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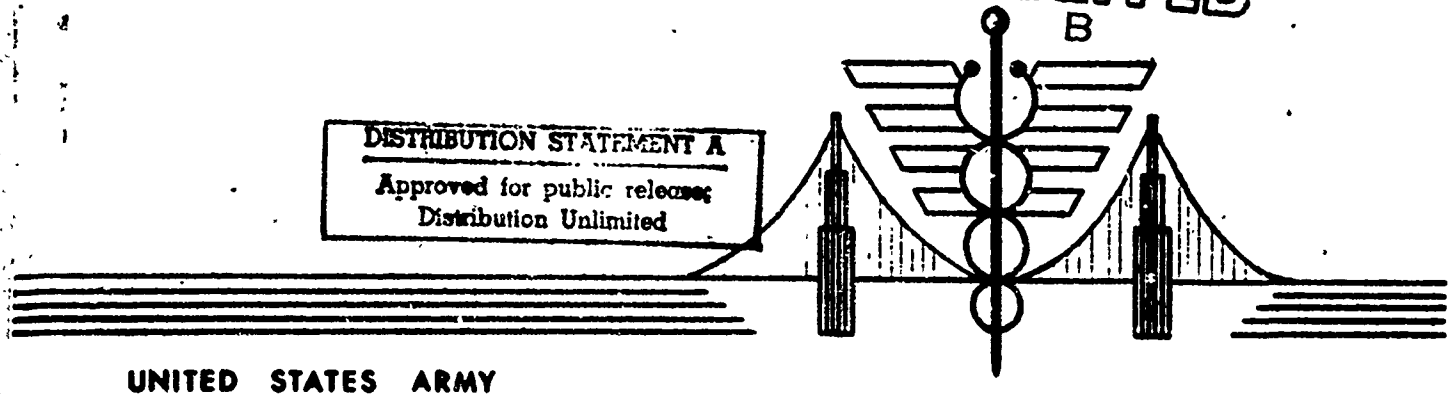
Letterman Army Institute of Research

SAN FRANCISCO, CALIF. 94129

ANNUAL PROGRESS REPORT, FY 1972

RCS SGRD-288(R1)

30 June 1972



UNITED STATES ARMY

MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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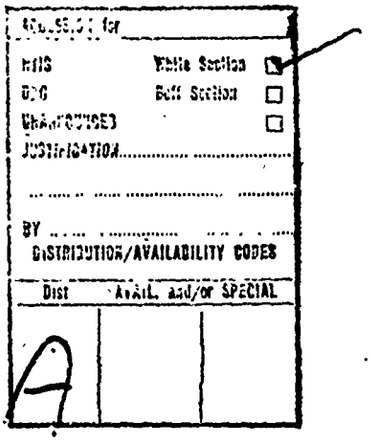
In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care,' as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council.



Item 24, (U) Dogs and sheep will be utilized as models. Experimental dorsal longitudinal sinus clots will be produced. I^{-131}-tagged fibrinogen will then be injected and renal brain scans taken and compared to control scans and correlated with clots present at autopsy.

Page 73, Item 23. (U) This study was initiated in 1968 at the written request of the military consultant in dermatology to the Surgeon General because tropical acne was the most common cause of evacuation for skin disease from Vietnam.

Item 24. (U) To develop new techniques to determine the composition and structure of minute quantities of lipids. Apply the evolved techniques to study surface skin lipids of normal men, uninvolved skin of acne patients, comedones and pustules of acne patients. Then study the influence of new medicaments on acne patients.

Page 191, Item 23. (U) This study attempts to determine why nuclear casualties become nauseated and vomit and to develop treatment and prevention of these symptoms.

Item 24. (U) Physiological and biochemical manifestations in laboratory animals will be systematically correlated with the observed clinical course following irradiation. Results of these studies will be evaluated for clues to the development of improved and new methods for diagnosis and treatment of acute and chronic radiation injury. Of particular interest are potential applications of dietary, chemical, and antibiotic therapy of radiation casualties and of radiation-treated patients. In addition, the program to provide pre-clinical evaluation of radiopharmaceuticals for diagnostic and therapeutic use in Army hospitals will be continued.

Letterman Army Institute of Research

Annual Progress Report, FY 1972
RCS SGRD-288(R1)

30 June 1972

ERRATA

Cover: LAIR Report No. 11

Page 8, Item 23. (U) Laboratory support of studies on cutaneous fungal infections, periodontal disease, wound healing, burns, and allergic responses in soldiers.

Item 24. (U) Leukocytes will be isolated from whole blood by agglutination of erythrocytes with dextran. The leukocytes will be separated on buffered, isotonic density gradients made with a high polymer of sucrose. Each cell type will be disrupted by shear or sonication, and the soluble and membrane-bound fractions will be injected into rabbits for the production of antibody.

Page 11, Item 23. (U) In severely wounded soldiers death may occur from shock despite alleged adequate whole blood replacement. Some evidence suggest oxygen is still not being delivered adequately in tissues. This study was initiated to demonstrate whether adequate oxygen is being delivered by the blood to tissues. Presently, no clinical laboratory technique is available, particularly for field use.

Item 24. (U) Adequate baseline studies will be performed using sheep blood to ascertain reproducibility and sensitivity of the technique. Effects of pH, PCO_2, hematocrit and other variables will be determined. The dissociation curve of sheep blood will be followed prior to, during, and following recovery from experimentally induced shock and the results correlated with the physiologic status of the model as determined by independent means.

Page 20, Item 23. (U) To develop an animal model using mice for producing chronic ileitis and colitis since diarrheal diseases, especially in tropical areas, rank second in cause of man days lost in soldiers.

Page 23, Item 23. (U) To develop an animal model to study the role of blood-brain-barrier in irreversible shock due to hemorrhagic shock as occurs in combat. Factors of sludged blood and thromboses are introduced.

UNCLASSIFIED
Security Classification

250

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Letterman Army Institute of Research
Presidio of San Francisco, California 94129

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP
N/A

3. REPORT TITLE
Letterman Army Institute of Research Annual Progress Report, FY 1972

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Annual Progress Report, FY 1972, 1 July 1971 - 30 June 1972

5. AUTHOR(S) (First name, middle initial, last name)

6. REPORT DATE
30 June 1972

7a. TOTAL NO. OF PAGES
251

7b. NO. OF REFS

8a. CONTRACT OR GRANT NO.
N/A

b. PROJECT NO.

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
LAIR Report No. 11

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
RCS SGRD-288(R1)

10. DISTRIBUTION STATEMENT
Approved for public release; distribution unlimited.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
U.S. Army Medical Research and
Development Command
Washington, D.C. 20314

13. ABSTRACT

This report is a compilation of work unit summaries of the investigative efforts of Fiscal Year 1972 at Letterman Army Institute of Research. These work units are devoted to basic and applied research in skin diseases of military importance; the effects of hemorrhagic shock on the heart and brain; the development of techniques for the early management of complex maxillofacial wounds and injuries; to identify diseases of the oral and maxillofacial tissues which are of special importance to the field soldier and to obtain basin information on these problems; advising, guiding, supporting, and encouraging clinical research at Letterman General Hospital.

DD FORM 1 NOV 68 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

UNCLASSIFIED
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Hemorrhagic shock						
Blood						
Brain						
Skin						
Skin diseases						
Fungal infection						
Friction blisters						
Human volunteers						
Maxillofacial injuries						
Clinical Investigations						
Experimental animals						

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☆U.S. GOVERNMENT PRINTING OFFICE: 1972 — 792 440/166

AD_____

HEADQUARTERS
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ANNUAL PROGRESS REPORT, FY 1972

1 July 1971 - 30 June 1972

RCS SGRD-288(R1)

FOREWORD

This report summarizes the investigative effort of Fiscal Year 1972 at the Letterman Army Institute of Research. Seven of our 23 investigators with doctoral degrees concluded their service at the laboratory this fiscal year so their work is presented in more detail. New, significant knowledge was gained concerning man's immunological response to cutaneous fungal infections, the effect of prolonged water exposure on man's skin, true oxyhemoglobin dissociation curves in hemorrhagic shock, mandibular bone grafting using cadaver cortical bone and autologous marrow, and identification of the most acceptable oral suture material presently available.

Those clinical investigations at Letterman General Hospital involving collaborative studies or major financial, personnel, laboratory, or animal resources support from LAIR are included.

William A Akers

WILLIAM A. AKERS
Colonel, Medical Corps
Commanding

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IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Program Element 6.11.01.A

Project Number 3A061101A91C

Task Area Number 00

INDEPENDENT LABORATORY IN-HOUSE RESEARCH

The Independent Laboratory In-House Research Program consists of research the Commander considers worthwhile toward solution of military medical problems that are not funded in other programs. Seven separate investigations are being conducted in this area.

Preliminary findings of research in hemorrhagic shock reveal light and fluorescent microscopy have demonstrated endothelial distortions in the cerebral capillaries and protein dye leaks in the blood-brain barrier of sheep in hypovolemic shock. These lesions appear to be an early and potentially reversible factor in shock. Preliminary findings indicate that simple protective therapy may be possible to prevent lesions and the development of irreversible shock.

A simple, rapid method for determining blood-oxygen affinity has been developed which requires small quantities of blood and uses equipment available in most medical facilities. This technique has great potential for evaluating acutely injured patients in combat medical facilities.

A laboratory animal model has been developed to study superior sagittal sinus thrombosis. Studies are in progress to develop methods for diagnosing this condition. This would be of great use in treating patients with acute head injuries.

Ways to separate and obtain pure samples of the five types of white blood cells are being developed. Findings indicate that this method is more accurate than current techniques.

An investigation is underway to determine if coliform induced colitis in mice is comparable to human chronic idiopathic ileitis and colitis and a suitable laboratory animal model is being perfected.

Studies to determine if neutrophil chemotactic variations in the normal individual influence susceptibility to inflammatory diseases could lead to a method for screening and identifying individuals susceptible to other inflammatory diseases.

In-vitro laboratory studies are being conducted on mammalian cell function. This work is directed toward identifying factors which could influence wound healing in patients.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6809	72 07 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
71 07 01	D.Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	380
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Studies on Cerebral Pathophysiology in Early Hemorrhagic Shock (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY.	16. PERFORMANCE METHOD
67 12	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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	72	2	7
FISCAL YEAR CURRENT	73	3	8

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

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ASSOCIATE INVESTIGATORS

NAME:

NAME:

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Hemorrhagic shock; (U) sheep; (U) cerebral blood flow; (U) blood-brain-barrier

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

25. (U) 71 07 – 72 06 Studies on cerebral pathophysiology in early hemorrhagic shock were continued. Techniques for observing pial (cerebral cortical) and retinal microvasculature were devised. Technique for sequential cerebral biopsy, light and electron microscopy and fluorescence microscopy are now being used. Light microscopy shows edema of the cerebral cortex, neuronal vacuolation and capillary endothelial distortions. Fluorescence microscopy shows leaks of protein bound dyes about capillaries (leak of blood brain barrier). Electronmicroscopy is pending. These microscopic changes are all potentially reversible and support our postulated early and important cerebral lesion in hemorrhagic shock.

$NaHCO_3$ treatment somewhat improved EEG and blood brain barrier in a series of experiments with pathologic verification and support the postulate that simple protective therapy is possible. A manuscript is being prepared.

* Available to contractors upon originator's approval

Studies on Cerebral Pathophysiology in Early Hemorrhagic Shock

Michael M. Bronshvag, MAJ, MC

PROBLEM:

Studies of hemorrhagic shock and especially of cerebral abnormalities in shocked organisms are directed towards answering basic questions:

1) Is there a structural lesion or physiologic derangement of the brain in the shocked but viable organism, as opposed to an analogous lesion observable only in the moribund organism?

2) Is such a lesion the cause of loss of protective (autoregulatory and homeostatic) mechanisms, contributing to impaired viability?

3) Is such a lesion the marker for borderline "irreversibility"? Is such irreversibility due only to impaired homeostasis or also to cerebral damage per se?

4) Can supportive treatment protect the brain and enhance viability while shock and its causes are being corrected?

We defined shock as hypotension plus systemic dysfunction caused by a disease or injury, and studied shock caused by atraumatic, acute blood loss ("hemorrhagic shock") in the adult, unanesthetized sheep. By doing this, we hoped to avoid possible artifacts induced by general anesthesia, coexisting tissue necrosis or infection, or chronic hypotensive state and secondary metabolic changes. We used sheep because they are docile and can be examined unsedated during blood loss.

Both our previous experimental experience and the clinical writings of others describe in hemorrhagic hypotension with shock, the sudden appearance on the EEG of continuous high voltage 1-3 ps delta waves, and we have also noted associated cortical suppression. This striking change, called the "slow wave point" by us, occurring in an awake and viable sheep, suggested that an early, primary cerebral lesion exists. We, therefore, used EEG changes as an important parameter in our studies.

As described below, we monitored EEG, blood-brain barrier and sequential histopathology, and believe we have described an early cerebral lesion of theoretical interest and perhaps clinical importance.

We have also begun investigation of simple pharmacologic agents, specifically trying to find one which would prevent or modify the early cerebral lesion in hemorrhagic shock and hopefully also improve viability of the organism.

APPROACH:

Unanesthetized adult sheep were bled from a femoral artery catheter to the slow wave point at the rate of 10-20 cc per minute. Average blood loss was 800-1000 cc or approximately 40 cc/kg of body weight. EEG was recorded bilaterally from frontal and occipital areas. Integrity of the vascular endothelium with regard to albumin ("blood-brain barrier") was measured by serial technitium^{99} -albumin brain scanning, and also by gross and fluorescent microscopic examination of cerebral tissue after the injection of Evan's blue (T1826) - an albumin-bound dye that fluoresces red in blue-free light. Tissues were taken for light and electron microscopic examination. In some animals 10 cc of isotonic $NaHCO_3$ was injected slowly into one carotid to see if it protected the ipsilateral cerebral hemisphere.

RESULTS AND DISCUSSION:

1. EEG - As previously discussed, the EEG changes (slowing to 1-3 cps high voltage activity) were of relatively sudden onset, and did not correlate very well with BP or amount of blood loss, although they were a sign of severe blood loss (mean 23.4 cc/kg to slow wave point). They probably represent the onset of some hemodynamic or metabolic event. Since in compensated hemorrhagic hypotension, systemic vasoconstriction is associated with cerebral vasodilation to maintain cerebral blood flow (cerebral autoregulation), so the slow wave point may represent that time at which cerebral autoregulatory mechanisms fail. An additional or alternative hypothesis is that the slow wave point represents that point at which a perverse metabolic process (ammonia or lactate buildup, endothelial edema or necrosis) causes cellular dysfunction. A metabolic process of this type might be singularly susceptible to simple pharmacologic treatment.

2. Vascular Permeability to Albumin - "Blood Brain Barrier" - As shown by technitium^{99} pertechnitate scanning (9 of 11 experiments), technitium^{99} - albumin couples (4 of 5 experiments), and Evans blue dye technique (gross inspection 9 of 10: fluorescence microscopy 3 of 4), a vascular leak occurs in the capillary endothelium to albumin-sized molecules. This type of lesion generates considerable theoretical and practical interest for it is not typical of anoxic-ischemic cerebral lesions, but is seen in CO_2 narcosis and hypertensive encephalopathy and strongly suggests[2] that a metabolic or vasospastic phenomenon in addition to simple oligenic hypoxia is present.

3. Results of $NaHCO_3$ Treatment - In hopes of reversing a postulated metabolic cerebral or metabolic cerebral endothelial lesion, several sheep had 10 cc of isotonic $NaHCO_3$ injected into the right carotid artery. Results were equivocal. (In no animal was unequivocal EEG improvement noted: in 4 of 10 brain scan protection against vascular leak was noted; and in 3 of 3 sheep, decreased Evans blue staining was noted.)

4. Neuropathology - Although a formal neuropathological description (which will be the basis of a manuscript is pending) the following changes have been noted in shocked but viable animals.

A. Light microscope - 1) Edema, extra-cellular and about blood vessels and neurons, similar to that seen in cases of vascular vasogenic protein leak (breakdown in blood brain barrier).. 2) Anoxic neuronal changes - nuclear and cytoplasmic vacuolations, chromatolysis, increased cytoplasmic eosinophilia. 3) Endothelial changes - ring hemorrhages and vascular distortion. These light microscopic changes are more compatible with an anoxic-metab-lic plus vascular (vasogenic lesion than with pure anoxic change.

B. Electron microscopic changes - pending

C. Fluorescence microscopy - Evan's blue, when activated, fluoresces red. Fluorescence microscopy has shown in several animals perivascular fluorescence compatible with vascular protein leak (breakdown of blood-brain barrier.)

DISCUSSION:

The neuropathology and pathophysiology of hemorrhagic shock in sheep has been examined. Evidence for a metabolic and/or vascular lesion exists in contrast to the pure anoxic-ischemic type lesion described in the literature. Vascular and metabolic lesions perhaps can be modified by simple pharmacologic treatment.

PLANS:

1. With the aid of a consulting neuropathologist, we will quantify and describe the light microscopic, electron microscopic and fluorescence micropic changes.

2. Test a series of simple pharmacologic compounds to see if they can protect the cerebral microvasculature (blood-brain barrier) from pathologic change and hopefully improve organism viability.

3. Using depth electrode technique, further examine EEG changes in hemorrhagic shock, looking for useful clinical parameters.

4. Using the Kowa fundus camera, examine vascular permeability to fluorescein of the retina (blood-retina barrier).

5. Using the Xenon^{133} desoburation technique and the Doppler flow-meter, to measure and correlate cerebral blood flow with the above changes.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6791	72 07 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
71 07 01		U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	382
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Characterization and Kinetic Studies of Discrete Classes of Granulocytes

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 002300 – Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 03		DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	1
	CURRENT 73		0

19. RESPONSIBLE DOD ORGANIZATION

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NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) leukocyte isolation; (U) leukopoiesis; (U) hematology

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

25. (U) 71 07 – 72 06 Differential marrow cell counts have been made on about 20 subjects. Marrow cores were embedded in plastic and 3μ sections were selectively stained. Counts of 10,000 – 20,000 cells were made on triplicate from each sample. Agreement was excellent in good sections, but not enough data has been obtained for definitive evaluation.

*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.

Characterization and Kinetic Studies of Discrete Classes of Granulocytes

Barbara Cheney, M.A., Medical Zoologist

PROBLEM:

An accurate enumeration of the cells of the bone marrow is essential to effectively study granulocyte kinetics. The granulocyte develops, proliferates, matures, and is stored in the marrow until it is needed. Rate of development and cell numbers in the different states will vary being dependent on body needs, e.g. normal state, infection, and hematological disorders.

Cell counts made from bone marrow aspirates are not satisfactory as many cells are damaged in the preparation, and, therefore, unidentifiable. It is necessary to obtain a marrow "core" and prepare this so that the cells are intact and countable in situ. Once normal values are established, changes in marrow cell count in abnormal states can be interpreted. Marrow section data will be correlated with other available techniques (1, 2, 3).

APPROACH:

The marrow samples have been obtained at the University of Washington Hospital and prepared in the laboratory of the Division of Hematology. Cell counting has been done at LAIR. Marrow cores are fixed in glutaraldehyde, decalcified in EDTA, and embedded in methyl-methacrylate. Sections, 3 micra thick, are cut and stained with eosine-y and azure II after reaction with Naoce-Kl.

The granulocytes are tallied in the following groups: blasts, promyelocytes, and myelocytes (proliferative compartment); metamyelocyte and bands (maturational compartment); segmented cells (storage compartment). Eosinophilic granulocytes are totalled separately from neutrophilic granulocytes. Normoblasts, megakaryocytes, lymphocytes and plasma cells are also counted. There is an "unknown" category for cells that cannot be identified for whatever reason.

RESULTS AND DISCUSSION:

The preparation of these slides and the cell differentiation is a lengthy, time-consuming process. Approximately twenty subjects have been counted to date in triplicate. Agreement between good sections has been excellent and I believe that this method of enumeration will be satisfactory.

While the marrow preparation was being developed, samples were obtained from patients hospitalized for blood dyscrasias. It is only now that specimens are underway from normal subjects.

FUTURE PLANS:

Marrow cell counts from "normals" are in progress. More data has to be added from patients. Granulocyte survival and turnover studies are underway at the University of Washington.

REFERENCES:

1. Harper, Laurence A., Megakaryocyte Quantitation. J. Clin. Investigation, 47:452-457, 1968.

2. Harper, Laurence A., Kinetics of Thrombopoiesis. J. Clin. Investigation, 47:458-464, 1968.

3. Harper, Laurence A., Thrombokinetics in Man. J. Clin. Investigation, 48:963-974, 1969.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6813	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY ACTY	6. TASK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	386
b. [redacted]	62110A	3A062110A826	00	217
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Development of a Rapid Clinical Procedure for Assessing Blood-Oxygen Affinity Curves (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 09	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA 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 72	1	13
FISCAL YEAR CURRENT 73	.75	12

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE

20. PERFORMING ORGANIZATION
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22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Blood-oxygen affinity curve; (U) oxyhemoglobin dissociation; (U) blood-gas transport

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

25. (U) 71 07 – 72 06 Data accumulated during the past year indicates that the technique can be successfully applied as a rapid clinical procedure for determining blood-oxygen affinity. Baseline studies of healthy volunteer subjects produced normal values for standard measures of the shape and position of the oxyhemoglobin dissociation curve (P_{50} $\Delta P_{50/\Delta}$ log pH, and the "n" value for Hill plot slope). The test requires only 0.20 of blood and can be completed in 5 or 10 minutes, making it ideal for routine applications. In addition, the required instrumentation is commonly available in most hospitals or clinics and relatively unskilled technical personnel can become facile with the technique after a few trials. This latter advantage results in part from the fact that volumetric accuracy is not necessary and also that the required information is translated to an electrical signal, thus permitting a permanent record of the test that can be retrieved and checked as required. Application of the technique to several clinical problems has been initiated and although the results do not as yet warrant definitive conclusions, several interesting observations have been obtained. For instance, volunteers receiving the antimalarial drug primaquine displayed a significant (both statistically and physiologically) decrease in blood-oxygen affinity commencing on about the fourth day after start of the drug, a shift which was correlated with a decrease in functional hemoglobin. All subjects displayed a predictable negative relation between functional hemoglobin and P_{50} whether on or off the drug. Several in vitro trials have suggested that epinephrine may alter oxygen affinity. Additional tests of this effect are planned. Working arrangements with LGH's Intensive Care Unit have been formulated to permit analysis of oxygen affinity with patients undergoing treatment in this facility.

(Available to contractors upon originator's request)

Development of a Rapid Clinical Procedure for Assessing Blood-Oxygen Affinity Curves

J. Ryan Neville, Ph.D., Research Physiologist

PROBLEM:

Interest in oxygen transport has recently been stimulated by the discovery that the position of the oxyhemoglobin dissociation curve is significantly altered by changes in the intracellular concentration of a number of metabolic intermediates, particularly 2, 3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP) (1). These relatively small chemical entities, whose traditional role has been involved with the storage and release of chemical energy for cellular metabolic processes, have been shown to combine with the protein chain associated with a deoxygenated heme group and to affect the allosteric properties of the entire 4-chain hemoglobin molecule. As a consequence of this alteration, there is a change in the ease with which oxygen molecules react with the remaining heme sites and this manifests itself by a shift of the oxyhemoglobin dissociation curve. With increasing concentrations of high energey organic phosphates the curve shifts to the right (decreased affinity) and an opposite shift (increased affinity) occurs when these molecules are absent or present in lower than normal concentrtrations. For example, in a number of anemic conditions, one finds a decreased oxygen affinity associated with an increase in erythrocyte DPG (2). The increased oxygen affinity of stored blood, first noted some twenty years ago, is now known to be associated with decreases in erythrocyte DPG. Several recent reports have noted a dramatic improvement in the condition of infants with respiratory distress when transfused with fresh adult blood (3,4). The physiologic basis for this improvement relates to the enhanced capacity of fresh adult blood to deliver oxygen to tissues by virtue of its low oxygen affinity compared with fetal hemoglobin.

Thus, the interest of the medical community in oxygen transport phenomena is well justified, not only because such knowledge sharpens the precision and scientific basis of known diagnostic and treatment procedures but also, perhaps more importantly, because it offers potential insight into morbid processes that are not well understood.

A comprehensive evaluation of the functional status of the oxygen transport system requires a knowledge of the shape and position of the oxyhemoglobin dissociation curve or blood-oxygen affinity. Consequently, the degree to which interest in this area can be translated into practical clinical routine is most likely to be

conditioned by the ease with which an individual's blood-oxygen affinity can be measured. Unfortunately, the classic experimental approach to this problem is not, for various reasons, suitable for the usual clinical setting. In view of this, as well as the potential importance of such information to effective medical care, this effort has been devoted to perfecting and evaluating a method that will permit routine measurement of blood-oxygen affinity.

APPROACH:

The general approach has been described previously (See LAIR Report No. 8, Annual Progress Report FY 1971, 30 June 1971.), and recent work has incorporated this approach without any essential modification. Briefly, when a sample of blood to be tested is mixed with a buffered solution containing a small quantity of glucose, oxygen and yeast cells, the yeast will consume oxygen at a constant rate, the value of which depends primarily upon the number of yeast cells and the temperature. By injecting this mixture into the chamber of an electrochemical blood-gas apparatus, this oxygen consumption is determined by following the time course of the change in partial pressure of dissolved oxygen, most conveniently accomplished with the use of a strip-chart recorder. The resulting record of oxygen tension versus time has two clearly distinguished features: 1) an initial linear portion where the blood remains saturated and only dissolved oxygen is consumed by the yeast; 2) a sigmoid portion that first becomes evident when the partial pressure is reduced to the point where combined oxygen begins to dissociate from the hemoglobin. During this latter phase, both combined and dissolved oxygen are removed by the respiration of the yeast until the solution is completely depleted of oxygen. Since the oxygen consumption during this last phase remains constant and equal to the oxygen consumption during the initial linear phase, it is possible to compute the existing per cent saturation of the blood at any point along the sigmoid portion of the tension-time trace. Actually, tedious computations are unnecessary and the record can be graphically analyzed by merely equating the time axis of the sigmoid portion of the trace into proportional units of per cent saturation. In other words, if the combined oxygen requires 5 minutes for its removal by yeast the hemoglobin in the blood sample would be 80 per cent saturated at one minute, 60 per cent saturated at two minutes, and similarly for any other time interval on the sigmoid trace. The trace height, of course, is directly proportional to the oxygen partial pressure. Thus, all the information necessary for determining the oxygen affinity of the blood sample under the conditions of the test (temperature and pH) is available from inspection of this single, continuous record.

RESULTS AND DISCUSSION:

A baseline study of 10 hematologically normal volunteer subjects has indicated that the technique yields reproducible oxygen affinity data that confirms published standards. For instance, the Bohr shift was found to coincide extremely well with the accepted ratio of .48 for d log P_{50}/d pH. Fig. 1 graphically displays representative data, each point being the mean of 5 separate determinations.

In Fig. 2, plot of the data from a typical tension-time curve according to the Hill equation gives the expected regression line with slope equal (in the case shown) to 2.6. The plot is actually slightly curvilinear, again as expected, because of the theoretical deficiency of the Hill concept, which is based on a one-heme model of the hemoglobin molecule. This particular treatment of oxygen affinity data remains common despite such an erroneous theoretical basis because of its convenience and the fact that the straight line equation is followed reasonably accurately in the physiologic range.

The individual P_{50} values (oxygen pressure at 50 per cent saturation corrected to pH 7.4) found for the group studied ranged from about 24 to 28 mm Hg, daily average group values usually being about 26.5 mm Hg. This average coincides very well with standard values of 26 to 27 mm Hg cited in the literature. Repeated daily observations of individual P_{50} values remained reasonably fixed within a range of $\pm$ 1.0 mm Hg, the differences between subjects apparently being real and probably related to differences in hemoglobin concentration levels (See below.).

Observations have been completed on 10 normal volunteer subjects who received 15 mg of primaquine daily for two weeks. Oxygen affinity of each subject was measured three days and immediately prior to administration of the antimalarial drug, as well as at the 4th, 7th, 11th, and 14th day after start of the drug regimen. Results are shown in Fig. 3. These values represent both a statistically and physiologically significant shift of the oxyhemoglobin dissociation curve to the right (lowered oxygen affinity) and are teleologically consistent with the concept of a compensatory mechanism aimed at maintaining tissue oxygenation in the face of diminished oxygen combining capacity. This latter value declined from a mean group value near 20.0 vol. per cent prior to the drug to 16.0 vol per cent at the termination of the study. Fig. 4 shows the good negative relationship found in these subjects between P_{80} and functional hemoglobin. Such a relationship appeared to be valid whether or not the subjects were on primaquine. There was no indication of a relationship between either methemoglobinemia or diaphorase activity and P_{80}. Methemoglobin has been reported to shift the curve leftward (5) in a fashion similar to that produced by carbon

monoxide. In this previous study, the concentrations of methemoglobin (produced by nitrite and ferri-cyanide) were excessive- up to 70 per cent of total pigment- and not comparable to the relatively small methemoglobin levels observed in this study- usually less than five per cent. Any tendency to shift leftward in our study would presumably have been counterbalanced by the opposite shift in the curve caused by low hemoglobin levels.

The importance to tissue oxygenation of the shift in oxygen affinity observed in this study can be appreciated by referring to Fig. 5. The upper curve (solid line) shows the average condition of the group oxygen affinity before the drug was administered. Going from a normal arterial PO_2 of about 100 mm Hg to a normal mixed venous PO_2 of about 40 mm Hg would result in the delivery of 4.8 cc of oxygen for each 100 cc of cardiac output. After the primaquine-related shift in oxygen affinity and the decreased carrying capacity of the blood had become established, the situation was that shown in the lower solid curve. Noteworthy is the fact that despite the decreased oxygen capacity, oxygen delivery to the tissues is unimpaired, the blood giving up 4.8 cc of oxygen with each 100 cc of cardiac output. Had the oxygen affinity remained fixed in its pre-drug position (dashed line), the observed decrement in the oxygen carrying capacity of blood would have resulted in a 27 per cent decrease in oxygen delivery to tissues, the blood in this instance delivering only 3.5 cc of oxygen per 100 cc output. Thus, a potential tissue hypoxia was avoided by the efficient expedient of altering the oxygen affinity rather than resorting to the less desirable means of increasing cardiac output.

Preliminary observations have indicated that adrenalin may alter the oxygen affinity of blood. Attempts to substantiate such an effect in the near future have been encouraged by several recently published findings indicating the possible presence of adrenergic receptors in the erythrocyte membrane. Adrenalin was found to block a propranolol-induced release of DPG absorbed on the red cell membrane (8) and also to cause a measureable change in the erythrocyte deformability (9). Both of these reported effects support a concept that endows red blood cells with an active role in the control of the microcirculation. The clinical implications of such a concept could become extremely important.

SUMMARY AND FUTURE PLANS:

Information now available suggests that the yeast technique for measuring oxygen affinity can be used profitably in a clinical setting. The method is easy to perform, requires a negligible (0.2 cc) sample of blood, and produces rapid results of acceptable accuracy. In addition to follow-up experiments aimed at measuring

the effect of adrenalin on the oxygen affinity, future plans call for a survey of oxygen affinity in patients admitted to the Intensive Care Unit at Letterman General Hospital. Also planned is a study to determine whether or not oxygen affinity may play a role during experimentally induced hemorrhagic shock in rats.

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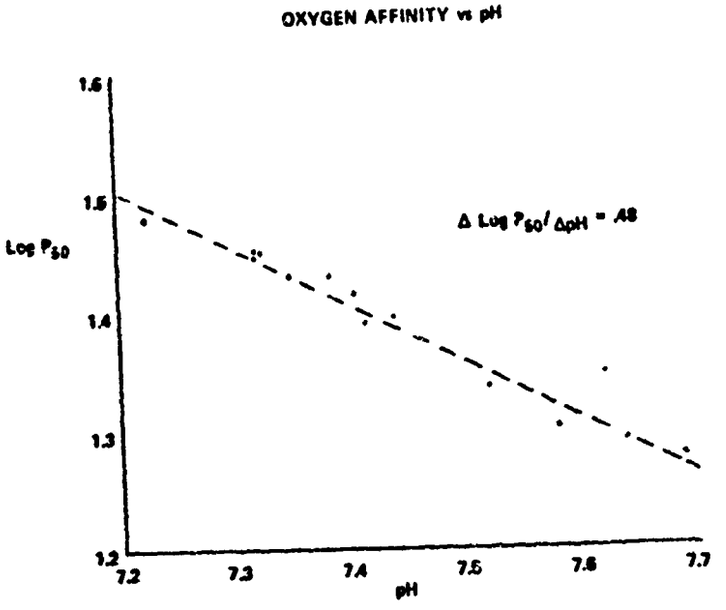
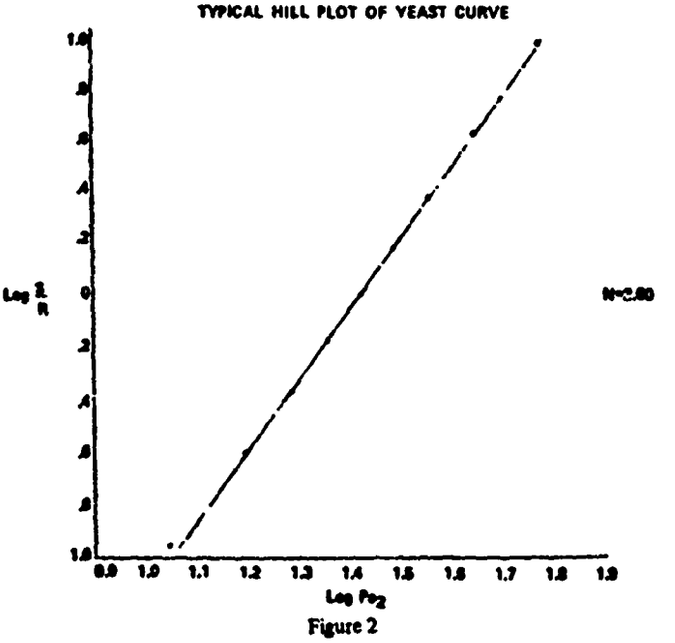


Figure 1



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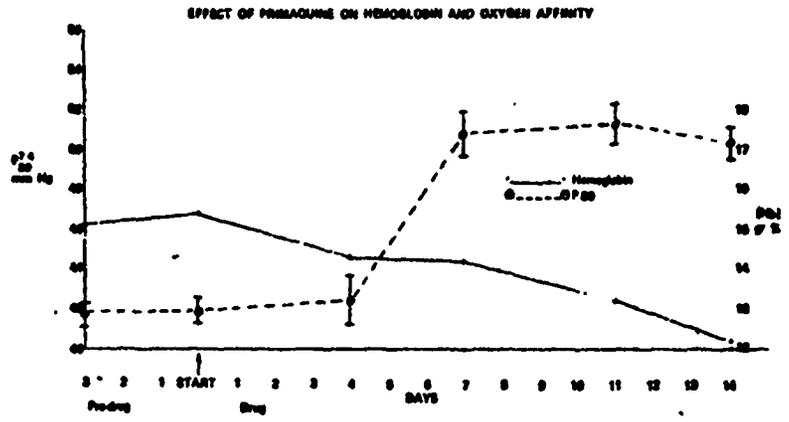

Figure 3

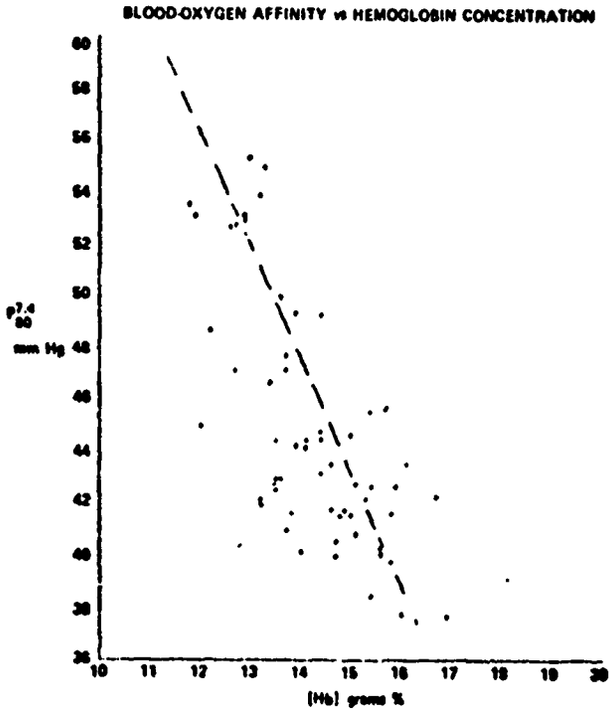

Figure 4

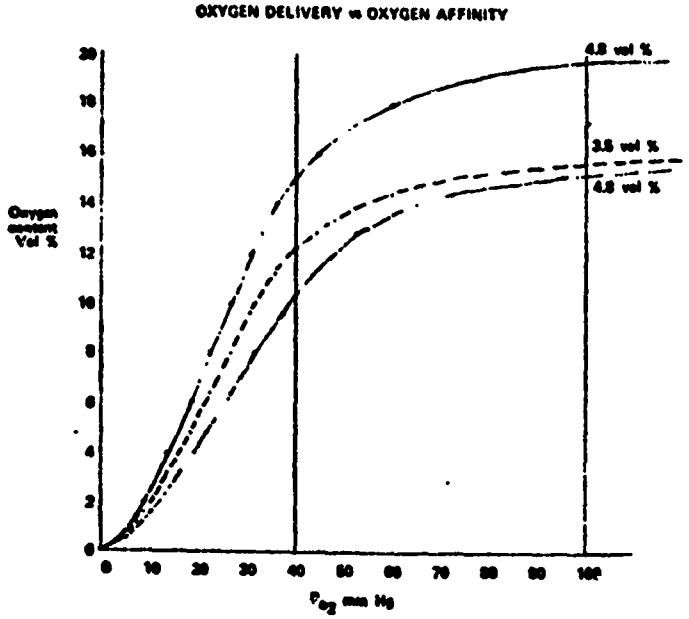
OXYGEN DELIVERY vs OXYGEN AFFINITY
Oxygen content Vol %
4.8 vol %
3.5 vol %
4.8 vol %
0
2
4
6
8
10
12
14
16
18
20
0
10
20
30
40
50
60
70
80
90
100
P_{O_2} mm Hg

Figure 5

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6926	72 07 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
72 03 16	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	387
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Pathogenesis of Coliform Induced Colitis in Mice (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 010100 – Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 03	73 01	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	.1	2
	CURRENT 73	1	3

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: 415-561-4714

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Experimental animal; (U) model; (U) mice; (U) colitis in mice; (U) bacterial colitis; (U) pathology

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

25. (U) 72 03 – 72 06 Analyses of the clinical and gross findings and study of selected cases microscopically indicates that the culture utilized was of low virulence. The animals went through a transient period of reduced food intake. Gross lesions were subtle and consisted of an increased moisture of the colonic content noted 7 days post exposure. No significant microscopic findings of a chronic nature were encountered. Bacterial populations of colonic contents indicated no trend toward homogenicity during the course of the study.

