.T. Primary dermal irritation resulting from the abrasive action when using M258A-1 Decontamination Kit (Study 6). Toxicology Series 14. Technical Note No. 81-25TN. San Francisco, California: Letterman Army Institute of Research, September 1981

7. FRUIN, J.T. Primary dermal irritation resulting from the abrasive action when using the M258A-1 Decontamination Kit (Study 7). Toxicology Series 15. Technical Note No. 81-26TN. San Francisco, California: Letterman Army Institute of Research, September 1981

8. JEDERBERG, W.W. and J.T. FRUIN. Primary dermal irritation potential of components of the M258A-1 Decontamination Kit (Study 8). Toxicology Series 21. Institute Technical Note No. 27TN. San Francisco, California: Letterman Army Institute of Research, November 1981

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 2348	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
80 08 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A875	BC	301 APC FLOA
b. CONTRIBUTING	62772A	3S162772A875	CD	301
c. CONTRIBUTING	STOG	80-7.2:1		

11. TITLE (Procede with Security Classification Code)*

(U) Skin Decontamination Technology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003200 CBR Warfare; 004900 Defense; 017100 Weapons Effects

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	2.2	198
FISCAL YEAR CURRENT 82	4.3	142

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Black, Kenneth E., LTC, MC, POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Models; (U) Chemical Defense; (U) Decontamination; (U) Skin; (U) Nerve Agents; (U) Vesicants; (U) Laboratory Animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) On the modern battlefield, both conventional and chemical (CW) casualties may have sublethal amounts of agent on their skin. Decontaminants and decontamination systems are needed to protect patients from further insult and to protect medical personnel from secondary exposure while treating them. Methodologies to measure sublethal levels of agents and standardized model systems that can be used instead of humans are needed for assessing degrees of contamination and efficacy of decontamination.

24. (U) Models will be developed and standardized to provide human-relevant data for skin decontamination studies. Quantitative and qualitative methods will be developed for determining which patients require decontamination and for assessing the efficacy of decontamination. Risks associated with CW agent exposure and decontamination will be assessed to aid in triage and treatment.

25. (U) 80 10 - 81 09. A collaborative study was conducted between LAIR and the US Army Medical Research Institute for Chemical Defense at Aberdeen Proving Ground, MD., to compare diethylmalonate to soman in shower decontamination trials of skin. The LAIR in vitro evaporation-penetration apparatus has undergone significant upgrading to allow for general use. Using this model the effects of skin storage time and temperature, air temperature, and permeation fluid on skin penetration have been determined. Studies have been initiated for human validation of several in vivo penetration models To include the hairless dog, weanling pig, and grafted athymic nude mouse.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3M162734A875	Medical Systems in Chemical Defense
WORK UNIT	301	Skin Decontamination Technology

The following investigations have been conducted under this work unit

STUDY NO. 2	Shower decontamination efficacy - in vitro determination
STUDY NO. 3	Skin permeability values in model systems and in man
PILOT STUDY	Establishment of a model

STUDY NO. 2 The use of simulants for hazardous chemicals such as nerve agents, permits studies to be done without the necessity of elaborate surety requirements which can cause considerable delay. Diethyl malonate and thickened diethyl malonate have been used as nontoxic simulants for the nerve agents soman and thickened soman in an in vitro study to determine the efficacy of shower removal of these chemicals from the skin surface. To validate the simulant, a study was conducted at Aberdeen Proving Grounds in collaboration with the Institute of Chemical Defense. Although differences were found in the disposition of soman and diethyl malonate applied to in vitro skin targets, decontamination efficacy of the two chemicals was not significantly different.

STUDY NO. 3 One of the goals of work unit is the development of a matrix of interrelated in vitro and in vivo models of skin penetration for use in developing substances to either protect or decontaminate the skin. This study compares the permeability of the skin of various animals to that of man, and compares in vitro and in vivo permeability values, as it is unlikely that a single species will be adequate for all needs. Therefore, several compounds with reported values for percutaneous penetration in man have been tested on the pig, hairless dog, grafted athymic nude mouse and in an in vitro system. Common laboratory animals such as conventional mice, rats, conventional dogs, guinea pigs and rabbits have very permeable skin, relative to man, and are not suitable as animal models.

PILOT STUDY. Higher priority research requirements prevented significant progress on the pilot study to establish a mouse model with a chronic subclinical Toxoplasma gondii infection. The model was to be used in a proposed study to assess recrudescence of disease resulting from percutaneous exposure to vesicants. This research has been discontinued to allow concentration of resources on more immediate requirements in skin decontamination models and methods and skin protection substances.

BODY OF REPORT

WORK UNIT NO. 301 Skin Decontamination Technology

STUDY NO. 2 Shower decontamination efficacy - in vitro determination

PROBLEM

The Armed Forces need to decontaminate non-ambulatory chemically contaminated casualties before they receive medical treatment for wounds. One purpose of decontamination is to protect medical personnel against exposure to detrimental levels of chemical agents. It is not yet possible for medical personnel in CBR protective clothing to provide necessary medical treatment to patients. Consequently, medical personnel must operate in a shirt sleeve environment.

Designers do not have sufficient information on non-ambulatory casualty decontamination to construct a prototype device for deployment and installation in fixed USAF facilities.

To obtain the necessary information, a decontamination bench model has been used to assess quantitatively the importance of several variables (water pressure and temperature, Triton X-100 concentration, nozzle type and shower time) on decontamination of the nontoxic agent simulants diethyl malonate and thickened diethyl malonate from pig skin in vitro. Diethyl malonate was chosen on the basis of similar physical properties to soman. Results of the initial decontamination studies indicated mean percutaneous penetration of simulant increased with showering. However, standard deviations were quite large and the ability of diethyl malonate to simulate the percutaneous penetration of soman was unknown. Therefore a second study was conducted to compare the percutaneous penetration of radiolabeled diethyl malonate to radiolabeled soman in shower decontamination trials of pig skin in vitro.

RESULTS AND DISCUSSION OF RESULTS

Soman and diethyl malonate were applied to pig skin in vitro at a chemical dose of 0.1mg/cm^2. Percutaneous penetration was measured by the appearance of radioactivity in Ringer's lactate solution on the visceral side of the skin. During a fifteen minute period immediately after application, the amounts of evaporation of diethyl malonate and soman, as measured by the amount of radioactivity recovered from vapor traps, were not significantly different. At the end of 15 minutes, levels of radioactivity on the skin surface and within the skin were higher after application of radiolabeled diethyl malonate than after application of radiolabeled soman. However, showering removed a

correspondingly larger amount of diethyl malonate than soman so that residues left on the skin surface (2.1% vs 1.1% of the applied radioactive dose, respectively) were similar. Furthermore decontamination efficiencies, calculated as the percentage of the skin residue that was removed by showering, were not significantly different between soman (69±14%) and diethyl malonate (75±7%).

During the 15 minute period immediately following application the percutaneous penetration of diethyl malonate was significantly greater than that of soman. However, both were less than 0.1% of the applied dose. Analysis of variance revealed no significant influence of showering or thickener on the percutaneous penetration of diethyl malonate or soman. Using an enzymatic analysis, the majority of soman removed by showering or scrubbing the skin was found to be inactivated. Any soman that had penetrated through the skin was below the detection limit of the enzymatic analysis.

CONCLUSIONS

During the fifteen minute period immediately following application, the percutaneous penetration of diethyl malonate was significantly greater than that of soman. However, both were less than 0.1% of the applied dose. Analysis of variance revealed no significant influence of showering or thickener on the percutaneous penetration of diethyl malonate or soman. Using an enzymatic analysis, the majority of soman removed by showering or scrubbing the skin was found to be inactivated. Any soman that had penetrated through the skin was below the detection limit of the enzymatic analysis. Decontamination efficiencies, calculated as the percentage of the skin residue that was removed by showering, were not significantly different between soman and diethyl malonate.

RECOMMENDATIONS

The decontamination efficiency for other chemical agents by showering should be determined.

PUBLICATIONS

None

STUDY NO. 3 Skin permeability values in model systems and in man

PROBLEM

Various in vitro and animal models for determining percutaneous penetration have been developed at LAIR and elsewhere. Many of these models were developed for specific projects. As a result, some models with limited capacity have been developed for predicting human skin permeability. No general validated model exists for predicting the skin permeability or metabolic fate of harmful chemicals that people may encounter. Systematic development of models for the study of percutaneous absorption is needed. Research on the barrier function of skin and mechanisms of percutaneous penetration has been meager. The effects of a number of factors (skin hydration, ambient temperature, solvent effects, structure and physical properties of the penetrant, etc) on skin penetration need further study. This technology is a critical requirement in developing products to protect or decontaminate the skin.

RESULTS AND DISCUSSION OF RESULTS

Various in vitro and animal models for determining percutaneous penetration have been developed in recent years at LAIR. The in vitro models employ excised skin and offer greater control of experimental variables than can be achieved in vivo. The animal models allow investigation of the dynamic processes that depend on a living dermis. Thus, for experiments where enzymes are to be studied or where a dynamic microcirculation is of interest, a living model is required.