*Available to contractors upon originator's approval

Pathogenesis of Coliform Induced Colitis in Mice

Robert M. Kovatch, MAJ, VC

PROBLEM:

This study was designed to document the sequential development of the lesions in the digestive tract of mice exposed to a coliform bacterium tentatively identified as a lysine decarboxylase-negative Enterobacter hafnia. The agent was obtained from the National Institutes of Health and was originally isolated from a mouse during an outbreak at Fort Detrick, Frederick, Maryland. The objective of the study was to document the morphologic changes in exposed mice and to compare the lesions to those of chronic idiopathic ileitis and colitis of man. It was hoped this small laboratory animal system might be developed to study the complex mechanisms active in the inducement of chronic idiopathic ileitis and colitis of man.

APPROACH:

The study will be divided into two phases (1) cesarean-derived, barrier maintained mice will be exposed to 2 levels (10^7 and 10^5) of a washed saline suspension of E. hafnia by gavage and by intrarectal instillation. From these four groups of animals, 2 animals will be killed at weekly intervals for 5 consecutive weeks. Gross and microscopic alterations will be evaluated to determine the most favorable parameters for reproducing the disease. (2) Utilizing information derived from phase I of the study, additional barrier maintained mice will be exposed to the appropriate number of bacteria and by the better of the routes of exposure determined in phase I of the study. Animals will be killed at 1, 3, 6 and 9 days and 2, 3, 4, 6, 12 and 18 weeks post exposure. The gross and microscopic evaluation of the developing lesion and their residua will be documented. The lesions will be compared to those reported for chronic idiopathic ileitis and colitis of man.

RESULTS AND DISCUSSION:

Sixty mice were divided into 5 groups (4 exposed and 1 control group) and exposed to 0.1 cc of a washed saline suspension of a coliform culture determined to contain 1.32×10^7 and 3.5×10^8 cells/cc. These suspensions were given by gavage and intrarectally. Control mice were given 0.1 cc of saline by both routes. Two animals from each group were killed at weekly intervals and autopsies were done. From one animal of each group fecal samples were taken from the colon for bacteriological determination. Tissues were selected and

fixed in 10% neutrol buffered formalin for microscopic examination. Analyses of the clinical and gross findings and study of selected cases microscopically indicates that the culture utilized was of low virulence. The animals went through a transient period of reduced food intake. Gross lesions were subtle and consisted of an increased moisture of the colonic content noted 7 days post exposure. No significant microscopic findings of a chronic nature were encountered. Bacterial populations of colonic contents indicated no trend toward homogenicity during the course of the study.

FUTURE PLANS:

During FY 1973/phase I of the study will be repeated utilizing another isolate currently being analyzed at the National Institutes of Health. Preliminary studies there indicate they now have an isolate of high virulence. Should phase I of the study show promise utilizing the new isolate, phase II of the planned study will be done.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6927	72 07 01	DD-DH&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
72 03 20	D.Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	388
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Detection of (Superior Sagittal Sinus) Thrombosis (In Dogs) Using Radionuclide Labelled Fibrinogen

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 – Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 03	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA 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 72	.1	4
	CURRENT 73		7

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE

ASSOCIATE INVESTIGATORS
NAME:
NAME:

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) cranial venous sinus occlusion; (U) I^{-131}–tagged fibrinogen; (U) large vessel occlusion

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

25. (U) 72 03 – 72 06 A method of inducing occlusion inside a tube in the dorsal longitudinal sinus of dogs has been devised. Partial tagging of fibrinogen with Te^{99} M-pertechnitate has been accomplished (approximately 50%); however, we are attempting to obtain 95% tagging. Some satisfactory Pho Gamma camera scanning has been achieved.

*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.

Detection of (Superior Sagittal Sinus) Thrombosis (In Dogs) Using Radionuclide Labelled Fibrinogen

Michael M. Bronshvag, MAJ, MC

PROBLEM:

Detecting vascular occlusive disease is most gratifying before it has caused infarction or embolization. Superior sagittal sinus (and other dural venous) occlusion is a good example of this, for it produces minimal clinical symptomatology (headache, raised intracranial pressure) or none, before extension of the clot into cerebral veins causes cerebral infarction. Demonstrating such clots presently depends upon angiography. Angiography carries inconvenience and risk. Intravascular clots are known to accumulate fibrinogen as fresh fibrin as they propagate or maintain their size. Propagating leg venous thrombi can be detected using I^{125} and I^{131} labelled fibrinogen and a point-imaging detector. However, virtual imaging (analogous to organ scanning) cannot be accomplished in humans using these radionuclides because of their high emission of alpha and beta particles relative to gamma emissions cause damage to tissues.

These isotopes can be used in animals to attempt clot imaging. If these techniques are successful, I^{123} can be substituted for human use since it is essentially a pure gamma emitter (but is quite expensive and therefore unsuitable for routine experimental use). Also Tc^{99M} may be used in humans and animals being a pure gamma emitter, having a 6 - 6 1/2 hours half-life, and being inexpensive; however, attempts to label it with fibrinogen have not been uniformly successful. Therefore, we plan experiments with I^{125} I^{131} and Tc^{99M} in dogs to devise a technique for clot detection by virtual imaging suitable for human use (I^{123} or Tc^{99M}).

APPROACH:

A. Operation: Adult dogs are anesthetized and a midline incision made in the head down to the skull with a Bovie coagulating scalpel. A hole is drilled through the skull in the midline in the superior sagittal sinus and a 2.5 - 5.0 cm length of 190 PE polyethylene tubing inserted. The drill hole is closed in the bone wax and the scalp wound sewn.

B. Preparation of Radionuclides: Iodine tagged fibrinogen is prepared by the method of alkaline incubation (ICI vol. 42, pg. 346, 1963). Technitium tagged fibrinogen is prepared by Clyde Cole, CPT,

MSC, Radiopharmacist, LAIR, by a modification of the TC-Albumin method of zirconium electrolysis (J. Nuc. Med. vol. 13, pg. 180, 1972).

C. Scanning: Radionuclide-tagged fibrinogen is injected into control and experimental animals (before and after tube placement); AP and vertex brain scanning is done by conventional techniques using the Pho Gamma III camera. A positive scan will show a virtual image of the experimentally induced clot.

D. Autopsy: All dogs are sacrificed subsequently and examined for success of tube placement, presence of clot, and also presence of cerebral infarction or surgical complications.

RESULTS AND DISCUSSION:

1. The technique for tube placement is successful without complications 50% of the time. More posterior placement of the tube should result in a higher success rate.

2. Tagging attempts with Tc^{99M} have resulted in a mixture of 50% tagged technitium and 50% clottable technitium-fibrinogen complex. Hopefully, empiric adjustment of the zirconium electrolytic procedure will result in higher tagged per cent.

3. In one animal to date injected with the Tc-Fibrinogen complex after successful tube placement, satisfactory clot imaging was obtained.

FUTURE PLANS:

1. Refinement of above techniques is planned. I believe a satisfactory new clinical tool will emerge.

2. Other workers have studied disappearance rates of fibrinogen as an indicator of intravascular thrombosis. LTC Logan (Hematologist and Chief, Clinical Research Support Division, LAIR), is studying this technique and we plan to collaborate with him to correlate scan and fibrinogen disappearnce studies.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	3. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6928	72 07 01	DD-DR&E(AR)636

2. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8b. DISP'N INSTR'NS	8c. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	389
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) The Relationship of Polymorphonuclear Neutrophil (PMN) Chemotactic Activity to Inflammatory Periodontal Diseases in Military Personnel

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 07	73 07	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA 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 72	.1	7
	CURRENT 73	1	4

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Hutchinson, R.A., LTC DC
NAME: Lilly, G.E., COL DC DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) periodontal disease; (U) periodontitis; (U) chemotaxis; (U) polymorphonuclear neutrophil; (U) white blood cell; (U) bacteria; (U) inflammation

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

23. (U) (1) Determine the variability of the PMN chemotactic response in military personnel. (2) Determine relationship between PMN chemotactic response and inflammatory periodontal disease. (3) Determine relationship between PMN chemotactic response in other specific infectious diseases in pediatric patients.

24. (U) White blood cell (WBC) migration (chemotaxis) will be measured in the laboratory using a Boyden chamber. The WBC chemotactic activity of cells from patients with severe inflammatory periodontal disease will be compared with the chemotactic activity of cells from healthy controls.

25. (U) None. New Study.

*Available to contractors upon originator's approval

DD FORM 1 MAR 88 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE.

The Relationship of Polymorphonuclear Neutrophil (PMN) Chemotactic Activity to Inflammatory Periodontal Diseases in Military Personnel

Major Thomas R. Tempel, DC

PROBLEM:

The primary objective of this study is to evaluate polymorphonuclear neutrophil (PMN) chemotactic response as a possible laboratory means of detecting the degree of inflammatory periodontal disease in a soldier. Our hypothesis is that variations occur in the chemotactic response of polymorphonuclear leukocytes in individuals, who are otherwise "normal"; and such differences account for variations in individual susceptibility, resistance, and response to infectious inflammatory diseases. The chemotactic response can be measured in the laboratory and variations in PMN chemotaxis can be correlated with the severity of inflammatory periodontal disease.

Present evidence favors the concept that oral bacteria are the most important etiologic agent in periodontal disease. The bacteria colonize as dental plaque adjacent to the gum tissue. PMNs are found in the normal gingival sulcus as well as the periodontal disease lesion adjacent to the dental plaque and are thought to play an important role in the patient's resistance to periodontal disease. Patients with reduced numbers of PMNs or PMN chemotaxis defects (neutropenia, agranulocytosis, and Chediak-Higashi disease) are known to be prone to severe destructive periodontal disease.

Recent studies have established that PMN chemotaxis defects occur in some individuals. Such individuals have altered inflammatory responses and are predisposed to infections. This abnormality has been recognized in specific diseases involving children and adults.

In view of recent findings concerning chemotaxis, this defect may conceivably be a factor in the etiology and course of other inflammatory diseases. Current laboratory procedures and findings offer a method for evaluating PMN chemotaxis which could prove of value in de-

determining etiology, course, and patient susceptibility to inflammatory diseases, and provide methods for monitoring and diagnosing such diseases.

In 1962 Boyden developed an in-vitro method of studying cell migration which is sensitive, reproducible, and yields quantitative data relative to PMN chemotactic response to a variety of chemotactic factors.

Studies during the past 10 years have clearly established the following:

1. PMNs respond to a chemotactic substance by unidirectional migration toward the greatest concentration of the attractant substance (chemotaxis).

2. Most bacteria elaborate a product that is directly chemotactic for PMNs. It is not known if fungi or mycoplasma elaborate a similar chemotactic product.

3. Antigen-antibody complexes (IgG and IgM) generate a chemotactic factor by activating complement. This factor has a molecular weight of 15,000 and is derived from C5 with the active fragment C5a.

4. PMNs from patients with Chediak-Higashi disease and diabetes have been shown to be defective in their ability to respond to chemotactic stimuli.

5. Recent evidence suggests that patients with inflammatory periodontal disease or stomatitis may show variations in PMN chemotactic response when compared to controls.

APPROACH:

Our hypothesis will be tested by correlating the periodontal disease index with the PMN chemotaxis index. The experimental methods and controls are outlined below.

Thirty patients with varying degrees of periodontal disease will serve as subjects in this study. Thirty additional individuals without periodontal disease will serve as healthy controls.

All subjects will be involved in the following experimental procedure:

1. Periodontal Disease Index:

a. Recording of personal data: age, rank, social security number, sex, and a brief medical and dental history.

b. Clinical dental examinations will be given and the degree of periodontal disease measured using the Ramjford periodontal disease index. A sample of dental plaque will be removed with a scaler and frozen in a preweighed plastic tube.

2. PMN Chemotaxis Index:

a. Ten (10) ml of blood will be drawn from the antecubital vein into a heparinized tube. Ten (10) additional ml of blood will be used to prepare fresh serum.

b. The heparinized blood will be sedimented in a 2 percent methyl cellulose solution. The plasma is separated and PMNs diluted in Hanks or Gey's solution. Cells are washed twice and concentration adjusted to 2.2 x 10^6 cells per ml by centrifugation, cell counts, and dilution. The final suspension of PMNs will be placed in the upper part of the Boyden chamber after the chemotactic substance is placed in the lower part.

c. The serum will be removed from the clotted blood and frozen if not used immediately.

d. Chemotactic stimuli:

(1) Bacterial chemotactic factor will be prepared from strains of human oral bacteria and purified by Biogel P-2 molecular sieve and Dowex I ion exchange column chromatography.

(2) Endotoxin from an oral gram negative microorganism will be used to activate fresh serum complement and the 15,000 MW C5a chemotactic factor purified on a G-2000 molecular sieve column.

(3) Two lambda of the dental plaque sample will be used to activate 1.0 ml of the patients' fresh serum. Serum inactivated at 56 degrees C for 30 minutes will be a negative control.

e. Millipore filters will be numbered and placed in the Boyden chamber.

f. The chemotactic stimuli or control will be placed in Gey's or Hanks solution in the lower part of the Boyden chamber; the PMN suspension in the same medium will be placed in the upper part.

g. The following experimental protocol will be followed for each subject. The number of PMNs in the upper part of the chamber remains constant and the PMN response to varying chemotactic stimuli will be evaluated in the following manner. Each chamber will be run in duplicate.

Chamber Number	Chemotactic substance		PMNs
1 & 2	Bacterial CTX	50 lambda	2.2×10^6
3 & 4	" "	200 "	"
5 & 6	" "	400 "	"
7 & 8	C5a Complement (C) derived CTX	50 "	"
9 & 10	" "	200 "	"
11 & 12	" "	400 "	"
13 & 14	heat inactivated C plus dental plaque	200 "	"
15 & 16	heat inactivated C plus dental plaque	200 "	"
17 & 18	patient serum plus dental plaque	200 "	"
19 & 20	Hanks medium control	200 "	"
21 & 22	saline control	200 "	"

h. The chambers are incubated for three hours at 37 degrees C, .5 percent CO_2 in high humidity.

i. Filters are washed, stained with hematoxylin, cleared and mounted on microscopic slides with a cover glass.

j. Cells will be counted on the bottom of the filter discs using 450X magnification and a grid. Ten random fields will be counted and averaged. The average number of PMNs per high power field is the chemotactic activity. The chemotactic activity minus the background cell migration is the chemotaxis index. Reading of slides is done in a single blind manner on coded slides. When the chemotaxis values are established, the values are decoded.

k. The chemotaxis index for each subject will be correlated with the periodontal disease index using statistical methods.

RESULTS AND DISCUSSION:

None. New study.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6929	72 07 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	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	391
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Some Effects of Hypertonic Medium on Cultured Mammalian Cells (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 04	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	.125	1
FISCAL YEAR CURRENT 73	.5	3

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

Cell growth; cell division; stimulation of cell growth

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

23. (U) Quantitate changes effected on mammalian cells by hypertonic treatment in order to determine if a reasonable means can be found to stimulate cell growth and division which would be applicable to closure of wounds.

24. (U) Cell culture experiments using biochemical, light microscopy and electron micrographic technique.

25. (U) None. New study.

*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.

DERMATOLOGY RESEARCH DIVISION

Program Element 6.21.10.A
Bio-Medical Irvestigations

Project Number 3A062110A822
Military Internal Medicine

Task Area Number 00

- - - - - - - - - - -

Project Number 3A062110A831
Tropical Medicine

Task Area Number 00

DERMATOLOGY RESEARCH DIVISION

The Dermatology Research Division studies the inter-related effects alone and in combination of water immersion, friction, heat and humidity, fungal and bacterial infections, and insect repellents on man's skin: The multi-disciplinary team approach is used (5 M.D.'s, 5 PhD's, 7 M.S.'s) in seeking solutions to pressing military dermatological problems.

One group designs instruments for producing and measuring various degrees of friction under scientifically controlled conditions and develops ways to prevent and treat blisters on the palms and soles of soldiers.

Another group studies prickly heat rash, its cause and prevention. Prickly heat rash is produced by putting Saran Wrap patches on a man's back for 2 to 4 days.

Mosquito repellents are being developed and tested on men that resist sweat-off and wash-off substantially longer than those now available.

Volunteers are infected on the forearm with a fungus that causes 'athletes foot'. Their response to the infection is closely studied, particularly the way they develop immunity. Such studies should lead to a vaccine, a better medicine to prevent such a skin disease, and more rapid, accurate, and economical methods to diagnose fungal infections.

Numerous basic scientific studies concern the way substances penetrate the skin barrier via the stratum corneum, and developing antifungal, antibacterial, anti-friction, insect repellents, and skin waterproofing substances to stay in the skin.

Dermatological research has been advanced greatly by having 30 full-time military medical research volunteers, permitting long, closely controlled experiments in the laboratory and field.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6800	72 07 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
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	155
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) More Effective Topical Repellents Against Malaria Bearing Mosquitoes (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 -- Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 11	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	20
	CURRENT 73	1	35

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Brodel, C., SP-4

NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) wash-off; (U) sweat-off; (U) human volunteers
(U) repellent; (U) mosquito; (U) skin; (U) stratum corneum; (U) persistence; (U) polymer formulations;

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

25. (U) 71 07 – 72 06 Wash-off vs sweat-off testing revealed that water wash-off is a much more severe test of a repellents' wash resistance than sweating.

*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.

More Effective Topical Repellents Against Malaria-Bearing Mosquitoes

William A. Akers, COL MC
Charles Brodel, SP4

PROBLEM:

Our objective is to develop a broad spectrum topical preparation which will repel malaria-bearing mosquitoes and other disease vectors, which will persist one or more days resisting removal by excessive evaporation, skin penetration, sweat and water, and abrasion. This year we had a 7-month gap in replacing the principal investigator so our activity was lower than desired. We tidied up some loose ends in evaporation studies of repellent-polymer formulations and in vivo tests of a few new chemicals and other formulations from industrial and academic laboratories. Our main studies compare the repellent loss from man's skin from sweating and water wash-off. We wish to develop and quantitate simpler, faster, and more economical screening methods- preferably in-vitro.

A new repellent mentioned last year, diethylbenzenesulfonamide (DBS), a crystalline material at room temperature, was supplied by Dow Corning Corporation. A cosmetically acceptable formulation contained 2 parts of DBS and 1 part of silicone polymer. Applied to give 0.25 mg/cm^2 of DBS to non-sweating forearms, the formulation protected against biting 19 hours. When the formulation was applied to give 1 mg/cm^2 of DBS and the entire 105 cm^2 repellent-treated arm area was rinsed every hour with 250cc of water at room temperature, the repellent protected 8 hours. This was remarkably superior to the performance of the present standard repellent, diethyl-meta-toluamide (m-DEET) which protected 5 hours on dry skin and only 2-3 hours when subjected to hourly rinses.

Unfortunately, the USA Environmental Hygiene Agency (USAEHA) toxicological tests in 2 species, rats and rabbits, clearly showed DBS at oral dosage of 500 mg/kg to be teratogenic by producing smaller fetuses and limb abnormalities.

APPROACH:

Formulating repellents with film-forming polymers, and additives in alcohol and other appropriate solvents will continue to be explored by cooperating industrial and academic laboratories; new systems showing water resistance, especially those showing both water resistance and cosmetic appeal, will be submitted to LAIR for mosquito challenge and water rinse testing on the arms of volunteers. LAIR feedback to cooperating polymer laboratories will aid in reformation to achieve the optimum compromise in necessary properties. Candidates showing improved sweat-off resistance (superior to simple DEET), cosmetic acceptability, and no skin irritancy will be considered for future field trials. Toxicological clearance will be obtained when necessary from the U.S. Army Environmental Hygiene Agency (USAEHA).

Wash-Off vs Sweat-Off of DEET: This pilot study compared the duration of DEET on men's forearms to washing with warm water and to sweating. DEET, 0.31 mg/cm^2, was applied to a 7x15 cm area on 4 volunteers' volar forearms, then was washed with water (temperature 34^{o}C, one liter per minute) for 3 minutes. The standard mosquito repellency test was conducted immediately. If the volunteer did not receive 2 bites, the 3 minute washing was repeated with subsequent repellency testing until the repellent failed.

The volunteer then had DEET, 0.31 mg/cm^2, applied to the 7x15 cm area on the opposite forearm. To cause sweating, he entered a controlled environment room (temperature 40^{o}C, relative humidity 48%) for 10 minutes, then exercised on a stationary bicycle for 10 minutes. On leaving the room, and without drying his forearm, he had the standard mosquito repellent testing performed. If the

volunteer did not receive 2 bites, he remained outside the chamber for 10 minutes, reentered the hot room, sat 10 minutes, bicycled 10 minutes, left the room, and was tested again. If he failed to receive any mosquito bites, he had the standard insect repellent test performed every 30 minutes until the repellent failed.

Ranking 4 Repellents: We wished to rank the 4 best mosquito repellents in use as to their persistence and resistance on man's skin against sweating, since this information is not available. We tested (1) DEET, (2) dimethyl phthalate, DMT, (3) Rutger's 612, R-612 (2 ethylhexanediol - 1, 3) and (4) Indalone. These were tested in 2 groups of 4 volunteers. Repellents were applied to 2 rectangular areas, 7x10 cm^2 on the volar wrist and proximal forearm. The site of application of each repellent was rotated within each group, resulting in a 4x4 Latin square design. Each half-hour after application the repellent treated areas were exposed to 200 Aedes aegypti mosquitoes. The tests were conducted in a controlled-environment room at 32°C and 90% RH. To induce and maintain sweating, the men participated in 3 ten-minute exercise periods each hour, usually on bicycles.

RESULTS AND DISCUSSION OF RESULTS:

We did in vivo and in vitro testing on approximately 20 chemicals or formulations supplied by contractors or industrial laboratories. One new chemical and a new formulation concept revealed distinct promise; but patent applications are underway so we cannot discuss them.

Wash-Off vs Sweat-Off of DEET: Three men received 2 bites after a single 3 minute warm water wash. The other volunteer was bitten after the second wash. After 40 minutes of 40°C and 48% RH, only one volunteer received 2 bites although all had sweat running-off the treated area at the end of each test period. One hour after they left the chamber 2 of 3 remaining men were bitten. These results confirm that water wash-off is a much more severe test of a repellents' wash resistance than

sweating. Wash tests are also simpler, faster, and more economical to perform.

Ranking 4 Repellents: The sweat test could not clearly separate DEET and Indalone as to their resistance to removal by sweating. No significant difference between DMT and R-612 was demonstrated. Both dry and sweating tests revealed no apparent difference between the sites of application on the wrist and proximal forewarms, but the difference between individuals still persisted.

Duration of Repellents

Means in Hours

	DEET	DMT	Indalone	R-612
Dry	6.2	2.9	2.2	3.9
Sweating	3.6	2.2	5.1	1.9

Ranking of Repellents

Dry				Sweating			
Group 1		Group 2		Group 1		Group 2	
1.	DEET	1.	DEET	1.	DEET	1.	Indalone
2.	R-612	2.	R-612	2.	Indalone	2.	DEET,DMT
3.	DMT	3.	DMT	3.	DMT,R-612	3.	R-612
4.	Indalone	4.	Indalone				

Group 1 was tested in the morning; group 2 in the afternoon.

We plan in vivo wash studies to rank these repellents. It is impossible to have people sweat the same amount using only heat or exercise as the stimulus. Sweat tests are more difficult to do than dry protection time tests, while wash tests are the quickest.

The previous principal investigator is working on the second draft of his technical report.

FUTURE PLANS:

Much information concerning the evaporation rate of DEET, when applied independently or in combination with various polymers, has already been gathered by utilizing a radio-labelled repellent and a sophisticated detecting apparatus. Operating this instrument manually demands around-the-clock attention so the apparatus is being automated. When completed the setup will permit more rapid screening of potential repellents with the most promising being tested on volunteers' forearms for data verification. Besides evaporation rates, repellent penetration rates through the stratum corneum will be studied. Basic studies concerning how polymers bind to the skin will be undertaken.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6804	72 07 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
71 07 01	K.Complete	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	156
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Investigation on the Pathogenesis, Prevention and Management of Tropical Acne (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 – Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 09	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	0	0
	CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

20. PERFORMING ORGANIZATION

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Presidio of San Francisco, CA 94129

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TELEPHONE:
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Griffin, T.B., M.D.
NAME: Henning, D., Ph.D.

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) acne; (U) skin; (U) tropical acne; (U) skin lipids

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

25. (U) 71 07 – 72 06 The study has been completed. The study group consisted of 11 patients with active tropical acne evacuated from the Republic of Vietnam and 3 patients with severe acne vulgaris who had never served in the tropics. The following aspects of tropical acne were investigated: (1) clinical and routine laboratory studies, (2) endocrinology, (3) histopathology, (4) microbiology, (5) biochemistry and (6) applied heat, humidity and frictional stress. All of the tropical acne patients had a previous history of mild to moderate acne vulgaris prior to their tour in Vietnam. The types of lesions which developed in these patients were clinically and histopathologically identical to those seen in severe pustular, cystic acne patients. The striking difference is the sparing of the face and the widespread distribution, including both the arms and the legs. Five of the tropical acne patients and one control acne patient were found to have low urinary testosterone levels. There was no direct relationship to the serum testosterone levels in these patients. In general, the urinary 17-hydroxycorticoids and the 17-ketosteroids were normal and no pattern or trend was apparent with the ACTH stimulation or Decadron suppression testing. One can only speculate on these paradoxical findings of testosterone values in patients with acne. The role played by androgens in the pathogenesis of acne still awaits clarification.

*Available to contractors upon originator's approval

Investigation on the Pathogenesis, Prevention and Management of Tropical Acne

Charles W. Lewis, LTC MC
Tommy B. Griffin, MAJ MC
David R. Henning, CPT MSC

The designation "Tropical Acne," is used to describe a severe form of cystic acne that typically affects military personnel assigned to a hot, humid climate. Recently, tropical acne became the major cause for evacuation for skin disease from RVN. It differs from acne vulgaris in that individuals affected are generally considered to be past the acne age. The onset is often explosive in nature involving the posterior neck, chest, back, buttocks, proximal extremities but sparing the face. Clinically the individual lesions resemble acne vulgaris in its severest form with primarily deep seated, painful cystic lesions that frequently result in severe scarring and is extremely resistant to therapy under the adverse tropical climate. Upon returning to a temperate climate, many cases resolve spontaneously.

The existing literature contains no detailed studies on the disease or the possible similarities and differences between tropical acne and common acne vulgaris.

Therefore, in April 1967 this collaborative study to investigate various parameters of tropical acne was undertaken at the Dermatology Service of Letterman General Hospital with the Letterman Army Institute of Research, Presidio of San Francisco, California.

APPROACH:

The study group was composed of 11 patients with active tropical acne evacuated from the Republic of Vietnam, and 3 patients with severe acne vulgaris who had never served in the tropics. All patients were

admitted to the Dermatology Service at Letterman General Hospital for comparative studies, received the standard hospital diet, were not allowed to bathe or shower until initial bacterial and lipid samples were obtained. The following aspects of tropical acne were investigated: (1) clinical and routine laboratory studies, (2) endocrinology, (3) histopathology, (4) microbiology, (5) biochemistry and (6) applied heat, humidity and frictional stress.

Clinical and Routine Laboratory Studies: Each patient received a complete history and physical examination, including an epidemiological family survey. Routine laboratory studies included complete blood count, urinalysis, Wintrobe erythrocyte sedimentation rate, urine culture, Venereal Disease Research Laboratory test for syphilis, fasting blood sugar, serum protein electrophoresis, total serum lipids and lipid fractionation, blood urea nitrogen, serum cholestrol, calcium, phosphorus, lactic dehydrogenase, serum glutamic oxalacetic transaminase, uric acid, bilirubin, alkaline phosphatase, serum vitamin A, and carotene. A chest xray was also obtained on each patient. CBC, ESR and urinalysis were repeated weekly times 3 and any abnormality of the above studies was evaluated as necessary.

Endocrinology: Thyroid evaluation included a T_3 uptake, RAI 131 thyroid scan and serum cholesterol. Additionally, several patients received PNI determinations. The pituitary and pancreas were evaluated by a glucose tolerance test with a concomitant plasma growth hormone level determination. Adrenal and gonadal evaluation was done by collecting an initial 24 hour urin for testosterone as described by Weigienka, et al., 17 ketosteroids, and 17 hydroxycorticosteroids and creatinine.

Histopathology: Biopsies of acne lesions in various phases of development were histologically processed and stained with hematoxylin and eosin, to elucidate the histopathology of the disease process.

Microbiology: The primary objective of the bacteriological studies was to compare skin surface flora

of patients with tropical acne, who had just returned from Vietnam, with the skin surface flora of patients who have severe cystic acne but who have not lived in a tropical environment.

Quantitation of microorganisms were obtained by serial dilution and spread plate techniques. Anaerobic culture was accomplished by evacuating and flushing Case-anaerobe jars three times with a mixture of 95% nitrogen and 5% carbon dioxide. Corynebacterium acnes were identified by typical colony morphology.

Biochemistry: Various skin sites of the tropical acne, control acne patients and uninvolved volunteers were extracted with ethyl acetate. The extract was evaporated to dryness and the residue quantitatively determined.

Results and Discussion:

Tropical acne developed in this group of patients after 2 weeks to 5 1/2 months (average 2.8 mos.) of exposure to a tropical climate. The average age at onset was 24.7 years (S.D.=8.27 yrs) with a range of 18 to 43 years. These men had all experienced acne vulgaris in their teens with an average age of onset of 15.5 years (S.D.=1.78). None of the patients previously had been completely free of acne, and 7 of the 11 patients had active acne lesions upon arrival in the tropical environment. Except for 1 patient, these 7 patients had minimal acne involving primarily the face or the face and upper back.

The severe cystic lesions developed on the skin over the neck, back, shoulders and chest in all patients. Eight of the 11 patients had involvement of the upper arms usually ending at the antecubital fossae, but one patient had lesions extending to his wrists. Five of the 11 also had cystic lesions over the buttocks and thighs. The skin surface of the face remained remarkedly clear of lesions in all patients.

A variety of tropical acne medications, soaps and systemic antibiotics (mainly Tetracycline) were

uniformly unsuccessful in halting the progression or inducing a remission of this disease process as long as the patient remained in RVN. However, upon departing RVN to a temperate climate, 3 patients experienced rapid clearing of lesions within 2 to 3 weeks.

Negative or normal values were obtained for the following: urine analysis, urine culture, serology, chemistry scan (SMA-12), glucose tolerance test, thyroid scan and chest xrays.

One-half of the patients have an elevated white blood cell count. The erythrocyte sedimentation rate was elevated in all patients studied, ranging from 13 to 37mm/hour. This finding plus the elevation of the gamma globulins in 11 of 14 patients and reversal of the A/G ratio in 4 of the 14 patients most likely represents the chronicity and the extensive low grade infection of acne lesions seen in these individuals. There was no consistent abnormality demonstrated by the electrophoretic pattern and most cases had normal findings.

Thyroid evaluation was essentially normal in all patients studied. Two individuals had borderline low values for the I^{131}, but gave normal results for PBI, T_3 and thyroid scans.

Vitamin A and carotene levels were normal except for 1 patient who had been taking one capsule daily of Vitamin A, 50,000 units for the past 6 months on his own initiative.

Only 4 patients with tropical acne had completely normal findings for serum lipids and fractionation. Various abnormalities were found primarily reflecting low values: total lipids low in 5 of 13; phospholipids low in 6 of 13; triglycerides low in 3 of 13 and elevated in one case; and cholesterol low in 3 of 13. There does not appear to be any specific pattern to these abnormally low values as some patients had only one fraction decreased, while others had 2 or 3 low values.

The baseline urinary testosterone value was low in 6 of the 14 patients. The one control patient with an extremely low level proved to have acquired gonadal dysfunction manifested by oligospermia and low testosterone levels in his urine. Unfortunately, at the time this patient was studied, serum testosterone determinations were not available to us. The follicle stimulating hormone in this patient was less than 50 mouse units, and his chromosome karyotype was normal.

Five of 11 tropical acne patients had low urinary testosterone levels; patients #5 and #11 having extremely low values on repeated determinations. Patient #5 had a normal serum testosterone and a low follicle stimulating hormone level of $\leq$ 15 mouse units. Patient #11 had a borderline low serum testosterone. The other 3 men with low urinary testosterone values had a low serum testosterone level, an elevated level, and in the third the test was not available at the time the patient was studied.

An additional patient had a normal baseline urinary testosterone, but low values following ACTH stimulation and Decadron suppression studies. The baseline urinary hydroxy- and ketosteroid determinations were normal in all but one who had a low value for 17-ketosteroids.

The ACTH stimulation tests were normal in all men studied except one, who also had low urinary testtosterone and normal serum testosterone levels. This patient did not show normal stimulation, but he did have normal suppression values.

The Decadron suppression tests were normal in all patients except two. These 2 men did not suppress below 4mg level of 17-hydroxycorticoids, which is considered to be the normal level for suppression. One of these patients, #8, also had the lowest serum testosterone value in this study.

Histopathology: Examination with the light microscope of biopsy specimens of comedos, including cysts and inflammatory lesions of various degrees of severity

from seven tropical acne patients revealed no apparent differences in the basic histopathologic changes when compared with biopsies taken from similar control patients with acne or with previously reported findings for acne vulgaris.

All biopsies from inflamed cystic lesions represent the end results of severe acne. When the follicular structure becomes dilated with a keratin plus to the point of rupture, the keratin fragments, lipid material and bacteria are dislodged into the dermal tissue. An intense foreign body reaction develops that is usually accompanied by some degree of abscess formation. Fibrosis was also noted in several specimens representing evidence of chronicity. None of the changes appear to be distinctive and can be seen in any severe type of acne.

Staphylococcus epidermitis and gram positive diphtheroids are resident organisms on all body sites examined. Corynebacterium acnes was isolated at least once from all body sites examined except the buttocks area. All Proteus species were isolated from only one of the patients.