This study compares the permeability of the skin of various animals to that of man, and compares in vitro and in vivo skin permeability values, as it is unlikely that a single species will be adequate for all needs. Therefore several compounds with reported values for percutaneous penetration in man are being tested on the weanling pig, hairless dog, grafted (human skin) athymic nude mouse, and in vitro. The usual laboratory animals (mice, rats, rabbits, guinea pigs) are characterized by a dense hair coat and thin epidermis and their skin is much more permeable than that of man. The preliminary results for three compounds tested in the various in vivo models is given in Table 1. Prior to the availability of human skin for grafting to the nude mouse, pigskin was used to develop procedures. The percutaneous penetration of the three compounds on the pigskin-grafted nude mice and on ungrafted skin of the nude mouse are included in the results given in Table 1. Except for the ungrafted skin of the nude mouse, all in vivo systems show promise of ranking the permeablity of compounds in the same order as in man. Similar results were obtained with the pig and human skin grafts on the nude mouse (Table 1). Additional compounds must be tested to complete the validation of promising in vivo models.

Table 1. Percutaneous penetration following topical application of radiolabeled malation, N,N-diethyl-m-toluamide and benzoic acid in various model systems and in man

	Mean Percent Penetration[1]					
	athymic nude mouse					
	ungrafted	pig graft	human graft	pig	dog	man
malathion	79	27	27	-[2]	-[2]	8.2[3]
m-deet	38	31	31	9.5	13	16[3]
benzoic acid	67	44	44	27	27	43[3]

[1]Mean percent penetration. Values are corrected for incompleteness of urinary excretion

[2]Data not yet available

[3]Data from Feldmann and Maibach

Several improvements in the LAIR in vitro evaporation-penetration model have been made to allow more general use. These include incorporation of commercially available Franz chambers which were modified to allow a flow-through penetration chamber, automation of penetration sample collection, replacement of piston pumps with more reliable cassette-type pumps, and installation of additional condensers for better control of ambient temperature. Using this upgraded model, the effects of skin storage time and temperature on skin premeability have been determined. Several permeation fluids (Ringer's lactate, Tyrodes solution and calf serum) have been tested for their effect on percutaneous penetration. Finally, the effect of variation of air temperature on percutaneous penetration has been determined. Studies have begun to validate this model using the same chemicals that are being tested in the in vivo models.

CONCLUSIONS

Based on the preliminary results, the pig, dog and human- and pigskin-grafted athymic nude mouse show promise of ranking the permeability of compounds in the same order as in man. Similar results were obtained with the pig and human skin grafts on the nude mouse. The LAIR in vitro evaporation-penetration model has undergone significant upgrading and is now ready for general use.

RECOMMENDATIONS

Additional compounds must be tested for their permeation in the promising in vivo animal models and the in vitro evaporation-penetration model to complete the validation process.

PUBLICATIONS

None

PILOT STUDY Establishment of a model

PROBLEM

Mustard agents are known to depress the immune system but little work has been done to determine if percutaneous exposure to sublethal doses of these agents will seriously impair immune functions. This pilot study was initiated to establish an animal model with a chronic subclinical Toxoplasma gondii infection. It would be used to test the hypothesis that exposure to sublethal doses of mustard agents would be sufficient to disrupt premunition immunity, causing recrudescence of unexpected clinical infections among patients convalescing from mustard injuries.

RESULTS AND DISCUSSION OF RESULTS

None. This investigation was discontinued before significant progress had been made.

CONCLUSIONS

None

RECOMMENDATIONS

This line of investigation is lower in priority than the acute agent injury and skin protection studies that require immediate attention. It does, however, address questions that have not been adequately answered. These problems should be reconsidered for investigation under contract or in-house when resources are available. This type of study would also be appropriate for assessing risk of recrudescent disease in conventional combat casualties.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DAOG 2881	81 10 01	DD-DR&E(AR)635

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A875	BD	302 APC TL07
b. CONTRIBUTING	62772A	3S162772A875	CE	302
c. CONTRIBUTING	STOG	80-7.2:1		

11. TITLE (Procede with Security Classification Code)*

(U) Good Laboratory Practice Training

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

016800 Toxicology; 012900 Physiology; 013300 Protective Equipment

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING 81	1.0	68
CURRENT 82	19.7	164

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: (415) 561-2963

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Powers, N.K., CPT, VC

NAME: Hanes, M.A., CPT, VC POC:DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Chemical Defense, LAIR; (U) Good Laboratory Practice - GLP; (U) Automated Data Collection; (U) Toxicology

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) New drugs to be used in defense of chemical warfare agents, new repellents for use against disease-carrying insects and arthropods, and compounds produced in the production and detonation of high explosives must be assessed for their toxic potential. Department of the Army must have in-house capabilities for conducting and monitoring contracts for toxicological testing of these products. This project is designed to provide experience, practice, special skills, and expertise required by toxicology research team members and support personnel to perform and monitor mammaliam toxicology studies in compliance with Food and Drug Administration and Environmental Protection Agency Good Laboratory Practice Regulations. Tests will include acute, subacute, and chronic oral, dermal, and irritation studies, and primary eye and dermal irritation, mutagenicity, and teratogenicity studies.

24. (U) The species and strain of choice will be determined for the selected test system. Compounds of both known and unknown effect will be tested. Clinical signs will be recorded and the appropriate specimens will be taken for chemical analysis. The use of automated data collection equipment and software (the TOXSYS) will be used whenever possible.

25. (U) 8010-8109. The capability to conduct acute oral toxicity in mice, acute dermal toxicity in rabbits, acute dermal and ocular irritation in rabbits, and dermal sensitization in guinea pigs in compliance with Federal GLP regulations was developed. Progress was made in generating reports with TOXSYS. Updated software was provided by the manufacturer and action was taken to permit direct interface of TOXSYS to mainframe computer. Data collection forms and procedures were developed. Training and procedure development for conducting teratology studies are just getting underway.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3M162734A875 Medical Systems in Chemical Defense

WORK UNIT NO. 302 Good Laboratory Practice Training

The following investigations have been conducted under this work unit:

STUDY NO. 1 Toxicology Group Training Protocol

EX-2 Acute dermal toxicity study and data collection

EX-3 Primary eye irritation study and data collection

EX-4 Primary dermal irritation study and data collection

EX-5 Dermal sensitization study and data collection.

STUDY 1. The capability to conduct acute dermal toxicity, primary eye and dermal irritation, and dermal sensitization studies in compliance with Federal Good Laboratory Practice (GLP) Regulations was developed under this study. In addition, the necessary Standard Operating Procedures (SOP) were prepared. Data collection forms and procedures were developed and a substantial number of them have been provided to other MRDC laboratories.

BODY OF REPORT

WORK UNIT NO. 302 Good Laboratory Practice Training

STUDY NO. 1 Toxicology group training protocol

PROBLEM

The U.S. Army Medical Research and Development Command has recognized a requirement for in-house capability to conduct toxicologic studies on an immediately responsive basis and on substances for which there is little or no interest from commercial or government testing facilities. The LAIR Toxicology Group was established to perform this mission. A toxicology testing program, conducted in compliance with proposed or existing Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) Regulations, requires considerable training, procedural development, quality control, and quality assurance. This work unit was established to train technicians and investigators in standard test procedures, to develop data collection and control procedures and necessary standard operating procedures (SOP), and to become familiar with the operation and use of the TOXSYS® data collection and reporting system. The required test capabilities include acute, subacute, and chronic oral and dermal studies, primary eye and dermal irritation studies, and mutagenicity and teratogenicity studies.

RESULTS AND DISCUSSION OF RESULTS

Training technicians and investigators to conduct acute oral and acute dermal toxicity, acute dermal and ocular irritation, and dermal sensitization studies has been completed. Data collection forms and data management procedures were developed. In conjunction with these studies, 170 SOPs were developed to comply with proposed and existing FDA and EPA regulations. Progress was made in collecting data with the TOXSYS® system and in generating reports. Although TOXSYS® performance has improved greatly, they are still collected manually to preclude data loss due to TOXSYS® malfunction.

CONCLUSIONS

The capability to conduct selected short- and intermediate-term toxicity testing has been developed in compliance with FDA and EPA Regulations.

RECOMMENDATIONS

Efforts to develop additional toxicology testing capabilities should be continued.

PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 6205	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D.CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A875	BC	303 APC FL10
b. CONTRIBUTING	62772A	3S162772A875	CE	303
c. CONTRIBUTING	STOG	80-7.2:1		

11. TITLE (Precede with Security Classification Code)*

(U) Toxicologic Assessment of Decontamination Materials

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

016800 Toxicology; 003200 CBR Warfare; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 81	0.1	18
CURRENT FISCAL YEAR 82	2.7	90

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE

Foreign Intelligence Not Applicable

ASSOCIATE INVESTIGATORS

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22. KEYWORDS (Precede EACH with Security Classification Code) (U) Toxicology; (U) Decontaminants; (U) Decontamination; (U) Skin; (U) Dermal; (U) Laboratory Animals; (U) Chemical Warfare

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) If chemical warfare agents are used, exposure and contamination of soldiers may be complicated by damage to their clothing or their bodies by conventional weapons fire. Also, especially with persistent agents, the risk of exposure may not be restricted to times or zones of active combat. Effective decontaminants and decontamination systems are needed. Decontamination should be harmless, or the extent of danger associated with decontamination should be known, even when open wounds are present. Methods will be developed to assess the relative safety of existing and proposed decontaminant materials.

24. (U) Studies will be conducted to identify changes in skin permeability, to determine toxicity accompanying wound contamination and to detect longer term (1-4 wk) systemic changes that might affect treatment, recovery, or ability to withstand subsequent chemical attack or decontamination. As they are identified, the safety and use parameters of chemicals for protecting skin from contact with chemical agents will also be evaluated.

25. (U) 80 10-81 09. Eight collaborative GLP studies were completed with the Toxicology Group under this work unit. Evaluations were made on the primary dermal irritation potential of the component solutions utilized in the M258A-1 Decontamination Kit. As assessed by the Draize test, the impact of occlusion, condition of the skin (abraded or nonabraded) age of the components, and immediate rinsing were evaluated. The components present a hazard of primary dermal irritation to the user. This hazard can be minimized by rinsing the site of application with saline. Further studies are needed to assess the systemic and dermal toxic effects of the components of the kit. Future GLP studies will be reported under a separate Work Unit.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3M162734A875 Medical Systems in Chemical Defense

WORK UNIT NO. 303 Toxicologic Assessment of Decontamination Materials

The following investigation has been conducted under this work unit:

STUDY NO. 1 Evaluation of the solutions contained in the M258A-1 Decontamination Kit for dermal and overt systemic toxicity

Eight collaborative GLP studies were conducted with the Toxicology Group under this work unit. The primary dermal irritation potential of the M258A-1 Decontamination Kit was evaluated by the Draize method. The impacts of occlusion, condition of the skin, age of the components, and immediate rinsing were explored.

The components present a dermal irritation hazard to the user, but is minimized by immediate rinsing. The data must be viewed with consideration for the primary purpose of the kit. Further evaluations must be made to assess whether or not the functional integrity of the skin is compromised and evaluate any systemic toxicity.

BODY OF REPORT

WORK UNIT NO. 303 — Toxicologic Assessment of Decontamination Material

STUDY NO. 1 — Evaluation of the solutions contained in the M258A-1 Decontamination Kit for dermal and and overt systemic toxicity

PROBLEM

The components of the M258A-1 Decontamination Kit present an unassessed hazard to the user.

RESULTS AND DISCUSSION OF RESULTS

Eight studies were conducted in compliance with the requirements of the Good Laboratory Practices Act. These studies consisted of evaluations of the primary dermal irritancy of the components of the M258A-1 Decontamination Kit by modifications of the method of Draize. Evaluation was made of the impact of fresh versus old components, occlusion versus nonocclusion of exposed sites, abraded versus unabraded skin, and the impact of immediate rinsing. The components of the kit were categorized as mild to severe primary dermal irritants under various conditions.

The irritancy was reduced by immediately rinsing the application site with saline.

CONCLUSIONS

The components of the M258A-1 Kit present a hazard to the user which may be minimized by rinsing the site of application with saline.

RECOMMENDATIONS

Provision should be made for the user to rinse away the components of the decontamination kit immediately after they are used as directed. Further evaluations must be made to determine the impact of these substances on the functional integrity of the skin.

PUBLICATIONS

FRUIN, J.T., and M.A. HANES. Primary Dermal Irritation Potential of Components of the M258A-1 Decontamination Kit (Study 1). (Toxicology Series 6). Institute Report No. 101. Presidio of San Francisco, California: Letterman Army Institute of Research, September 1981

FRUIN, J.T., and M.A. HANES. Primary Dermal Irritation Potential of Components of the M258A-1 Decontamination Kit (Study 2). (Toxicology Series 8). Technical Note No. 81-21TN. Presidio of San Francisco, California: Letterman Army Institute of Research, September 1981

FRUIN, J.T., and M.A. HANES. Primary Dermal Irritation Potential of Components of the M258A-1 Decontamination Kit (Study 3). (Toxicology Series 9). Technical Note No. 81-22TN. Presidio of San Francisco, California: Letterman Army Institute of Research, September 1981

FRUIN, J.T., and M.A. HANES. Primary Dermal Irritation Potential of Components of the M258A-1 Decontamination Kit (Study 4). (Toxicology Series 11). Technical Note No. 81-18TN. Presidio of San Francisco, California: Letterman Army Institute of Research, September 1981

FRUIN, J.T., and M.A. HANES. Primary Dermal Irritation Potential of Components of the M258A-1 Decontamination Kit (Study 5). (Toxicology Series 13). Technical Note No. 81-24TN. Presidio of San Francisco, California: Letterman Army Institute of Research, September 1981

FRUIN, J.T. Primary Dermal Irritation Resulting From the Abrasive Action When Using the M258A-1 Decontamination Kit (Study 6). (Toxicology Series 14). Technical Note No. 81-25TN. Presidio of San Francisco, California: Letterman Army Institute of Research, September 1981

FRUIN, J.T. Primary Dermal Irritation Potential of Components of the M258A-1 Decontamination Kit (Study 7). (Toxicology Series 15). Technical Note No. 81-26TN. Presidio of San Francisco, California: Letterman Army Institute of Research, September 1981

JEDERBERG, W.W., and J.T. FRUIN. Primary Dermal Irritation Potential of Components of the M258A-1 Decontamination Kit (Study 8). (Toxicology Series 21). Technical Note No 82-27TN. Presidio of San Francisco, California: Letterman Army Institute of Research (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOG 6206	81 10 01	DD-DR&E(AR)36

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D.CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A875	BD	304 APC TL04
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7-2:1		

11. TITLE (Precede with Security Classification Code)*

Toxicity Testing of Phosphinate Compounds

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

016800 Toxicology; 012900 Physiology; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	1.9	78
FISCAL YEAR CURRENT 82	5.7	124

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: POC:DA

NAME:

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Toxic substance; (U) Phosphinate; (U) Toxicology; (U) Toxicology testing; (U) Mutagenicity; (U) Laboratory Animal

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The family of phosphinate compounds has demonstrated efficacy in reversing the action of chemical warfare agents (CWA) that act as anticholinesterases. Proposed pathways of reversal are: 1) initial blocking of CWA and cholinesterase bonding, or 2) subsequent reactivation and bond breaking of the cholinesterase after bonding. Similar phosphinate compounds (i.e., oximes,2-PAMC1) have been proven effective when given parenterally, especially in conjunction with atropine in mammals. Knowledge of the immediate and long-term toxic potentials, if any, is consequently required in the anticipated development of phosphinate compounds as antidote for chemical warfare agents. Soman, Sarin, and Tabin have been shown to act as anticholinesterases. These studies are intended to determine the toxicity potential of specific phosphinate compounds using non-mammalian systems.

24. (U) The Ames Salmonella/Mammalian Microsome Mutagenicity Test and the Drosophila melanogaster sex-linked recessive lethal test will be the initial tests employed. Depending on the outcome of these and the results of tests conducted elsewhere, further testing may include acute, subacute, and chronic oral, parenteral, and dermal toxicity.

25. (U) 8010-8109. Considerable difficulty has been encountered in testing the phosphinate because of the rapid hydrolization in aqueous solutions. By using DMSO as the solvent for the Ames test, the compound was not exposed to water until it mixed with the test strains, thus permitting sufgicient exposure before compound disintegration. Twenty-six phosphinates have been tested and found to be negative for mutagenicity by the Ames test. A delivery method to test these compounds in D. melanogaster utilizing acidified buffer and liposome is under development. Acute oral toxicity in mice will be determined by dissolving the compounds in Tween, then diluting in acidified buffer.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3M1627734875	Medical Systems in Chemical Defense
WORK UNIT NO.	304	Toxicity Testing of Phosphinate Compounds

The following investigation has been conducted under this work unit:

STUDY NO. 1 The Ames Salmonella/Mammalian Microsome Mutagenicity test of phosphinate compounds (GLP Study Nos. 80012, 81002, 81012, 81013, 81014, and 81015)

Study No. 1. Twenty-six compounds were tested for mutagenic potential (Ames Mammalian Microsome Mutagenicity test). All compounds were tested with microsome activation tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538, on triplicate plates. It was determined that some of the tested substances were mutagenic.

BODY OF REPORT

WORK UNIT NO.	304	Toxicity Testing of Phosphinate Compounds
STUDY NO.	1	The Ames Salmonella/Microsome Mutagenicity test of phosphinate compounds (GLP Study Nos. 80012, 81002, 81012, 81013, 81014, and 18015)

PROBLEM

The defense against chemical warfare agents (CWA) is directed toward a number of specific elements which include early detection, physical barriers, post-exposure treatment, and pre-exposure protection of individuals with specific drugs.