A significant number of gram negative organisms were recovered from acne lesions, normal appearing skin and the nares. E. coli was recovered from 4 tropical acne patients in 13 of 72 cultures and in 1 control patient in 5 of 20 cultures. Proteus mirabilis was identified in one culture from 2 tropical acne patients and Aerobacter aerogenes(Enterobacter aerogenes) on 4 cultures from one tropical acne and one control patient. Staphylococcus aureus, coagulase positive, was isolated in 6 of these 10 patients from at least one or more acne lesions.

Conclusion:

All of the tropical acne patients had a previous history of mild to moderate acne vulgaris prior to their tour in Vietnam.

The types of lesions which developed in these patients

were clinically and histopathologically identical to those seen in severe pustular, cystic acne patients. The striking difference is the sparing of the face and the widespread distribution, including both the arms and the legs.

Five of the tropical acne patients and one control acne patient were found to have low urinary testosterone levels. There was no direct relationship to the serum testosterone levels in these patients. In general, the urinary 17-hydroxycorticoids and the 17-ketosteroids were normal and no pattern or trend was apparent with the ACTH stimulation or Decadron suppression testing.

One can only speculate on these paradoxical findings of testosterone values in patients with acne. The complexities of androgen metabolism are currently being extensively investigated by many research laboratories. The role played by androgens in the pathogenesis of acne still awaits clarification.

Future Plans:

The study is concluded. The final report is being prepared.

LABORATORY DATA

No.	Age	Total Lipids 500 - 800 mg%	Phospholipids 200 - 350 mg%	Triglycerides 50 - 250 mg%	Cholesterol 150 - 250 mg%
Controls					
1	20	472*	181*	122	169
2	23	457*	208	70	179
3	20	300*	236	12*	200
Tropical Acne					
4	39	805	205	207	256
5	20	354*	228	8*	118
6	21	–	–	–	–
7	18	640	162*	152	127
8	43	560	257	151	233
9	24	480*	196	118	147
10	24	688	164*	26*	163
11	21	727	134*	422*	200

*Abnormal values for LGH Laboratory

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6814	72 07 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
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	159
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Studies on Blistering Produced by Mechanical, Thermal and Chemical Agents: Kinetics of Blister Fluid Proteins (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 - Biochemistry; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 12	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	.1	0
	CURRENT 73	.1	1

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER.

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) biochemistry; (U) blister fluid; (U) albumin; (U) fibrinogen; (U) immunoglobulins

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

25. (U) 71 07 – 72 06 Radial gel immunodiffusion micro techniques have been perfected for albumin, fibrinogen, immunoglobulins IgG and IgM. Because of manpower limitations the project was held in an inactive state in FY 72.

*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.

Studies on Blistering Produced by Mechanical, Thermal, and Chemical Agents: Kinetics of Blister Fluid Proteins

Peter Schmid, Ph.D.

PROBLEM:

A variety of bullous and vesicular diseases are regularly seen in military populations. At Fort Ord, every day more than 1% of the population of this training center reports to sick call because of friction blisters. Various insects and plants throughout the world have chemicals which, when they come in contact with skin of soldiers, produce blisters. Other bullous and vesicular diseases seen by military dermatologists are pemphigus vulgaris, erythema multiforme and dermatitis herpetiformis. Once formed, bullae rupture or are torn more or less rapidly and may become secondarily infected by fungi, staphylococci or streptococci.

In previous investigations in this laboratory, new microtechniques have been developed to determine four specific proteins: albumin, fibrinogen, immunoglobulin IgG and IgM. Subsequently, the concentration of these proteins were determined in friction blister fluid and cantharidin blister fluid.

The mechanisms and dynamics of friction blister fluid formation are largely unknown. Little is known about the repair process, i.e., the reconstitution of the epidermis following trauma.

APPROACH:

For friction blister studies, friction blisters are made on each palm and heel with our latest friction blister machine, and the kinetics of formation of the blister recorded. Friction blister fluid is withdrawn at 1, 4, 8, 16, 24, and 72 hours following blistering. Two cantharidin blisters are made and the fluid harvested at 8 and 24 hours and a small sample of venous blood collected. The blister fluids and serum are then analyzed for albumin, fibrinogen and immunoglobulin IgG and IgM by the radial immunodiffusion technique.

The protein content of blister fluid in skin diseases like poison ivy dermatitis, tinea pedis, and erythema multiforme can be analyzed in the same manner.

RESULTS AND DISCUSSION:

Because of manpower limitations, the project was held in an inactive state in FY 72.

FUTURE PLANS:

Kinetic studies for albumin, fibrinogen, IgG and IgM proteins can begin when manpower is available and trained.

Initiate determination of albumin, fibrinogen and immunoglobulins in blister fluid obtained from suction blisters of normal skin and skin exposed to various aqueous solutions.

Initiate determination of albumin, fibrinogen and immunoglobulins in clinically important bullous eruptions.

The 2500 watt solar simulating xenon lamp and monochromator can now be used to raise sunburn and phototoxic blisters. Initiate analysis of blister fluid proteins obtained from phototoxic blisters.

Develop microtechniques for the determination of glucose and other small molecules.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6815	72 07 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
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	160
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Code with Security Classification Code)

(U) Clinical Evaluation of Alpha-Cyanoacrylates and Treatment of Friction Blisters

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 – Clinical Medicine; 012600 – Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 05	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA — 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 72	.1	1
FISCAL YEAR — CURRENT 73	.1	2

19. RESPONSIBLE DOD ORGANIZATION

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NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) isoamyl-cyanoacrylate; (U) toxicity; (U) topical; (U) systemic; (U) volunteer; (U) blister therapy; (U) pentyl; (U) fluoralkyl

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

25. (U) 71 07 – 72 06 Phase I. Cutaneous reactivity and sensory response to applying cyanoacrylate homologues to raw friction blister bases were evaluated utilizing the following cyanoacrylate monomers initially: n-butyl, isobutyl and n-heptyl spray, and isoamyl and isobutyl liquid. Liquid isoamyl cyanoacrylate proved to be the monomer of choice. Phase II. Various methods for treating experimentally produced blisters of the heels with cyanoacrylate homolouges were tried. The monomers selected were liquid isobutyl and isoamyl cyanoacrylate. Isoamyl cyanoacrylate produced greater comfort and less halo of inflammation than the isobutyl monomer. Phase III. Cyanoacrylate treatment was compared to the usual treatment for friction blisters and proved more satisfactory than Neosporin Cream and moleskin. Phase IV. Field trial was conducted in collaboration with the Podiatry Service, US Army Hospital, Fort Ord, California. Forty-seven of the 119 soldiers developed foot blisters during the march and 35 recruits with 39 blisters volunteered to be treated with isoamyl cyanoacrylate. The results were excellent for relieving pain, preventing infection, and permitting the continuation of training.

*Available to contractors upon originator's approval

Clinical Evaluation of Alkyl Alpha-Cyanoacrylates and Treatment of Friction Blisters

Colonel William A. Akers, MC, LAIR
and Fred Leonard, Ph.D., USA Biomechanical
Research Laboratory

PROBLEM:

We need a practical treatment for a medical corpsman or the soldier to use to lessen the pain and to minimize or prevent infection of torn or denuded blisters of the hands and feet. Cortese (1966) chose to test the alpha-alkyl cyanoacrylates since they offered some desirable characteristics which may be useful to accomplish our objective.

Cyanoacrylates were first developed as "tissue glues" to provide a quick method of closing wounds without sutures. A tissue adhesive should (1) wet and spread freely on the tissue surface and (2) polymerize rapidly to form a solid with sufficient deformability to reduce the buildup of elastic stresses during formation of the bond. The higher members of the n-alkyl a-cyanoacrylate homologous series (n-butyl to n-octyl) wet, spread freely, and rapidly polymerize on protein surfaces like the liver, kidney, spleen, and omentum. The lower members of the homologous series - methyl, ethyl, and propyl show a greater affinity for non-protein surfaces like distilled water, saline, and dextrose solutions.

Polymerization occurs at room temperature without additional components such as special catalysts, external heat, or solvents. Bonding action results from anionic polymerization which is initiated by weakly basic substances including alcohols, amines, and water. Water is an excellent initiator; and in most bonding applications monomer polymerization is caused by trace amount of water on the adherent surfaces. Polymerization is mildly exothermic. To stabilize a monomer in a tube, bottle, or can sulfur dioxide (SO_2) is added in trace quantities to inhibit premature polymerization.

APPROACH:

Subjects: The volunteers were all soldiers on active duty and ranging in age from 18 to 28.

Phases: The study was divided into four phases. As different monomers became available they were tested in a similar manner.

Phase I: Cutaneous reactivity and sensory response to applying cyanoacrylate homologues to raw friction blister bases were evaluated utilizing the following cyanoacrylate monomers initially: n-butyl, isobutyl and n-heptyl spray, and isoamyl and isobutyl liquid. Experimental friction blisters were rubbed on volunteers' palms and the inner surface of their heels. The blisters were de-roofed at 2 hours and each monomer was applied to a single blister base in at least 5 volunteers while intact and de-roofed blisters on each volunteer served as controls. The volunteers' subjective response to applying the adhesives was recorded. Observations were made almost daily for 12 days then at 3-4 day intervals until the film fell off, noting the persistence and physical characteristics of the polymerized film, and untoward cutaneous reactions including signs of local and regional inflammation and infection of the blister base.

Phase II: Various methods for treating experimentally produced blisters of the heels with cyanoacrylate homologues were tried. The monomers selected from Phase I were the liquid isobutyl and isoamyl cyanoacrylate. Blisters were rubbed on paired symmetrical sites on the inner heels (2 sites each heel) of each volunteer in order to compare the monomers. Three separate studies were done. First the liquid cyanoacrylates were applied to the tops of blisters in 5 volunteers immediately after blistering in an effort to prevent filling of the blisters; in a second group of 5 volunteers the blister tops were removed 2 hours after blistering, and the liquid cyanoacrylate monomers were applied to the denuded bases. A third group of 5 volunteers had the blister tops partially removed and reflected; the liquid cyanoacrylates were applied to the entire blister bases, and the tops were then replaced. Daily evaluation of test sites were made as in Phase I.

Phase III: Cyanoacrylate treatment was compared to the usual treatment for friction blisters at

Fort Ord, California. Bilateral, symmetrical blisters were raised on the inner surface of the heels; and isoamyl cyanoacrylate was applied to the blister bases on the right heel, and Neosporin Cream(R) covered with moleskin (later Bandaids(R)) was applied to the blister bases on the left heel. Follow-up examinations were made almost daily as in other studies.

Phase IV: A field trial was conducted in collaboration with the Podiatry Service, US Army Hospital, Ft. Ord, California. One company of new recruits, prior to their first 12 mile march which occurs during their 4th week of basic training, were examined and only those without foot blisters before the march were used in the study. With the soldier's permission, at the end of the march all torn or denuded blisters were treated with the liquid plastic. Intact blisters were not treated. The soldiers were followed for 7 days at the routine sick call. If any soldier developed an infected blister he would have been admitted to the hospital, treated appropriately, and his course documented. During the 7 days follow-up period, the recruits made several 3-1/2 miles marches and one 5 mile march.

RESULTS AND DISCUSSION:

Twelve studies involving 90 volunteers with 265 blisters were done in the laboratory. In the Phase I study, if the blister top was ruptured, torn, or removed, applying a cyanoacrylate proved to be the most satisfactory method of management. Of the various cyanoacrylates tested in the early studies liquid isoamyl cyanoacrylate proved to be the monomer of choice for the following reasons: (1) it produced the least amount of stinging when applied to the raw blister base, (2) it was the easiest to apply (just put a drop on, spread with a cotton-tipped applicator), (3) it produced the least halo of inflammation and (4) one application lasted 6 to 30 days, with a mean of 20 days before peeling off.

In the Phase II experiment isoamyl cyanoacrylate produced greater comfort and less halo of inflammation than the isobutyl monomer. Applying the cyanoacrylate to the intact blister top did not prevent the blister from filling. Attempts to cement a partially detached roof smoothly to the raw blister base failed, because the cyanoacrylate rapidly polymerized. This worked only with pentyl cyanoacrylate

which polymerizes more slowly, 3-5 seconds. The re-attached top was generally uncomfortable, feeling like a rock in the shoe.

For the Phase III experiment, the cyanoacrylate method proved more satisfactory than Neosporin Cream and moleskin since: (1) moleskin was extremely difficult to keep in place on walking and had to be replaced by Bandaids(R) after 48 hours, (2) local evidence of inflammation occurred at the sites treated by moleskin but did not occur at any sites treated with cyano-acrylate.

In the field trial (Phase IV), 47 of the 119 soldiers developed foot blisters during the march and 35 recruits with 39 blisters volunteered to be treated with isoamyl cyanoacrylate. The results were excellent for relieving pain, preventing infection, and permitting the continuation of training. On one large blister (4x5.5cm) on the sole of one recruit, the plastic film failed to adhere to 1/3 of the base so he had the plastic re-applied the next day. No infections resulted. Usually the infection rate in a training company after a 12 mile march is 8 percent.

Cyanoacrylate films are freely permeable to oxygen. The film is also permeable to water as evidenced by stinging of the blister base when the volunteers took showers or washed their hands. Until we noted the above, cutaneous sensitivity was tested by applying 70 percent ethanol to the treated and raw blister bases, and the bases treated with plastic became less sensitive than the control by 72 hours. The palms proved more tender to pressure than the heels, but the heels developed halos of erythema which were infrequently seen on the plams. The palms and heels were scored separately to reflect such differences. Volunteers noted a gritty sensation of the heel blister bases on walking when the adhesives were sprayed on. Evidently the spray splatters some thicker droplets which a man can perceive on walking which was not appreciated when only palmar blisters were used. Simply applying a drop of isoamyl, spreading with a cotton-tipped applicator, produces a thinner and more comfortable film.

Raw blister bases become more inflamed than an intact blister or a blister treated with cyanoacrylate. A drained, intact blister is the most comfortable, but

a blister base treated with cyanoacrylate is less painful on walking than a raw blister base or a blister base treated with Neosporin Cream(R) and Bandaids(R).

The only infection occurred during the second day of Experiment 11 when one patient developed increased tenderness, oozing, and erythema of all 4 blister bases. Staphylococcus aureus (3/4) and Staphylococcus epidermitis (1/4) were recovered from his blisters. Healing was rapid using soap and water cleansing and Neosporin Cream(R).

Cracking occurred less frequent and less deep with the plastic film than with the natural crust of a blister. A flexible film that does not crack readily makes the best artificial top. Isoamyl and pentyl cyanoacrylates are superior in this respect (no cracks up to 144 hours) whereas butyl and isobutyl crack and begin to peel within 48 hours.

Healing of the raw blister bases occurred within 120 hours despite the monomer used and we never observed any interference with healing. Volunteers always preferred cyanoacrylate therapy to no treatment; and only in Experiment 11 was therapy with an antibiotic cream and a dressing equal to the cyanoacrylate application.

FUTURE PLANS:

Larger scale field trials will be done. Also, other viscosities of isoamyl cyanoacrylate will be prepared and tested.

TABLE

EXPERIMENT	DATE	PHASE	SITE	MONOMER	VOLUNTEERS	BLISTERS No.	BLISTERS Total
1	Apr 67	I	Palms		16	3	48
				n-butyl spray # 96	(5)		
				n-butyl spray # 99	(4)		
				n-heptyl spray # 149**	(7)		
			Heels	n-butyl spray	4	3	12
2	Feb 68	I	Palms	n-butyl spray isobutyl spray isoamyl spray isoamyl** n-heptyl spray	5	5	25
3	Feb 68	II	Heels	isoamyl** control	5	2	10
4	Feb 68	II	Heels	isoamyl** reattached	5	2	10
5	Feb 68	II	Heels	isoamyl** intact control	5	2	10
6	Feb 68	III	Heels	isoamyl** neosporin + bandage	5	2	10
7	Mar 68	II	Palms	isoamyl	5	2	10
8	Apr 68	I	Heels	isoamyl** isobutyl	5	2	10
9	Sep 68	I	Heels	isobutyl 125 c.p.† isobutyl 215 c.p.† isoamyl**	8	3	24
10	Oct 68	I	Heels	isobutyl isobutyl + $Na(HCO_3)_2$ isoamyl**	7	3	21
11	Jul 69	IV	Feet	isoamyl	35		39
12*	Mar 71	I,II,III	Palms Heels	isoamyl** pentyl** fluoralkyl neosporin + bandage**	12	4	48
13*	Jun 71	I, III	Palms Heels	isoamyl 200 c.p.† isoamyl 300 c.p.† ** pentyl** neosporin + bandage	8	4	32
					125		309

*Hemograms, urinalyses, and blood chemistries (SMA-12) done pre-application, 1 week, and 1 month later on all volunteers revealed no evidence of systemic toxicity from topical application.

** Best of test

† c.p. – centipoise

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6911	72 07 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
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	161
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Biosynthesis of Proteins and Lipids in Human Skin with Particular Emphasis on Prickly Heat (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002300 - Biochemistry; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 06	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA 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 72	.1	18
CURRENT FISCAL YEAR 73	1	5

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code): (U) biochemistry; (U) prickly heat; (U) protein synthesis; (U) lipid synthesis; (U) skin; (U) human volunteers

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

25. (U) Because of manpower limitations the project was held in an inactive state in FY 72.

*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 ANNY USE) ARE OBSOLETE.

Biosynthesis of Proteins and Lipids in Human Skin With Particular Emphasis on Prickly Heat

Peter Schmid, Ph.D.

PROBLEM:

No adequate hypothesis presently exists which explains the primary events causing miliaria. Much data suggests that bacteria may be involved, but contrary evidence exists indicating that bacteria are not important. Other work suggests that high level of salt in the sweat is responsible for producing miliaria but experimental testing did not confirm this hypothesis. Still other work suggests that lack of adequate lipid in the stratum corneum permits keratin to swell thereby blocking the sweat duct orifice.

From measuring the sweat rate per gland and the dimensions of the duct, linear flow rates of sweat secreted through an unobstructed duct to the surface can be calculated to reach values up to 100 mm/min. Physically obstructing the duct and/or lowering the sweat flow rate may have far reaching consequences on the metabolism of the sweat gland apparatus because solutes such as ammonia may no longer clear the duct rapidly and may become toxic to the sweat gland apparatus.

This work unit attempts to test a new hypothesis: that the differential rate of protein and lipid synthesis of the cells lining the sweat duct, spinous and granular layers of the epidermis leads to distortion and occlusion of the sweat duct and thus to miliaria.

APPROACH:

Miliaria is produced on one-half of the body surface by the method of Sulzberger, Griffin, et al. Volunteers are injected intravenously with a sterile solution of 200 $\mu C^{3}H$ glycine and 100 $\mu C^{14}C$ acetate. The experimental and control area on the back are scraped lightly to remove the outermost layer of stratum corneum and the dust-like scales collected. Scrapings are performed every second day over prolonged periods of time.

The dry scales are extracted with lipid solvents and phosphate buffer. Lipid, soluble proteins and insoluble material is analyzed for ^{3}H and ^{14}C by liquid scintillation spectrometry.

RESULTS AND DISCUSSION:

Because of manpower limitations, the project was held in an inactive state in FY 72.

FUTURE PLANS:

Continue and terminate development of methodology.

Initiate measurement of samples from pilot study.

If necessary, modify procedures developed.

Measure protein synthesis and lipid synthesis of volunteers who were subjected to the induction procedure for experimental miliaria.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6912	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REG'RADING	8A. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 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	62110A	3A062110A822	00	162
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procode with Security Classification Code): (U) Studies on the Effects of Heat & Humidity Upon the Human Skin with Particular Emphasis on Prickly Heat & Consequent Disabling Dermatoses (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 08	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA — 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 72	2	23
	CURRENT 73	1	20

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

21. GENERAL USE

22. KEYWORDS (Procode EACH with Security Classification Code): (U) miliaria; (U) prickly heat; (U) rash; (U) sweating; (U) heat fatigue; (U) heat retention; (U) stratum corneum

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

25. (U) 71 07 – 72 06 A sensitive silicone sweat impression technique was developed and utilized for therapeutic studies. Topical antibiotics and cooling lotions do not affect the rate of sweat recovery from experimental prickly heat. Peeling agents, causing desquamation of horny layer in 5 to 7 days, accelerate the recovery of sweating. However, these agents initially cause profound decrements in sweat delivery. Cellophane tape stripping, either preceding or following the induction of experimental prickly heat, will affect only a partial recovery in sweat delivery. These studies indicate that simple removal of the stratum corneum (horny layer) or topical antibiotics will not provide satisfactory therapy.

*Available to contractors upon originator's approval

Studies on the Effects of Heat and Humidity Upon the Human Skin with Particular Emphasis on Prickly Heat and Consequent Disabling Dermatoses: Summary of 3 Years of Studies

Major David R. Harris, MC

PROBLEM:

The torment of itching that precludes sleep is familiar to everyone who has suffered from prickly heat rash (miliaria). Recently, a laboratory model for producing experimental miliaria, using occlusive polyethylene wraps over a 72 hour period, was developed at the Letterman Army Institute of Research (LAIR). Using this model, it was shown that clinical and experimental prickly heat is associated with long-lasting decrements in sweat delivery. Significantly, in World War II investigators observed that heat fatigue and exhaustion were associated with transient reductions in sweating. Subsequent collaborative experiments by LAIR personnel at the U.S. Army Research Institute of Environmental Medicine, Natick, consistently demonstrated profound heat fatigue associated with widespread post prickly heat sweating deficiencies. Prickly heat caused disability in both combat and support personnel in RVN. The results of questionnaires submitted by LAIR to most battalion surgeons in RVN engaged directly in military patient care revealed neither an effective treatment nor a unified approach to the treatment of miliaria or other sweating disorders. Moreover, prickly heat rash serves as a prototype to investigate cutaneous diseases associated with assaults of heat and humidity upon the stratum corneum including: the sweat retention syndrome associated with heat fatigue, disabling disorders of the feet, including warm water immersion foot and paddy foot, bacterial and fungal infections, and tropical acne. All of these diseases are closely linked with the occupational hazards of the fighting man in a tropical climate.

With this background, the main thrust of this miliaria investigative program has been toward: (1) development of a human model; (2) elicitation of the causal mechanisms; (3) investigation of preventive and therapeutic modalities.

APPROACH:

1. Development of the human model. A technique was perfected to produce experimentally the clinical equivalent of miliaria rubra over multiple sites on the backs of volunteers. Test sites were dressed for 72 hours with a 3" x 5" occlusive dressing composed of polyethylene film and 1/4" thick polyurethane foam, held in close proximity to the skin with an adhesive bandage. Following a sweat stimulus, these occluded sites revealed confluent prickly heat for 24 to 48 hours followed by sweating decrements lasting up to 3 weeks (Sulzberger, M.B. and Harris, D.R.: Miliaria and anhidrosis. III. Multiple small patches and the effects of different periods of occlusion. Arch Derm, in press).

2. Elucidation of causal mechanisms

a. Development of a sweat impression technique. Initially, increments and decrements in heat lamp stimulated sweating were measured by air flow hygrometry. Subsequently, our studies showed that with this method, day-to-day measurements varied greatly at the same control sites over the back and were dependent on a large number of variables. These included the ambient air temperature and humidity, skin color and distance of the skin from the heat lamp, the moisture content in and the flow rate of the air passing over the sweating site, and the length and temperature of the hygrometry tubing. Because these factors were difficult to control, we evaluated a number of sweat indicator and impression techniques which measure the number of actively secreting eccrine units and not the rate of sweat delivery. A technique was developed utilizing a thin silicone film sweat impression and a constant humidity chamber for a controlled sweat stimulus. Subjects are heated for 25 minutes in an environment of 118°F and 30% RH. A 10 minute active exercise program is carried on during the stimulus period. With vigorous sweating continuing in the chamber, 1 cc of a type II silicone base, class 3 light body impression material (Kerr Manufacturing Co., Romulus, Michigan 48174) is mixed with 1 drop of catalyst and spread in a thin film over the skin with a wooden applicator stick. This hydrophobic material withdraws from emerging sweat droplets and as polymerization occurs, an impression of active eccrine units is fixed (Harris, D.R.; Polk, B.F. and Willis, I.: Evaluating sweat

gland activity with imprint techniques. JID 58: 78-84, 1972). In this manner, an accurate quantitation of day-to-day eccrine sweating over the same site can be achieved.

b. Histological studies. Three approaches were utilized for the histological evaluation of experimental prickly heat. Six volunteers had pairs of symmetrical occlusive dressings affixed for 8, 24, 48 and 72 hours (total of 8 dressings per man). At the end of each occlusive period, test sites were biopsied, formalin fixed, cut and prepared for light microscopy in the usual manner. Each specimen was serially sectioned, stained with the H&E and the PAS techniques and compared with control specimens. Second, number 15 gauge needle specimens were prepared in the usual manner for electron microscopy, being fixed with gluteraldehyde and osmic acid and slowly dehydrated in increasing concentrations of alcohol to 100%. This tissue was embedded in plastic, cut in thin 1 micron sections, stained with methylene blue and examined with both light and electron microscopic techniques. Third, 1 cm^2 free hand-split thickness shave specimens were fixed in sodium bromide and dehydrated in 70% alcohol solution. This tissue was prepared and examined under the scanning electron microscope at Johnson and Johnson Research Division, New Brunswick, New Jersey.

c. Selection and Characterization of Individuals Prone and Resistant. Two field studies were conducted in which each of 29 volunteers was physically stressed in a warm environment (average 83°F) for a 72 hour period after being wrapped over 1/2 of the trunk with an occlusive dressing. Twice each day, volunteers engaged in strenuous exercise by marching at a brisk rate over hilly country for a total of 5 miles. All subjects developed dripping sweats. Following removal of the occlusive dressings, the severity of miliaria was determined clinically. Then, at 4 consecutive weekly intervals each volunteer was evaluated for sweat delivery at control and miliaria sites in the manner previously outlined. Individuals who manifested sweating decrements at miliaria sites over the test period significantly greater than the group mean were designated "prone to miliaria." Conversely, individuals who had decrements significantly below the norm were designated "resistant to miliaria." These 2 groups were then compared with one another with regard to severity of experimental miliaria, past history and family history of the disease, sweat electrolyte

concentration (measured by flame photometry and skin chloride electrode), skin pH with and without occlusion and total cutaneous microflora counts.

d. Role of the stratum corneum (horny layer). To determine the possible role of a horny layer blockade in the induction of experimentalmiliaria and hypohidrosis, several cellophane tape stripping experiments were performed. In each, the technique was the same. Individual lengths of cellophane tape were applied to the test site with 2 firm thumb strokes and then gently peeled off. Separate pieces of tape were used for each stripping. In separate trials employing 5 volunteers each, the following experiments were performed:

(1) Separate sites stripped 3, 6, 9, 12 and 18 times immediately before induction of miliaria;

(2) Separate sites stripped 3, 6, 9, 12 and 18 times immediately after the induction of miliaria;

(3) Separate sites stripped 12 times 24 hours after induction of miliaria;

(4) Separate sites stripped 6 and 12 times immediately after the induction of miliaria;

(5) Separate sites stripped to glistening and 1/2 glistening 4 hours after induction of miliaria.

3. Therapeutic Trials. Multiple patches of miliaria were induced in groups of 5 volunteers each. The following classes of topical agents were then applied by the investigator at control and test sites twice daily for 7 days:

a. Topical antibiotic (Neosporin-G^{R} cream)

b. Peeling solutions (resorcinol 14, salicylic acid 14, lactic acid [85%] 14, ethanol qs 100 and Retinoic Acid 0.1% in ethanol [55%])

c. Cooling lotion (Witch Hazel NF)

Each subject was evaluated for decrements or increments in sweat delivery at test and control sites at 3 day intervals for periods of 15 to 20 days.

RESULTS AND DISCUSSION:

1. Causal Mechanisms

a. Histological Studies. Examination of multiple sites occluded for periods between 8 and 72 hours, revealed neither clinically apparent experimental miliaria or long lasting sweat decrements before 48 hours of occlusion. The development of miliaria and hypohidrosis following this period correlated closely with the development of changes within the epidermis and the stratum corneum as determined with light microscopy. The epidermis showed edema and acute and chronic inflammation around the eccrine duct at the epidermal-dermal junction. These findings were identical to previous histological reports of the naturally occurring disease. At the same time, specimens fixed for electron microscopy showed varying degrees of edema within the granular layer of the epidermis and in the fully formed stratum corneum. In some specimens the cellular ultrastructure within the horny layer was dispersed. Pioneer studies using surface scanning electron microscopy (SEM) revealed no uniform picture. While some miliaria samples showed apparent surface plugs at the ostea of sweat ducts, some control site specimens also showed plugged ostea. After examining several thousand scans, this investigator, collaborating with Dr. Christopher Papa of Johnson and Johnson Research Division, concluded that baseline SEM studies should be undertaken first defining the range of normal sweat gland ostea.

b. Characterization of individuals prone and resistant to miliaria. Eight individuals, in 2 groups of 4 each, were selected from a group of 30 volunteers either as "prone" or "resistant" to experimental miliaria on the basis of being at the extremes of sweating decrements at test sites. Whether prone or resistant, there were no group differences in the expression of clinical disease when the occlusive wraps were removed. Nor were there significant differences between the 2 groups in reporting a past history of miliaria, in sweat electrolyte concentrations, or in the skin pH and bacterial flora at control and occluded sites. Overall, differences in skin pH were seen in both the prone and resistant groups at control compared to unwrapped miliaria sites. An average rise in pH from 5.6 to 6.4 was noted from the unoccluded to the occluded patches. Similarly, a 2-1/2 log average increase in aerobic bacteria was

seen in all the volunteers after 72 hours of occlusion. We conclude for the present that we have no clear picture of what factor or factors dispose a "prone" individual to prickly heat. Nor do we have assurance, given any set of environment circumstances, that the same individual will remain consistently "prone" or "resistant" to experimental or naturally occurring prickly heat.

c. Stratum corneum stripping studies. When multiple strippings of the horny layer were taken either before or after occlusion, a consistent trend toward greater sweat gland counts was seen. In one experiment, stripping 72 hour occluded sites 9, 12 or 18 times returned the sweat gland count to 29% of normal from a baseline of 9% of normal ($p<.05$). Similar increments were noted at sites stripped 3, 6, 12 or 18 times before occlusion. Subsequent experiments showed that the effect of stratum corneum removal on sweat delivery was most apparent at 7 days following stripping to glistening when a brisk desquamation of stratum corneum was seen. However, a factorial analysis of the effects of stripping following induction of experimental prickly heat revealed statistically significant increases in sweat delivery in only 1 of 4 experiments ($F<0.05$). In summary, stripping can increase sweat impression counts at miliaria sites from 8 to 20% depending on how and when it is done. Certainly, normal sweat delivery is not reinstituted.

Nonetheless, these stripping experiments coupled with the histological studies showing edematous disruption of the horny layer and deeper epidermal-dermal junction inflammation only in association with sweat decrements, strongly suggest that at least a partial horny layer blockade to the free egress of sweat is the anatomical event initiating prickly heat.

d. Therapeutic trials. Two considerations dictated the selection of agents for therapeutic trials. First, historically, a multitude of so-called "peeling" agents have been advocated for prickly heat since World War II. There efficacy has always been in doubt because most of these agents contain substances which, while causing drying and peeling, also evaporate in warm climate, cooling the prickly skin. Since a stratum corneum blockade apparently plays a role in the etiology, two peeling agents which were found to cause a brisk desquamation in from 5 to 7 days were chosen for initial clinical trials. Results showed

that this type of agent initially causes a decrement in sweat delivery ranging from 30 to 70% of baseline at both control and previously occluded sites. Subsequently, return to normal sweat counts at miliaria sites was accelerated over the 14 to 21 day normal and coincided with the onset of horny layer desquamation at 5 to 7 days. Whether these agents will beneficially effect either comfort or performance in the field has yet to be determined. Certainly, routine use of any agent which can initiate profound sweat decrements cannot be advocated.

The use of topically applied antibiotics has been a standard therapeutic approach since O'Brien, Acton and Lyson implicated staphylococci in the etiology of miliaria. We have shown, as have others, a tremendous increase in cutaneous microflora both in the naturally occurring state and after induction of experimental miliaria and hypohidrosis under occlusive wraps. With this background, MAJ T. Griffin and COL W. Akers of LAIR proceeded to demonstrate some beneficial effect using either topical neomycin or chloramphenicol prophylactically to prevent post-miliarial hypohidrosis.

The only controlled therapeutic study of topical antibiotics showed neomycin and Kanomycin^{R} lotions to be clinically effective when only the clinical expression of disease and patient comfort were evaluated. Thus, we chose to evaluate a readily available preparation, Neosporin-G^{R} cream, containing the same concentration of neomycin as in previously reported studies. There was absolutely no effect on sweat delivery either at control or miliaria test sites over a 2 week period of evaluation.

We conclude that effective therapy as defined by the reestablishment of normal sweating at a physiological level may be impossible to achieve by means presently available. If the initiating event in miliaria is damage to the structural integrity of the stratum corneum, then a complete turnover in the horny tissue averaging 14 days will be necessary for return to normal sweat delivery. All of our results thus far serve to substantiate this premise. On the other hand, if effective therapy is to be defined by relief of discomfort through the natural course of the disease, then a unified approach to therapy will be more readily obtainable.

FUTURE PLANS:

Work will be directed toward more accurately defining and initiating effective therapy. Present prophylactic means of prevention are unrealistic in terms of the massive number of personnel at risk in certain environments. The following research priorities are offered:

1. Development of a long-lasting model. To test the effectiveness of therapeutic modalities in terms of patient comfort, performance, and long-lasting hypohidrosis, experimental prickly heat must be maintained without significant clinical or physiological recovery for at least 10 days to 2 weeks. To achieve this goal, the research subject will be placed in an environment of 85°F, 80-90% RH, with controlled daily exercise immediately after induction of experimental miliaria.

2. Consideration of potential therapeutic agents. Included for consideration are agents which buffer skin pH (citric and ascorbic acid), anionic wetting agents, antibholinergic substances (hexopyrronium bromide and Probanthine^{R} solution), and substances demonstrating duel solubility (cholesterol containing immulsions, polyethylene glycol). In addition, a number of newer, more potent broad spectrum topical antimicrobials bear consideration. These include Miconazole^{R} cream, Haloprogin^{R} cream, zinc and sodium Omadine and isoquinalinium bromide.

Normal and Abnormal Variations in Eccrine Sweat Delivery and Distribution

Major Isaac Willis, MC

PROBLEM:

Utilizing our new silicone imprint technique for identifying and counting active eccrine units, the objectives of this study were to determine the following: (1) if imprints of the same anatomical site were obtained at weekly intervals over 4 to 8 weeks, would significant variability in the function of a particular unit be detected, and (2) the usefulness of this technique in delineating the borders of skin tumors and evaluating the progression and/or regression of skin lesions.

Accomplishing the first objective would validate the superiority of this technique over conventional methods and would also serve to resolve previous questions concerning the possibility of "cyclic" or "periodic" functioning of sweat units that would render a single imprint using any technique unreliable. Accomplishing the second objective would provide not only a clinical means to uncover subclinical invasion or presence of tumors, but will be of use in "permanently" recording the spread or healing of lesions either under clinical or investigative therapy or simple observation, provided that they interfere with sweat delivery.

APPROACH:

1. To accomplish our first objective, 5 adult volunteers were utilized. To insure that imprints were taken from the exact same sites each time, a flexible plastic triangular mold bound by 3 permanent skin lesions (nevi, scars, etc.) was made for each test area and a 2 cm square was cut from the center of the mold where the imprint material was to be applied to the skin. Test areas included the forehead, flexor surface of the forearm, upper back, and medial surface of the leg. Sweating was induced and imprints were taken as described in our manuscript.

Initial imprints were projected onto sheets of paper where sweat ostia "light spots" could be outlined. Subsequent projected imprints could then be superimposed on old outlines to see if the same ostia were

functioning. Each ostia was coded and its weekly activity was recorded. A total of 4 to 8 weeks of imprints was obtained for each site in each subject.

2. The second objective was accomplished using patients with the following skin lesions: (a) superficial basal cell epithelioma, (b) sclerosing basal cell epithelioma, (c) epithelial nevus, (d) early keloid, (e) borderline tuberculoid leprosy.

These impressions were examined by direct inspection since ostia openings are easily seen and this observation would be sufficient for our purposes.

RESULT AND DISCUSSION:

1. The numbers of active eccrine units determined by repeated skin imprints of the same anatomical site did not vary by more than 5 percent. Approximately 10 percent of all units identified were found to function poorly in the initial imprint. This pool of poorly functional units remained poorly functional throughout the study.

We concluded that a single impression using this technique could be relied upon to detect >90% of all functional sweat units. These data indicate that sweat glands do not undergo cyclic or periodic function. Counts obtained with this method agree closely with those of Kuno who did in vitro histologic studies to determine the number of sweat glands.