Phosphinate compounds have demonstrated efficacy in reversing the action of those CWA that act as anticholinesterases. Two possible mechanisms of this action have been presented: initial blocking of CWA and cholinesterase bonding, or subsequent reactivation and bond breaking of the cholinesterase after bonding. Knowledge of any toxicologic, mutagenic, or other potential of these compounds is essential for evaluating their use in man. Initial studies should be aimed at determining the level of hazard associated with the compounds and to accumulate a data base for a drug clearance petition later.

The Ames test is an inexpensive, relatively reliable test to predict mutagenicity. There is a high correlation between mutagenic activity and carcinogenicity. The Ames test is routinely included in the initial battery of toxicity tests applied to therapeutic agents.

RESULTS AND DISCUSSION OF RESULTS

Compounds were tested using the Ames Salmonella/Mammalian Microsome Mutagenicity test at the concentrations indicated. All compounds were tested with and without microbial activation using tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538, using triplicate plates at the concentrations indicated below:

Toxicity Testing of Phosphinate Compounds (Continued)

Substance	Chemical Code No.	Levels Tested
4-nitrophenyl methyl phenyl phosphinate	37	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl diphenyl phosphinate	73-A	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl dimethyl phosphinate	83	3.2×10^{-4} - 1 mg/plate
4-chlorophenyl methyl phenyl phosphinate	53	3.2×10^{-4} - 1 mg/plate
4-chlorphenyl diphenyl phosphinate	91	3.2×10^{-6} - 10^{-2} mg/plate
4-nitrophenyl isopropyl (phenyl)phosphinate	103B	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl ethyl (phenyl) phosphinate	113	3.2×10^{-4} - 1 mg/plate
phenyl 4-nitrophenyl (methyl)phosphinate	103A	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl 2-methoxy phenyl (methyl) phosphinate	36	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl 4-nitrophenyl (methyl)phosphinate	21	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl bis (2-thienyl)phosphinate	41	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl 2-furyl (methyl)phosphinate	72	3.2×10^{-4} - 1 mg/plate
4-cyanophenyl bis (2-furyl)phosphinate	82	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl bis (2-furyl)phosphinate	87	3.2×10^{-4} - 1 mg/plate

3-nitrophenyl dimethylphosphinate	111	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl 4-methoxyphenyl (methyl) phosphinate	47A	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl 4-methylphenyl (methyl) phosphinate	73BM	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl di-n-butyl phosphinothioate	107	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl 4-chlorophenyl (methyl) phosphinate	47B	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl bis (chloromethyl) phosphinate	16	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl phenyl (trichloromethyl) phosphinate	51	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl dinitrophenyl dichloromethyl(phenyl) phosphinate	77	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl 4-trifluoromethylphenyl(methyl) phosphinate	86	3.2×10^{-4} - 1 mg/plate
4-nitrophenyl 3-trifluoromethylphenyl(methyl) phosphinate	4	3.2×10^{-4} - 1 mg/plate
4-fluorophenyl methyl (phenyl)phosphinate	44	3.2×10^{-4} - 1 mg/plate
4-methylsulfinylphenyl methyl(phenyl) phosphinate	96	3.2×10^{-4} - 1 mg/plate

More than twice the spontaneous reversion rate, coupled with a dose-response, was the criteria for classifying a compound as mutagenic. None of the 26 phosphinates tested demonstrated mutagenic potential.

CONCLUSIONS

We concluded that the phosphinates tested did not demonstrate mutagenic activity.

RECOMMENDATIONS

We recommend these compounds be considered for further toxicologic and efficacy testing.

PUBLICATIONS

1. SAUERS, L.J., F.R. PULLIAM, and J.T. FRUIN. The mutagenic potential of: 4-nitrophenyl methyl phenyl phosphinate, 4-nitrophenyl diphenyl phosphinate, 4-nitrophenyl dimethyl phosphinate, 4-chlorophenyl methyl phenyl phosphinate, 4-chlorophenyl diphenyl phosphinate. Toxicology Series 3. Institute Report No. 99. San Francisco, California: Letterman Army Institute of Research, July 1981

2. SAUERS, L.J., F.R. PULLIAM, and J.T. FRUIN. The mutagenic potential of 4-nitrophenyl isopropyl (phenyl) phosphinate, 4-nitrophenyl ethyl (phenyl) phosphinate, phenyl 4-nitrophenyl (methyl) phosphinate, 4-nitrophenyl 2-methoxyphenyl phosphinate, 4-nitrophenyl 4- nitrophenyl (methyl) phosphinate. Toxicology Series 4. Institute Report 100. San Francisco, California: Letterman Army Institute of Research, July 1981

3. SAUERS, L.J., F.R. PULLIAM, and J.T. FRUIN. The mutagenic potential of: 3 nitrophenyl dimethylphosphinate, 4-nitrophenyl 4-meth-oxyphenyl(methyl)phosphinate, 4-nitrophenyl 4-methylphenyl (methyl)phosphinate, 4-nitrophenyl di-n-butylphosphinothioate. Toxicology Series 16. Institute Report 102. San Francisco, California: Letterman Army Institute of Research, September 1981

4. SAUERS, L.J., F.R. PULLIAM, and J.T. FRUIN. The mutagenic potential of: 4-nitrophenyl bis(2-thienyl)phosphinate, 4-nitrophenyl 2 furyl (methyl)phosphinate, 4-cyanophenyl bis(2-furyl)phosphinate, 4-nitrophenyl bis(2-furyl)phosphinate. Toxicology Series 17. Institute Report 104. San Francisco, California: Letterman Army Institute of Research, September 1981

5. SAUERS, L.J., F.R. PULLIAM, and J.T. FRUIN. 4-nitrophenyl 4-chlorophenyl(methyl)phosphinate, 4-nitrophenyl bis(chloromethyl)

phosphinate, 4-nitrophenyl phenyl(trichloromethyl)phosphinate, 4-nitrophenyl dinitrophenyl dichloromethyl(phenyl)phosphinate. Toxicology Series 18. Institute Report 105. San Francisco, California: Letterman Army Institute of Research, September 1981

6. SAUERS, L.J., F.R. PULLIAM, and J.T. FRUIN. The mutagenic potential of: 4-fluorophenyl methyl(phenyl)phosphinate, 4-nitrophenyl 4- trifluoromethylphenyl(methyl)phosphinate, 4-nitrophenyl 3- trifluoromethylphenyl(methyl)phosphinate, 4-methylsulfinylphenyl methyl(phenyl)phosphinate. Toxicology Series 19. Institute Report 106. San Francisco, California: Letterman Army Institute of Research, September 1981

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 6787	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 01 07	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A875	BC	305 APC FL1B
b. ~~CONTRIBUTING~~	62772A	3S162772A875	CD	305
c. ~~CONTRIBUTING~~	STOG	80-7.2:1		

11. TITLE (Procede with Security Classification Code)

(U) Applied Skin Protection Technology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry; 012100 Organic Chemistry; 003200 CBR Warfare

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 01 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	1.0	54
FISCAL YEAR CURRENT 82	2.0	78

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

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NAME: Westrom, Dale R.,M.D., Ph.D., MC

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Nerve Agent; (U) DFP; (U) Chemical Defense; (U) Enzymes; (U) Skin; (U) Protection

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Even properly trained, equipped and prepared troops in a chemical warfare environment are at risk. In a surprise attack agents may contact exposed skin. They may also gain access through breaks or tears in chemical warfare protective garments or by osmosis in areas where sweat or water has saturated garments. This work unit will address technology development and early feasibility studies on topical substances to supplement chemical warfare protective clothing and equipment to prevent dermal exposure to nerve, blister and other percutaneously-active toxic agents, and developing substances for safely decontaminating injured soldiers.

24. (U) Models and methods for assessing the efficacy of skin protective substances will be developed. Emphasis will be placed on methods that will detect and quantify sublethal dose levels that decrease performance and ability to return to duty, and on obtaining information in a form that will allow valid prediction of operational efficacy. Substances to be evaluated will include commercially available topical substances (salves, creams and ointments) to prevent agent access to exposed skin, active substances (enzymes, chemicals, etc.) that are presently available, are produced under contract, or are developed in-house to deactivate, degrade or immobilize agents, and substances to improve the skin's innate ability to resist penetration or inactivate agents during penetration.

25. (U) 81 01 - 81 09. Assay systems using a fluoride specific ion analyzer of pH stat titration were developed for assessing nerve agent hydrolysis. These systems will be vital to future screening of substances for their ability to neutralize organophosphorous-type nerve agents. Using these systems, imidazole was shown to be active in hydrolyzing diisopropylfluorophosphate while nucleotides, nucleosides, sugars and [illegible]ches proved to be inactive.

* [illegible] contractors upon originator's approval.

DD FORM 1 MAR 84 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3M162734A875	Medical Systems in Chemical Defense
WORK UNIT NO.	305	Applied Skin Protection Technology

The following investigations have been conducted under this work unit:

PILOT STUDY Substances to enhance organophosphate neutralization

Chemicals are being screened as to their ability to neutralize organophosphates. DFP is being used as the substrate. Of the chemicals tested, imidazole has shown the highest activity. Among the compounds which showed no activity are nucleotides, nucleosides, sugars and starches. The neutralization rate was monitored by a fluoride specific ionanalyzer or by pH stat titration. The development of the assay systems is vital to this and future studies in the area.

BODY OF REPORT

WORK UNIT NO. 305 Applied Skin Protection Technology

PILOT STUDY Substances to enhance organophosphate neutralization

PROBLEM

On the integrated battlefield the use of chemical agents may be rampant. Among the greatest chemical threats are the nerve agents. Any method that can neutralize the agent, either during or after exposure, is of high potential value. Possible uses are as prophylactics and decontaminants.

RESULTS AND DISCUSSION OF RESULTS

A variety of chemicals have been screened for their efficacy in catalyzing (mechanism undetermined) the neutralization of a fluorophosphate (DFP) by heterolytic cleavage of the phosphorus-fluorine bond. Imidazole was the only compound shown to have activity at biological pH. Amino acids, nucleotides, nucleosides, sugars, and carbohydrates all uniformly showed no activity. The in-house development of the ability to assay for organophosphate neutralization was achieved by both fluoride measurement with specific ion electrodes and acid production with a pH stat titrator.

CONCLUSIONS

None

RECOMMENDATIONS

Further work in the area is necessary. A more comprehensive study protocol is indicated and is being prepared.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOE 6308	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A878	BA	161 APC EL06
b. ~~CONTRIBUTING~~				
c. CONTRIBUTING	STOG	80-7.2:4		

11. TITLE (Precede with Security Classification Code)

(U) Laser Technology - Ocular Bioeffects

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

009600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 07	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	8.7	636
FISCAL YEAR CURRENT 82	14.2	588

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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NAME: Stuck, D., DAC POC:DA

21. GENERAL USE

Foreign Intelligence not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U)Human Vol;(U)Ocular Hazard;(U)Laser Safety;(U)Flash Effect;(U)Pursuit Tracking;(U)Lab Animal;(U)Erbium;(U)GaAs;(U)Neodymium;(U)Multi Pulse

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Provide bioeffects data base for documenting improvement of safety standards as applied to laser training devices. Evaluate ocular hazards of near infra-red lasers considered for future laser training devices. Study environmental factors that influence laser designator operators' performance in combat.

24 (U) Determine in the laboratory the ED_{50} levels for pulsed repetitive laser exposures. Study the effects of multiple flashes on the performance of laser designator operators under simulated combat conditions. Correlate laboratory results with actual field conditions using a modified .0W missile launcher

25 (U) 8010-8109. Determined the Ocular damage thresholds (ED_{50} or energy/pulse levels) for exposure to repetitive pulse (pulse duration of 170 µs , PRF of 10 hz), frequency doubled neodymium laser radiation (532 nm): 1 pulse, 2.8 µJ; 10 pulses, 1.6 µJ; 100 pulses, 1.1 µJ. Determined the ocular damage threshold (ED_{50} levels) for exposure to a single pulsed (20 nsec) dye laser (912 nm): 5.5 µJ. Determined corneal damage thresholds (ED_{50} levels expressed in peak radiant exposures) for a single long pulse exposures (200 µs FWHM) to erbium laser radiation (1.732 µm) for 3 radiance diameters: 34, 26, and 22 J/cm^2 for irradiance diameters (1/e intensity points) of 475, 750, and 920 µm respectively. Flashes produced significant disruption in tracking performance; preliminary data from the TOW system seem to be in accord with that found with the BLASER laboratory simulator.

Available to contractors upon originator's approval

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT NO. 3E612777A878 Health Effects of Military Lasers

WORKUNITNO. 161 Laser Technology - Ocular Bioeffects

The following investigation has been conducted under this work unit:

STUDY NO. 1 Acute effects of laser exposure: ocular effects at 1.732 μm

Corneal dose-response relationships were determined by exposing Rhesus monkeys to erbium laser radiation at 1.732 μm. The ED_{50}s (the effective dose required to produce a corneal lesion, as observed with the slit lamp biomicroscope, 50% of the time) were determined for three irradiance diameters with the erbium laser operating in the long pulse mode. The emission duration was 200 μs Full-Width-Half-Maximum (FWHM). The ED_{50}s (expressed in peak radiant exposure) were 34, 26, and 22 J/cm^2 for irradiance diameters (1/e intensity points) of 475, 750, and 920 μm, respectively. The ED_{50} for the production of an acute corneal lesion is dependent on the corneal irradiance diameter for exposure conditions in this range. Corneal lesions produced by this laser involved the full corneal thickness. The nature of the response and the dose required to produce the minimal response correlate with the relative absorption properties of the ocular medium. These data, when combined with others, support inclusion of a wavelength dependence for permissible exposure limits in this spectral region.

BODY OF REPORT

WORK UNIT NO. 161 Laser Technology - Ocular Bioeffects

STUDY NO. 1 Ocular effects at 1.732 μm

PROBLEM

Current and proposed military laser systems operate in the infrared region of the electromagnetic spectrum beyond 1.4 μm. In the spectral region from 1.0 to 3.0 μm, the absorption coefficients of the outer ocular media (cornea, aqueous, lens, and vitreous) vary over three orders of magnitude. Although limited data are available for specific exposure conditions, the wavelength dependence of the dose-response relationships relevant to Army systems has not been adequately defined. Permissible exposure limits have been defined in TB MED 279; however, bioeffects data for exposure conditions in this spectral region may warrant change in permissible exposure limits and impact on the design and employment of military systems.

The erbium laser, yttrium lithium flouride (Er in YLF), operating at 1.732 μm is being considered for use in an "eye safe" laser rangefinder. A 1.732 μm erbium laser rangefinder is currently being developed by a private contractor for the U.S. Army Night Vision and Electro-Optics Laboratory, Fort Belvoir, Virginia. The system is being developed for use as a training device that would preclude operational restrictions in the training environment. A major consideration in its conception and design was that it would be an "eye safe" system. For wavelengths greater than 1.4 μm, the transmission is near a minimum at 1.732 μm. The ocular effects at this wavelength were evaluated to expand the data base for laser safety considerations.

RESULTS AND DISCUSSION OF RESULTS

An erbium laser operating at 1.732 μm was fabricated in this laboratory. The 0.25 by 3.0 inch rod was inserted into an elliptical cavity and was pumped by an EGG FX-42C3 flash lamp.

Energy input to the lamp was approximately 425 joules (C = 262 μf and V = 1800 volts). The total energy out was approximately 200 mJ. The laser emission duration was 200 μsec (FWHM). Rhesus monkey eyes were used to determine the dose-response relationships as a function of corneal irradiance diameter. The ED_{50}s (the effective dose required to produce a corneal lesion 50% of the time as observed by slit lamp biomicroscope) were determined for three corneal irradiance diameters and estimated for the fourth. Four lenses were used to obtain a range of corneal irradiance diameters. The corneal plane was located in the

experimentally determined focal plane, a distance f_p from the lens. The intensity profile at the corneal plane was approximately gaussian and the beam diameters given ($d_{1/e}$) are at the 1/e intensity points. The reported radiant exposure is the peak radiant exposure obtained by dividing the total incident energy by the area defined by the 1/e diameter. A summary of the experimental data obtained is given in the Table.

CORNEAL EFFECTS AT 1.732 μm

F_p(cm)	$d_{1/e}$(cm)	ED_{50} TIE*(mJ)	ED_{50} H_e(J/cm^2)
14.3	475	60	34
18.2	750	115	26
22.2	920	145	22
30.5	1200	175	16

*TIE = Total Incident Energy

No corneal lesions were produced for the 1200 μm beam diameter; therefore, the ED_{50} is indicated as a "greater than." No lenticular effects were observed for these exposure conditions and subjects have been evaluated two months after exposure. No corneal lesions were observed at 24 or 48 hours that were not observed at one hour. Some lesions near the ED_{50} were observed only at one hour and not at 24 or 48 hours after the exposure. Lesions produced at doses 1.5 to 2.0 times the ED_{50} resulted in a stromal scar which extended throughout the entire corneal thickness. Two months after exposure the stromal scar was less dense but still visible.

CONCLUSIONS

For the exposure conditions evaluated to date, the doses required to produce an acute ocular effect at 1.732 μm are well above the maximum permissible exposure (10 mJ/cm^2). The ED_{50} for the production of an acute corneal lesion is dependent on the corneal irradiance diameter for diameters ranging from 500 to 1200 μm. This work supports a previously drawn conclusion that the doses required to produce a biomicroscopically visible corneal lesion for laser radiation in the 1.0 to 3.0 μm region of the electromagnetic spectrum exhibit a wavelength dependence which correlates with the relative absorption properties of the cornea.