2. The findings from imprints taken in areas of skin lesions revealed that each lesion interfered with sweat delivery. In the case of both basal cell epitheliomas that were later removed, areas of non-functioning sweat units corresponded to areas of actual tumor involvement. In the case of the borderline tuberculoid leprosy lesion, a partial return of sweat function was found after two months of treatment.

Results indicate that this is a useful clinical technique.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6912	72 07 02	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
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	03	163
b. CONTRIBUTING	62110A	3A062110A831	03	002
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Composition of Lipids (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	60
	CURRENT 73	1	55

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

20 PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) biochemistry; (U) lipids; (U) fatty acids; (U) glycerides; (U) tropical acne; (U) soldiers

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

25. (U) 72 07 – 72 06 We have developed a second generation high pressure liquid chromatograph for separation of complex skin lipids. Concurrent with this work, many solvent systems were tested to optimize solvent properties with regard to skin irritation, toxicity, solubility, and corrosion properties as well as solvent mixture stability.

*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 1339-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Composition of Lipids

Peter Schmid, Ph.D.

PROBLEM:

Tropical acne and severe acne conglobata were leading causes of evacuation for skin disease (varying between 20-30%) of U.S. soldiers from the Republic of Vietnam. New LAIR statistics indicate quite clearly (Army and Navy data) that acne outpatient visits are consistently above 10% of total visits and are thereby the most time consuming and costly item in military dermatology.

The role of lipid metabolism of sebaceous gland cells during the various stages of differentiation is largely unknown and very little modern data is available which does or does not suggest changes in lipid composition and lipid metabolism in acne.

If progress in understanding the normal function of this lipid secreting organ is to be made, new techniques for analyzing microquantities of lipid components of human sebaceous glands must be developed.

APPROACH:

Thirty samples of 18 hospitalized patients with tropical acne have been collected and are analyzed at present by conventional analytical methods by an Army contractor.

New and more effective treatment methods for acne vulgaris and tropical acne depend on progress in understanding the regulation of the metabolism of sebaceous glands. To understand lipid metabolism of sebaceous glands requires knowledge of the composition of sebaceous lipids. Analysis of surface lipids, pooled from many people or from individuals, have been performed. However, it is essential to analyze the contents of a small number of individual sebaceous glands and comedones. This in turn requires handling, separating, and quantitating very small amounts of exceedingly complicated lipid mixtures into its myriad components.

RESULTS AND DISCUSSION:

Quantitative removal of very small amounts of lipid from the skin and subsequent meaningful analysis

required an exceptional amount of work on new solvents in FY 72. For optimization of collection and analysis of lipids, skin irritation, toxicity, solubility properties of lipid components, corrosion problems with "inert" stainless steel, stability of solvents as well as reactions of solvents with lipid components had to be studied.

Our lipid solubility studies indicated that methods currently in use for removal of non-lipid matter from lipid extracts are totally inadequate. For this reason, we have developed, tested and integrated a system to collect, purify and separate small quantities of lipids quantitatively from skin.

In continuation of work begun in FY 71, a comparison of several solvent mixtures to extract lipids from the skin surface of human volunteers was performed. We now have the basic knowledge to formulate effective solvent systems which show little skin irritation. Our results confirm our previous suggestion that most skin lipid analysis in acne patients were performed with inadequate lipid extracts.

FUTURE PLANS:

The acne and tropical acne project is discontinued at the end of FY 72, however, several publications will be prepared in FY 73.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6915	72 07 01	DD DR&E(AN)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
71 07 01	D.Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	167
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Evaluation of Promising Antifungal Agents in Soldiers in a Combat Zone (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 – Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	0
FISCAL YEAR CURRENT 73	1	7

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NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) dermatology; (U) dermatophytosis; (U) drugs; (U) therapy; (U) mycology; (U) patients; (U) diagnoses; (U) skin

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

25. (U) 71 07 – 72 06 Lessening demands resulted in no field studies conducted in the combat zone. Data on incidence on skin diseases in 20-30 year old active duty soldiers was obtained from the 95th Evac Hospital, RVN, and William Beaumont General Hospital, El Paso, Texas. The incidence of fungal infections was third in both RVN and El Paso. The need for epidemiological data to predict epidemic of skin sidease is emphasized.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68

Evaluation of Promising Antifungal Agents in Soldiers in a Combat Zone

Dermatology Outpatient Data System

LTC H. Earl Jones, MC

Biographical and clinical data are collected on each patient seen in certain Army dermatology outpatient clinics. The individual data cards are coded, key punched, sorted, and then analyzed by computer to determine the incidence of each disease seen. Using available climatological information from the Environmental Control Center and the biographical information from the data cards, the incidence of disease can be correlated with the age, sex and race of the surveyed population and the environment. The effect of the individual's occupation and factors pertinent to the local elevation and terrain can also be analyzed.

RESULTS AND DISCUSSION:

An 18 month study has been completed from William Beaumont General Hospital (WBGH) in El Paso and the 95th Evacuation Hospital in DaNang, Vietnam. The incidence of skin disease in both dermatology clinics is listed in decreasing order in Table 1. Dependent women, children, and elderly retired individuals seen at WBGH are being compared to a young, healthy male population in Vietnam. Thus, the incidence of skin disease cannot justly be compared and this is reflected in the high indicence of disease prevalent in dependents and the elderly at WBGH and the reverse in Vietnam. Utilizing the computer, 20-30 year old, active duty males can easily be compared; and by so doing, differences in disease incidence can be attributed to factors peculiar to each locality. Table 2 lists such a comparison and demonstrates that the diseases frequently seen in the total WBGH population attributable to dependents and retired patients are thus eliminated.

Certain diseases are apparently more common in the tropics than the arid El Paso area and vice versa. Some diseases seen in RVN can be attributed to the war-time situation in RVN.

WBGH is located in a desert with a persistent low humidity (20-30%) and temperatures fluctuating from 30° to 100°F while the 95th swelters in the tropics with the humidity rarely falling below 80% and reaching 99% almost daily. From the climatological data and the specific age-sex disease incidence, certain conclusions can be reached.

Humidity seems to greatly favor the development of infectious diseases of the skin, i.e., both fungal and bacterial. This hypothesis is supported by the incidence studies observed in this study. Comparing the incidence of fungal infection by type and anatomic area involved in the opposite climatic types, no increased incidence of tinea pedis is observed in the humid tropics. This may be explained by the fact soldiers wear boots and socks which always provide a local, very high relative humidity for the feet irrespective whether the man is in the desert or the tropics. The ambient relative humidity that "bathes" the glabrous skin in the tropics converts the skin into a fertile garden for fungal growth. Ringworm of the glabrous skin increased 11-fold as noted from analyzing this data reflecting the change in moisture content of the glabrous skin.

Miliaria is seen at the 95th during the hot, humid summer but never in El Paso, where the temperature is as great, if not greater, than in DaNang. The low relative humidity in El Paso does not permit hydration of the stratum corneum and resultant plugging of the sweat pores because of rapid evaporation of surface water that arises from either transepidermal loss or sweat thus no miliaria occurs in the desert climate. That such an environment affects the hydration dynamics of the stratum corneum seems confirmed by the reverse dry air situation present in El Paso. Dry skin

dermatoses are frequent in El Paso but never seen in the tropics of DaNang (see Table 3). The incidence of miliaria in DaNang and dry skin dermatosis in El Paso are cyclic and both diseases can be related to the opposite relative humidity and/or temperature changes.

Certain other diseases may be more frequent in Vietnam (see Table 4), such as vitiligo, alopecia areata and urticaria, and it is generally believed that these disorders relate in some way to anxiety or other emotions. This may relate to the war-time stress on the soldiers. Warts, atopic dermatitis, psoriasis, seborrheic dermatitis and numerous other disorders do not show any different incidence (see Table 2). Acne vulgaris does not show any great difference in incidence, but the tropical-type cystic acne of the trunk and extremities was seen much more frequently at the 95th.

This data is also being analyzed for the effect of occupation (MOS) on the incidence of skin disease but the results are not available at this time.

FUTURE PLANS:

1. The data obtained from the WBGH and the 95th Evac study will be completely analyzed.

2. A similar data collection system will be used to determine the incidence of skin disease at 4 large Army dermatology clinics (WRAMC, BAMC, FAMC, LGH). This trial study will operate for 2 years and will accomplish 3 goals presently beyond the scope of any Army data system:

a. Both biographical and clinical data on the incidence of skin disease in several large military clinics will be available for the first time. A disease outbreak and/or epidemics at an early stage can possibly be detected by monitoring this system.

b. Numerous valuable research studies will be conducted on the accumulated data.

c. A review of a physician's progress will be provided to his chief of service.

Since the 4 hospitals are situated in widely different climatic types we should be able to analyze the age, sex, occupation specific incidence of any skin diseases and determine the effect different ambient temperature and humidity combinations may have on the frequency of skin disease.

From this unique experience we shall be able to construct a predictive temperature-humidity graph from which the risk of developing various skin diseases can be predicted. This should be of great interest to the Army and other American military components.

TABLE 1

DISEASES CODED ALL CLINIC VISITS

	WBGH, El Paso, Texas Jan 1970 – Jun 1971 TOTAL 9,819			95th EVAC, Da Nang RVN May 1970 – July 1971 TOTAL 4,605		
	DISEASES	NO.	% TOTAL	DISEASES	NO.	%TOTAL
1	Warts, all	1896	19.03	Warts, all	729	15.83
2	Acne, all	1082	11.02	Acne, all	466	10.19
3	Actinic keratosis	505	5.14	Dermatophytosis, all	371	8.06
4	Nevi	442	4.50	Pseudofolliculitis barbae	289	6.28
5	Dermatophytosis, all	371	3.78	Penile ulcer (? chancroid)	221	4.79
6	Contact derm	360	3.67	Miliaria	199	4.32
7	Atopic derm	288	2.93	Pyodermia, all	178	3.86
8	Basal cell carcinoma	274	2.79	Contact derm	167	3.63
9	Psoriasis	258	2.63	Urticaria	126	2.74
10	Dry skin	234	2.38	T. versicolor	123	2.67
11	Dyshidrosis	222	2.26	Psoriasis	106	2.30
12	Seborrheic derm	202	2.06	Atopic derm	95	2.06
13	Seborrheic keratosis	174	1.77	Dyshidrosis	95	2.06
14	Urticaria	162	1.65	Alopecia areata	82	1.78
15	Misc. dermatosis & dermatides	162	1.65	Monilia	71	1.54
16	Pityriasis rosea	154	1.57	Herpes - progenitalis	68	1.48
17	Sebaceous cyst	139	1.42	Seborrheic derm	56	1.22
18	Skin tags	115	1.17	Misc. dermatosis & dermatides	51	1.11
19	Pseudofolliculitis barbae	114	1.16	Insect bites	48	1.04
20	Lichen simplex chronicus	110	1.12	Molluscum contagiosum	41	0.89
21	Alopecia areata	108	1.10	Sebaceous cyst	40	0.87
22	LE, discoid	104	1.06	Lichen planus	40	0.87
23	Pyoderma, all	93	0.76	Pityriasis rosacea	39	0.85
24	Tinea versicolor	75	0.76	Hand & foot eczema	37	0.80
		TOTAL	77.57		TOTAL	81.24

TABLE 2

DISEASES CODED 20–30 YEAR OLD ACTIVE DUTY MALES

	WBGH, El Paso, Texas TOTAL 1,752			95th EVAC, Da Nang RVN TOTAL 3,015		
	DISEASES	NO.	% TOTAL	DISEASES	NO.	% TOTAL
1	Warts, all	335	22.37	Warts, all	560	18.57
2	Acne, all	150	8.56	Acne, all	317	10.50
3	Dermatophytosis, all	103	5.88	Dermatophytosis, all	243	8.06
4	Pseudofolliculitis barbae	85	4.85	Pseudofolliculitis barbae	239	7.93
5	Gonorrhea	61	3.48	Penile ulcer, chancroid	145	4.83
6	Nevi, all	60	3.42	Miliaria	141	4.68
7	Contact derm	52	2.97	Pyoderma, all	122	4.05
8	Seborrheic dermatitis	40	2.28	Contact derm	105	3.48
9	Urticaria	38	2.17	Urticaria	94	3.12
10	Dyshidrosis	37	2.11	Tinea versicolor	89	2.95
11	Psoriasis	36	2.05	Alopecia areata	63	2.09
12	Sebaceous cyst	36	2.05	Psoriasis	62	2.06
13	Tinea versicolor	33	1.88	Atopic derm	60	1.99
14	Dry skin, xerosis	32	1.83	Dyshidrosis	60	1.99
15	Pityriasis rosea	31	1.77	Monilia	46	1.53
16	Misc. dermatosis & dermatides	31	1.77	Seborrheic derm	38	1.26
17	Herpes - progenitalis	29	1.66	Herpes - progenitalis	37	1.23
18	Lichen simplex chronicus	26	1.48	Molloscum contagiosum	32	1.06
19	Atopic derm	20	1.14	Insect bites	29	0.96
20	Pyoderma, all	20	1.14	Sebaceous cyst	29	0.96
21	Molloscum contagiosum	20	1.14	Hand & foot eczema	25	0.83
22	Skin tags	14	0.80	Misc.dermatosis & dermatides	25	0.83
23	LE, discoid	14	0.80	Erythema multiforme	24	0.80
24	Alopecia areata	14	0.80	Nevi, all	20	0.66
25	Balanitis	13	0.71	Vitiligo	20	0.66
26	Erythema multiforme	9	0.51	Balanitis	19	0.63
27	Pediculosis pubis	8	0.46	Lichen planus	18	0.60
28	Drug eruptions	7	0.39	Pityriasis rosacea	18	0.60
29	Lyphogranuloma U.	7	0.39	Drug eruptions	15	0.49
30	Lichen planus	7	0.39	Keloids	14	0.47
31	Ulcer, other	6	0.34	Lichen planus	13	0.43
32	Basal cell carcinoma	6	0.34	Corn & callouses	13	0.43
33	Intertrigo	6	0.34	Lichen simplex chronicus	11	0.37
34	Granuloma annulare	6	0.34	Nummular eczema	10	0.30
35	Nummular eczema	5	0.28	Syphilis, infection	8	0.27
36	Dermatofibroma	5	0.28	Syphilis, late	8	0.27
37	Pruritis	5	0.28	Lymphogranuloma U.	7	0.23
38	Photosensitive derm., all	3	0.17	Granuloma annulare	5	0.14
39	Insect bites	3	0.17	Pruritis	5	0.14
40	Corns & callouses	3	0.17	Striae	4	0.12
41	Miliaria	2	0.12	Polymorphic light eruption	4	0.12
42	Penile ulcer, (? chancroid)	1	0.06	Onychomycosis	3	0.09
43	Vitiligo	1	0.06	Photocontact derm.	2	0.06
44	—	1	0.06	No diagnosis		1.29
	TOTAL		81.40			94.89

Table 3. Effect of Climate and/or Elevation on Incidence of Disease in 20-30 year/old Males

	Incidence per 100 clinic visits		Vietnam as
	WBGH	95th Evac	% of WBGH
Dermatophytosis, all	5.88	8.06	137%
T. pedis	3.88	2.82	73%
T. cruris	1.08	1.56	144%
T. corporis	0.24	2.65	1105%
Other tinea	0.68	1.03	152%
Pyoderma, all	1.14	4.05	356%
Impetigo	0.24	1.39	580%
Ecthyma	0.24	0.63	263%
Furuncles	0.5	1.76	309%
Cellulitis	0.12 (2 cases)	0.27	225%
Miliaria, all	0.12 (2 cases)	4.68	3900%
Dry Skin Dermatosis	1.83	0	absolute

Table 4. Effect of Climate and/or Elevation on Skin Disease in 20-30 year/old Active Duty Males

	Incidence per 100 clinic visits		Vietnam as
	WBGH	95th Evac	% of WBGH
Pseudofolliculitis Barbae	4.85	7.93	164%
Contact Dermatitis	2.97	3.48	117%
Atopic Dermatitis	1.14	1.99	175%
Tinea Versicolor	1.88	2.95	157%
Urticaria	2.17	3.14	145%
Alopecia Areata	0.80	2.09	262%
Vitiligo	0.05 (1 case)	0.63 (20 cases)	1260

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6913	72 07 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
71 07 01	D.Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	168
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) The Effects of Prolonged Water Exposure on Human Skin (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 04	72 12	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	17
FISCAL YEAR CURRENT 73	1	20

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21. GENERAL USE

20. PERFORMING ORGANIZATION

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ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) warm water immersion foot; (U) paddy foot; (U) human volunteers; (U) skin; (U) skin damage; (U) water composition

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

25. (U) 71 07 – 72 06 The experimental model utilizing the small cups has been perfected. The experimental model is now available for use in evaluating the relative effectiveness of potential prophylactic agents.

*Available to contractors upon original DA 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

The Effects of Prolonged Water Exposure on Human Skin

Major Isaac Willis

PROBLEM:

Warm water immersion foot and its acute, severe variant "paddy foot" caused severe disability among troops fighting in the Meking Delta of Vietnam and previously in troops located in the Pacific Islands during World War II. Oddly, warm water immersion foot also occurs in artic areas among soldiers wearing waterproof footgear. The severity and extent of morbidity in both instances were found to be related to unavoidable prolonged water exposure under combat conditions. Field trials revealed that temporary restoration of the skin's integrity occurred when predisposed areas were daily removed from inundated conditions and allowed several hours to dry. However, a lack of knowledge as to underlying pathologic changes induced by water exposure not only limited attempts at specifically effective prophylactic and therapeutic methods, but also knowledge as to the exact extent of temporary or permanent skin damage in those so exposed.

APPROACH:

Design of a Method for Continuous Water Exposure. This was accomplished by using clear Plexiglass^R to make a cup of 5 ml capacity that covered a surface area of 3.97 cm^2. Each cup also contains an approximately 16-gauge flexible plastic tube connecting from its side for use in introducing and removal of water samples while the cup is affixed to the skin. This flexible tube may be bent upon itself or plugged to seal off the cup and its contents. The open end of the cup is first affixed to a 2 x 2-inch strip of pliable cellophane tape (Mystik Tape^R) using plastic rubber (Duro-Plastic Rubber^R) allowing 24 hours for the rubber to dry. After sterilizing the cup in ethylene oxide, the cellophane tape covering the open end of the cup is cut-away immediately prior to use. Two coating of aerosol Medical Adhesive B^R are sprayed on the skin around the test site and 30 second later the test cup, affixed to its cellophane tape, is placed in position. Elastoplast^R is used to cover and support the entire base and edges of the test site.

Method of Testing. Subjects: Thirty normal young adult volunteers were utilized. The legs and backs served as test sites. A maximum of either 3 sites on each leg or 10 sites on the back could be tested simultaneously.

Water Samples: These consisted of (1) sterile water for injection, USP, and (2) sterile buffer solutions of 0.1 M N-2 Hydroxyethylpiperaxine-N'-2 Ethanesulfonic Acid (HEPES) at pH 3.5 and pH7.5.

Preparation of Subject and Application of Test Materials: A 14-day period in which the use of all antibacterial soaps and cosmetics was proscribed preceded application of test materials. Volunteers discontinued all bathing for 24 hours immediately prior to testing. Water cups that had been prepared and gas-sterilized with ethylene oxide, were then affixed to the skin. Using sterile 5 ml syringes, 4 ml water samples were divided via Swinnex 25 Millipore^{R} filters to the test cups, leaving a lml air space inside the cup to allow the water to move about. Water samples delivered in this manner were placed on culture media to detect any bacteria contamination that may have occurred despite careful handling of cample. The tube used for delivery of water to each cup was bent upon itself and secured in position using tiny winding strips of adhesive tape. Test sites were randomized and each sample was tested in duplicate. One of each duplicate sample was changed every 6 to 12 hours while others remained unchanged for the duration of the test period (72-144 hours).

Controls: These consisted of (1) an empty water test cup affixed and sealed as above and (2) a 3.97 cm^2 Saran^{R} wrap occluded site affixed at its edges in the same manner as the cup.

Methods of Evaluating Test Responses. Clinical: Preliminary observations for erythema were made at 12-hour intervals through the transparent plastic. Edema could not be adequately evaluated until the cups were removed. Final test responses recorded at 2 hours after removal of test materials were graded on a 5-point scale:

0 - no reaction

1+ - mild diffuse erythema or moderate spotty erythema

2+ = moderately intense confluent erythema

3+ = moderately intense erythema plus moderate edema

4+ = erythema, intense edema, and vesiculation

Histology: Biopsy specimens obtained from 72 and 144 hour test and control sites were formalin fixed and prepared by routine hematoxylin-eosin staining for light microscopic examination.

Bacterial: Baseline bacterial samples were obtained in 18 subjects from 4 different sites located between areas demarcated for water testing and controls. These were obtained immediately prior to application of the test and control materials. Samples from the test and con trol sites in 9 of these subjects were obtained at 72 hours and in the remaining 9 subjects at 144 hours. Water samples from the cups were also cultured at this time.

Samples were collected using a mechanical scraping technique (detailed later in this report) in which phosphate buffered saline, pH 7.9, containing 0.1% Triton X-100 served as the collecting medium. Total bacterial counts were calculated from diluted samples grown after 5 days of aerobic and anaerobic inculbation on Casman agar. MacConkey agar was used to isolate gram negative bacteria, and Baird-Parker agar to isolate staphylococci and micrococci.

Method of Assaying the Effects of Antibacterials on Test Responses. Ten subjects were utilized in this experiment. One of each duplicate test site received 0.1 ml of syringe-delivered Neosporin-G^{R} cream both at 12 hours and immediately preceding testing. The cream was spread evenly over each 3.97 cm^2 test site using sterile wooden applicator sticks.

In 10 other subjects, crystalline Chloromycetin Ophthalmic^{R} was added to one of each duplicate set of water samples to give a concentration of 0.5 mgm/ml.

Observations were recorded as in the previous studies.

Method of Demonstrating Changes in Eccrine Sweating. The numbers of freely functioning eccrine sweat units

per cm^2 were determined at water test, control, and adjacent normal untested skin sites in every subject. Within 15 minutes after removal of test materials, subjects were first placed in an environmental chamber at a temperature of 110° F and 50% relative humidity. Fifteen minutes later, functioning sweat units were "permanently" recorded using a silicone impression material (Kerr Syringe Elasticon). Repeated imprints were taken at 24 hour intervals for 3 days and then at weekly intervals for 2 weeks to detect any delayed change in the number of functioning sweat units.

RESULTS AND DISCUSSION:

Clinical Responses. Observations revealed that simple continuous exposure to water for 72-144 hours, without any additional potentially adverse environmental factors, induced striking inflammation in skin. The degree of inflammation seen at water test sites could not be correlated with the pH of test samples. The pH of the buffer solutions did not vary by more than 0.5 unit throughout testing, while that of water changed from a pH of 6.7 to a more acidic pH of about 5.0.

The degree of clinical inflammation due to water exposure was 2 to 3 times greater than that observed at hydrated Saran^R and empty cup control sites (Table 1). These control sites showed only mild to moderate maceration, and diffuse inflamed pin-point papules that became accentuated upon attempts to induce thermal sweating (typical of miliaria). In contrast, the water test sites showed moderate to marked maceration, confluent erythema and edema. Pyoderma was never observed at any of the test sites. Hairs were thickly coated with a yellowish waxy material at almost all water and pH 7.5 buffer exposed sites. The same type but a much lesser amount of material was seen on hairs of pH 3.5 buffer exposed sites.

Skin responses after changing water samples every 6 to 12 hours were just as intense as those induced at sites where water samples remained unchanged for 72 to 144 hours.

The induction of dermatitis was not influenced by location, i.e., leg versus back. However, the back

proved to be a more suitable test area because of more available testing space and less chance of trauma to the cups.

Histological Observations. Histologic studies of specimens from water-induced dermatitis revealed the following: (1) a swollen thickened horny layer, (2) moderate acanthosis, (3) moderate to marked edema of the upper dermis, (4) a mild to moderate increase in perivascular mononuclear cells throughout the dermis, and (5) scattered pigment and pigment-laden macrophages in the upper dermis. These findings were quite different from those seen in specimens from the Saran^{R} and empty cup control sites.

Observations of Deposits on Hairs: Microscopic examination of gram-stained smears of the yellowish waxy material seen on hairs in water and buffer test sites revealed only masses of predominantly gram-negative rod-shaped bacteria.

Observations of Antibacterial Treated Sites: Water exposed skin sites that were either pre-treated with Neosporin-G^{R} cream or treated with Chloromycetin^{R} during the test developed inflammation at the same time and to the same degree as untreated test sites.

Eccrine Sweat Function: The most striking and unexpected finding was in regard to sweat delivery. While both of the control, empty cup and Saran^{R}, test sites showed moderate to severe decrements in the number of freely functioning sweat units after only 72 hours of occlusion, not a single water test site showed a decrement after even 144 hours of exposure. In fact, most water test sites showed a slight increase in the number of freely functiong sweat units (Table 2).

Bacteriology: The total numbers of aerobic and anaerobic organisms cultured from water test sites and from the Saran^{R} covered and empty cup control sites were compared with the numbers of organisms cultured from adjacent normal skin areas prior to testing. Except for the pH 3.5 buffered water test site where the bacterial counts ranged from approximately the same to a slight decrease in number, all other test sites showed manifold qualitatively

similar increases in the numbers of both aerobic and anaerobic organisms. In general, the greatest increase in counts occurred at the sterile water test sites followed by counts from the pH 7.5 buffer sites, the empty cup controls and Saran^{R} covered sites. Although sterile water and pH 7.5 buffer showed the greatest increase in bacterial counts, the degree of inflammation at these sites were greater in only a few subjects than that seen at the lower pH 3.5 site where bacterial counts were much lower (Table 1). Except at all test and control sites in two subjects, Staphylococcus aureus organisms were not isolated on selective media. Although retrieved water samples grew out organisms in numbers of 1×10^{1} to 1×10^{7} per cc of sample, these were not consistently higher for one sample over another.

SUMMARY:

Continuous 72 to 144 hour exposure to water using the experimental model described in this study resulted in a clinical and histologically proven subacute dermatitis. This dermatitis differs notably from responses induced by conventional Saran^{R} wrap type hydrating methods in the following ways: (1) Its onset and severity were not found to be dependent upon increases in skin pH and bacterial population. (2) Although hydration and maceration of the horny layer were marked, evidence of neither post-hydration anhidrosis nor miliaria could be found.

Results of this study indicate the need for re-evaluating our concepts concerning the pathogenetic relationship of prolonged hydration to such clinical dermatoses as warm water immersion syndromes (paddy foot), dyshidrotic eczema, miliaria rubra, housewive's eczema, contact irritant dermatitis and infections seen in tropical climates and warm inundated terrains. It is proposed that water, itself, may induce an irritant dermatitis through its direct toxic effect on viable cells following sufficient hydration and maceration of the horny layer.

FUTURE PLANS:

Utilizing this experimentally induced dermatitis to serve as a model for water-induced dermatoses, this technique may now be used to evaluate and compare the

effectiveness of potential water protective agents for prophylactic use in predisposed individuals.

Table 1

Test Responses to Hydration

Test Site	Reaction Rate	Reaction Intensity
Sterile Water	25/30	3+
	5/30	2+
Buffer (pH 3.5)	19/30	3+
	11/30	2+
Buffer (pH 7.5)	6/30	4+
	20/30	3+
	4/40	2+
Empty Cup Occlusion	3/30	2+
	27/30	1+
Saran^{R} Wrap Occlusion	6/30	2+
	24/30	1+

Table 2

Effects of Hydration on the Number of Functioning Sweat Units per cm^2

Test Site	Percent Change (Range)
H_2O for Inj., USP	+1 to +10%
Buffer at pH 3.5	+3 to +10%
Buffer at pH 7.5	-2 to +8%
Empty Cup	-30 to -80%
Saran	-75 to -100%

Bacterial Sampling Technique

Major Isaac Willis, MC

PROBLEM:

While practically every available technique for sampling the cutaneous bacterial flora will give satisfactory qualitative information, almost none of these have been proven reliable for quantitative study. In our investigations where quantitative data was desired, it became necessary to have a reliable sampling technique. The only currently available technique for "reliable" quantitative sampling requires a trained technician who can skillfully duplicate his manual scrubbing method. We have no such person; and at the same time, I believed that a mechanical rather than manual method of sampling could be developed that would permit consistent replication thereby giving more reliable quantitative data.

APPROACH:

The horizontal arm of a linear friction blister machine was modified so that its rubbing end would contain an aperature for vertical insertion of a Teflon^{R} spatula. A rectangular plexiglass cup was devised to hold the bacterial collecting medium (phosphate buffered saline, pH 7.9, containing 0.1% Triton X-100). With the horizontal arm (adjustable by using a level on the arm) holding the vertical spatula in place, a given number of strokes (scrubs) could be made at a given skin site. The amount of downward pressure on the spatula tip touching the skin could be adjusted by sliding a metal weight along the horizontal arm of the machine.

RESULTS AND DISCUSSION:

Quantitative data obtained from samples of adjacent and bilateral normal skin sites using this new mechanical sampling technique have repeatedly demonstrated that the bacterial counts generally do not vary nearly as much as reported in the literature where other techniques have been used. Moreover, the sampling technique can be employed by almost any person without special training.

This technique fulfilled my needs for bacterial analysis of test sites in the study of the effects of prolonged water exposure on human skin. Since the initial studies, machined stainless steel spatulas and sampling cups have been made so that this equipment is more durable and can be sterilized more quickly (autoclaving).

This technique will also be of use in quantitative and qualitative studies of corneocytes

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6790	72 07 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
71 07 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A831	00	001
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Delayed Type Skin Reaction and Lymphocyte Transformation in Cutaneous Diseases (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 010100 - Immunology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 04	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	3	51
FISCAL YEAR CURRENT 73	3	50

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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TELEPHONE: 415-561-5485

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) delayed hypersensitivity; (U) human volunteers; (U) skin (U) intradermal skin test; (U) lymphocyte transformation; (U) leukocytes; (U) phytohemagglutinin

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

25. (U) 71 07 – 72 06 Lymphocyte transformation has been perfected for use with tuberculin (PPD) and fungal (Trichophytin) antigens. The antigen dose, culture time, culture volume and other parameters that effect the test's reproducibility and reliability have been optimized.

Using multiple PPD and Trichophytin skin test and LTT in 250 human subjects we have shown:

1. The response to a skin test is a continuum and an arbitrary cutoff of 5 mm or 10 mm for a positive test cannot be used to interpret reaction from the immunologic viewpoint.
2. A skin test may boost or possibly sensitize the susceptible recipient and in experimental and/or clinical situations where this is not permissible, in vitro tests such as LTT must be substituted.
3. Two classes of allergic reactions may occur to the same antigen and with trichophytin the immediate reaction is antagonistic to the delayed reaction.
4. The presence of DTH to trichophytin protects the human from experimental infection with Trichophyton mentagrophytes.
5. Virgin subjects acquire DTH from the 1st experimental infection and are resistant to further experimental infection. Thus, specific acquired immunity is demonstrated in the human.
6. The presence of immediate hypersensitivity to Trichophytin renders the host more susceptible to natural and experimental dermatophyte infections.
7. Atopic humans are genetically predisposed to develop immediate sensitivity from natural and experimental dermatophyte infections and account for 50%+ of chronic dermatophyte infections observed in our surveys.

* Available to contractors upon originator's approval

Delayed Type Skin Reaction and Lymphocyte Transformation in Cutaneous Diseases

LTC H. Earl Jones, MC

PROBLEM:

To develop a reliable in vitro test for studying delayed type hypersensitivity (DTH) in certain cutaneous diseases (fungal infections, contact dermatitis) and the chronology of the immunologic response of subjects infected with the fungus Trichophyton mentagrophytes and/or other organisms.

Fungal antigens prepared from T. mentagrophytes will be screened by lymphocyte transformation (LT). Ideally we hope to look for antigenic fractions which could be used as immunogenic compounds to be administered to experimental animals and eventually to immunize humans to develop DTH and resultant immunity to infection with dermatophytic fungi.

APPROACH:

Prerequisite for all related and further research has been developing the LT technique as a reliable tool. Using the commonly accepted delayed hypersensitivity antigen, purified protein derivative of tuberculin (PPD), the LT technique has been studied at LAIR for three years. Applying the fundamental principles learned from the study of PPD-induced LT in subjects with and without tuberculin sensitivity, a similar study of trichophytin skin sensitivity and the response of the subject's lymphocytes to trichophytin will be accomplished. This will obviate the booster effect that repeated skin testing may have in analysis and interpretation of the fungal infection model.

The LT technique using lymphocytes from subjects infected with the fungus will be employed as a screening procedure to analyze new antigen preparations for immunologic reactivity.

RESULTS AND DISCUSSION OF RESULTS:

Lymphocyte Transformation with Tuberculin. Numerous problems were encountered with the LT technique. One of the most serious was chemical contamination of glassware used in the experiment with chromic acid, detergents and/or other chemicals which affect the lymphocyte response. This difficulty was overcome by using plastic disposable containers whenever possible and the thorough, diligent washing of essential glassware. Purified protein derivative (PPD) of the tubercle bacillus as commercially available for skin testing contains phenylolic preservatives which are toxic to lymphocytes in vitro. We now use PPD prepared free of phenylolic preservatives by the Parke-Davis Company. This PPD has proven to be an excellent tuberculin for in vitro lymphocyte studies, is nontoxic, and does induce specific LT in subjects with DTH skin test reactions.

The dose response curve of lymphocyte cultures has been determined for the Parke-Davis PPD and the optimum concentration is 30 mg/cc of culture volume. From time-response studies we determined 6 days to be the optimal time for harvesting the PPD antigen-stimulated lymphocyte cultures. The optimum concentration of lymphocytes per unit volume of the culture is 5×10^5 per cc, using 30 mg of PPD/cc as a stimulus and a 6 day harvest time. The response of the individual's lymphocytes to PPD has been found to be consistent in replicate cultures performed on the same day, or weeks and months apart, and the accuracy range is within 10%.

Relationship of the Lymphocyte Transformation Response to the Skin Test Response. To determine the minimal PPD concentration necessary to elicit a DTH skin test response and to quantify that response, multiple skin tests were done in patients at Letterman General Hospital and in volunteers at LAIR. Commercial available PPD, 1st and intermediate strength, were administered simultaneously to the volar surface of one forearm unless there was a history of tuberculin sensitivity in the past; if so, only the indicated dose of PPD was

used for skin testing. If no response to either skin test or only a minimal response to the intermediate test, 7 days later the individual was tested again using 2nd strength PPD on the opposite forearm. All skin tests were read at 20 minutes, at 24 and 72 hours, at 7 and 10 days following testing. The results of these skin tests are listed in Table 1. The LT was performed on each subject using various doses of PPD. Each culture contained 5 x 10^5 lymphocytes per cc and all cultures were harvested on the 6th day. Table 2 compares the degree of LT as related to the skin test reactivity. A direct linear relationship exists between the minimal strength of PPD required to elicit a skin test reaction, the size of the response, and the individual's LT response to PPD.

Because the individual's response to PPD is a continuous phenomenon with a normal curve of distribution in the population, there cannot actually be a cut-off of skin test positivity at any arbitrary level. The skin test responses were not labelled as positive or negative using the arbitrary 5 mm criteria for a positive test commonly used for epidemiologic testing of individuals for tuberculosis. We believe this is logical from an immunologic point of view since an individual at 4.5 mm would be negative according to those criteria, whereas someone with a similar quality skin test response measuring 5 mm in diameter would be positive. Our LT studies support this hypothesis in that any individual who manifests a response to any one of the concentrations of PPD, including 2nd strength, also had a significant degree of LT. Conversely, 2nd strength PPD negative individuals had no degree of LT. From this evidence, we recommend that the epidemiologic term positive and negative not be applied to skin test responses if this response is to be considered from the immunologic point of view. Rather, the individual's response should be measured in millimeters and characterized as to the erythematous, edematous, and/or indurated quality of the lesion.

From the above studies of sequential PPD skin testing we were suspicious that a booster effect

may have been obtained by the quantity of antigen injected. We retested part of the 36 subjects one week later and another group 2.5 months later. Using intermediate PPD for the followup skin test, 2 of 36 individuals manifested larger reactions which would have been interpreted as positive by epidemiologic criteria, whereas they were negative on the first test. Presently we are studying this phenomenon in experimental animals, but our Moen-Chase guinea pigs develop DTH so exquisitely that they all become sensitized to the minute quantities of phenylolic preservatives included in the commercial PPD preparation.