RECOMMENDATIONS

Although additional experimental data are needed for long exposure durations and larger corneal irradiance diameters for infrared laser exposures from 1.4 to 3.0 μm, a generalized wavelength correction to current permissible exposures appears necessary based upon the relative absorption properties of the ocular media.

Several portions of the protocol addressing the ocular effects at 1.732 μm remain to be completed. These include: a) determine if a retinal effect can be produced with a collimated beam, b) evaluate and describe the histology of selected corneal lesions and retinal lesions (if produced), c) evaluate potential corneal and lenticular effects for repeated exposures of a collimated beam, and d) determine ED_{50} for single and repeated Q-Switched exposures. These areas are currently being investigated and it is recommended they be completed.

The relationship between the ED_{50} for corneal injury and the corneal irradiance diameter should be determined at other infrared wavelengths for both short (nanosecond) and long (second) exposure durations.

PUBLICATIONS

None

ABSTRACT

PROJECTNO. 3E612777A878 Health Effects of Military Lasers

WORK UNIT NO. 161 Laser Technology - Ocular Bioeffects

The following investigation has been conducted under this work unit:

STUDY NO. 1 Ocular and dermal effects of infrared laser radiation

This study was withdrawn and replaced by the master protocol titled, "Acute Effects of Laser Exposure," Study No. 1, "Ocular Effects at 1.732 μm."

WORK UNIT NO. 161 Laser Technology - Ocular Bioeffects

STUDY NO. 1 Ocular and dermal effects of inrared laser radiation

PROBLEM

Current and proposed military laser systems operate in the infrared region of the electromagnetic spectrum beyond 1.4 μm. Although limited data are available for specific exposure conditions, the wavelength dependence of the dose-response relationships relevant to Army systems has not been adequately defined. Permissible exposure limits have been defined in TB MED 279; however, bioeffects data for exposure conditions in this spectral region may warrant change in permissible exposure limits and impact on the design and employment of military systems.

RESULTS AND DISCUSSION OF RESULTS

None

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

None

ABSTRACT

PROJECT NO. 3E162777A878 Health Effects of Military Lasers

WORK UNIT NO. 161 Laser Technology - Ocular Bioeffects

The following investigation has been conducted under this work unit:

STUDY NO. 2 Project MILES

This study was withdrawn and replaced by the master protocol titled, "Acute Effects of Laser Exposure," Study No. 1, "Retinal Effects From 800 to 900 nm."

BODY OF REPORT

WORK UNIT NO. 161 Laser Technology - Ocular Bioeffects

STUDY NO. 2 Project MILES

PROBLEM

Laser devices are widely deployed within the Army. Use of these devices exposes personnel to laser radiation. It is essential that the ocular and dermal hazards of these lasers be completely understood and that adequate bioeffects data base be available upon which to formulate the standards for safe use of the lasers.

RESULTS AND DISCUSSION OF RESULTS

None

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

None

ABSTRACT

PROJECT NO. 3E612777A878 Health Effects of Military Lasers

WORK UNIT NO. 161 Laser Technology - Ocular Bioeffects

The following investigation has been conducted under this work unit:

STUDY NO. 2 Acute effects of laser exposure: retinal effects from 800 to 900 nm

Retinal dose-response relationships were determined for ocular exposure in the Rhesus monkey to repetitive pulse frequency doubled neodymium laser irradiation (532 nm). The duration of each pulse was 170 ns, and the pulse repetition rate was 10 Hz. The ED_{50}s (total intraocular energy/pulse) were: 1 pulse, 2.8 μj/pulse; 10 pulses, 1.6 μJ/pulse; 100 pulses, 1.1 μJ/pulse.

The ED_{50} for retinal damage in the Rhesus monkey was determined for exposure to radiation from a dye laser operating at a wavelength of 912 nm. The pulse duration was 20 ns. For a single pulse exposure the ED_{50} was 5.5 μJ total intraocular energy.

BODY OF REPORT

WORK UNIT NO. 161 — Laser Technology - Ocular Bioeffects

STUDY NO. 2 — Acute effects of laser exposure: retinal effects from 800-900 nm

PROBLEM

Laser devices are widely deployed within the Army. Use of these devices exposes personnel to laser radiation. It is essential that the ocular hazard of these lasers be completely understood and that an adequate bioeffects data base be available upon which to formulate standards for safe use.

RESULTS AND DISCUSSION OF RESULTS

Dose response data were obtained for exposure to repetitive pulse trains from a frequency-doubled neodymium laser, emitting 170 ns duration pulses at a wavelength of 532 nm. The laser was a continuously pumped, acousto-optically Q-Switched neodymium device with an intracavity frequency-doubling crystal. The pulse repetition frequency of the laser was 10 Hz. Dose response relationships were also determined for a pulsed dye laser emitting at a wavelength of 912 nm. The dye laser was pumped by the 532 nm beam of a frequency-doubled neodymium laser. The dye used in this experiment was IR140 dissolved in DMSO. The pulse duration was 20 ns. A dichroic beamsplitter, having high reflectivity at the laser wavelength and high visual transmittance, directed the laser beam into the eye of the monkey while permitting continuous viewing of the retinal exposure site via fundus camera. The mirror and fundus camera were aligned so that the laser beam passed through the center of the ocular pupil and coincided with the crosshairs at the retina, facilitating selection of the exposure site. A constant proportion of the beam energy was diverted into a reference detector for dosimetry. The energy at this detector was correlated to the energy at the eye by placing a calibrated radiometer at the eye position and determining the ratio of the energy received by the two detectors. The exposure duration was controlled by an electronic shutter, and neutral density filters were used to attenuate the beam energy to the desired exposure level.

The animals used in these experiments were Rhesus monkeys. The animals were anesthetized and the pupils dilated for exposure. The eye was held open by a lid speculum during the exposure, and corneal clarity was maintained by periodic irrigation with a normal saline solution.

Thirty exposures were placed in a rectangular array in the extramacular retina at doses that varied from the level required to produce an immediately visible lesion to 3 log-units below that level. The exposure sites were examined via ophthalmoscope one hour after exposure. The criterion for damage was the observation of a lesion at this examination. The data were evaluated by probit analysis to determine the ED_{50} for each exposure condition.

The ED_{50} and associated 95% confidence limits were determined for exposure to pulse trains from the frequency-doubled neodymium laser of 1, 10, and 100 pulses. These data are tabulated. The following definitions apply:

PRF = pulse repetition frequency

t = duration of each pulse in the train

T = total exposure duration

N = number of pulse per exposure (N = PRF x T)

ED_{50} = ED_{50} expressed as total energy per exposure

ED_{50}/pulse = ED_{50} expressed as energy per pulse

ED_{50}/pulse = ED_{50}/N

95% limits = 95% confidence limits for the ED_{50}/pulse

TABLE

Frequency-doubled neodymium laser - wavelength 532 nm

PRF = 10 Hz t = 170 ns

T	N	ED_{50} (μJ)	ED_{50}/pulse (μJ)	95% limits (μJ)
140 ns	1	2.8	2.8	2.5-3.2
1 s	10	16	1.6	1.3-2.0
10 s	100	107	1.1	0.8-1.7

The ED_{50} for exposure to a single pulse from the dye laser emitting at 912 nm was 5.5 μJ. The 95% confidence limits were 4.6-6.7 μJ.

Previous work in this laboratory has led to the derivation of an empirical relationship equating the ED_{50}/pulse of a pulse train to the ED_{50} for a single pulse and the number of pulses (N) in the pulse train. The relationship $ED_{50}/\text{pulse} = KN^{-1/4}$, where K is the ED_{50} for a single pulse, was valid for the near infrared wavelengths previously reported. The data for the frequency doubled neodymium laser also reflects that relationship.

Little data exist giving the ED_{50} for wavelengths between 694.2 nm (ruby) and 1064 nm (Nd). As a result, the ED_{50}s in the wavelength region have commonly been estimated by interpolation between the ED_{50} at 694.3 and 1064 nm. The ED_{50} for 912 nm reported here is almost an order of magnitude lower than that obtained by the interpolation.

CONCLUSIONS

Repetitive pulse lasers pose a significantly greater ocular hazard than do continuous wave or single pulse lasers. The energy per pulse required to produce ocular damage is inversely proportional to the fourth root of the number of pulses. This relationship holds for visible, as well as near infrared, laser radiation. The ED_{50} for ocular damage by lasers operating near 900 nm is significantly lower than the value obtained by interpolation between the ED_{50}s at 694.3 and 1064 nm.

RECOMMENDATIONS

It is recommended that the provisions of the Army laser safety standards as applied to repetitive pulse lasers be changed to more accurately reflect the bioeffects data setting C_R equal to $N^{-1/4}$. It is recommended that more bioeffects research be performed to determine the wavelength dependence of ED_{50} between the wavelengths of 694.3 and 1064 nm.