Trichophyton Skin Test and Lymphocyte Transformation. We previously determined that 10 ug of Cruickshank's trichophytin will elicit delayed hypersensitivity in subjects who have had fungal infections but does not elicit a skin response of any type in women and children with no history of fungal infection. We undertook a clinical-skin test survey of 180 men. Each volunteer in this survey completed a questionnaire involving basic biographical data including his personal history of fungal infections, atopy, and other relevant skin conditions. The physical examination included examining the feet for possible tinea pedis. Each subject had his feet and groin cultured for dermatophytes and was skin tested on his arm with trichophytin, 10 ug. Test sites were examined at 20 minutes, at 24, 48, and 72 hours, and at 7, 10, and 14 days. The diameter of the response was measured and the presence of erythema, edema, induration, vesicles, papules and ulceration at the test site was noted. The skin test results (Table 1) permit the subjects to be divided into three classes by the response at the skin test site at 72 hours: Type I - nonreactors; Type II - immediate reactors, plus or minus delayed hypersensitivity; and Type III - delayed hypersensitivity of varying intensity.

Table 2 shows the distribution of DTH by size of the skin test response which follows a normal biological curve of distribution. Table 3 records at 72 hours the diameter of the skin test response and relates this to the presence or absence of

erythema, edema, induration, and vesicles. This scatter-gram demonstrates that the intensity of the skin test response is directly related to the size of that response. This is to be expected. This again supports our concept that skin test responses are a continum and that an arbitrary value of 5 mm or 10 mm cannot be used for immunologic purposes as a criteria of positive or negative.

That the trichophytin skin test may have a booster effect is demonstrated by the fact 31% of those subjects showing no reaction at 72 hours later manifested some skin test reactivity when examined at 7 or 10 days. This later response was observed at the same skin test site that did not show any reaction at the 72 hour reading. The subjects manifesting the booster effect had a history of fungal infections more frequently than did the skin test nonreactors who did not boost with repeated trichophytin skin tests (see Table 4). This suggests that the skin test acts as a booster and not a primary sensitizer. Repeated skin tests in subjects who showed the booster effect at 7 days resulted in a larger diameter skin test response with each subsequent skin test. More importantly we observed that the intensity of the subsequent skin test was progressively greater. Experimental infections with T. mentagrophytes in these booster susceptible subjects behave similarly to subjects with manifested DTH reactions of even larger magnitude. This indicates that in the susceptible human the skin test may act as a booster and alter the immunologic status of the recipient.

Of the individuals susceptible to the booster effect from trichophytin, 50 percent have a personal history of respiratory atopy. Individuals with respiratory atopy comprise only 23% of the surveyed population. Therefore, it appears that subjects with atopy may more readily lose an acquired delayed hypersensitivity than normal individuals.

Lymphocyte transformation studies using our PEBC antigens have confirmed that individuals with weak reactions to trichophytin do undergo

specific lymphocyte transformation but to a lesser degree than subjects manifesting stronger skin test reactions.

Relation of Immediate Skin Test Response to Delayed Skin Test Reactivity. Although the subjects in the immediate reactor subclass have obviously been infected with a dermatophytic fungus (i.e., they developed a specific allergic sensitivity) only 64% of them manifest DTH to the same antigen when the same skin test site is examined at 72 hours (see Table 1). The average size of the DTH reaction in the immediate reacting group is about one-half the size in the subjects with only delayed hypersensitivity. The immediate reactor subclass has approximately twice the frequency of atopy as do either nonreactors or the frequency of atopy as do either nonreactors or the group average. The DTH reactors are significantly below the overall average of personal atopy for all subjects. This suggests that immediate reactions to trichophytin is associated with atopy; and when this occurs, the delayed reactions are less than in individuals without immediate reactions and atopy. Conversely, subjects with no history of atopy are likely to develop strong DTH following dermatophyte infection.

The effects of immediate reaction on the latter response (Table 5) was examined by dividing those subjects showing immediate reactions into two groups, those with a large size response and those showing only a moderate size reaction. There is a lesser frequency of DTH associated with the large size immediate reactions than with the moderate size immediate reaction. By mixing chlorophenaramine (CTM) with the trichophytin skin test antigen, we partially inhibited the immediate reaction, and noted the size and intensity of the delayed reaction to increase in 80% of the subjects with large immediate reactions. In subjects with large immediate reactions, CTM successfully unmasked an occult delayed hypersensitivity in 3 subjects. The only subject with a moderate reaction without some degree of delayed hypersensitivity had his occult DTH unmasked by CTM. Chlorophenaramine inhibits the effect of

histamine released from the trichophytin-IgE interaction, and by so doing, reduces the hyperemia of the immediate reaction with a consequent reduction in wash-out of the injected trichophytin. This illustrates one mechanism by which immediate reactions may inhibit or decrease the size and intensity of the DTH response.

Forty-three percent of individuals with an immediate reaction had a personal history of atopic respiratory disease. Those with no personal history of atopy more frequently had positive family histories of atopy than individuals who only manifested DTH response to trichophytin. Table 6 illustrates this amply and also depicts the results of skin testing with a battery of antigens that frequently elicit immediate hypersensitivity in allergic subjects. As expected, those subjects with atopy reacted frequently and with multiple positive responses to the ten screening test antigens whereas those individuals with no immediate reactions reacted infrequently. Persons with immediate reactions to trichophytin but no personal history of atopy reacted intermediately, indicating that they have a fundamental increased propensity to develop immediate type antibody upon immunization with the appropriate antigen. This tendency probably accounts for their immediate reactions to trichophytin as well.

Considering the history, physical examination, and the allergy skin test responses, the subjects can be classified into 3 types: 1. the skin test nonreacting group, the "fungal virgins"; 2. the delayed hypersensitivity reactive group who characteristically had a fungal infection in the past, but only rare occurrences: and 3. the immediate skin test reaction group, most of whom have persistent or frequent recurrent fungal infections. Individuals with immediate reactions to trichophytin may become infected at a younger age and these individuals have more persistent and/or recurrent fungal infections as substantiated on their physical examination (see Table 7). Dermatophytes were recovered 3 times more frequently from those subjects with immediate reactions compared to the DTH positive subjects or atopic subjects with no immediate reactions.

Experimental Fungal Infection. The experimental model dermatophyte infections described previously and discussed elsewhere in this report display 4 stages during the experimental infection: Stage 1 is the toxic phase, Stage 2 is the spreading infection stage, Stage 3 is the development of ringworm, and Stage 4 is the healing phase. When subjects of each of the clinical skin test subgroups described above are infected one sees strikingly different responses: 1. DTH positive subjects seem to be relatively immune to the experimental infection; 2. fungal virgins with no existing skin test reactivity undergo the same toxic phase of the infection and the lesions continue to spread until the onset of delayed hypersensitivity limits further enlargement of the lesions; 3. the skin test booster group seems to be protected in a manner identical to those subjects manifesting DTH; 4. subjects with immediate reaction to trichophytin and with a history of poor resistance to infection are susceptible to infection with small numbers of spores and the individual's lesions become large and persist several times longer than subjects with DTH almost following the protracted course seen in the primary infected virgin. This would not be expected since many of these individuals manifest both delayed hypersensitivity and immediate hypersensitivity. Perhaps the immediate hypersensitivity interferes with the coexisting delayed hypersensitivity in a manner similar to that observed in skin testing and thus fails to afford protection to the individual.

Some of the "fungal virgin" group had asthma or hay fever at younger ages. During the course of infection these subjects developed delayed hypersensitivity and appeared to be healing; however, at about 30 days, 2 of the 4 such subjects developed immediate sensitivity and concomitantly lost control of the infection and large, gyrate, serpiginous, ringworm lesions erupted that have persisted for at least 90 days. This confirms the concept that immediate wheal and flare sensitivity to trichophytin is a liability to the individual manifesting.

Of the 129 subjects in the survey on which complete data is available, 18 (14%) had chronic and/or persistent recurrent fungal infections. This is a familiar figure since most male populations surveyed have a 10 to 20% incidence of athletes foot. Since atopy is present in most populations at about the same frequency, it seems likely that the percentage of individuals found in surveys to have athletes foot may coincide with the percent of individuals in the population who manifest immediate reactivity to trichophytin. In this survey, two-thirds of the 18 subjects with chronic or recurrent tinea pedis had a personal history of either asthma or hay fever.

In summary, it seems that delayed hypersensitivity confers relative immunity to dermatophyte fungal infections in man. This immunity may be antagonized by coexistent immediate hypersensitivity to trichophytin.

Lymphocyte Transformation and Trichophytin Antigens. During this fiscal year LT tests with several antigens prepared from T. mentagrophytes was attempted. LT was obtained using Cruickshank's antigen at 400 ug/cc of culture; but such large amounts would soon exhaust our supply of trichophytin. A growth supernatant antigen and a propylene glycol extractable antigen similar to Cruickshank trichophytin were toxic to lymphocytes in vitro. A pyridine extractable glycoprotein antigen prepared from T. mentagrophytes was found to specifically stimulate lymphocytes of subjects with trichophytin sensitivity. Small quantities (ugm) of PEGB-P1 and PEGB-P4 would induce transformation in sensitive subjects.

Cytotoxicity. A technique was developed in which HeLa cells are cultured in T flask and aliquots of potential toxic materials are added to the flask to assay for cytotoxicity. From extracts of T. mentagrophytes grown in culture, 4 mycotoxin-like fractions have been prepared. Assaying these mycotoxin-like materials for their ability to destroy HeLa cells, we found that mycotoxin fraction 2 and 3 are as toxic if not more so than aflatoxin B_1. Further progress with this system has been slow due to difficulties in quantitating the technique and in producing sufficient quantities of pure mycotoxins.

FUTURE PLANS:

We will continue to develop the LTT to detect DTH to trichophytin *in vitro* which is absolutely essential in understanding the developing immunologic phenomenon in virgin subjects infected with *T. mentagrophytes*. Obviously, multiple skin tests in these subjects may significantly influence their developing immune-mechanisms. The LTT will be used to select volunteers as experimental subjects to receive the model infection thereby avoiding skin testing and the consequent possibility of a booster effect.

The LTT will permit studying the intricate relationships between the sensitized lymphocyte and circulating antibody. We have demonstrated in subjects with immediate hypersensitivity and natural or experimental fungal infections that IgE antibody specific for trichophytin is antagonistic to delayed hypersensitivity in some manner. By isolating the lymphocyte, the IgE, and the fungal antigens *in vitro* and using the LTT as a detector of the interactions among these biologic materials, we should be able to define the mechanism by which IgE exerts its antagonistic effect on the cell mediated immune response.

Better understanding the above relationships will provide better ways to combat fungal infections such as treatment with antihistamines, desensitization, or the using of transfer factor. New screening diagnostic techniques will be developed to detect carriers of pathogenic dermatophytes and/or subjects prone to develop progressive or persistent dermatophyte infections prior to exposure in a high risk area. This will greatly reduce morbidity and duty time loss because of fungal skin diseases such as athletes foot, jocky itch and ringworm. This should culminate in the development of an immunization program for use in military and civilian populations.

Table 1. Trichophytin Skin Test Responses

Skin Test Class	#	Age	DTH	Average Size 72 hr Response	Personal Atopic History
Non-reactors	53	29.9	0	0	22%
DTH reactors	99	31.5	100%	11 mm	9%
Immediate reactors	28	29.2	64%	6 mm	43%
Average - all subjects	180	30.5	65%	7 mm	23%

Table 2

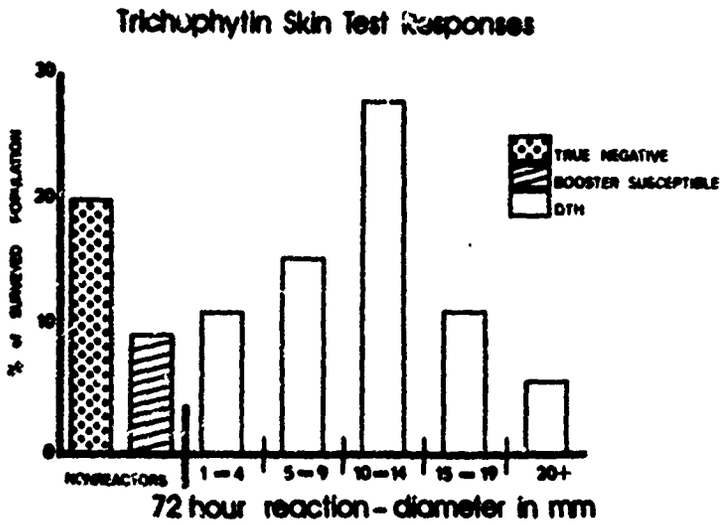

Table 3

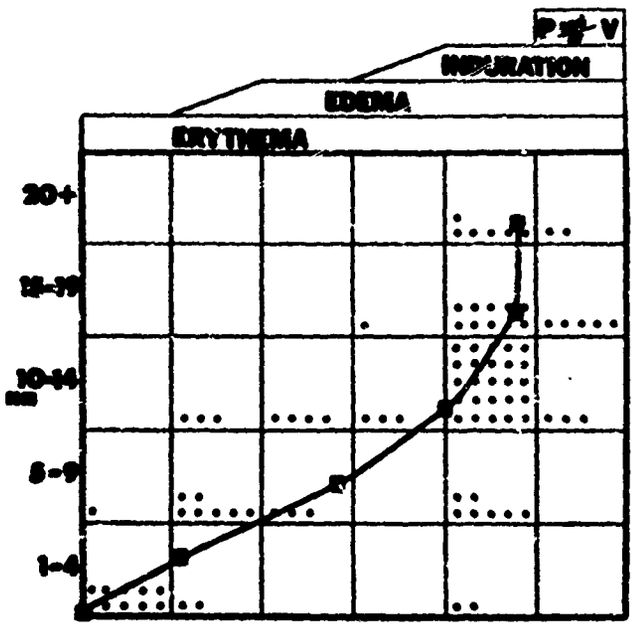

Table 4. BOOSTER EFFECT OF TRICHOPHYTIN SKIN TEST

	Number	Historical or Physical Evidence of Fungal Infection	Personal Atopic History
Susceptible to booster	12.39 (31%)	10/12	6/12 (50%)
Not susceptible to booster	27/39 (69%)	17/27	4/27 (15%)

Table 5. TRICHOPHYTIN: EFFECT OF IMMEDIATE REACTION ON THE DELAYED RESPONSE

	Size of Immediate Skin Test Response Large	Moderate
Average Wheal/Flare	21/49 mm	13/29 mm
Number Subjects	13	15
72 hr Response		
Antigen Only	38%	93%
Antigen & CTM	62%	100%
Antigen & CTM - 72 hrs		
Size & Intensity	80%	30%
Unmask occult DTH	3 subjects	1 subject

Table 6. IMMEDIATE SKIN TEST RESPONSE: RELATION TO ATOPY

Trichophytin Reactor Class	Family Hx Atopy	10 Test Allergy Screening Series* # with + Test	Average # +/Subjects
Immediate Reactors			
Personal Hx Atopy	50%	6/6	4.2/subject
No Personal Hx Atopy	21%	8/13	1.9/subject
No Immediate Reactions + DTH	8%	4/14	0.9/subject

*Antigens: 1. Mixed trees 2. Mixed grasses 3. Plantain 4. Mixed ragweed 5. Mixed Western weeds 6. House dust 7. Mold mixture 8. Egg 9. Milk 10. Wheat 11. Control

Table 7. CLINICAL EVIDENCE OF FUNGAL INFECTION

	DTH + Non-Atopic	Atopic Subjects (no immediate reaction)	Immediate Reactors ± Atopy
Number Subjects	91	19	28
Average Age Onset	16.8	14.3	12.7
Reoccurrences			
None	76%	72%	19%
2-5 Episodes	20%	11%	8%
Persistent or recurrent	2%	17%	73%
Physical Exam			
Normal	40%	53%	11%
Possible Tinea	40%	16%	29%
Tinea	19%	31%	61%
Culture +	26%	7%	63%

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6783	72 07 01	DD-DRAE(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
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A831	00	004
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 - Biochemistry; 010100 - Microbiology; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 11	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	4	46
	CURRENT 73	5	50

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL
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TELEPHONE: 415-561-5181

21. GENERAL USE

20. PERFORMING ORGANIZATION

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Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: King, R.D., CPT MSC
NAME: Jaeger, J.R., DAC DA

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Arthroderma benhamiae; (U) Trichophyton mentagrophytes; (U) enzymes; (U) antigens; (U) immunogens; (U) virulence; (U) guinea pigs; (U) skin

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

23. (U) 1) Elucidate biochemical mechanisms of infection. 2) Perfect assay of enzymes capable of hydrolyzing substrates found in stratum corneum; characterize isolated enzymes by physical-chemical procedures. 3) Correlate virulence, enzyme production, and nutritional requirements of fungus with morphological and physiological variations in vitro and in vivo. 4) Determine antigenic activity of isolated enzymes. 5) Isolate and purify fungal antigens and fungal toxins. 6) Devise novel therapeutic and preventive measures by perfecting antigen isolation procedures for use in immunization studies.

24. (U) 1) Enzymes – determine fungal enzymic activity against stratum corneum components; isolate and purify fungal enzymes. 2) Antigens (Vaccine) – isolate and pruify cellular fractions capable of confering immunity to experimental and natural Dermatophyte infections in animals and man. 3) Toxins – isolate and characterize the mycotoxins produced by Trichophyton mentagrophytes to determine their biologic function and their role in disease.

25. (U) 71 07 – 72 06 1) Strains of T. mentagrophytes and A. benhamiae have been studied for their ability to produce the following enzymes: collagenase, elastase, keratinase, lipase, phospholipase, protease and urease. 2) A purified keratinase preparation from T. mentagrophytes has elicited positive delayed hypersensitivity responses in guinea pigs and humans. 3) Cell walls have been isolated from T. mentagrophytes; cell wall fractions are being characterized chemically and immunologically 4) Cell membrane and cytoplasmic antigens have been isolated and are being characterized. 5) All sub-cellular fractions of the fungus are being evaluated for their ability to confer immunity in experimental animals and in human volunteers in conjunction with Project No. DA OC 6794, Work Unit 006.

*Available at contractor 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

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6792	72 07 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
71 07 01	H. Terminate	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A831	00	005
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Development of Methods for Diagnosing and Combatting Susceptibility to Fungal Infections (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
010100 - Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 04	72 02	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: NA 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 73	0	0
	CURRENT 72	1	10
	BUDGET		

19. RESPONSIBLE DOD ORGANIZATION
NAME:* Letterman Army Institute of Research
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RESPONSIBLE INDIVIDUAL
NAME: Akers, W.A., COL MC
TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Kornfeld, L. Ph.D.
TELEPHONE:
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

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

25. (U) 71 07 - 72 06 Study terminated due to resignation of investigator.

*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.

Development of Methods for Diagnosing and Combatting Susceptibility to Fungal Infections

Lottie Kornfeld, PhD

GENERAL PROBLEM:

Fungal infections of the feet, groin and trunk are among the most common military medical problems in the humid tropics. Clinical and experimental observations indicate that the inflammatory nature of dermatophyte infections is due to a state of delayed hypersensitivity to the infecting fungus and/or its products. The hypersensitive state may persist for long periods after remission of a primary infection, so that an intense and more rapid inflammatory response occurs following reinfection with the same or an antigenically related fungus.

If immunoprophylaxis of the dermatophytoses is to be successful, it must prevent, or at least reduce, the development of the hypersensitive state. Before approaching the ultimate goal, certain fundamental questions must be answered. To what extent does the immune response of the host affect the pathogenesis of the fungal infection? Does alteration of the host response alter the course of the disease? Which components/products of the fungus are responsible for eliciting the immunological responses? Is a sensitive and specific *in vitro* test available for detecting delayed hypersensitivity to dermatophytes?

APPROACH:

A series of 4 experiments were performed to gain insight into these questions. The principle investigator departed in March 1972.

PROBLEM #1:

To evaluate various fractions prepared from *Trichophyton mentagrophytes* as potential skin test antigens for detecting delayed hypersensitivity to this fungus.

METHOD: Lyophilized materials prepared by CPT Reinhardt were dissolved in physiological saline and

sterilized by millipore filtration. Further dilutions to give the desired concentrations were prepared aseptically in sterile saline. The substances tested are listed in Table 1. Groups of normal guinea pigs and guinea pigs which had recovered from experimental infection with T. mentagrophytes were injected intradermally with 0.1ml quantities of all test materials. Skin reactions were observed 30 minutes later and after 24 and 48 hours. Reactions were scored by the usual criteria: the diameter of erythema was measured; intensity of erythema and intensity of induration were evaluated on a scale of 0 to 3. For comparison with a known skin test antigen, all animals were tested simultaneously with Cruickshank's trichophytin (CT).

RESULTS:

Immediate reactions to the test materials were never observed. Delayed reactions are summarized in Table 2. It can be seen that the following preparations exhibited both specificity and sensitivity under our experimental conditions and compared favorably with CT (100 µg):

Precipitate of culture supernate	100 ug
Partially purified keratinase	100 ug
170-671-CT1	100 ug
170-771-SEP1	100 & 10 ug

PROBLEM #2:

To determine whether skin testing of normal guinea pigs with Cruickshank's trichophytin (CT) induces delayed hypersensitivity demonstrable by subsequent skin tests.

METHOD:

3 normal guinea pigs were each given 4 weekly intradermal injections of 100 ug CT. Skin reactions were recorded after 24 and 48 hours.

RESULTS:

Mild erythema without induration (mean diameter 9 mm) was observed 24 hours after the initial skin tests, but the reactions did not persist for 48 hours. However, intense erythema with induration (mean diameter

15.6 mm) was seen both 24 and 48 hours after the second set of skin tests. The severity of the skin reactions was essentially unchanged on further testing. Although only a small number of animals were used, the results of this study indicate that a single skin test with 100 ug CT was sufficient to induce delayed hypersensitivity to this antigen in our guinea pigs.

PROBLEM #3:

To test sera from volunteers and experimental animals for the presence of complement-fixing antibodies to Trichophyton mentagrophytes.

APPROACH:

Complement fixation tests were carried out essentially as outlined by Campbell, et al, in "Methods in Immunology," 1964. Overnight fixation at 4°C was found to be most suitable in this system. Lyophilized T. mentagrophytes "disrupted mycelium and cell sap," was rehydrated (1 mg/ml) and used in 1:8 dilution as antigen. Serum obtained from rabbits injected repeatedly with T. mentagrophytes mycelium was used for the titration of antigen and as a positive control. The titer of the immune rabbit serum was 1:128.

RESULTS:

a. Sera from 12 guinea pigs obtained 4 months after infection with T. mentagrophytes were negative in 1:2 dilution.
b. Sera from 6 guinea pigs obtained 7 months after fungal infection were negative in 1:4 dilution and showed slight complement fixation (of doubtful significance) in 1:2 dilution.
c. Sera from 6 human volunteers obtained before and during experimental fungal infection did not fix complement specifically in 1:2 dilution.

PROBLEM #4:

To select breeders for the LAIR guinea pig colony on the basis of ease of acquiring contact sensitivity to dinitrochlorobenzene (DNCB).

METHODS:

The prospective breeders were brought to LAIR in October and December 1971 from the University of California at Davis, where a breeding colony had been maintained since March 1970. This colony was established with male and female Moen-Chase guinea pigs obtained directly from Dr. Merrill W. Chase of Rockefeller University, New York.

The methods employed for sensitization and skin testing were essentially those recommended by Dr. Chase, wit one notable exception. Dr. Chase tests and selects only prospective male breeders, we subjected both males and females to this procedure.

Sensitization:

A fresh solution of DNCB in alcoholic saline (42 ug DNCB/ml 0.83% ethanol in saline) was prepared by dissolving the chemical in an appropriate volume of 83% ethanol and diluting 100-fold with sterile saline. Three intradermal injections (0.1ml each) were given on the shaved right foreflank so that the three sites were approximately 1 inch apart and formed a triangle.

Skin Testing:

Ten days later, one drop (about 0.03 ml) of a 5% solution of DNCB (freshly prepared by dissolving a weighed amount of chemical in a small quantity of acetone and diluting with fresh corn oil to the required volume) was applied to the shaved back. Skin reactions were scored after 24 and 48 hours according to the following criteria:

0 negative
+ faint pink spots
1+ faint pink - confluent
2+ pale pink
3+ bright pink
4+ deep pink, thickened

77 guinea pigs (Group 1) were sensitized on 29 October and skin tested on 8 November 1971; 53 guinea pigs (Group 2) were sensitized on 10 December and skin tested on 20 December 1971.

RESULTS:

24 and 48 hour skin reactions of all (130) animals were compared. 79 animals (61%) were selected for inclusion in the breeding colony

FUTURE PLANS:

Dr. Chase has stated that about one-third of the animals should be rejected. On this basis, the following tentative criteria for the selection of breeders have been adopted: at least a 2+ reaction either 24 or 48 hours after DNCB challenge. It may be advisable to apply more stringent criteria to the next generation of animals.

Table 1

Experimental T. mentagrophytes skin test antigens

Designation	Description
Disrupted mycelium and cell sap	
Precipitate of culture supernate	Ammonium sulfate preciptate of extracellular supernate from fungus grown on plantar skin
Partially purified keratinase	DEAE column effluent of above material
170-671-CT1	Corresponds to Extract II described by Cruickshank, et al: JID 35: 219,1960
170-871	Nucleoprotein fraction
170-871-BCF1	"Cruickshank fraction" dialyzed, lyophilized, pH 9.5 borate
170-871-CAS1	"Cruickshank fraction" calcium acetate soluble
170-871-CT2	"Cruickshank trichophytin" 10 August 71
170-871-NPS1	Neutral polysaccharide fraction 18 August 71
170-771-SEP1	Schweizer's reagent of residue from two ethanol extractions

TABLE 2

Skin reactions of infected and control guinea pigs to experimental *T. mentagrophytes* antigens

Antigen	Dose μg/0.1 ml	Infected guinea pigs: Number Animals	Erythema 24 hr	Erythema 48 hr	Induration* 24 hr	Induration* 48 hr	Control guinea pigs: Number Animals	Erythema 24 hr	Erythema 48 hr	Induration 24 hr	Induration 48 hr
Quickshank trichophytin	10	6	9.5	10.2	0	0	10	4.7	0.8	0	0
Disrupted mycelium and cell sap	500	6	17.3	13.2	2.0	2.0	10	17.0	13.4	1.0	1.2
	100	6	8.2	5.0	0	0.9	10	7.8	3.6	0	0
	10	6	5.0	2.8	0	0	10	6.1	2.6	0	0
	1	6	3.8	1.7	0	0	10	5.8	2.3	0	0
Quickshank trichophytin	10	6	6.0	3.9	0	0	3	4.0	2.7	0	0
Precipitate of culture supernate	100	6	12.0	11.3	1.3	0.5	3	3.3	1.0	0	0
	10	6	4.5	3.9	0.5	0	3	2.7	1.7	0	0
	1	6	1.9	0.9	0	0	3	2.0	1.0	0	0
Partially purified keratinase	100	6	10.0	10.0	1.7	1.0	3	2.7	3.0	0	0
	10	6	5.2	3.6	0.7	0	3	2.0	2.0	0	0
	1	6	2.6	1.6	0	0	3	1.7	1.0	0	0
Quickshank trichophytin	100	6		12.5		1.8	4		1.8		0
	10	6		5.8		1.0	4		1.5		0
170-671-CT1	100	6		12.5		2.3	4		2.0		0.3
Quickshank trichophytin	100	8	12.5	12.6	1.7	1.6					
170-871-NPF1	100	4	6.25	5.75	0	0	4	5.00	3.25	0	0
	10	4	4.25	0.50	0	0	4	4.25	1.50	0	0
170-871-BCF1	100	4	11.75	13.00	2.8	3.0	4	10.75	8.25	2.3	1.5
	10	4	9.00	7.75	2.0	1.5	4	4.75	2.25	2.0	1.3
170-871-CAS1	100	4	9.75	6.25	0.8	0.8	4	3.75	1.00	0	0
	10	4	4.75	2.00	0.5	0	4	4.00	0.50	0	0
170-871-CT2	100	4	5.00	1.75	0	0	4	2.75	2.00	0	0
	10	4	4.25	0.75	0	0	4	2.25	0	0	0
170-871-NPS1	100	4	3.75	1.75	0.5	0	4	1.75	0	0	0
	10	4	5.50	3.50	1.5	0	4	4.00	2.25	0	0
170-771-SEP1	100	4	14.00	13.25	2.2	1.8	4	3.50	0.75	0.8	0
	10	4	12.00	10.00	1.5	0.8	4	3.75	1.75	1.3	0

*Mean diameter in mm
**Mean intensity (on scale of 0 to 3)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6794	72 07 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
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A831	00	006
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Experimental Fungus Infections in the Skin of Man: A Therapeutic Model (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 - Microbiology; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 11	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	2	25
FISCAL YEAR CURRENT 73	2	30

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Presidio of San Francisco, CA 94129

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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Inv Civilian)

NAME: Jones, H.E., LTC MC

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Reinhardt, J.H., CPT MSC

NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U)dermatophytosis; (U) experimental infection; (U) skin; (U) human volunteers; (U)Trichophytin mentagrophytes

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

23. (U) 1) Perfect a reproducible, experimental fungal infection of the skin of humans. 2) To determine the minimum number of fungal spores required to produce persistent infections in humans with and without pre-existing immunity to dermatophytic fungi. 3) To correlate the degree of immunity in human volunteers with the severity of the induced infections. 4) To prepare a protective vaccine that will prevent or minimize the severity of experimental and natural infections. 5) To evaluate prophylactic and therapeutic anti-fungal agents.

24. (U) 1) Purified spores of Trichophyton mentagrophytes RVN D-1 are inoculated onto normal skin and covered with a wet gauze patch and a piece of Teflon® film for 96 hours. 2) Resulting infections are evaluated using standardized lesion grading criteria. 3) The immune status of human volunteers is determined by skin testing and by in vitro methods such as lymphocyte transformation. 4) Prophylactic and therapeutic agents are evaluated using controlled, double-blind studies.

25. (U) 71 07 – 72 06 1) Over 300 persistent infections have been initiated on 140 human volunteers. The natural history of the untreated lesions in volunteers with and wothout pre-existing immunity has been carefully documented in all cases. 2) One (1) spore from Trichophyton mentagrophytes RVN D-1 can produce severe, persistent ringworm (tinea corpus) in 100 percent of volunteers without immunity 3) Over fifty (50) spores per cm² of skin are required to produce progressive disease in volunteers with pre-existing immunity. 4) The effectiveness of various immunogenic preparations and methods of immunization are being studied. Attempts to induce immunity by vaccination to prevent dermatophyte infections (athletes foot and/or "Jungle Rot" are being persued in conjunction with Project No. DA OC 6783, Work Unit 004.

*Available to contractors upon originator's approval

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6921	72 07 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
	K Complete	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A831	00	009
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Yeast Flora of Interdigital Areas of the Foot (05)

12 SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 07	72 07	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	i	1
	CURRENT 73	0	1

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Presidio of San Francisco, CA 94129

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TELEPHONE: 415-561-5181

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Dermatology Research Division
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Halde, Carlyn, Ph.D.

NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

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

25. (U) 2253 cultures were obtained from the toe webbs of 27 volunteers. Sample size varied from one subject to the next invalidating quantative data. Definitive mycology was performed. Approximately 40% of the fungi recovered were members of the genera cryptococcus, Torulopsis, Rhodotorula, and Candida. The remaining 60% consisted primarily of Aspergillus and Penicillium. All organisms recovered were identified to species using established taxonomic criteria. Comparisons of genera and species are in progress.

*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.

Yeast Flora of Interdigital Areas of the Foot

CPT Michael McGinnis, MSC

PROBLEM:

Objectives: (a) Determine both qualitatively and quantitatively the fungal flora of the interdigital areas of the "normal" human foot. (b) Determine which taxa shifts, if any, occur due to dermatophyte infected feet as compared to the "normal" foot. (c) Evaluate possible implications as regards the presence and types of yeast found versus a predisposing disease state, i.e., dermatophytosis.

Hypothesis: A representative survey of the fungal population of the "normal" human foot may or may not be relatively constant. If findings indicate a rather uniform population of fungi (taxonomically and numerically) then gross variations in these species or numbers might be correlative with the presence of dermatophytoses.

Background: While many investigators have studied the microbial flora of the human skin, few have indicated mycologically the full taxonomic range of fungi encountered. Former investigators have classified fungi recovered from various anatomical positions as Genera and Types. To our knowledge, the present investigation is the only study which has attempted to speciate all fungi recovered from the human foot.

APPROACH:

Experimental Design: In-house, informed, volunteers were utilized. Sampling involved the following procedures. The right and left interdigital spaces of each foot from each volunteer were scraped, and resulting scrapings diluted and placed upon the proper media for growth of yeasts and molds. Upon obtaining growth in pure culture of the organisms enumerated, each was identified to species by the appropriate methods.

Methodology: Using sterile technique, the interdigital spaces of both right and left feet from each volunteer were scraped by means of a sterile scapel. The scrapings from the interdigital spaces of the right foot were pooled in 10ml of sterile distilled water. The same procedure was followed for the scrapings of the left foot. In addition, scrapings were spread directly on Dermatophyte Test Medium (DTM), 2 plates per foot, to detect possible presence of dermatophytes.

The dilutions were shaken on a vortex mixer and a 1ml aliquot withdrawn from each tube. This aliquot was added to 0ml sterile distilled water and shaken. The tubes were then diluted tenfold and ready for inoculation onto each of the various media to be used.

The media used for initial isolation of the yeast and mold fungi were as follows:

For isolation of mold forms: (1) Martin's Media; (2) Sabouraud's Dextrose Agar.

For isolation of dermatophytic fungi: Dermatophyte Test Medium

Physiological conditions were varied so as to include the entire temperature range for a maximum recovery of fungi.

Martin's Media and Sabouraud's Dextrose Agar (MM and SDA) were used primarily for isolation of the mold fungi. These media were incubated at room temperature, approximately 25°C.

Yeast extract malt extract agar (YM) was inoculated primarily for recovery of yeast fungi. Plates were incubated at both room temperature (25°C) and at 32°C.

Dermatophyte Test Medium (DTM) was inoculated directly from foot scrapings and incubated at 32°C.

Plates were inoculated by pipeting a 0.5 ml aliquot from the 10-fold dilution tubes directly onto the media desired and spreading the inocula

out with a sterile glass spreader thus giving uniform dispersion of the fungi.

The plates and tubes were allowed 4-5 days growth time and then examined for growth of fungi. Each colony was then counted and subcultured on the basis of being either a yeast or mold.

Yeast fungi were subcultured to YM plates and incubated at the temperature from which the original isolate had been growing. If mold fungi were present, they were incubated at room temperature on SDA. Colonies appearing on DTM were likewise placed on the proper media at the correct temperature range.

These colonies were then allowed to develop; finally each was transferred to an appropriate medium for maintenance as a pure stock culture.

All yeast fungi were identified by the methods of Lodder*. All mold fungi were identified by the investigators with some speciations being accomplished through the use of additional texts.

RESULTS AND DISCUSSION OF RESULTS:

From the 26 volunteers sampled, 1886 specimens were recovered. Approximately one-half of these were yeast fungi, with the other 50% being mold forms. Of the mold forms, the vast majority of species were in the Genera Aspergillus and Penicillium. The remaining molds were representative of common saprophytes from all 3 classes of the eumycota. The yeast fungi have fallen into the following genera: Candida, Cryptococcus, Phodotorula, Torulopsis, and Trichosporon.

In general, all fungi recovered and identified are very constant from volunteer to volunteer.

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*Lodder, J. (ed.). The Yeasts - A Taxonomic Study. 2nd ed., North-Holland Publishing Co., Amsterdam. 1970.

The presence of dermatophytes is being carefully examined for comparison of fungal recovery versus those volunteers having no dermatophytes. Until all data is fully analyzed, little correlation can be drawn.

An inverse relationship of population numbers between yeasts and molds is constant in each volunteer. Large yeast counts were associated with small mold counts, and low yeast numbers with high mold numbers. Further analysis is in progress.

FUTURE PLANS:

The same volunteers are being rescraped for a quantitative survey of fungi present. This will show if any population shifts have occurred in the individuals since the original sampling data. These results will indicate possible factors involved in explaining the relative constancy of species of yeasts and molds thus far encountered from individual to individual.

The future may hold promise for investigations of the virulence, pathogenicity, and infectious properties of dermatophytes as revealed by presence and types of yeasts found on the human foot via these ecological type studies.

MAXILLOFACIAL SCIENCES DIVISION

Program Element 6.11.02.A
Defense Research Sciences, Army

Project Number 3A061102B71R
Dentistry

Task Area Number 04

- - - - - - - - - - -

Program Element 6.21.10.A
Bio-Medical Investigations

Project Number 3A062110A825
Oral & Maxillofacial Sciences

Task Area Number 00

MAXILLOFACIAL SCIENCES DIVISION

This division's research program is directed toward developing methods for reducing the morbidity associated with traumatic injuries to the maxillofacial area and oral diseases prevalent in soldiers.

During the period covered by this report, research has been conducted in 16 different areas. Major advances have been made in the following research studies: Mandibular Bone Grafts; Reaction of Oral Tissues to Suture Materials; Prevention of Post Extraction Alveolitis; Oral and Maxillofacial Wound Infection; and Survey of Oral and Maxillofacial Injuries in the Federal Dental Services.

A new mandibular bone grafting method has been developed employing a combination of surface decalcified lyophilized allogenic bone and autologous bone marrow. Laboratory findings show this technique to have distinct advantages over current clinical methods of mandibular bone grafting. Preliminary clinical trials on six patients indicate confirmation of laboratory findings.

Research on sutures has identified polyglycolic acid suture as the most satisfactory suture for oral wound closure currently available.

Studies on alveolar osteitis reveal that presence of adjacent soft tissue inflammation does not counterindicate the extraction of teeth and that patients receiving estrogen and progesterone therapy have a marked predilection for alveolitis subsequent to tooth extraction.

Oral and maxillofacial wound infections have a more diverse group of bacteria involved than previously reported and organisms previously considered not to be pathogens appear to be responsible for some of these infections. Additional findings suggest that prophylactic antibiotics may be contraindicated in maxillofacial surgery.

Studies have been initiated in the five Federal Services to identify the types of maxillofacial injuries and problems associated with the management of such injuries occurring in personnel deployed in various situations.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6780	72 07 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
71 07 01	D. Change	U	U	NA	NL	X YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY		3A061102B71R	04	135
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Oral Disease in Military Populations (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 - Biology; 012900 - Physiology; 010100 - Microbiology; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	7	65
	CURRENT 73	7	70

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
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20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Maxillofacial Sciences Division

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

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Cutcher, J.L., LTC DC

NAME: Tempel, T.R., MAJ DC DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) effect of electric stimulation on bone repair; (U) endodontics; (U) alveolitis; (U) bacteremia; (U) bone repair; (U) military patient; (U) fluorescent bacteria

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

25. (U) 71 07 – 72 06 Investigations are being conducted in six sub-projects: Endodontics in Military Dentistry; Prevention of Post-Extraction Alveolitis; Control of Bacteremia Associated with Tooth Extraction; Oral Fluorescent Bacteria in the Military Population; Bone Repair; Effect of Electric Stimulation on Bone Repair.

See specific DD Form 1498 for current status of each subject.

*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.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6801	72 06 30	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
71 07 01	K. Completed	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A0621102B71R	04	137
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Oral Fluorescent Bacteria in Military Populations (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 - Microbiology; 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 06	72 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	.5	4
	CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
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20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
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ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) oral; (U) bacteria; (U) fluorescent; (U) tongue; (U) disease; (U) wound infection

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

25. (U) 71 07 – 72 06 Bacteriologic samples obtained from the posterior half of the tongue dorsa of 202 subjects yielded cultures showing orange-pink fluorescence under ultraviolet light in 43.1 percent of the population studied. Current smokers accounted for 56.3% of the total positive cultures obtained. Present evidence indicates that these fluorescing colonies consist of a member of the genus Neisseria, growing in synergistic combination with a gram positive pleomorphic cell form. No relation of these organisms to clinical oral disease has been established. This study has been completed.

*Available to contractors upon originator's approval

Oral Fluorescent Bacteria in Military Populations

Lieutenant Colonel J.L. Cutcher, DC
Judith B. Richey, B.S.
Robert H. Heflich, B.S., M.S.

PROBLEM:

The human oral cavity is the site of one of the most luxuriant and varied bacterial floras known. The bacterial components of this flora have been specifically implicated in systemic and local diseases, e.g., bacterial endocarditis, actinomycosis and alveolar osteitis. In general, however, practically nothing is known about the relationship of endogenous oral microorganisms to human diseases.

Recent dermatologic reports involving the implication of diphtheroid bacteria, which fluoresce under ultraviolet light, in the etiology of erythrasma have led to the observation that bacteria causing similar fluorescence are present on the dorsum of the tongue of some indiviuals. The purpose of this study is to determine the incidence, nature and significance of such fluorescent bacteria in the oral cavities of military personnel.

APPROACH:

A total of 200 subjects will be evaluated bacteriologically to determine the presence of bacteria which fluoresce under ultraviolet light when grown on a special laboratory culture medium containing porphyrin.

Bacteriologic samples are obtained by means of a calcium alginate swab from the posterior aspect of the dorsum of the tongue. Such samples are immediately streaked onto an enriched agar plating medium devised to enhance fluorescence of bacteria capable of metabolizing porphyrins. All cultures are incubated at 37° C in 4 percent carbon dioxide for a minimum of 24 hours, before fluorescent screening procedures with long-wave ultraviolet light (Wood's light) are performed. All cultures are screened at 24, 28, and 72 hours after initial incubation. Patients are questioned concerning current and prior smoking habits, in order to determine if such habits are related to the presence of these bacteria.

Qualitative biochemical and morphological studies are also being conducted on selected fluorescent isolates in order to identify the specific microorganisms involved.

RESULTS AND DISCUSSION:

Bacteriologic samples obtained from the posterior half of the tongue dorsa of 202 subjects yielded cultures showing orange-pink fluorescence under ultraviolet light in 43.1 percent of the population surveyed.

When apparently well isolated, fluorescing colonies were carefully picked using a stereoscopic microscope, 2 different cell forms frequently could be seen following gram staining:

1. A gram negative coccoid form, often kidney-shaped and Neisseria-like in appearance. Oxidase testing of the mixed culture gave varying results.

2. A gram positive pleomorphic cell form, occurring in short chains of coccobacillary cells on solid medium. Fluorescent colonies could be subcultured onto serum agar plates, but this frequently resulted in loss of fluorescence. When a fluorescent colony was subcultured onto a trypticase soy agar blood plate, fluorescence was not observed.

With respect to tobacco smoking habits of the subjects:

1. Forty-nine of the 107 subjects classified as "current smokers" were positive for in vitro fluorescence (45.8 percent).

2. Nineteen of the 39 subjects listed as "previous smokers" were positive for fluorescence (48.7 percent).

3. Nineteen of the 56 subjects tested listed as "tobacco abstainers" were positive for colony fluorescence (33.9 percent).

These results do not indicate a statistically significant relationship between use of tobacco and presence of fluorescent bacterial colonies.

Operational circumstances prevented correlation of positive in vitro fluorescence with clinical fluorescence of each subject's tongue. Observations on a limited

population, however, suggest that a tongue exhibiting clinical coral pink fluorescence will usually provide fluorescent laboratory cultures.

We have been unable to specifically identify these fluorescent organisms. While it is possible that diphtheroid bacteria may be involved, gram negative Neisseria-like colonies appear to play a larger role in colony fluorescence in terms of occurrence and relative predominance within the colony. In any case, no lingual pathology was observed in any of the subjects studied.

In view of the relatively high incidence of these bacteria, it is probable that they are non-pathogenic members comprising the "normal flora" of many individuals. Consequently, no further studies to determine possible pathogenic complications of these bacteria are contemplated.

FUTURE PLANS:

This study has been completed.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6804	72 06 30	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
71 07 01	K. Complete	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	04	141
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Control of Bacteremia Associated with Tooth Extraction (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 06	72 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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: 72	1	6
FISCAL YEAR CURRENT: 73	0	0

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)

NAME: Cutcher, J.L., LTC DC
TELEPHONE: 415-561-4042
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Jones, J.C., MAJ DC
NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) bacteremia; (U) tooth extraction; (U) military patients; (U) topical antiseptics

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

23. (U) Bacteria are known to invade the bloodstream of 50-85 percent of all people who have teeth extracted. Such bacteremias may have serious consequences in certain patients. Studies are directed toward developing methods which are practical in military situations for reducing the incidence of such dentally-induced bacteremias and thereby reducing the complications which may result from such bacteremias.

24. (U) Reduction of the number of oral bacteria associated with the tooth to be extracted, by use of local antiseptic solutions. A collaborative study with Letterman General Hospital evaluating the effect of different local antiseptic solutions on the incidence of bacteremia subsequent to tooth extraction.

25. (U) 71 07 - 72 06 Studies on 301 patients to date have demonstrated a statistically significant reduction in incidence of bacteremia associated with tooth extraction when a phenolated antiseptic mouthwash solution is employed as an oral rinse and as a sulcus irrigating agent, prior to extraction. Additional studies on two generically different commercial mouthwash solutions have been delayed by Food and Drug Administration actions relating to the efficacy of these solutions. In view of this situation, and because the original objective of this project has been attained, this study is considered to be complete.

* Available to contractors upon originator's approval

Control of Bacteremia Associated with Tooth Extraction

Lieutenant Colonel J.L. Cutcher, DC
Major John C. Jones, DC

PROBLEM:

Transitory bacteremia, which is known to occur subsequent to as much as 85 percent of all tooth extractions, may have serious consequences in certain patients, particularly when the viridans group of streptocci are involved. Recent studies indicate that one-third of reported cases of subacute bacterial endocarditis can be directly associated with prior dental procedures.

It has been established that bacteremias associated with oral procedures are caused by the invasion of the vascular system by microorganisms present in the oral cavity. Previously reported investigations under this project conducted in this laboratory have demonstrated that applying a phenolated topical oral antiseptic solution can result in as much as a 72 percent reduction in incidence of bacteremia following tooth extraction. This study was undertaken to determine the effect of 2 different mouthwashes, each generically distinct from the solution used in earlier studies, on the incidence of postextraction bacteremia.

APPROACH:

A total of 201 patients will serve as subjects for this study. Patients will be selected from the routine daily workload of people presenting to the Oral Surgery Service, Letterman General Hospital. Patients are placed, by means of random selection, into one of 3 groups of 67 subjects, as follows: Control group--no oral rinse or sulcus irrigation is performed on these patients; Mouthwash group A--a mouthwash solution containing .05 percent cetyl pyridinium chloride is used as an oral rinse and for sulcus irrigation in this group of patients; Mouthwash group B--receives a mouthwash solution containing thymol, eucalyptol, methyl salicylate, boric acid, methol and ethanol.

Laboratory bacteriologic evaluation of pre- and post-operative blood samples is employed to determine incidence of positive blood cultures.

RESULTS AND DISCUSSION:

Recent efforts to evaluate other selected commercial antiseptics (see Approach) have been hampered by uncertainty resulting from Food and Drug Administration (FDA) actions concerning efficacy of these formulations. A total of 48 patients had been surveyed prior to these FDA actions, but resultant data were insufficient for statistical analysis. In view of this situation, and because the original hypothesis and purpose of this investigation have been demonstrated on studies conducted earlier under this project title, this investigation is now classified as completed.

FUTURE PLANS:

This study is completed.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPO	CONTROL SYMBOL
DA OC 6805	72 07 01	DI	(AR)6-1A

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	04	143
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Endodontics in Military Dentistry (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 01	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	10
FISCAL YEAR CURRENT 73	1	6

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

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RESPONSIBLE INDIVIDUAL

NAME: Akers, W.A., COL MC

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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)

NAME: Robert, R., CPT DC

TELEPHONE: 415-561-4042

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Lilly, G.E., COL DC

NAME: Freeze, K., SP4 DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) endodontics; (U) root canals; (U) dentistry; (U) human teeth; (U) canine teeth; (U) military oral health

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

25. (U) 71 07 – 72 06 Studies evaluating selected dental materials and methods of filling root canals have been accomplished and a high pressure mechanical syringe has been developed. We found that of the materials and techniques developed and studied to date, resorbable materials and high pressure mechanical syringes are the most promising for reducing the time necessary to accomplish root canal filling. Two papers have been presented at national scientific meetings.

*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

Endodontics in Military Dentistry

Colonel Gilbert E. Lilly, DC
Captain Richard C. Robert, DC
SP-4 Kenneth D. Freeze, B.S.

PROBLEM:

Endodontics involves the treatment of the internal portion of the tooth, and more specifically, the pulp chamber and root canal and their contents - the dental pulp. Due to the unique anatomy of the tooth when the pulp degenerates subsequent to bacterial invasion associated with caries or other forms of trauma, it has limited regenerative powers. The apical foramen of the tooth is the only avenue of entrance of vascular supply and viable mesenchymal tissue. This foramen is so narrow that when the pulp becomes nonvital, it prevents connective tissue ingrowth and thus precludes phagocytosis and regeneration. As a result, the nonviable root canal acts as a reservoir of irritating material which incites a chronic periapical inflammatory reaction.

Debridement and obliteration of the root canal space with a nonresorbable inert material will effectively eliminate this dead space and its irritating contents. Such treatment results in restoration of the periapical tissues to a normal status of health, and retention of the tooth as a functional member of the masticatory apparatus for a prolonged period of time.

Currently, debridement and obliteration are accomplished by opening the pulp chamber through the crown of the tooth and debriding and enlarging the root canal with mechanical instruments. The two basic instruments used in the removal of the contents of the canal and its enlargement are small hand instruments, the reamer and file. These instruments are used to clean and enlarge the root canal prior to insertion of a filling material. Various materials are employed in the filling and sealing of root canals. The most popular method combines the use of a sealing cement and a solid material, such as a silver point, with the use

of hand instruments.

Presently the treatment time associated with endodontic procedures averages a total of 3 to 5 hours per posterior tooth, and requires 2 to 4 or more separate dental appointments over a 7 to 14 day period. In addition, special professional skill, beyond the capability of many practicing dentists, is required to accomplish endodontic therapy on posterior teeth. These time and professional skill factors currently limit the application of endodontics in many military situations.

Endodontic treatment has the advantage of maintaining the individual tooth as a functional member of the dentition. Such a tooth, if properly treated, is superior to any prosthetic replacement. In military dentistry, it has the added advantage of obviating the need for exodontia and subsequent prosthetics; and by so doing reduces the dental workload and non-effective time for the patient.

The purposes of this study are to: 1) simplify endodontic treatment; 2) reduce the number of professional manhours required to accomplish such treatment; 3) increase the endodontic capability of the Army Dental Corps; and 4) reduce the number of tooth extractions required.

APPROACH:

Two aspects of endodontic treatment are under investigation:

a. Debridement and enlargement of the root canals.

b. Filling and obliterating the root canal space.

Studies in each of these areas have been divided into 5 phases of investigation:

(1) In vitro studies on extracted human teeth.

(2) In vivo studies on animal teeth.

(3) In vivo toxicity studies on animals.

(4) Human clinical trials.

(5) Feasibility studies on large troop populations.

In vitro studies on extracted human teeth and in vivo studies on animal teeth have been conducted in the following areas:

a. Debridement and enlargement of the root canal.

(1) Chelating agents, e.g., ethylene-diamine-tetracetic acid (EDTA) and ethylene-bis (iminodiacetic) acid, as well as other chemicals, such as HCl and H_2SO_4 to debride, decalcify, and enlarge the root canals.

(2) Mechanical means (rotary instruments and ultrasonic instruments) to debride and enlarge the root canals.

b. Filling and obliterating the root canal space.

(1) Preformed materials (gutta percha, silver points) and liquid materials (zinc oxide-eugenol cements, polyketone cements, chloropercha and eucopercha pastes, iodoform pastes).

(2) Methods of delivering filling materials.

RESULTS AND DISCUSSION:

To date, studies have been limited to in vitro experiments on extracted human teeth and in vivo investigations on animals.

In vitro studies on extracted human teeth have evaluated various mechanical, ultra sonic, and chemical means for enlarging the root canal. None of these methods have proven feasible. Rapid mechanical methods for enlarging the root canal cannot be accurately controlled and frequently result in perforation of the root. Ultrasonic methods are not sufficiently efficient to remove adequate amounts of odontogenic material in a reasonable time frame. Chemical agents also require a prolonged time interval to be effective and then only facilitate other methods of enlargement. In addition, the in vivo tissue toxicity of effective chemical agents precludes their clinical use.

In vitro studies on extracted human teeth have been conducted in evaluating selected debriding agents. Debriding agents were retained in the tooth for 15 minutes prior to flushing with saline. Effectiveness of the debridement was determined by examination with a dissecting microscope after splitting the tooth. Detectable difference in debridement between various proteclytic enzymes and hydrogen peroxide could not be ascertained by this system.

In vitro studies with various filling materials and delivery methods have been conducted and a high pressure mechanical syringe has been designed and fabricated. Effective sealing of the root canal can be rapidly accomplished with fluid materials using the high pressure mechanical syringe without extensive debridement or canal enlargement. The particle size of resorbable root canal filling materials is too large to permit use in this system and the effect of nonresorbable materials on the periapical tissues has not yet been determined.

FUTURE PLANS:

In vivo laboratory studies to evaluate periapical toxicity of nonresorbable root canal sealers delivered with the high pressure mechanical syringe are planned.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6795	72 07 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
71 07 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	04	144
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Prevention of Post-Extraction Alveolitis (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 01	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	10
FISCAL YEAR CURRENT 73	1	7

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Jones, J.C., MAJ DC

NAME: Lilly, G.E., COL DC DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) alveolar osteitis; (U) mandibular third molar; (U) extraction; (U) antiseptic mouthwash; (U) military dentistry

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

23. (U) Although the incidence has not been established in the US Army, available data suggest that at least 2 percent of all tooth extractions are followed by local infection of the tooth socket (alveolitis or "dry socket"). Ninety percent of the cases of alveolitis that do occur follow the removal of a mandibular third molar (wisdom tooth). The objective of this study is to develop a simple, effective method of preventing this complication, that is practical in military situations.

24. (U) Prevention of post-extraction alveolitis by use of a topical antiseptic to temporarily reduce the bacterial population of the oral cavity. A collaborative clinical study between Letterman General Hospital, Letterman Army Institute of Research and Oakland Naval Hospital.

25. (U) 71 07 – 72 06 To date, data have been obtained on 912 patients and 1452 mandibular third molar extractions. Alveolitis occurred in 90 instances, or 6.2 percent of all mandibular third molar extractions. The sample is still too small to provide statistically significant data on the effectiveness of the topical antiseptic. Significant findings do reveal that the presence of pericoronitis at the time of extraction does not increase the incidence of alveolitis; the incidence of alveolitis in teeth with pericoronitis was 5.83 percent as compared to 6.33 percent in cases without pericoronitis. Women taking oral contraceptives have a marked predilection for alveolitis; the incidence of alveolitis in women taking contraceptives was 17.2 percent as compared to an incidence of 4.82 percent for women not taking contraceptives.

*Available to contractors upon originator's approval

Prevention of Post-Extraction Alveolitis

Colonel Gilbert E. Lilly, DC
Major J.C. Jones, DC
Colonel Donald B. Osbon, DC

PROBLEM:

The incidence of alveolitis (dry socket) is not established in the US Army. Available data suggest that approximately 2 percent of all tooth extractions, and 5 to 6 percent of all mandibular third molar (wisdom tooth) extractions, are followed by this complication. Furthermore, 90 percent of the dry sockets that do occur are in the mandibular third molar region. Based on the current Army population and dental treatment required, 40,000 cases of alveolar osteitis are estimated to occur annually. This results in an annual loss of 160,000 patient man-hours and creates a dental workload of 80,000 separate additional appointments.

While the precise etiology of alveolitis has not been established, 3 contributory factors are recognized; (1) the general systemic status of the patient (age, nutrition, systemic disease, and medications); (2) local trauma; and (3) local microorganisms. The systemic status of the patient and the amount of local trauma are factors which are unique to each patient and each extraction. Because of this, their inherent variability makes them extremely difficult to control or standardize in studying a series of patients. Although the specific types and numbers of microorganisms vary from one case to another, their presence is constant. Topical oral antiseptics are known to drastically reduce the oral bacterial population for periods of up to 1 1/2 hours.

This study tests the hypothesis that a topical antiseptic can effect a reduction in the oral bacterial population during the time interval in which surgery and subsequent clot formation occur, and that such a reduction is a critical factor in preventing alveolar osteitis.

APPROACH:

Patients requiring mandibular third molar extractions are randomly placed in one of 2 groups: Experimental and Control. Patients in the experimental group have their mouths and gingival sulci in the mandibular second and third molar areas lavaged with an antiseptic mouthwash prior to third molar extraction. Patients in the control group receive no such pre-extraction lavage. In all other respects, patients in the control group and experimental group receive comparable treatment before, during and after surgery. Basic information concerning the patient, the extraction and group is recorded on a special card. Postoperatively, all patients are evaluated for alveolar osteitis. The investigator conducting the examination is not aware of the patient's group allocation (experimental or control), and findings are recorded on a separate data card. For the purpose of the investigation, alveolar osteitis is defined as any case with localized post-extraction pain which requires treatment with a local obtundent dressing.
Collected data is processed with electronic data processing equipment as the basis for determination of the incidence of alveolar osteitis. Approximately 3,000 mandibular third molar extractions will be needed for a valid sample. This collaborative study involves Letterman Army Institute of Research, Letterman General Hospital, and Oakland Naval Hospital.

RESULTS AND DISCUSSION:

To date, data have been obtained on 912 patients and 1452 mandibular third molar extractions. Alveolitis was reported in 90 instances, or 6.2 percent of all mandibular third molar extractions. At this time, the sample is not of sufficient size to provide significant data on the effectiveness of the topical antiseptic. Significant findings reveal: a) that the presence of pericoronitis at the time of extraction does not increase the incidence of alveolitis; the incidence of alveolitis in teeth with pericoronitis is 5.82 percent as compared to 6.33 percent in cases without pericoronitis; b) women taking oral contraceptives have a marked predilection for alveolitis; the incidence of alveolitis in women taking oral contraceptives is 17.2 percent as compared to an incidence of 4.82 percent for women not taking contraceptives.

FUTURE PLANS:

This study will be continued until sufficient cases are included to permit statistical verification of findings. To further explore the hypothesis that lowering the bacterial count intraorally may lessen the incidence of alveolitis, an extension of the use of antiseptic mouthwash will be instituted on 1 July 1972. Patients in the experimental group will be provided with 8 ounces of mouthwash to be used four times a day for 2 days, beginning the day after surgery.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6910	72 07 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
71 07 01	D. Change	U	U	NL	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	04	146
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Effect of Electric Stimulation on Bone Repair (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 03	74 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	10
	CURRENT 73	2	19

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
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21. GENERAL USE

20. PERFORMING ORGANIZATION

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TELEPHONE: 415-561-5160
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Hackett, P. SP4
NAME: Jones, J.C., MAJ DC DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) mandibular fractures; (U) bone repair; (U) piezoelectricity; (U) wound healing

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

25. (U) 71 07 – 72 06 Initial studies are being directed toward developing a laboratory model and technology. Preliminary findings indicate that direct electric current in the 10 micro amp range can accelerate bone repair.

*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.

Effect of Electric Stimulation on Bone Repair

Colonel Gilbert E. Lilly, DC
Major J.C. Jones, DC
SP-4 Perry B. Hackett

PROBLEM:

Accepted treatment for fractures and bone grafts of the jaws usually involves wiring the jaws together. Because of this, it is necessary to hospitalize the patient until bone repair has progressed to the point that the wires can be removed and the patient can eat a normal diet and is not in danger of aspirating foreign material into the lungs. Such care requires prolonged hospitalization and results in loss of many manhours. A number of authors have reported and measured electric potentials which are apparently due to a piezoelectric effect in human and other mammalian bones when bones are subjected to mechanical stress in in vitro laboratory studies. More recently, in vivo laboratory studies and selected applications on humans have resulted in reports of increased and more rapid bone deposition in bones subjected to small amperage direct electric current. The exact amount of current necessary and the relation of bone formation to the implanted electrodes are not well established. This study is being undertaken to: (a) determine the effect of electric current on bone repair, (b) determine the most appropriate amount and method of delivering electric stimulation to the mandible to accelerate bone repair, and (c) reduce the hospitalization time and military manhours lost due to mandibular fractures.

APPROACH:

Five bilateral mandibular surgical defects were prepared by way of an aseptic extra-oral surgical approach on 8 dogs. Each defect was 1 mm wide and 1 cm apart, cut through the inferior border of the mandible. Each defect was extended to, but not through, the mandibular canal. Direct electrical current across the center defect was furnished by a circuit similar in design to that of Lavine et. al., using a 1.4 volt E400 Eveready Ch battery connected in series with a 170 K OHM 10

resistor (resistance measured prior to use). This power unit was sealed in Silastic Silicone A (Dow Corning Silicone, type A). The copper wire battery leads were fastened to the voltage source with conducting silver epoxy cement (Emerson & Cuming, Gardena, Calif.) One lead had a surface exposure for current monitoring after implant. Platinum electrodes (0.020 inch diameter) were soldered to the copper wire and insulated with shrink tubing. The electrodes were implanted in drill holes (0.5 cm diameter) on either side of the center cut of each defect series. A pair of electrodes were implanted on each side of the mandible, but only those on one side were connected to a power source. Osteogenic activity at the defects was evaluated radiographically and histologically. Dogs were sacrificed 2 and 4 weeks after surgery.

RESULTS AND DISCUSSIONS:

Numerous technical problems have been encountered in this study related to the electric circuitry and the animal model. The copper wire leads separated in 3 cases due apparently to metal fatigue, the result of the dogs' constant movement of the neck and jaws. In 2 other cases, circuit failure resulted from interruption of the platinum electrode-copper wire union. Inflammation and infection at the cutaneous entry site of the circuit has been a continual problem necessitating early sacrifice of some animals.

In 2 animals maintained for 4 weeks with intact electric circuits, we have microscopically observed increased osteogenic activity and osteoid formation in defects between the electrodes as compared to contralateral defects and bilateral defects not receiving electrical stimulation. The electric potential in these circuits varied from 5 ma to 10 ma during the course of this experiment. Due to the numerous problems encountered, these findings have been fragmentary and cannot be considered meaningful.

In view of the problems encountered, primary effort has been directed toward developing more reliable and sophisticated electric circuitry and solving problems encountered in the animal model. In cooperation with Rosemount Engineering Corporation, Minneapolis, Minnesota, we have developed an electric power unit and

circuitry which is deperdable and capable of wider ranges of electric outp it. In order to avoid the problems encountered with electrode and wire lead failures, this system uses multi-stranded platinum leads and continuous electrodes. In cooperation with Tecna Corporation, Emeryville, California, we have modified a microporous polyurethane percutaneous lead for monitoring of the circuit. This system should permit prolonged exposure of the electric leads without local inflammation or infection.

FUTURE PLANS:

Employing newly developed technical and animal model modifications, a series of 20 dogs will be studied, evaluating osteogenesis at various electric stimulation rates.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6911	72 07 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
71 07 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	04	147
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Bone Repair (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 03	74 03	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	2.5	25
	CURRENT 73	3	38

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
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ADDRESS:

RESPONSIBLE INDIVIDUAL

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21. GENERAL USE

20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Payne, T.F., CPT DC

NAME: Lilly, G.E., COL DC

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) avulsion wound; (U) bone repair; (U) radioisotopes; (U) mandibular bone grafts; (U) fractures

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

24. (U) Laboratory studies on dogs relating radioisotope activity, biochemical, microscopic, clinical, radiographic and physical strength findings.

25. (U) 71 07 – 72 06 Two experimental models are being developed to study mandibular bone graft strength is correlated with the actual force required to fracture a graft site under simulated physiologic stress. The defects are through-and-through defects analogous to grafts which have been performed in humans. The second model correlates graft strength with the force required to shear a graft plug from a round-punch defect. This approach allows comparison of various grafting techniques within a single bone. Presently, the model is being applied to the tibia. Both models have been adapted for strength testing in the Instrom Materials Testing Apparatus.

*Available to contractors upon originator's approval

Bone Repair

Colonel Gilbert E. Lilly, DC
Major J.C. Jones, DC
Captain Richard C. Robert, DC
Captain Thomas F. Payne, DC

PROBLEM:

Accepted treatment for fractures and bone grafts of the jaws usually involves wiring the jaws together. In the military, the patient is hospitalized until bone repair has progressed to the point that the wires can be removed, the patient can eat a normal diet, and he is not in danger of aspirating foreign material into the lungs. Such care requires prolonged hospitalization and results in many man-days lost. Currently, the most reliable clinical criteria for determining adequacy of bone repair are physical manipulation, and, in the laboratory, microscopic evaluation. Both of these methods are subjective.

This study is being undertaken to determine more reliable quantitative and qualitative methods for assaying bone repair to develop more objective laboratory and clinical endpoints for studying and evaluating bone healing.

APPROACH:

Preliminary studies were undertaken to develop a suitable model for through-and-through bone defects in order to compare various grafting techniques on a histological, radiological, and physical basis. Defects were prepared on dog radii, tibias, and mandibles, and various graft and stabilization techniques attempted.

Graft strength has been determined by two techniques. The first is based on the force required to fracture a graft plug from a round-hole defect 6 mm in diameter. This type of defect is small and uniform in morphology permitting different types of graft materials to be compared within a single bone. Due to ease of access and surface area of bone available, the tibia has been used for this study.

The second strength determination has attempted to simulate the physiologic force required to fracture a through-and-through mandibular graft site. A downward force was applied to the anterior portion of the mandible, perpendicular to the body of the mandible.

Both of these strength determinations are being adapted to an Instrom Materials Testing Apparatus.

RESULTS AND DISCUSSION.

Grafts were attempted on the radii of 4 dogs to determine the suitability of the radius as a model for through-and-through defects. The radius was found to be unsatisfactory because of overgrowth of fibrous connective tissue and bone. In addition, graft immobilization proved to be a significant problem. Mandibular grafts were attempted using an Iowa Bi-Phase Stabilization apparatus for external fixation. Two dogs received grafts and were stabilized with the Iowa Bi-Phase. Although through-and-through grafts on one of these dogs were successful, fixation again proved to be a problem. Most of the difficulty could be traced to the malfunction of teeth in the upper and lower arches due to misalignment of the mandible after grafting. It was determined that functional trauma to the grafts could not be overcome without removal of the mandibular teeth.

After the adoption of an edentulous mandible as the model for study, removal of teeth from mandibular arches of dogs was begun. To date, 7 dogs have had their lower teeth extracted. Similar procedures will be carried out on 13 more dogs in order to obtain a total of 20 for the study.

Tibiae from 2 dogs have been obtained for the preparation of round plugs for grafting. The plugs are obtained by cutting cores with a 6 mm trephine. These cores are presently being surface decalcified for use as grafts, according to procedures described in Work Unit No. 063, "Early Restoration of Mandibular Continuity".

Permission has been obtained from the U.S. Public Health Service Hospital of San Francisco to make use of their Instrom unit to make strength measurements on

the grafts.

FUTURE PLANS:

Mandibles of the remaining dogs necessary for study will be made edentulous. Grafts will then be performed in order to determine the time-rate of healing of the various types of grafts. Attempts will be made to correlate through-and-through defects with the round punch defect in order to obtain comparison of grafts with the round punch model.

150

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6920	72 07 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
71 07 01	D. Change	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	060
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Early Management of Oral and Maxillofacial Wounds (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 012900 - Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 11	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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	72	10	77
FISCAL YEAR CURRENT	73	12	90

19. RESPONSIBLE DOD ORGANIZATION

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

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TELEPHONE: 415-561-5160

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Jones, J.C., MAJ DC

NAME: Cutcher, J.L., LTC DC DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code) (U) bone; (U) maxillofacial wounds; (U) wound incidence; (U) alloplastics; (U) debridement; (U) surgery; (U) dentistry; (U) avulsion wounds; (U) sutures

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

25. (U) 71 07 – 72 06 Investigations are being conducted in eight sub-projects: Incidence of Oral and Maxillofacial Injuries; Early Restoration of Oral Integrity; Tissue Reaction to Sutures; Early Restoration of Mandibular Continuity; Mandibular Bone Grafts; Oral and Maxillofacial Wound Infections; Repair of Salivary Gland Ducts; Actinic Blocking Agents for Protection of Lip Mucosa.

See specific DD Form 1498 for current status of each sub-project.

* 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

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6807	72 07 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
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	061
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Early Restoration of Oral Integrity (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

017100 - Weapons Effects; (U) 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 11	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	6
	CURRENT 73	1	8

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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TELEPHONE: 415-561-5160

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Cutcher, J.L., LTC DC

NAME: Jones, J.C., MAJ DC DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) wounds; (U) treatment; (U) oral; (U) maxillofacial; (U) weapons effects; (U) combat; (U) dimethylsiloxane

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

25. (U) 71 07 – 72 06 To date, a synthetic material has been used in 14 human battlefield casualties, to temporarily replace missing soft tissue of the maxillofacial area. Preliminary findings indicate that this material and method of management are effective in the treatment of large maxillofacial avulsion wounds. No evidence of untoward reaction has been found. Preliminary laboratory studies indicate that delayed primary closure of selected large grossly contaminated maxillofacial wounds may be preferable to initial primary closure.

*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

Early Restoration of Oral Integrity

Colonel Gilbert E. Lilly, DC
Lieutenant Colonel J.L. Cutcher, DC
Major J.C. Jones, DC

PROBLEM:

When high velocity missiles strike the body, tissues are severely damaged, and large portions frequently are avulsed. Such large wounds in some cases cannot be closed primarily without severe distortion of anatomic features, and the traumatized tissues are not favorable sites for immediate grafts. When avulsion wounds involve the maxillofacial area, its integrity may be lost. This can lead to: problems in feeding; frequent requirements for intravenous or nasogastric nutrition; extensive nursing care; and psychologic problems, all being major factors in early patient management. Current initial therapy for such wounds, when they are large and primary closure is impossible, consists of suturing skin to mucosa with no attempt to close the defect.

The currently accepted treatment is to initiate immediate primary closure of maxillofacial wounds subsequent to debridement for esthetic reasons. In other areas of the body, battle wounds are usually closed several days after initial debridement, stabilization and initiation of tissue regeneration. Despite antibiotics and adequate debridement, some maxillofacial wounds break down after initial primary closure often increasing disfigurement and scarring. Advances in total patient care, antibiotic therapy, and materials to dress and support maxillofacial wounds suggest that delayed primary closure of selected maxillofacial wounds should be considered.

APPROACH:

Previous laboratory studies on dogs indicated that large orofacial avulsion wounds could be effectively managed by fabricating a silicone prosthesis directly in the wound to replace missing tissue and support the remaining tissues in their normal anatomic relation. To evaluate this method of management under realistic

conditions on patients with fresh battlefield wounds, permission was obtained to conduct a clinical study in the Republic of Vietnam.

Patients selected for this study had large orofacial avulsion wounds which could not be closed primarily or had orofacial wounds in which primary closure was considered inadvisable. Patients with concomitant injuries, which might compromise maxillofacial wound management, were not included in the study. Conversely, patients with concomitant injuries which might be adversely affected by the maxillofacial wound management, were also excluded. Wounds were debrided conservatively, consistent with accepted surgical principles for maxillofacial injuries. Fractures, if present, were treated in a conventional manner with reduction, fixation, and soft tissue coverage. Soft tissues were closed in layers, taking care to preserve their anatomic position and relation as much as possible. Those portions of the wound which could be closed primarily, with skin to skin or mucosa to mucosa, were so managed. In areas of large avulsion, when possible, skin to mucosa closures were accomplished. Liquid RTV 382 dimethylsiloxane was then mixed with stannous octoate catalyst (10 drops catalyst/one ounce of RTV 382 dimethylsiloxane) and poured into the defect. This mixture self-vulcanizes in approximately 5 minutes. A prosthesis was formed which replaced the avulsed tissue and was well adapted to the wound. Subsequent to vulcanization, the prosthesis was trimmed with scalpel and scissors, if necessary. In some cases, sutures were passed through the prosthetic device and into the soft tissues to insure adequate retention.

Patients received antibiotics postoperatively, usually consisting of parenteral penicillin and streptomycin for at least 10 days. Analgesics were prescribed as needed. Postoperatively, the wounds were irrigated twice daily with saline. This irrigation was accomplished by gently lifting the margins of the silicone prosthesis and flushing both intra- and extra-orally, using a large Luer syringe. Early self-feeding was encouraged, except in cases where a nasogastric feeding tube was in place. In other respects, postoperative care was routine. Studies on delayed primary closure of maxillofacial wounds are in progress on rabbits. Bilateral maxillofacial wounds are produced on the cheek of each animal

with blank-shell explosive charges. These injuries traverse and destroy a portion of the masseter muscles but do not fracture facial bones.