PUBLICATIONS

1. LUND, D.J., B.E. STUCK, and E.S. BEATRICE, Biological research in support of project MILES. Institute Report No. 96. Presidio of San Francisco, CA, Letterman Army Institute of Research, San Francisco, July 1981

ABSTRACT

PROJECT NO. 3E162777A878 Health Effects of Military Lasers

WORK UNIT NO. 161 Laser Technology - Ocular Bioeffects

The following investigation is being conducted under this work unit:

STUDY NO. 6 Antipersonnel optical countermeasures

EX-1 The effects of repetitive, small spot incoherent flashes on pursuit tracking performance under simulated field conditions

Lasers represent a significant new light hazard in the combat environment. Directed laser light in the visible spectrum may produce flash blindness or retinal damage to soldiers engaged in visual tasks who may be intentionally exposed to high intensity light which would disrupt the performance of those tasks. Infrared high energy laser radiation may vaporize the surface of optics, while visible and near infrared laser sources may produce retinal burns. These effects can be simulated in a laboratory scenario using non-laser white light flashes. Ten trained volunteers used a viscous-damped mount optical tracking device to track targets at a constant angular velocity of 5 mrad/sec under low ambient light and bright light conditions. Pursuit tracking performance data were collected under simulated field conditions, which included scale model targets and terrain, and a full-size bunker which houses a simulated laser designator optical tracking device. The preliminary findings suggest that the deleterious effects of the small spot flash on training performance, were reduced compared with earlier work where a full field flash was used.

AD-A123 769 LETTERMAN ARMY INSTITUTE OF RESEARCH ANNUAL RESEARCH PROGRESS REPORT FY 1981(U) LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANCISCO CA J D MARSHALL 4/4

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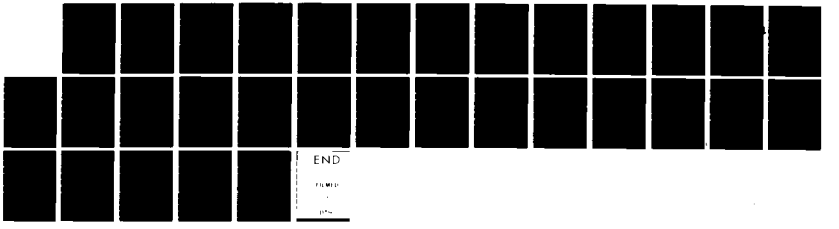

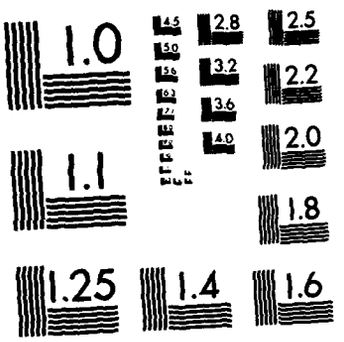

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

BODY OF REPORT

WORK UNIT NO. 161 Laser Technology - Ocular Bioeffects

STUDY NO. 6 Antipersonnel optical countermeasures

PROBLEM

Optical countermeasures may be designed to take advantage of weaknesses in optical tracking systems. Actions may also be taken to incapacitate the operators of these systems. It is important that the U.S. Army understand the physiologic and behavioral effects of countermeasures directed against soldiers in the field. Guidelines that will maximize the likelihood of a successful mission should be based on information that has considered the type of countermeasure, the specific military tasks, environmental conditions, and individual differences among soldiers. This information should be obtained under environmental conditions that are representative of those expected during combat. These data can be used to develop methods of protecting troops against optical countermeasures.

RESULTS AND DISCUSSION OF RESULTS

Lasers represent a significant new light hazard in the combat environment. Directed laser light in the visible spectrum may produce flash blindness or retinal damage to soldiers engaged in visual tasks (who may be intentionally exposed to high intensity light, which would disrupt the performance of those tasks). Infrared high energy laser radiation may vaporize the surfaces of optical devices. The resulting reradiation from the optical surface will also produce flash effects. This study evaluated the surface of multiple, small, retinal image diameter (100 μ), white light or chromatic flashes on pursuit tracking performance. Ten trained volunteers used a viscous-damped mount optical tracking device to track targets at a constant angular velocity of 5 mrad/sec under low ambient light and bright light conditions. Pursuit tracking performance data were collected under simulated field conditions, which included scale model targets and terrain, and a full size bunker which houses a simulated laser designator optical tracking device. The collection of these data has just been completed. The preliminary findings suggest that the deleterious effects of the small spot flash on training performance, relative to earlier work where a full field flash was used, was reduced. Upon completion of the data analysis a more exact comparison between the two studies can be made.

CONCLUSION

Preliminary evaluation of small spot flash data indicate considerably reduced effects on tracking performance as compared to earlier studies of large retinal area flash effects.

RECOMMENDATIONS

None can be made until the data analysis is completed.

PUBLICATIONS

None

ABSTRACT

PROJECT NO. 3E162777A878 Health Effects of Military Lasers

WORK UNIT NO. 161 Laser Technology - Ocular Bioeffects

The following investigation has been initia ed under this work unit:

STUDY NO. 6 Antipersonnel optical countermeasures

EX-2 Field evaluation of the Hughes aircraft optical jamming simulator

Preliminary data have been collected on three male volunteers in a study designed to examine flash effects upon pursuit tracking performance utilizing a modified ground TOW anti-armor weapon system. The modifications to the TOW include the addition of an Optical Jamming Simulator. This device consists of hardware (and software) which are attached to the TOW launcher and permit the operator to be exposed to optical countermeasures (flashes) while he is tracking targets under actual field conditions. The operator's response, in terms of degraded tracking performance, is directly monitored by an infrared sensor in the TOW optical sight and by an attached silicon vidicon CCTV camera. The initial studies were conducted at Camp Parks, CA, under cloudy and sunny-bright lighting conditions (1100-1500 hrs). The volunteers tracked a jeep-mounted, one-meter square target at distances of 300-600 meters. The target moved from left-to-right at speeds of 10-20 mph. On several trials, randomly chosen, the subjects were presented with a series of five 3-microsecond white light flashes, at a frequency of 20 Hz. The flashes were produced by a Xenon short-arc flashlamp, which when focused via the system's optics resulted in a 500 μ spot on the retina. Initial results indicated that flashes produced brief (2-3 sec) disruptions in tracking performance during which time the center of the target was reportedly obscured by both the flash and its afterimage. These results, in many respects, parallel those of our laboratory simulations utilizing the BLASER (GLLD-type) simulator. The retinal flashes utilized in this study were an order of magnitude below permissible safe exposure levels, and much lower than levels produced by military laser devices. Brief disruptions were produced even though target size was relatively large and its movements predictable. The effects of manipulating target size, speed, ambient lighting conditions, area of retinal illumination, wavelength, or locus of retinal exposure are not yet known.

BODY OR REPORT

WORK UNIT NO. 161	Laser Technology - Ocular Bioeffects
STUDY NO. 6	Antipersonnel optical countermeasures
EX-2	Field evaluation of the Hughes aircraft optical jamming simulator

PROBLEM

Operators of visual tracking devices may be exposed to optical countermeasures intended to disrupt their performance. Previous work from this laboratory, utilizing the BLASER simulator, indicate disruptions in tracking performance following the presentation of brief, large and small spot flashes. For the U.S. Army to assess the physiologic and behavioral effects of countermeasures that could be directed against soldiers in the field, data should be obtained under environmental conditions and with weapons systems similar to those used in combat. Information obtained under these conditions would serve to validate and expand our laboratory findings and serve as a baseline with which to pursue methods of providing protection for troops against the threat of optical countermeasures.

RESULTS AND DISCUSSION OF RESULTS

Preliminary data have been collected on three male volunteers in a study designed to examine flash effects upon pursuit tracking performance utilizing a modified ground-TOW anti-armor system. The modifications to the TOW include the addition of an Optical Jamming Simulator. This device consists of hardware (and software) attached to the TOW launcher that permit the operator to be exposed to optical countermeasures (flashes) while he is tracking targets under actual field conditions. The operator's response, in terms of degraded tracking performance, is directly monitored by an infrared sensor in the TOW optical sight and by an attached silicon vidicon CCTV camera. The initial studies were conducted at Camp Parks, CA, under cloudy-bright and sunny-bright lighting conditions (1100-1500 hrs). The volunteers tracked a jeep-mounted, one-meter square target at distances of 300-600 meters. The target moved from left-to-right at speeds of 10-20 mph. On several trials, randomly chosen, the subjects were presented with a series of five 3-microsecond white light flashes at a frequency of 20 Hz. The flashes were produced by a Xenon short-arc flashlamp which when focused via the system's optics resulted in a 500 μ spot on the retina. Initial results indicate that flashes produced brief (2-3 sec) disruptions in tracking performance during which time the center of the target was reportedly obscured by both theflash and its afterimage. These results, in many respects,

parallel those of our laboratory simulations. The retinal flashes utilized in this study were an order of magnitude below permissible safe exposure levels and much lower than levels produced by military laser devices. Brief disruptions were produced even though target size was relatively large and its movements predictable. The effects of manipulating target size, speed, ambient lighting conditions, area of retinal illumination, wavelength, or locus of retinal exposure are not yet known.

CONCLUSIONS

This study used brief, small-spot flashes that were an order of magnitude below permissible safe exposure levels and considerably lower than levels typically produced by military laser devices. The flashes produced brief disruptions in pursuit tracking performance under bright ambient lighting conditions and with predictable target behavior utilizing a modified TOW launcher. The effects of manipulating target size, target movement predictability, ambient lighting, area of retinal illumination, flash wavelength, and retinal exposure locus are not yet known.

RECOMMENDATIONS

This study should be expanded t include a greater number of subjects to determine flash effects upon operator performance for a pursuit tracking task utilizing the TOW weapons system. Furthermore, the effects of manipulating target size, target movement predictability, ambient lighting, area of retinal illumination, wavelength, and retinal exposure locus should be investigated.

PUBLICATIONS

None

ABSTRACT

PROJECT NO. 3E162777A878 Health Effects of Military Lasers

WORK UNIT NO. 161 Laser Technology - Ocular Bioeffects

The following investigation is being conducted under this work unit:

STUDY NO. 6 Biomedical factors affecting laser designator operator performance

EX-5 Evaluation of the effects of four different compressed practice tracking schedules on laser designator operator performance

In previous studies using the BLASER simulator it has taken four training days before the trackers' performance stabilized so that the experimental variables could be studied. In the future BLASER studies, considerable time and money could be saved if the training period could be reduced. To date, 30 male volunteers from various units at the Presidio of San Francisco and Fort Ord have been trained under five different training schedules. Except for the usual four-day training schedule, the remaining schedules are variations of massed (a relatively large number of trials in a short period of time) or distributed (a relatively small number of trials spaced over time) which require only two days of training. The results thus far show both between-and within-group variability. However, before final comparisons among the four compressed training schedules can be made, 10 additional soldiers must be trained.

BODY OF REPORT

WORK UNIT NO.	161	Laser Technology - Ocular Bioeffects
STUDY NO.	6	Biomedical factors affecting laser designator operator performance
EX-5		Evaluation of the effects of four different compressed practice tracking schedules on laser designator operator performance

PROBLEM

With the increased use of laser rangefinder/designators in the battlefield, knowledge regarding biomedical factors that can affect the operators' performance is needed. The BLASER simulator at LAIR can provide valuable information to assist in preparing soldiers who will operate these devices. Considerable time is spent in training volunteers to operate the BLASER tracking device under the current distributed practice schedule. If this training period could be shortened, the time saved would allow for more efficient use of volunteer/experimenter time and permit more rapid assessment of the experimental variables of interest. These data will also be useful to commanders responsible for training laser designator operators.

RESULTS AND DISCUSSION OF RESULTS

Thus far 30 male volunteers from various units at the Presidio of San Francisco, CA, have been trained using five different training schedules:

1) Distributed Practice - 30, 15 sec trials/day for 4 days

2) Massed Practice (1-min trial) - 2 days of 15, 1 min trials/day

3) Massed Practice (30 sec trial) - 2 days of 30, 30 sec trials/day

4) Mass Distributed (1-min trial) - 22, 1-min trials on the first day followed by 32, 15 sec trials on the second day

5) Mass Distributed (30 sec) - 45, 30 sec trials of the first followed by 30, 15 sec trials on the second day.

The results thus far show both within-group and between-group differences. However, before statistical comparisons can be made among the groups, 10 additional soldiers will have to be trained.

CONCLUSION

The remaining 10 solders must be run before analysis of the data can begin.

RECOMMENDATIONS

The remaining 10 soldiers must be run before analysis of the data can begin.

PUBLICATIONS

None

FOR REVIEW

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 3371	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BA	243 APC HL19
b. CONTRIBUTING				
c. CONTRIBUTING	STOG	80-7.2:5		

11. TITLE (Precede with Security Classification Code)*

(U) Ballistic Injuries

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

008800 Life Support; 016200 Stress Physiology; 017100 Weapons Effects

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 08	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	0.1	20
FISCAL YEAR CURRENT 82	3.7	185

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Letterman Army Institute of Research
ADDRESS:* Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
NAME: Marshall, J.D., COL, MSC
TELEPHONE: (415) 561-3600

20. PERFORMING ORGANIZATION

NAME:* Letterman Army Institute of Research
Division of Combat Casualty Care
ADDRESS:* Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Bellamy, Ronald F., COL, MC
TELEPHONE: (415) 561-5816
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Fackler, Martin, COL, MC
NAME: Surinchak, J.S., SFC POC: DA

21. GENERAL USE

Foreign Intelligence Not Applicable

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Wound Healing;
(U) Military Trauma; (U) Animal Model; (U) Ballistic Trauma

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Ballistic injuries caused by ultra high velocity missiles (2 km/sec) are likely to occur in the battlefield of the future. The purpose of this work unit is to characterize ultra high velocity missile wounds and to determine whether or not traditional concepts of wound debridement will be adequate to manage such wounds.

24. (U) Anesthetized pigs will be wounded in their buttocks with 1-gram projectiles fired with a velocity of 2 km/sec. Histological criteria will be established allowing the assessment of tissue injury. Possible release of vasoactive substances from injured tissue will be studied by performing appropriate biochemical assays on effluent blood. Debridement techniqes will be tested, possibly including the use of an argon laser-assisted quartz scalpel.

25. (U) 80 10 - 81 09 A previous study under this work unit which proposed using the laser scalpel was not carried out due to the unavailability of this instrument. A wound ballistic laboratory in the basement of LAIR is nearly complete. The characteristic wound of ultra high velocity missiles has been demonstrated in studies using cadavers. Studies using living tissue will begin shortly.

MILITARY TECHNOLOGY CERTIFIED UNDER
SECTION 224 OF PUBLIC
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*Available to contractors upon originator's approval

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ABSTRACT

PROJECT NO. 3M161102BS10 Research on Military Disease, Injury, and Health Hazards

WORK UNIT NO. 243 Ballistic Injuries

The wound ballistics of ultrahigh velocity missiles was demonstrated in anesthetized pigs and gelatin blocks. One-gram steel rods were shot at velocities of up to 2 km/sec. These missiles create very distinctive wounds of entrance which are rather similar in appearance to the wound of exit of high velocity military assault rifle rounds.

BODY OF REPORT

WORK UNIT NO. 243 Ballistic Injuries

PROBLEM

Certain newly fielded weapon systems depend upon ultrahigh velocity projectiles for their destructive potential. Work done by Charters and Charters several years ago suggests that whenever the velocity of a projectile exceeds wave speed in the target, the site at which the missile impacts will be characterized by a huge defect. The wound ballistics of ultrahigh velocity missiles is not known and, on the basis of Charter and Charter's work, might be expected to be quite different from wounds created by ordinary high velocity military assault rifle rounds. Thus, it is possible that established standards for wound management will have to be modified to adequately treat casualties with these wounds.

RESULTS AND DISCUSSION OF RESULTS

During the fiscal year, a wound ballistics laboratory was constructed in the basement of LAIR. A devise designed to fire ultrahigh velocity missiles was obtained. Briefly, this consists of a 30-caliber barrel with a 50-caliber receiver. Steel rods 1 gram in mass and 5.54 mm in diameter (22 caliber) are mounted within a discard sabot. The powder charge is on the order of 240 grains. Impact velocities ranged between 5800-6200 ft/sec (1.8 to 1.9 km/sec). Several experiments were carried out (under the auspices of an approved pilot protocol) at the end of the fiscal year in which anesthetized swine were shot in various anatomical locations with this device. When the target is in fleshy tissue, such as the buttock, an enormous wound of entrance is created. The permanent wound track is much less prominent and frequently the missile does not have a wound of exit. When the target is located in tissue overlying bone, such as the skull, the wound of entrance is much smaller. However, destruction deep to the wound of entrance is truly fearsome. In one experiment in which the missile struck rib initially and then hit the heart, the left ventricle was disintegrated. Gelatin blocks have also been used as targets, but here the results are much less impressive. It is probably that the very large wound of entrance seen with ultrahigh velocity missiles results from the formation of an unusually powerful temporary cavity at the site of impact. Much energy is deposited into the tissue at this point because the missile is supersonic in the initial portion of its trajectory. The drag coefficient of a supersonic projectile is, of course, much greater than for a subsonic projectile.

CONCLUSIONS

Ultrahigh velocity missiles create distinctive wounds of entrance.

Ballistic Injuries (Cont)

RECOMMENDATIONS

Work must continue until it can be determined whether or not the SOP for soft tissue wound management needs to be altered when one deal with ultrahigh velocity missile wounds. For this to be accomplished, it will be necessary to develop a chronic model for wound ballistic research.

PUBLICATIONS

None

APPENDIX A

PUBLICATIONS ACCESSIONED - FISCAL YEAR 1981

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