Alternate methods of delayed primary closure managing the wounds are being evaluated. These methods include topical antibiotics, daily saline lavage, and no therapy other than the dressing. Five days postoperatively, the wounds are closed with sutures. The wounds are evaluated clinically and histologically for adequacy of wound healing 14 days after injury.

RESULTS AND DISCUSSION:

A total of 14 patients with combat maxillofacial avulsion wounds have been treated in the manner outlined, with a temporary prosthetic device prepared in the wound from RTV 382 dimethylsiloxane. The small number of patients treated in this preliminary study with wounds of such a diversified nature does not allow any valid conclusions to be reached concerning this method of management. We have not detected any evidence of local or systemic toxicity associated with the in vivo vulcanization and retention of RTV 382 dimethylsiloxane in the wound; nor have we observed any evidence that this material has contributed to wound infection or secondary breakdown. This is true even though the material was used in 3 patients subsequent to initial wound breakdown. The silicone prosthesis, fabricated in vivo, closely adapted to the wound and provided a splint which effectively replaced lost tissues and supported the remaining soft tissues. This method of management is not intended to be used to primarily close maxillofacial wounds. In selected maxillofacial avulsion wounds, however, where primary closure is impossible or cannot be accomplished without severe distortion of facial tissue, this method may prove valuable.

Dimethylsiloxane can be autoclaved and has a prolonged shelf life. Because it closely adapts to the wound with its rubbery consistency, support to the mobile soft tissues is provided.

Delayed primary closure of experimental wounds in rabbits indicates that, of the methods evaluated, topical treatment of the wound with bacitracin ointment and dressing with a thin sheet of silicone is the most acceptable method for delayed primary closure.

FUTURE PLANS:

The clinical portion of this study using RTV silicone prosthesis will be continued. Based on our experience this year, however, we anticipate that the number of wounds needing this type of management will be limited due to the reduced number of combat casualties.

Additional studies evaluating alternate methods of delayed primary closure are planned in the animal experimental model.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6808	72 07 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
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	062
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Incidence of Oral and Maxillofacial Injuries (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 09	75 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	8
CURRENT 73	1.5	12

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) oral; (U) maxillofacial; (U) wounds; (U) injuries; (U) incidence; (U) treatment

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

23. (U) This investigation was undertaken to obtain information concerning the relative incidence and types of maxillofacial injuries treated by the Federal Dental Services. This information is considered essential for determination of the Federal Dental Services' commitment in this area which may be used as a guide for staffing, support required, orientation of training programs and direction of research activities.

24. (U) Survey of oral and maxillofacial wounds at selected hospitals of the Federal Services. Reports are submitted through attending oral surgeons. Data are tabulated and organized by electronic data processing equipment.

25. (U) 71 07 – 72 06 To date, approximately 1,000 cases have been reported and transcribed to data cards. No tabulation of data has been done as of this report.

*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

Incidence of Oral and Maxillofacial Injuries

Colonel Gilbert E. Lilly, DC
Major J.C. Jones, DC
Lieutenant Colonel J.L. Cutcher, DC

PROBLEM:

Previous studies conducted by this Unit based on data on over 10,000 oral and maxillofacial injuries in Vietnam, revealed that the incidence of such injuries is much higher than previously reported (as high as 24 percent of all admissions for trauma at one major hospital reporting over 1100 maxillofacial injuries in a one year period). In addition, the nature of these injuries and resulting complications required more sophisticated treatment and supportive care than that traditionally associated with oral and maxillofacial injuries. These findings indicated: a) a greater dental commitment than previously realized; b) a requirement for greater support by the Medical Department; c) the need for more extensive training in certain areas; d) a requirement for redirection of research programs to deal with identified problems.

Current methods of reporting injuries incurred in the Federal Services are not sufficiently detailed to provide identifiable and usable data on maxillofacial injuries. Information on the type, number, treatment, and hospitalization time associated with maxillofacial injuries occurring within the United States is needed, since the majority of the population serviced by the Federal Dental Services is located within the United States. Such information is necessary to determine the Federal Dental Services' commitment in this area, which may be used as a guide for staffing, support required, orientation of training programs and direction of research activities.

The purpose of this study is to determine: 1) The commitments of the Federal Dental Services to the management of maxillofacial injuries and to establish the support required to meet these commitments. 2) The number and type of maxillofacial injuries at selected Federal hospitals. 3) The nature of maxillofacial

injuries, the treatment required, complications associated with such injuries and identification of specific problem areas.

APPROACH:

The oral surgery services at selected hospitals of the Air Force, Army, Navy, Public Health Service, and Veterans Administration would be furnished survey forms to be completed on all cases of maxillofacial trauma treated at their respective facilities. A representative from each service would be delegated as a coordinator to collect and review the data and forward it to Maxillofacial Sciences Division, Letterman Army Institute of Research. The data would be processed and tabulated by electronic data processing equipment. Accrued information would be made available to the Chiefs of the Federal Dental Services and, upon their approval, promulgated in the usual manner. Subsequent to the approval by the Chiefs of the Federal Dental Services, it is proposed that this study be initiated 1 January 1972 and continue for a period of 3 years until 31 December 1974.

RESULTS AND DISCUSSION:

To date, over 1,000 cases have been reported and the forms returned to LAIR. Data on these have been transcribed to key punch cards. The data has not yet been tabulated since it is anticipated that this will be done on an annual basis.

FUTURE PLANS:

Tabulate and analyze accumulated data on an annual basis. Continue data collection for two more years.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6809	72 07 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
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	063
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Early Restoration of Mandibular Continuity (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 017100 - Weapons Effects

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 06	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	.5	11
	CURRENT 73	.5	5

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) maxillofacial; (U) weapons effects; (U) early treatment; (U) mandible; (U) osseous avulsion wounds; (U) oral

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

24. (U) Laboratory studies on dogs and clinical studies to verify laboratory findings.

25. (U) 71 07 – 72 06 Laboratory studies have indicated that mandibular continuity can be effectively temporarily restored, and soft tissues supported, with preformed silicone segments. Preformed silicone mandibles have been fabricated and are being evaluated in clinical trials. Two patients have been treated with this device. Preliminary findings indicate that continuity has been effectively restored and the soft tissues supported without evidence of untoward reaction.

Available to contractors upon originator's approval

Early Restoration of Mandibular Continuity

Colonel Gilbert E. Lilly, DC
Major J.C. Jones, DC
Lieutenant Colonel J.L. Cutcher, DC

PROBLEM:

When missiles strike the body, they frequently result in avulsion of tissues. The limited number of missile injuries experienced in civil life make such injuries essentially unique to the military.

Current information indicates that 96 percent of all maxillofacial injuries resulting from hostile action (RHA) in Vietnam are caused by missiles, and that 37 percent of these involve facial fractures. The mandible is the most common of the facial bones fractured (19 percent of all facial fractures). Seventy-five percent of these mandibular fractures are comminuted, and 55 percent involve avulsion of osseous fragments.

Major avulsion wounds of the mandible are usually treated by stabilization of the remaining osseous fragments and primary closure of the soft tissues. Such treatment does not usually provide adequate support for the soft tissues and results in their collapse into the defect and subsequent soft tissue deformity which is, because of scarring, difficult to reconstruct. Kirschner wires placed in the osseous stumps prior to soft tissue closure have been used in an attempt to provide support for the soft tissues. Such wires do not, however, provide sufficient support, and they literally cut through the tissues and usually become exposed in a period of 2 to 4 weeks.

This program is directed toward developing a method of management for large osseous avulsion wounds to reduce morbidity and man-days lost.

APPROACH:

Unilateral discontinuity defects were prepared surgically in the body of the mandible of 8 dogs. Each defect measured 3 cm in length. Immediately after preparation of the osseous defects, mandibular continuity

was restored with a block of precontoured vulcanized medical grade silicone, number 373, wired in place with trans-osseous stainless steel wires in 4 of the animals and a Kirschner wire in the other 4 dogs. The soft tissues were closed primarily over the silicone and Kirschner wire. The mandibles of each animal were then stabilized with an external pin fixation appliance. One animal from each group was sacrificed at 4, 6, 8, and 10 weeks after the surgical procedure. At sacrifice, radiographs were taken of each experimental site and the tissues recovered for microscopic study. Subsequent to fixation in formalin and decalcification, histologic sections were prepared from each surgical site.

Precontoured human mandibles have been prepared from vulcanized medical grade silicone and made available to selected oral surgeons in the United States and Vietnam for use on humans.

RESULTS AND DISCUSSION:

Clinically, the soft tissues overlying the osseous defects restored with preformed vulcanized silicone were supported in their normal anatomic position in all animals. The soft tissues overlying the silicone prosthesis appeared to heal normally even though in one case intraoral wound dehiscence occurred. Microscopically, the silicone prosthesis was surrounded by a thin fibrous connective tissue capsule containing a mild inflammatory infiltrate of lymphocytes and plasma cells. Later postoperative specimens obtained at 6, 8, and 10 weeks exhibited fewer inflammatory cells. The fibrous connective tissue capsules in these later specimens were well vascularized and did not show evidence of progressive fibrosis and thickening.

Clinically, the soft tissues overlying the defects restored with Kirschner wires initially appeared to heal in a normal manner. After 6 weeks, however, there was evidence of soft tissue deformities and, in one case, the wire became exposed into the oral cavity. In the latter instance, a severe inflammatory reaction was associated with the exposed wire necessitating its removal. Microscopically, there was a dense inflammatory infiltrate composed of plasma cells, lymphocytes, and neutrophils surrounding the wires. This inflammatory

infiltrate was present in all specimens, but it was more severe in the specimen with wire exposure. There was increased soft tissue fibrosis and bone resorption surrounding the wire in later specimens.

To date, preformed silicone mandibles have been used in 2 humans. In both cases, the silicone prosthesis was placed at the time of removal of a large benign central tumor of the mandible (ameloblastoma and myxoma). Subsequent to placement of the prosthesis, the retained mandibles were immobilized with intermaxillary fixation. In both cases, the postoperative period was uneventful and the silicone mandibles adequately supported the soft tissues in their normal anatomic position. There was no evidence of suppuration or other untoward reaction. In both of these cases, intraoral soft tissue dehiscence occurred 2 months after the prosthesis was placed. This dehiscence was not associated with increased inflammatory reaction in either case and appeared to be directly due to excessive bulk of the silicone prosthesis. Three months after placement, both prosthetic appliances were removed due to persistent but uncomplicated intraoral dehiscence. In both cases, this removal and subsequent healing were uncomplicated. The soft tissues overlying the prosthesis were stabilized at this point and did not collapse after removal of the internal appliance.

Based on the animal studies and limited human clinical trials, the silicone prosthesis appears to more adequately support the soft tissue than does Kirschner wire and is associated with less local tissue reaction.

Although the clinical results have been less than ideal, due to intraoral dehiscence, this appeared, in the opinion of the surgeons, to be the result of inadequate soft tissue coverage of the appliance and could have been avoided by reducing the bulk of the prosthesis prior to placement. The lack of tissue reaction associated with intraoral dehiscence is a phenomenon which has been noted by other observers in association with intraoral submucous silicone prostheses. We are unable to explain this lack of reaction, but consider it to be an observation of great interest and of possible clinical significance.

FUTURE PLANS:

Based on the clinical and laboratory findings, this appliance and method of management appear to offer advantages to alternate methods for temporarily restoring mandibular continuity. Additional clinical studies are planned with attention to method of coverage so as to avoid wound dehiscence.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6909	72 07 01	DD DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8b. SPECIFIC DA & CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	064
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Mandibular Bone Grafts (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 03	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	4	32
FISCAL YEAR CURRENT 73	4	36

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

20. PERFORMING ORGANIZATION

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NAME: Osbon, D.B., COL DC DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) mandibular bone grafts; (U) maxillofacial; (U) bone induction; (U) avulsion wounds

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

24. (U) Laboratory studies on dogs investigating new methods for mandibular bone grafting and clinical studies to verify laboratory findings.

25. (U) 71 07 – 72 06 Laboratory studies on dogs indicate that mandibular bone grafts with surface decalcified lyophilized allogenic bone and autologous marrow are well accepted by the host, appear to be stronger microscopically than marrow grafts, are easier to fabricate and place than marrow grafts, and do not have secondary complications requiring removal of the metal framework used in marrow grafts. Clinical trials have been initiated. Studies on 5 patients point to preliminary findings which are similar to laboratory results. Two papers have been presented at national scientific meetings and a paper has been presented at the 1972 Army Science Conference.

*Available to contractors upon originator's approval

Mandibular Bone Grafts

Colonel Gilbert E. Lilly, DC
Major John C. Jones, DC
Colonel Donald B. Osbon, DC

PROBLEM:

A survey of 9,139 patients with maxillofacial injuries received in Vietnam reveals that in 9.4 percent of patients with mandibular fractures due to hostile action, avulsion of a significant portion of the mandible occurred.

Many of these patients required bone grafts to restore the mandibles. A review of 89 patients who required such mandibular bone grafts at selected army hospitals revealed that the hospitalization time for these patients averaged 509 days, with a range of 138 to 1,392 days. The patients in this group who were discharged from the military service had an average of 25 percent disability related to their mandibular wounds. Graft failures, or complications requiring additional grafts, occurred in 12.4 percent of these patients. These findings indicate the continued need for research directed toward reducing the morbidity and hospitalization time associated with mandibular bone grafts.

Recent work by Boyne and associates with autologous hematopoietic marrow has resulted in the development and clinical acceptance of a method for mandibular grafting that uses marrow fragments. Since the marrow fragments used in this technique have no inherent allostructural support, it is necessary to use a Vitallium metal framework to bridge the defect in order to support the graft and provide internal stability to the mandibular fragments. This framework is lined with a cellulose acetate micropore filter that serves to isolate the transplant from connective tissue invasion but allows for fluid interchange. Although rapid repair and earlier mobilization have been reported with this technique, the metal framework can be difficult to fabricate and adjust and may require surgical removal after the graft has healed.

Urist and associates recently have reported that

decalcified or surface decalcified allogenic bone or dentin will induce mesenchymal cell differentiation into osteoblasts and formation of new bone. They have labeled this process the "bone induction principle" (BIP) and have related it to the topochemistry of bone or dentin matrix collagen. With these findings, they have theorized that bones, unlike most human organs, retain the primordial capacity to induce regeneration of lost parts.

This study was undertaken to determine the feasibility of using surface decalcified allogenic bone for mandibular bone grafts and, if feasible, to develop methods for accomplishing such grafts.

APPROACH:

Bilateral osseous defects of the same size were created surgically in the inferior border of the body of the mandibles in 23 dogs. Each defect was 3 cm long and extended superiorly to the inferior alveolar canal. In group one, each of 17 dogs had one mandibular defect restored with fresh autologous marrow obtained from the iliac crest. These grafts were stabilized with a Vitallium crib lined with Millipore filter in the method described by Boyne. The contralateral defect in each of these animals was restored with surface decalcified allogenic bone obtained from the mandibles of other dogs and secured in place by intraosseous stainless steel wires.

Surface decalcified allogenic bone was prepared in the manner described by Urist by placing donor specimens in 0.6N HCL for 2 hours at 25 C. After surface decalcification, each specimen was washed for 30 minutes in normal saline solution and placed in neutral 70 percent ethanol at 20 C for 48 hours to defat the specimen. The specimens were then lyophylized after exposure to liquid nitrogen for 15 minutes and stored at -10 C in vacuum containers.

The dogs were sacrificed at 2, 3, 4, 5, 7, 9, 10, 11, 12, and 14 weeks postoperatively--2 dogs at each interval, except on the last 3 dates when only one animal was sacrificed. Selected animals received radioactive ^{18}F intravenously (doses were calculated

to give 10μ curie/kg at time of sacrifice or photoscan) at various intervals before sacrifice and were scanned with a positron photoscanner. Osseous core samples were obtained from these animals after sacrifice and ^{18}F activity was determined by well counting. The mandibles were recovered by block section and processed for histologic study. After decalcification, step serial sections, stained with hematoxylin and eosin, were prepared in all instances.

In group two, each of 6 dogs was treated in the same way as the first, except that the mandibular defects were restored with autologous marrow grafts on one side and with a combination of surface decalcified bone and autologous marrow fragments on the other side. in the latter instance, a hollowed-out tray was formed from the surface decalcified bone and the marrow cavity and the interface was packed with autologous marrow. The surface decalcified bone was held in position with stainless steel intraosseous wires. These dogs were sacrificed at 2, 3, 6, and 7 weeks after grafting. Two dogs were sacrificed at 3 and 6 weeks and one each at the other times. The specimens were prepared in the same manner as previously described for the animals in the first group. The animals were monitored by ^{18}F photoscans at regular intervals and scintillation well counts at sacrifice.

RESULTS AND DISCUSSION:

TWO WEEKS POSTGRAFT--Surface decalcified grafts: Endosteal host bone proliferation had resulted in initial fusions between the graft and the host bone.

Marrow grafts: In some specimens, the transplanted marrow fragments were associated with early evidence of osteogenesis. Other specimens revealed no detectable osteogenic activity. None of the transplanted fragments was fused with each other or the host bone. Endosteal bone proliferation was comparable to that observed in surface decalcified specimens. An inflammatory infiltrate was observed at the external margins of the graft adjacent to the Millipore filter and Vitallium mesh.

Surface decalcified grafts with marrow fragments: The specimen showed early evidence of fusion with the host

bone associated with considerable endosteal proliferation. Osteogenic activity was noted around some of the marrow fragments. There was no evidence of an inflammatory infiltrate.

THREE WEEKS POSTGRAFT--Surface decalcified grafts: Fusion between the graft and host was more advanced. There was evidence of osteoclastic activity and initiation of graft replacement by the host. No inflammatory infiltrate was observed.

Marrow grafts: All grafts showed some evidence of osteogenesis in relation to the transplanted fragments. The amount of osteogenic activity varied considerably from one specimen to another. Fusion between the separate fragments and between the host bone and the marrow fragments was observed in only one instance. An inflammatory infiltrate was present at the margins of the graft associated with the Millipore filter and Vitallium mesh. In these areas, osteogenic activity was not evident.

Surface decalcified grafts with marrow fragments: Endosteal bone proliferation was evident, with fusion between the host bone and surface decalcified graft. Marrow fragments showed limited osteogenesis. No inflammatory infiltrate was noted.

FOUR TO SEVEN WEEKS POSTGRAFT--Surface decalcified grafts: Microscopic evaluation revealed progressive remodeling and fusion of the graft with the host bone. No inflammatory reaction was evident.

Marrow grafts: Marrow grafts showed fusion between the fragments and with the host bone at 4 weeks, with progressive osteogenesis and fusion at later dates. The grafts were trabecular in character and hematopoietic activity was present in some grafts. Endochondral ossification within the graft was noted frequently, and in some instances was extensive. The margins of the grafts adjacent to the Millipore filter and Vitallium mesh showed fibrosis and contained a chronic inflammatory infiltrate. In these areas, osteogenic activity was limited.

Surface decalcified grafts with marrow fragments: Six- and 7-week specimens showed fusion between the host

bone and graft. Marrow fragments showed active osteogenesis with endochondral ossification in some areas. Extensive remodeling was present in some grafts. No inflammatory infiltrate was present. No further histologic evaluation of this type of graft was performed at longer postoperative time intervals.

NINE TO TEN WEEKS POSTGRAFT--Surface decalcified grafts: All specimens showed fusion of the graft to the host bone with extensive remodeling and progressive incorporation into the host mandible.

Marrow grafts: Although the grafts were undergoing progressive osteogenesis, they were still trabecular, and many contained areas of hematopoietic activity. Cartilage was observed in some areas. An inflammatory and fibrotic reaction to the Millipore filter and Vitallium crib was noted in most instances. There was no evidence of a cortical plate.

ELEVEN TO 14 WEEKS POSTGRAFT--Surface decalcified grafts: Grafts were well incorporated into the host mandibles and showed continued remodeling. There was no evidence of rejection.

Marrow grafts: Fusion with host bone was extensive. The grafts were trabecular and hematopoietic activity was noted in some areas. Some specimens exhibited reaction to the Millipore filter and Vitallium mesh, and only one graft had a well-developed cortical plate.

^{18}F ACTIVITY--Positron photoscans of animals revealed increased ^{18}F activity in all grafts at all intervals after 2 weeks. The resolution of these photoscans was not sufficient to permit quantitative or qualitative differentiation between the various grafts.

Scintillation well counts of core samples revealed increased ^{18}F activity at all graft sites as compared to controls. Maximum ^{18}F activity was observed in all types of grafts 4 weeks after placement of the graft. This level of activity appeared to correlate with microscopic findings as to maximum osteogenic activity. In general, ^{18}F activity was higher in grafts composed only of marrow fragments, as compared with those containing surface decalcified bone. As remodeling progressed, grafts containing surface decalcified bone

had ^{18}F activities that more closely approximated activity levels observed in pure marrow grafts.

The surface decalcified allogenic bone grafts used in this study were well accepted by the host. We were unable to detect any evidence of rejection of this material. Microscopically, the decalcified material appeared to be tolerated in the same fashion as autologous nonviable bone. New bone fused directly to the allogenic graft, followed by gradual resorption of the nonviable graft and progressive replacement by new bone. The microscopic, radioisotopic, and clinical end points used in this study were not sufficiently sensitive to permit determining the induction effect of the surface decalcified bone on the mesenchymal tissue of the host. Conversely, they did not reveal any inhibition of host bone osteogenic activity. Therefore, from our results we cannot support or refute the induction principle reported by Urist for surface decalcified allogenic bone.

The transplanted marrow fragments did appear to maintain their viability, as shown by the microscopic presence of intact osteocytes within the lacunas of the fragments. The apposition of osteoid directly on these viable fragments, which were not directly associated with host bone, is in agreement with Boyne's findings that fresh autologous marrow has osteogenic potential. However, our findings would appear to show that this potential is retarded for a time after transplantation. The endochondral bone formation observed in association with transplanted marrow fragments has not, to the best of our knowledge, been reported previously for such grafts to the mandible. Endochondral bone formation occurs frequently in osseous repair in other parts of the body. This formation appears to be the result of incomplete vascularization of the repair site before osseous proliferation.

The inflammatory reaction associated with the Millipore filter and Vitallium mesh, which was consistently observed in the first 9 weeks after grafting, appeared to inhibit osteogenesis in the local area. This finding conflicts with those of Boyne when he used a similar technique. We have conferred with Boyne on possible technical differences but have been unable to explain this variance.

Although the surface decalcified graft with autologous marrow fragments appeared to have little advantage over the grafts composed entirely of surface decalcified bone, in a human discontinuity defect the addition of autologous marrow fragments with osteogenic potential is considered desirable.

The use of surface decalcified allogenic bone with autologous marrow fragments has advantages over the autologous marrow graft supported with Vitallium mesh lined with Millipore filter. The surface decalcified bone unites structurally with host bone, is removed by physiologic processes, and has shown no evidence of inciting local inflammatory reactions. In addition, the use of allogenic human mandibles would simplify fabrication problems associated with metallic frameworks, and such mandibles are much easier to contour and adapt than metal frameworks.

The ^{18}F activity at the graft sites, as measured by positron photoscans and scintillation well counts, is not yet a sufficiently sensitive measurement to relate the significance of emission variations to the total repair process.

FUTURE PLANS:

Additional studies have been initiated on the experimental animal model described in this report to determine if variations in the amount of surface decalcification have any effect on bone repair. Clinical studies have been initiated to verify laboratory findings.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6811	72 07 01	DD DRAP (AR)635

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. DOWN SECURITY	7. REGRADING	8A. DISB'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D.Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	066
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Tissue Reaction to Sutures (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 012900 - Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 06	73 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA — 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: 72	.5	4
FISCAL YEAR — CURRENT: 73	.5	2

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

20. PERFORMING ORGANIZATION

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NAME: Jones, J.C., MAJ DC DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Maxillofacial wounds; (U) sutures; (U) tissue reaction; (U) oral surgery; (U) mouth; (U) mucous membranes

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

25. (U) 71 07 – 72 06 The oral tissue response to 18 different suture materials has been evaluated histologically in dogs. The monofilament suture materials and the multifilament resorbable suture material, polyglycolic acid, were associated with milder tissue responses than the nonresorbable multifilament suture materials studied, even though some of the latter materials were impregnated with antibiotics. Antibiotic impregnation did appear to improve the relative tissue response as compared to untreated nonresorbable multifilament sutures. Bacteriology studies reveal that polyglycolic acid suture inhibits the transmission of bacteria compared to other multifilament sutures. Clinical studies indicate that polyglycolic acid sutures are well tolerated by the patient and oral tissues. These findings suggest that the polyglycolic acid suture is the material of choice for suturing oral wounds. Four manuscripts have been published and an additional manuscript has been submitted for publication. Six papers have been presented at national scientific meetings.

*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

Tissue Reaction to Sutures

Colonel Gilbert E. Lilly, DC
Colonel D.B. Osbon, DC
Lieutenant Colonel J.L. Cutcher, DC

PROBLEM:

Because of military urgencies requiring evacuation and transfer of patients, and the frequent necessity to wire the jaws together, prolonged retention of oral sutures is often necessary. In such situations, particularly when battlefield wounds are involved, it is critical that the sutures used to maintain closure do not themselves contribute to tissue irritation and wound breakdown with resultant increased morbidity.

In previous studies on dogs, resorbable multifilament polyglycolic acid (PGA) sutures were associated with less severe tissue reactions histologically than multifilament nonresorbable sutures. The histologic findings in these previous studies suggested that bacterial transmission by the PGA suture was less than with the other multifilament suture materials and that this was a factor accounting for the milder tissue reaction associated with the PGA sutures.

This study was undertaken to clinically verify the previous animal studies and to determine if the PGA suture did inhibit bacterial transmission.

APPROACH:

In the clinical portion of this study, 12 periodontal flaps and 220 surgical flaps associated with the bilateral removal of third molars were evaluated in 122 patients. Periodontal flaps were sutured with alternate 4-0 silk and 4-0 PGA sutures. Third molar flaps were sutured with 4-0 silk and on the contralateral side with 4-0 PGA sutures. All sutures were on swedged 3/8 circle cutting needles. Sixty-one percent of the patients were males and 80 percent of the patients were in the second and third decades of life (see Table 1). Slightly over 68 percent of the surgical procedures were in the mandibular area and 90 percent were third molar flaps. The sutures were removed four to seven days after they were placed and 88 percent were removed on either the fourth

or fifth postoperative day. At the time of suture removal, the response of the tissues to the PGA and silk sutures was compared in each patient as to appearance, retention of sutures, flap position, evidence of complications, and patient complaints related to the sutures.

TABLE I

CLINICAL STUDY

Patients		Location of Sutures		Type Flap		Suture Removal	
Males	61%	Maxilla	31.8%	Periodontal	9.8%	<5 days	88%
Age		Mandible		3rd Molar			
15-30	86.2%		68.1%		90.2%	>6 days	12%

The ability of sutures to transmit bacteria was evaluated in vitro by a specially designed bacteriologic culture chamber. Sterile thioglycollate medium was placed in the central chamber and the exterior chamber was partially filled with a similar medium containing a mixed culture of oral microorganisms. The interface between the two chambers was impervious except at the site occupied by the suture. The chambers were incubated aerobically at 37° C and the presence or absence of growth determined by visual examination after twenty-four hours. The bacterial transmission features of PGA, silk, steel, and gut sutures were evaluated in this system.

RESULTS AND DISCUSSION:

All of the PGA sutures were retained in position in 96.7 percent of the cases and in no case were they all lost (see Table II). The flaps were maintained in their proper position in 95 percent of the cases. In comparison, contralateral flaps sutured with silk were retained in position in 95.9 percent of the cases, and some of the silk sutures were lost in 7.2 percent of the patients. Local complications related to the sutures were noted in five cases with PGA sutures and in nine cases with silk. Only one of the 122 patients had any complaints related to the PGA suture. In the clinical judgment of the surgeon, the reaction

associated with the PGA sutures was judged to be the same as that associated with silk in 78.7 percent, better than silk in 13.9 percent, and worse than silk in 7.4 percent of the patients surveyed. In the laboratory study, steel and gut sutures exhibited no evidence of bacterial transmission at twenty-four hours (see Table III). Twenty-five percent of the PGA sutures and 74.1 percent of the silk sutures exhibited bacterial transmission. This difference in bacterial transmission was significant with a P value of < 0.01.

TABLE II

CLINICAL RESULTS

	Suture Retention	Flap Retention	Local Complications	Patient Acceptance
PGA	119 (96.7%)	116 (95.1%)	5 (4.1%)	121 (99.2%)
Silk	112 (91.8%)	117 (95.9%)	9 (7.4%)	120 (98.4%)

TABLE III

BACTERIAL TRANSMISSION

Suture	Number of Trials	Negative	Positive	Percent Positive
Silk	27	7	20	74.1
PGA	24	18	6	25.0
Gut	12	12	0	0.0
Steel	12	12	0	0.0

The in vitro bacteriologic findings indicate that the PGA sutures do not transmit bacteria as readily as silk sutures. In our opinion, these findings add support to our previously expressed contention that bacterial transmission is a major factor responsible for the tissue reaction to intraoral sutures.

In view of the bacteriologic findings in this and previous studies, we are convinced that PGA does inhibit bacterial transmission and that this is the major

factor accounting for decreased oral tissue reaction as compared to multifilament nonresorbable sutures. Although neither polyglycolic acid or its in vivo hydrolytic breakdown product, glycolic acid, are bactericidal, it is theorized that within the microcosm between the interstices of the PGA suture, the glycolic acid concentration is sufficient to result in a pH that is not compatible with bacterial growth.

Although the clinical observations made in this study fail to demonstrate a clinical superiority of PGA suture as compared to silk, the nature of the population (predominately young adults), the procedures (elective surgery), and the suture retention time (usually five days or less) resulted in a situation where, in most cases, the host resistance was sufficient to preclude demonstration of clinical differences. The clinical findings indicate that PGA sutures are not toxic, are well tolerated by the patient, and technically provide and maintain tissue approximation.

Because of the microscopic and bacteriologic findings, in cases where longer suture retention is required and/or tissue resistance is lowered, we believe the PGA suture is preferable to silk.

The physical handling characteristics of the PGA suture are similar to silk and other multifilament suture materials and therefore make it clinically more acceptable than the monofilament suture materials currently available. Although the PGA suture is resorbable, the resorption time is sufficiently long (10-20 days in the oral cavity) to make suture removal necessary. The major disadvantage to the clinical use of PGA suture, in our opinion, is its pale color which makes visualization difficult.

This comparative clinical and bacteriologic study of PGA and silk sutures revealed no significant clinical difference in the tissue reaction to the two sutures studied. The bacteriologic findings and previous histiologic findings indicated that PGA sutures inhibited bacterial transmission. This inhibition of bacterial transmission is thought to be a major factor responsible for reaction of oral tissues to sutures. The bacteriologic, histologic, and clinical handling features of PGA sutures appear to make them superior to

silk for oral sutures which are retained for extended periods or in patients with lowered resistance.

FUTURE PLANS

At this time, no additional studies are planned. As additional suture materials become available for use in the oral cavity, they will be evaluated.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6920	72 07 01	DD-DRA-(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
71 10 18	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	067
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Repair of Salivary Gland Ducts (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 08	73 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	.5	2
	CURRENT 73	.5	3

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

DA

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) combat surgery; (U) oral injuries; (U) salivary gland duct repair

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

25. (U) 71 10 – 72 06 Preliminary studies have indicated extreme difficulties in developing a laboratory model that is analogous to human clinical situations. A model has been developed but preliminary results on two dogs indicate further refinement is necessary.

*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.

Repair of Salivary Gland Ducts

Colonel Gilbert E. Lilly, DC
Major J.C. Jones, DC
SP-4 Perry B. Hackett

PROBLEM:

High velocity missile wounds involving the face frequently result in the laceration or transection of major salivary gland ducts. Various methods have been suggested for the repair of these injuries to permit normal salivary drainage, promote normal gland repair and function and prevent salivary fistula formation on the face. Although the most frequently advocated and accepted method of repair is to suture the cut ends of the duct together over a polyethylene tubing stent, there is still a question as to how the stent should be placed. The adequacy of such repairs has not been defined. The treatment of a severed duct, where primary repair is not possible, is not well established. This study is directed toward developing methods for managing major salivary gland duct injuries that will decrease patient morbidity and promote normal salivary gland function.

APPROACH:

Adult mongrel dogs were used in this experiment. All dogs were anesthetized and both submandibular ducts were surgically exposed and transected approximately 2 cm from the orifice. In 6 dogs the ducts were repaired by suturing them over polyethylene tubing stents. Three dogs had the tube removed from the duct immediately on one side, and 2 weeks postoperatively on the other. Three other dogs had the tube removed from 1 side at 4 weeks and the other side at 6 weeks postoperatively. Three dogs did not have a primary repair of the severed duct. In these animals, one gland was drained by means of a tube through the distal end of the duct. The other side was drained by a larger diameter polyethylene tube through a stab incision. The proximal stump of the duct was not surgically approximated to the distal stump in these

dogs. The adequacy of repair and gland function was evaluated by sialography at weekly intervals, and by microscopic examination of the glands and ducts at sacrifice. All dogs were sacrificed at 12 weeks postoperatively.

RESULTS AND DISCUSSION:

Retention of the polyethylene catheter in the animals has been a major problem. Despite placement of deep sutures, the catheters frequently have been lost prior to the end of the experimental period for retention. This problem in the experimental model has precluded obtaining meaningful data on these different methods of management. Histologic and sialographic studies in animals that retained catheters for a minimum of 2 weeks indicated major differences in the catheterized salivary glands as compared to contralateral untreated control salivary glands with resected ducts.

Control glands histologically exhibited marked diffuse acinar degeneration and contained an inter- and intralobular inflammatory infiltrate of lymphocytes and plasma cells. Catheterized glands contained a mild intralobular inflammatory infiltrate and minimal acinar degeneration. Sialograms were essentially normal for the experimental glands. Sialograms of control glands revealed marked stricture of the salivary gland ducts and limited definitive filling of the glands with resection and are currently being evaluated in the experimental model.

FUTURE PLANS:

Additional studies are planned to develop a predictable animal model.

Preliminary trials with silicone catheters have indicated less problems with rejection and are currently being evaluated in the experimental model. A microporous polyurethane catheter has recently become available which may offer advantages to other types of catheters.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6923	72 07 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
71 10 18	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	068
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Oral and Maxillofacial Wound Infection (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 08	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	2	10
	CURRENT 73	3.5	20

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Osbon, D.B., COL DC

NAME: Hutchinson, R., LTC DC DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) wound infection; (U) antibiotic susceptibility testing; (U) pathogenic bacteria

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

24. (U) Clinical and laboratory study of infected human oral and maxillofacial wounds, identifying all microbial genera cultured from such infections, and delineating the antibiotic susceptibility patterns of all microorganisms involved. Such information is collated with the clinical course of infection in order to evaluate the effect of drug therapy.

25. (U) 71 10 - 72 06 Preliminary fundings on 50 patients indicate a wide spectrum of microorganisms isolated from oral and maxillofacial wounds (30 different species have been isolated to date, 15 of which are gram negative). Over half of all cultures contained at least one gram negative strain of bacteria. Gram positive bacteria resistant to penicillin G were cultured from 52 percent of all patients. The diverse nature of the antibiotic susceptibility patterns of these microorganisms underlines the need for culture and sensitivity testing in hospital practice of oral surgery.

*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.

Oral and Maxillofacial Wound Infection

Lieutenant Colonel J.L. Cutcher, DC
Colonel Donald B. Osbon, DC
Judith B. Richey, B.S.
Lieutenant Colonel R. Hutchinson, DC

PROBLEM:

Gram negative bacteria play a major role in postoperative surgical infections of combat wounds in regions other than the oral cavity. Moreover, gram negative organisms are now partially supplanting the gram positive bacteria formerly regarded as the major factors in secondary breakdown of maxillofacial combat wounds, even in the presence of standard, accepted antibiotic therapy. Oral and maxillofacial wound infection results in prolonged hospitalization of affected patients since wound healing is invariably delayed. In addition, such infection frequently has a deleterious effect on restoration of facial function and esthetics which often have already been seriously compromised.

A meaningful approach to effective therapeutics should entail a comprehensive knowledge of the types of microorganisms involved in oral and maxillofacial wounds.

The purpose of this investigation is to survey the microfloras of oral and maxillofacial infections, and to establish guidelines for therapy by determining the microbial population characteristics and antibiotic sensitivity patterns of these infections.

APPROACH:

This study will be performed on selected patients having oral and maxillofacial infections who present for treatment at the Department of Dentistry, Letterman General Hospital.

Prior to definitive therapeutic measures or oral manipulations, bacteriologic samples will be obtained from the inflammatory site and inoculated directly into selected culture media for immediate incubation. Pertinent data relating to the patient and the lesion will

be recorded and forwarded to the Oral Microbiology Laboratory, Maxillofacial Sciences Division, Letterman Army Institute of Research, along with the bacteriologic specimen.

Specimens will be evaluated for identification of aerobic, facultative, and anaerobic microorganisms involved in these infections, and for determining bacterial antibiotic susceptibility and resistance patterns according to the Kirby-Bauer method.

All laboratory findings relating to antibiotic sensitivity data and culture characterization will be collated with the clinical course of each infection.

RESULTS AND DISCUSSION:

Preliminary findings on 50 patients to date indicate that the spectrum of microorganisms isolated from oral and maxillofacial infections is broader than generally reported (30 different species, 15 of which are gram negative, have been isolated thus far).

Total pure culture isolations from these 50 cases number 182. Of these, 24.7 percent consist of gram negative bacteria, indicating the increasing significance of this type of bacteria in these infections. Fifty-two percent of all patients exhibit cultures containing gram positive bacteria resistant to penicillin G. To date, such bacteria have been exclusively members of the genus Staphylococcus, with S. epidermidis predominating.

At least one gram negative strain of bacteria was cultured in 56 percent of all samples obtained.

In general, the in vitro antibiotic susceptibility patterns and culture characteristics of these microorganisms are extremely varied, indicating the need for culture and sensitivity testing in the hospital practice of oral surgery. Moreover, it is becoming increasingly apparent that continued empirical use of antibiotics such as penicillin, in dental and maxillofacial infections, raises the distinct possibility of iatrogenic or nosocomial infections due to resistant bacteria.

FUTURE PLANS:

Clinical and laboratory studies should be continued in FY 73 to obtain a comprehensive understanding of the culture characteristics and antibiotic susceptibility patterns of microorganisms involved in these infections.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6922	72 07 01	DD-DRAE AR(SA

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
71 10 18	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A825	00	069
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Actinic Blocking Agents for Protection of Lip Mucosa (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 09	73 06	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: NA

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 72	.5	4
	CURRENT 73	.5	4

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Willis, I., MAJ MC DA

21. GENERAL USE

22. KEY WORDS (Precede EACH with Security Classification Code)

actinic damage; (U) lip mucosa; (U) actinic blocking agents

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

24. (U) In vivo laboratory studies on human volunteers to evaluate the protective effect of commercially available agents when applied to the lips. A Xenon Solar Simulator will be used which emits an ultraviolet light spectrum similar to that received on the earth's surface. By using graded time selected exposures the actinic blocking agent which gives the most durable protection when applied to the lip will be ascertained.

25. (U) 71 10 – 72 06 Previous studies demonstrated limitation for this study in design of the solar simulator and the need for careful focusing of the beam to insure an even intensity in the field of exposure. Familiarization and modification of the Xenon Solar Simulator are currently underway and evaluation of agents on human volunteers has been started.

*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.

Actinic Blocking Agents for Protection of Lip Mucosa

Captain Thomas F. Payne, DC
Colonel Gilbert E. Lilly, DC
Major Isaac Willis, MC

PROBLEM:

Military personnel are frequently exposed to sun and wind for prolonged periods of time during combat or training exercises. Such exposure, particularly in light-complexioned individuals, can result in painful sunburned or chapped lips. Severely sunburned lips in addition to personal discomfort may interfere with speech and limit the effectiveness of military operations involving voice transmission. Moreover, actinic radiation received by chronic exposure to sunlight is known to be a causal factor of lip carcinoma. Commercial agents are available that are purported to effectively protect the lip mucosa from sun and wind. However, there are conflicting reports concerning the effectiveness of the various active ingredients. No comparative evaluation of the effectiveness of actinic blocking agents on the lip has been done. At present, no agent is available as a standard military formulary item.

The purpose of this study is twofold: first, to determine the effectiveness of selected commercially available actinic blocking agents for the protection of the lip mucosa against actinic radiation, and second, to evaluate additional vehicles for application should the penetrative features or adhesiveness of the commercial preparations prove unsatisfactory.

The Xenon Solar Simulator provides the source of actinic radiation to be used in this study. This source emits a spectrum which is nearly identical to that received on the earth's surface from natural sunlight, including ultraviolet, (290-360 nm). A radiometer measures the energy delivered at various wavelengths.

The unit of dosage in evaluating human response to exposure to ultraviolet light is called the minimum erythema dose (M.E.D.). This is defined as the lowest time related exposure of ultraviolet light which will

produce erythrema with a sharply defined border on a test site. The time required to produce an M.E.D. may be varied by altering the intensity of the ultraviolet beam or varying the distance from the test site to the beam aperture. It is desirable in this study to have the M.E.D. range in the area of 50 to 60 seconds.

The ideal actinic blocking agent would possess the following characteristics:

a. The agent should be nontoxic.

b. Protection against a 3 M.E.D. exposure 8 hours after application.

c. The agent should not cause drying of the lips, but should offer protection against wind-burned and chapped lips.

d. The agent should be cosmetically acceptable.

e. The agent should not have an unpleasant taste.

Since all of the agents to be tested in this study are commercially available and cleared by the F.D.A., they are assumed to be nontoxic.

APPROACH:

The effectiveness of various actinic blocking agents will be determined in the following manner: Groups of 10 human volunteers will participate in each experiment. The first experiment will evaluate the range and consistency of the M.E.D.'s of the subjects, both on the lower lip and the ventral surface of the forearm.

The average M.E.D. will be adjusted to fall in the 50 to 60 second range by altering the intensity of the light source. When a reasonable intensity setting has been determined, then all settings on the Xenon Solar Simulator will remain constant for the entire experiment. This will be done by determining the M.E.D. to the nearest 5 seconds and repeating the determinations at 1 week, 2 weeks, 1 month, and 2 months. Careful attention to the condition of the lip at the time of exposure is important because dryness may have a significant effect on the M.E.D. The experiment will

give background information on the consistency of the M.E.D. for each individual as well as the M.E.D. range between individuals. A correlation between the M.E.D.'s, as determined on the ventral surface of the forearm and the lower lip, will also be made.

The second experiment will identify the agent which best protects the lip against ultraviolet light. Groups of 10 volunteers will have their individual M.E.D.'s determined. The agent to be tested will be applied to the lip and 5 hours later the site exposed to a 1, 1.5, and 2 M.E.D. dose. Agents, if any, which provide 2 M.E.D. protection after 5 hours will be tested at the 3 M.E.D. level and the time interval between application and testing prolonged until a protection profile for each agent is completed. In the event that no agent gives 1.5 M.E.D. protection after 5 hours, then the test will be repeated 3 hours after application.

RESULTS AND DISCUSSION:

Previous experiments for determining M.E.D.'s have been discarded because it was discovered that the intensity of the UV beam was not consistent for all parts of the field. This precludes using multiple sites with a single exposure. These experiments did provide familiarization with the machine and experience in applying radiation to mucosa and skin. As opposed to skin, lip mucosa when subjected to an exposure much over 1.5 to 2 M.E.D. tends to crack with healing by eschar taking place in 1-2 weeks. Some difficulty is encountered in seeing erythrema on lip mucosa, but this is overcome by extending the strip to be exposed over the vermilion border, thus marking the exposure site.

FUTURE PLANS:

To complete the project as outlined.

CLINICAL RESEARCH SUPPORT DIVISION

Program Element 6.11.02.A
Defense Research Sciences, Army

Project Number 3A061102B71P
Radiobiology

Task Area Number 09

Biochemistry

Task Area Number 01

CLINICAL RESEARCH SUPPORT DIVISION

Clinical Research Support Division terminated its existence as a division of LAIR with the end of this fiscal year. During this year a wide range of clinical research projects were supported with monies, laboratory equipment, supplies and technical personnel. Primary technical support was afforded through the fully staffed and equipped biochemistry laboratory. Another major factor contributing to the success of the clinical research projects was the competent advice and guidance provided by this division as research review prior to initiation of projects.

The Pho/Gamma III scintillation Camera was the largest and most complex piece of equipment used in support of clinical and in-house research, and it will continue to be used to an even greater extent as more studies are initiated for its use.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6812	72 07 01	DD-DRAE(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
71 07 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71P	01	091
b. [redacted]	61101A	3A061102B71P	09	951
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Clinical Management of Patients Undergoing Radiation Therapy (05)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine; 013900 - Radioactivity

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	72 09	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	50
FISCAL YEAR CURRENT 73	.025	0

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER.

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) radiation; (U) ionizing radiation; (U) radiation treatment; (U) radiation lethality

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

25. (U) 71 07 – 72 06 From two to fourteen days after x-ray exposure, mice exhibited greater concentrations of serum triglycerides than did sham-irradiated, pair-fed controls. Lipoprotein lipase and glyceride synthetase, enzymes which are associated with the assimilation of esterified fatty acids from the circulation, were more active in homogenates of adipose tissue from x-irradiated mice than from corresponding homogenates of sham-irradiated controls. The fatty acid esterifying capacities of liver homogenates were unaffected by exposure to sub-lethal doses of x-irradiation. Thus, the hypertriglyceridemia of x-irradiated mice is most likely due to a reduced uptake of triglycerides by extrahepatic tissues rather than an increased output by the liver.

The radioprotective action of SKF-525A is associated with an enhanced recovery of erythrocytes, and a histologically active bone marrow on the 12th to 15th days after x-irradiation. The concentration of lipid peroxides, as determined by uv absorption at 233 nm and by fluorescence, did not differ between x-irradiated and sham-irradiated mice. We conclude that SKF-525A most likely protects x-irradiated mice by stimulating the recovery of the hematopoietic system and not by inhibiting the formation of lipid peroxides.

*Available to contractors upon originator's approval

Clinical Management of Patients Undergoing Radiation Therapy

John D. Benson, CPT, MSC

PROBLEM:

The concentrations and pattern of serum triglycerides in animals that survive x-irradiation differ markedly from those in animals which eventually succumb. Consequently, serum triglycerides, or some fraction thereof, could be useful in the prognostic evaluation of radiation casualties or of patients undergoing treatment with ionizing radiation. An understanding of the mechanism responsible for the correlation between serum triglycerides and radiation lethality may suggest more effective measures for the prevention and treatment of radiation injury.

If the peroxidative decomposition of structural lipids is one of the initial biochemical events leading to radiation-induced cell death, pharmacologic agents which modify the formation of lipid peroxides may significantly affect radiation lethality. Such agents may, therefore, be useful both for enhancing the clinical effectiveness of radiation therapy and for modifying the undesirable side effects of radiation therapy.

APPROACH:

Male, LAF_1 mice were exposed to sub-lethal doses of x-rays and sacrificed every three days for a 14-day post x-ray period. The following variables were measured in these and sham-irradiated mice: 1) serum triglycerides, 2) lipoprotein lipase and fatty acid esterifying activity of adipose tissue homogenates, and 3) fatty acid esterifying activity of liver homogenates. The enzymatic activities provided an estimate of the tissue's capacity to assimilate and esterify fatty acids.

To evaluate the role of lipid peroxidation in radiation lethality, female, LAF_1 mice were pretreated with drugs which either stimulate (phenobarbital) or inhibit (SKF-525A) chemically-induced lipid peroxidation. Thirty day mortalities were used to evaluate the radioprotective effect of these drugs. Tissue concentrations of lipid peroxides, blood cell concentrations, and histological changes of body tissues were monitored in both drug pretreated and saline treated mice.

RESULTS AND DISCUSSION:

<u>Serum Triglycerides</u>

From 2 to 14 days after x-ray exposure, serum triglyceride levels were greater in x-irradiated than in sham-irradiated mice.

Adipose Tissue Enzymes

Lipoprotein lipase and fatty acid esterifying activities of epididymal adipose tissue homogenates were generally lower than the corresponding activities from pair-fed, sham-irradiated controls. These results suggest that the hypertriglyceridemia of x-irradiated mice may be partially due to a reduced uptake of fatty acids into the adipose tissues.

Liver fatty acid esterification

The fatty acid esterifying capacities of liver homogenates from x-irradiated mice did not differ from those of sham-irradiated controls; thus, an increased hepatic synthesis of triglycerides was not responsible for the hypertriglyceridemia.

Drug Pretreatment

Intraperitoneal injections of 50 mg/kg of SKF-525A given one hour prior to x-ray exposure, slightly, but consistently reduced the LD 50/30 of x-irradiation in female, LAF_1 mice (dose reduction factor = 1.1). A similar protective effect was noted when the drug was injected within one hour post irradiation; no radioprotection was observed, however, when the drug was injected one week before or one week after x-ray exposure. Thus, the protective effect of SKF-525A depended upon its presence in the tissues during or shortly after x-ray exposure.

SKF-525 inhibits the chemically induced formation of lipid peroxides in vivo; we sought to determine whether the radioprotective effect of this drug was being mediated by a similar mechanism. Lipid peroxides in whole homogenates of liver and spleen and in liver microsomes were estimated by the formation of conjugated dienes (uv absorption at 235 nm) and of fluorescent lipids. The content of lipid peroxides from spleen or liver was not affected by x-irradiation in the period from 1 to 12 days following exposure. In addition, the in vitro capacities of liver microsomes from x-irradiated mice to form peroxides was not significantly different from those of sham-irradiated mice.

Blood Cells

Hematocrits, reticulocyte and leukocyte concentrations of SKF-525A treated, x-irradiated (600 r) mice were significantly greater than those of saline-treated, x-irradiated mice on the 14th and 16th day after exposure in two separate studies.

Pathology

Histological and gross examination of 8 tissues (brain, liver, heart, kidney, intestine, spleen, bone marrow, pancreas) from 1) SKF-525A treated sham-irradiated, 2) SKF-525A treated x-irradiated, 3) saline treated, sham-irradiated and 4) saline treated, x-irradiated revealed the following findings: 1) SKF-525A-treated mice exhibited inflammatory reactions in the peripancreatic tissues; 2) all irradiated mice regardless of drug treatment exhibited cellular degeneration in the germinal center of the spleen and depletion of the myeloid element in the bone marrow as early as two days post irradiation; 3) Myeloid regeneration in the bone marrow and return of function in the spleen appeared on the 11th day after x-irradiation and was more pronounced in the SKF-525A treated group.

On the basis of these experiments we conclude that SKF-525A most likely protects x-irradiated mice by stimulating the recovery of the hematopoietic system and not by inhibiting the formation of lipid peroxides.

FUTURE PLANS:

We intend to characterize the triglyceridemia of x-irradiated mice and to determine the serum concentrations of other lipid fractions. The in vivo incorporation of ^{14}C fatty acids into tissue lipids will be determined and correlated with our in vitro results. The effect of SKF-525A on erythrocyte membrane stability will be ascertained and correlated with the radioprotective effects of this drug.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6916	72 07 01	CD-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	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71P	01	092
b. CONTRIBUTING				
c. CONTRIBUTING				

11. (U) (Precede with Security Classification Code)

In-vitro Interactions of Digitoxin-I-125 and its Metabolites with Cholestyramine and Tetraethylenepentamine(05

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 09	73 01	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 72	1	5
	CURRENT 73	.5	5

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) digitoxin; (U) adsorption; (U) cholestyramine; (U) tetraethylenepentamine; (U) beagle

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

23. (U) 1) To verify the significant adsorption and/or binding phenomena of digitoxin by steroid-binding resins in vitro in order to more quickly and effectively reduce the levels in patients treated with digitoxin. 2) Produce metabolites of digitoxin in the beagle in order to study the role of these metabolites play in digitoxin intoxication and the manner in which they may be removed from the blood stream by resin adsorption.

24. (U) Laboratory studies evaluating 1) in vitro interactions of digitoxin and cholestyramine and tetraethylenepentamine; 2) in vitro interactions of digitoxin and its metabolites with cholestyramine and tetraethylenepentamine.

25. (U) 71 09 – 72 06 Preliminary in vitro examination of colestipol and cholestyramine as possible therapeutic agents for digitalis toxicity has been completed. Experiments controlling pH, ionic strength, temperature, dilution, and metabolic competitors have delineated the optimum conditions of digitoxin "binding" by both resins in vitro.

Considering digitoxin only, the "binding" is dependent on a pH range of 5.8 to 8.1 with maximum binding at 7.2 – 7.4 and increases with decreasing ionic strength due to buffer with maximum uptake in distilled water at pH 6.8.

The binding is independent of temperature, is inversely proportional to sample volume, and is effected by competitive binding from bile salts. Chromatographically pure digitoxin is reversibly assimilated into a complex with either resin, depending upon environmental conditions.

The effect of either resin when administered orally to 5 beagles following an induced intoxication was tested. The results indicate that either resin can lower a toxic serum level of digitoxin significantly more effectively than a cereal placebo, and both resins are the proficiency of both statistically. The same data suggests that the lowering effects is only accomplished when the serum levels are elevated beyond the acceptable maintenance range of 26.4 ± 7.9 ng/ml. A second experiment demonstrated a statistically insignificant lowering of the serum glycoside level when the resins were administered on day 5 of a 6-day maintenance program.

The results of the in vivo studies indicates that a species other than digitoxin itself is associated in a consistently stable complex with the resins. Chromatographic studies in vivo blood samples indicate the rapid appearance of polar digitoxin metabolites attributed to both hepatic and extra-hepatic metabolism.

*Available to contractors upon originator's approval

In Vitro Interactions of Digitoxin and Its Metabolites with Cholestyramine and Colestipol

Michael J. Gray, SP4

PROBLEM:

The series of digitalis glycosides in common use today, including digitoxin and digoxin, possesses a high, positive inotropic activity but also a very low therapeutic ratio. Knowledge concerning normal digitalization, maintenance routines and computed physiological half-lives of digitalis glycosides has not effectively reduced the incidence of digitalis toxicity to a stastically acceptable level. The most promising new approach to this old problem involves the recent postulation that digitalis glycosides and their metabolites undergo enterohepatic circulation. A significant lowering of the incidence of toxicity could be anticipated with the discovery of an agent capable of interrupting the enterohepatic circulation and removal of the glycoside from circulation.

Long term studies of the steroid content of serum and duodenal aspirates in patients with hypercholesterolemia being treated with cholestyramine have revealed the possible glycoside-binding capabilities of the same agent. Such patients simultaneously received digitalis. The statistically significant lower levels of glycoside found in the serum of the drug group compared to placebo controls suggests that cholestyramine and a similar agent, colestipol, may lower toxic levels of digitalis glycosides undergoing enterohepatic circulation.

APPROACH:

In Vitro

A simple mixing procedure with constant shaking was devised to determine the binding capacity of each resin for digitoxin. Four concentrations of digitoxin were prepared from weights of crystalline digitoxin: 25, 50, 75, and 100 ug/ml. Each solution contained 5 uc/ml digitoxin-^{3}H. The solvent systems employed were 0.15M Tris-Tris HCl, pH 7.2, and deionized distilled water, pH 6.8, each containing 1.0% ethanol to effect solution. Four different weights of each resin were studied: 100, 200, 400, and 500 mg. The total volume of reaction solution ranged from 1 to 4 ml.

Sixteen numbered plastic tubes were divided equally into four groups representing 100, 200, 400 and 500 mg of resin. Each

weight of resin was prehydrated in an excess of solvent at 37 degrees C. over a 12 hour period, before the tubes were centrifuged at 1500 g's and the supernatant discarded. Into tubes 1-4, 5-8, 9 - 12 and 13 - 16 was pipeted respectively 0, 1, 2 and 3 ml of the same solvent used to hydrate. Into all sixteen tubes was pipeted 1 ml of one of the digitoxin solutions. The tubes were capped, shaken in a paint mixer for 30 min, centrifuged the supernatant decanted and filtered, added to scintillation cocktail, and cooled for two hours prior to counting. Each concentration of digitoxin prepared was run in duplicate with each solvent using one of two resins.

In Vivo

Six male beagles, ranging in weight from 14 to 25 pounds were studied based on successful metabolic analysis by the SMA12 series. The dogs were fasted 24 hours prior to and during the experiment. Each animal, serving as a control for himself, underwent treatment following digitalization on three separate days, receiving either Cholestyramine, Colestipol, or a placebo of cereal. A 7 French urinary catheter was placed in the bladder on the morning of each experiment. Loading doses of 0.2 mg/10 pounds of body weight digitoxin sp. A. 5 uc/.1 mg were administered in the right cephalic vein. The specific treatment, as a slurry of 1.0 g resin/20 ml in distilled water, was administered initially by gastroscopy at 1.0 g/10 lb and 1.0/10 lb q 3 hours over six hours. Blood samples were drawn from either jugular vein at 1 minute, 0.5 hours, 1 hour, 3, 3.5, 6, 6.5 and 12 hours post-injection. Urine specimens were collected separately during the first six hours post-injection and feces obtained up to 24 hours post-injection. Total radioactivity determined in all samples was converted to ng glycoside/ml serum.

Determination of Radioactivity - All determinations of radioactivity were performed with a liquid scintillation counter (Nuclear Chicago Mark II analyzer). Serum samples (0.2 ml) were digested using 2.0 ml NCS tissue stabilizer and 200 ul 30% hydrogen peroxide to decolorize. Chomluminescence was halted by neutralizing with 100 ul glacial acetic acid and 10.0 ml scintillation cocktail was added. An equal volume of urine was decolorized with 100 ul 30% hydrogen peroxide and allowed to stand for 48 hours at room temperature before 10 ml of cocktail was added.

Total individual feces samples were homogenized with an equal weight of 10% alcohol and a 10 ml aliquote centrifuged an 2000 g's for 50 minutes. The supernatant and pulp washings were pooled and a 2.0 ml aliquote prepared for counting by decolorizing with 500 ul 30% hydrogen peroxide over a 48 hour period. Again 10 ml of cocktail was added and the samples allowed to equilibrate to chamber

temperature before counting. Quenching in all cases was corrected for by both sample blanks and external standardization.

Digitoxin -^{3}H and Labelled Metabolites - Serum specimens from all six dogs representing resin treatment and placebo were separately pooled. The same was done for urine and feces, however feces homogenates were lyophilized and reconstituted to a smaller volume before pooling. Serum, urine, and feces pools representing one minute, three hours, and six hours sampling time were then extracted, the chloroform and water phase separated by centrifugation and filtration, and each phase washed twice. Preoperative column chromatography was carried out as described by Beerman et al. (1971). The 20% ethanol-chloroform phase after column elution was then applied as a base-line streak to a 20 x 20 cm sheet of silica gel and developed with solvent system I (consisting of methyl isobutyl ketone - diisopropyl ether-methanol (80:20:10). Standards of digitoxin, digoxin, digoigenin, and digitoxose were run as parallel strips. Horizontal strips with Rf's similar to the standards were cut from the sample sheet and eluted. The extract fortified with a standard of similar Rf was then applied as a spot to a second silica gel sheet and developed in a double-phase, right angle system developing with solvent system I as the first phase and ethyl acetate-butanol (90:10) as system II. The carriers were visualized by spraying with a solution of acetic anhydride-sulfuric acid-methanol (5:5:50), and charring for one minute at 110° C. and visualizing under UV. Each spot was then cut from the sheet and, suspended in cocktail and counted.

The water phases of all samples were first deproteinized then lyophilized. The dried sample was then redissolved in 500 ul 25% methanol and the previously described systems of preparative and analytic TLC applied. To study the possible alteration of the digitoxin molecule during extraction 0.1 mg of digitoxin with sp. A of 50 uc/mg was incubated with either 10 ml distilled.water or freshly prepared canine serum for six hours and then analyzed as described.

RESULTS:

Digitoxin in vitro - Table 1 lists the results of one phase of the in vitro study, showing % bound for each resin weight in distilled water as a mean ± S.D. for all four amounts of digitoxin. A greater degree of binding was obtained in the distilled water and increased with increased resin weight, but decreased with increased dilution. Subsequent washing of the resin with 10 ml volumes of the original solvent removed almost all of the drug (98.0%) by the sixth wash. Removal of digitoxin from colestipol by solvent extraction was accomplished with 40% less volume than that required to remove a similar amount from cholestyramine.

Reduction of glycoside level - The serum values expressed as ng/ml glycoside for each dog receiving a similar treatment were combined and plotted. Table 2 shows the results of the three treatments when applied to a single loading dose of digitoxin. A significant glycoside binding effect is exercised by both resins when the serum level is $\geq$ 50 ng/ml. The slopes of each treatment curve are dissimilar only during the first hour of treatment and thereafter are similar until treatment stops at 6.0 hours. Statistically, there is no difference between the capacities of either resin to lower glycoside level. There is, however, a pronounced difference between resin therapy and the placebo. The resins are capable of lowering the blood level to a statistically normal (26.4 hg/ml $\pm$ 7.9) within six hours compared to the more than twelve hours with placebo. The resin values fall below 50 ng/ml within one hour.

The level in urine rises sharply to almost 300 ng/ml by 3.0 hours, levels off from 3.0 to 6.0 hours, and falls to less than 100 ng/ml at the twelve hour mark in all three treatments. There is an increase in fecal glycoside concentration in resin therapy but data at present is incomplete. So the fall in serum glycoside levels witnessed in a six hour resin therapy program can probably be attributed to an increase in fecal excretion possibly attributed to the binding capacity of the resins, for there is no significant alteration of urine volume or glycoside content.

Detection of Metabolites - Recovery of tritiated digitoxin from the chloroform extracts of serum and water following the addition of the drug in vitro was: Water, 99.6%, unincubated serum, 98.0%, and incubated serum, 86% digitoxin, 14% metabolites. Although chromatographic analysis using a two solvent right angle system cannot be taken as firm proof of the identity of all of the radioactive compounds in the digitoxin "spot" with digitoxin, the failure so far to obtain any different results by using several solvent systems adds support to the contention that this spot did indeed contain only digitoxin. The extent and pattern of digitoxin metabolism was determined by the ratio in each experimental sample, expressed as percent, of the radioactivity in the digitoxin spot to the total activity on the chromatographic plate.

In each serum sample, at least 90% of the total radioactivity was recovered in the chloroform phase. Radioactivity in the water phase is largely associated with molecules of digoxin, digoxigenin and a similar metabolite dihydrodigoxigenin known to migrate with the front in both solvent systems used. In the chloroform phases most of the activity can be associated with digitoxin; however, even in the 1-5 minute samples there rapidly appears an abundance of metabolites. Table 3 shows the levels of digitoxin and metabolites

in chloroform extracts of three separate sample periods. It is apparent that as digitoxin undergoes absorption, metabolism, or excretion, an overall equilibrium between the present drug and its metabolites is constantly readjusting. That radioactivity not attributable to the major metabolites used as standards is largely distributed in lipid Rf's (cholesterol, triglycerides) or either at the origin or front. Several other metabolites of digitoxin, however, with more complicated alterations of molecular structure, are listed in the literature as having Rf's similar to these remaining areas of activity.

DISCUSSION:

The in vitro experiments suggest that the binding of digitoxin is not favored in the ionic medium present in the gastrointestinal tract. Whatever binding does occur more than likely involves weak ionic bonds, which if in a medium at low ionic strength could be considered a Zwitter ion effect. The in vivo experiments, indicate that despite predictions, both resins are capable of complexing some form of digitoxin and effectively removing it from enterhepatic circulation. It does not seem possible that the glycoside being bound is digitoxin itself. Both resins are ion-exchange in nature and, besides the probable saturation of the resin with steroids and bile salts, digitoxin is not an ionic entity. Either digitoxin is being combined in a complex which, as a more suitable unit, is being irreversibly bound, or it is a metabolite of digitoxin, presumably an ionic species, rather than the parent drug which is being bound.

The first case suggests a plausible incorporation of digitoxin and/or its metabolites into a micelle. With subsequent binding of the micelle itself, of a transfer of the solubilized glycoside into the binding site with the micelle existing as a cofactor or intermediate. A similar mechanism involving micelle formation is postulated in the binding of cholesterol and other steroids in vivo. The second case, equally plausible, suggests that a polar metabolite of digitoxin is being bound. Results obtained from the TLC studies of serum samples indicate an abundance of polar metabolites being circulated.

A third possibility of trapping can be eliminated as a major contributor to the lowering effect. The trapping effect, being a purely nonchemical, physical phenomenon, depends so largely on momentary conditions in the lower tract that no consistent results could be expected from one animal on two different days, much less a group of five animals on three different days.

The results of the in vivo study bear final analysis. According to the curves produced by graphing mean ng/ml ± S. D. against time, the significant binding occurs in the first segment of the experiment, 0.0 to 1.0 hours. This makes sense since it is during this time that the largest amount of glycoside is being circulated. This is expressed in the graph by the dissimilarity of the slopes of each treatment in this period. If as the in vitro suggests a given weight of resin consistently binds a fixed percentage of the drug, then the net amount of glycoside removed from circulation during the period of the first hour would be several orders of magnitude greater than that removed during subsequent periods when the gross amount of glycoside in circulation is considerably less. During the later periods, although a similar percentage is being removed from circulation, the actual quantity of glycoside removed is not significant enough to effect a deviance from the normal.

Although it is not likely that a clinical situation will arise where intoxication is verified within one hour of drug administration, the in vivo experiment performed dose demonstrate that a reduction in the serum level can be achieved if the amount of drug in enterohepatic circulation is significantly high. Furthermore, a maintenance level of 50 ng/ml (considered the borderline of toxicity) indicates a level of tissue absorption quite different from what 50 ng/ml indicates in this experiment. It remains to be seen whether there are sufficient amounts of digitalis glycosides in enterohepatic circulation when 50 ng/ml is a maintenance serum level.

The observations of this study suggest a very convenient theory implying that the regimen rather than the dosage will be the critical factor in obtaining maximum lowering effect of the resin. Almost of equal importance is the fact that there is a specific amount of glycoside required in enterohepatic circulation critical in affecting significant reduction of the serum level. If this prerequisite is adhered to strictly in the lowering effect the treatment of toxicity will not jeopardize adequate maintenance.

COLESTIPOL		CHOLESTYRAMINE	
	Total % bound		Total % bound
mg Resin		mg Resin	
100	26.5 ± .80	100	42.0 ± .85
200	42.0 ± .83	200	64.5 ± .84
400	56.3 ± .59	400	81.7 ± .61
500	62.4 ± .37	500	95.6 ± .41

All values pertain to maximum % bound in 1 ml sample volume

TABLE 1

$t_{\frac{1}{2}}$ hrs.	PLACEBO	CHOLESTYRAMINE	COLESTIPOL
1 min	196.0 ± 10.3	209.0 ± 9.4	198.0 ± 8.9
0.5	78.7 ± 7.6	61.2 ± 3.3	53.5 ± 5.9
1.0	60.7 ± 7.8	44.6 ± 4.1	39.3 ± 2.9
3.0	47.2 ± 8.2	34.2 ± 3.4	30.9 ± 4.9
3.5	43.4 ± 10.5	32.0 ± 4.6	27.7 ± 4.1
6.0	35.4 ± 8.5	26.7 ± 4.8	23.4 ± 2.7
6.5	31.7 ± 6.3	25.1 ± 5.4	22.4 ± 2.2
12.0	26.9 ± 6.7	21.0 ± 4.7	17.8 ± 2.8

TABLE 2

	1-5 min.	3.0 hr.	6.0 hr.
Digitoxin	42.0%	56.0%	41.0%
Digoxin	2.0	17.0	9.0
Digitoxigenin	34.0	4.0	6.0
Digoxigenin	9.0	8.0	9.0
Digitoxose	2.0	8.0	7.0
Unidentified	11.0	7.0	28.0

TABLE 3

CLINICAL RESEARCH SERVICE
LETTERMAN GENERAL HOSPITAL

Program Element 6.21.10.A
Bio-Medical Investigations

Project Number 3A062110A826
Clinical Investigations

Task Area Number 00

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 7109	72 07 18	DD-DR&E(AR)636

DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. D.STR'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 08 10	H. Terminate	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
PRIMARY	62110A	3A062110A826	00	004
CONTRIBUTING				
CONTRIBUTING				

TITLE (Precede with Security Classification Code): (U) Clinical Investigations - Letterman General Hospital and Letterman Army Institute of Research

SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 - Clinical Medicine

START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD

CONTRACT/GRANT
DATES/EFFECTIVE: EXPIRATION:
NUMBER:
TYPE: a. AMOUNT:
KIND OF AWARD: f. CUM. AMT.

16. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72		88
	CURRENT 73		0

RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Inst. of Research
ADDRESS: Presidio of San Francisco, CA94129

RESPONSIBLE INDIVIDUAL
NAME: Akers, W.A., COL MC
TELEPHONE: 415-561-5181

c. GENERAL USE

20. PERFORMING ORGANIZATION
NAME:
ADDRESS:

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:
TELEPHONE:
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

KEYWORDS (Precede EACH with Security Classification Code)

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

25. 71 07 - 72 06 Thirty-eight clinical investigation studies being conducted at Letterman General Hospital at the end of FY 72 will continue but will now be funded by OMA funds. Project number 3A062110A826 has been deleted from this laboratory's project numbers as of 1 July 1972.

* 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.

STUDY OF THE EFFECT OF AVAILABILITY OF A COMPUTER PROGRAM TO SUGGEST THE DIGOXIN DOSAGE AND ATTENDANT TOXICITY/EFFICACY

Carl C. Peck, Major, MC
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Carroll M. Martin, Major, MC
Darrell T. Combs, Major, MC

PROBLEM

One problem always in digoxin therapy concerns the efficacy and toxicity of digoxin when administered to outpatients suffering from chronic congestive heart failure or atrial fibrillation. We assessed digoxin therapy in this group with two recently introduced innovations: 1) A computer program (DIGDOX-I), to enable the clinician to accurately administer proper dosages; and 2) serum digoxin levels determined by radioimmuno-assay.

Digoxin therapy relies upon generally effective "recommended" dosages derived from previous studies of "digitalizing" and "maintenance" dosages.(1) These recommendations often result in an alarming toxicity rate - up to 20% in hospitalized patients (2) with a potential mortality up to 30%(3). "Under-digitalization" may also be attendant(4). Serum digoxin levels can now be measured and Smith(5) has shown that serum digoxin levels correlate with toxicity better than do dosages.

Estimates of the influence of variations in renal and thyroid function as well as body size on digoxin pharmacokinetics have been developed(6, 7) but the average clinician may find it difficult to relate the estimates to the state of digitalization in a given patient. Computer assistance has been developed (8) but no prospective study evaluating the utility of such a program has been reported.

This study tested the DIGDOS-I Program (DIP) to predict serum digoxin levels as well as the outcome of decisions

based on its application in terms of digoxin efficacy and toxicity. A prospective trial in which the physicians of randomly selected patients were offered DIGDOS-Assistance (DA) was conducted. The capabilities of DA were compared with a group of internists and cardiologists familiar with digoxin therapy.

APPROACH

The DIGDOS-I Program (DIP) was developed by Sheiner and has been described in detail elsewhere(9). Briefly, the pharmacokinetic model upon which it is based draws heavily from the data of Jellife (6). It utilizes six patient characteristics to derive estimates of body size, thyroid and renal function (sex, age, height, weight, designation of clinical thyroid status as hyper-, eu-, or hypothyroid, either BUN or serum creatinine). Entries for "desired" serum digoxin levels as well as all previous digoxin dosage information are required. From these data, the program is capable of generating the following:

a. Predicted instantaneous serum digoxin level
b. Predicted mean serum digoxin level (at steady state)
c. Suggested digoxin dosage schedule designed to achieve the "desired" serum digoxin level

Patients drawn from the Letterman General Hospital Cardiology Clinic, were suffering from chronic congestive heart failure or supraventricular tachycardia, and all required digoxin for ammelioration of signs and symptoms of their disease. Burroughs Welcome Lanoxin from Lots No. 364-A, 376-A, was used.

Based on decisions made by their primary physicians (MD) regarding digoxin therapy, patients were assigned to 1 of 4 groups on entry into the study:

Group I — No previous digoxin therapy within 30 days. Deemed in need of initiating digoxin therapy.

Group II On previous but "inadequate" digoxin therapy. Deemed in need of an increase in dose of digoxin.

Group III On previous and "adequate" digoxin therapy. No change in dosage required.

Group IV On previous digoxin therapy. Reduction of dosage advisable.

Each patient was randomly assigned to the Control or Experimental group. Because of the expected high number of Group III patients, this group was randomized separately from Groups I, II and IV. Control patients were managed thereafter exactly as the MD saw fit. Data on experimental patients was immediately submitted to DIP, following which the DIP generated predictions and dosage change suggestions were offered to the MD for his consideration. Experimental patients received DA suggested doses except when computer "advice" was not followed - DIGDOS-refused (DR). When this occurred, the MD continued to manage his patients as he saw fit, unassisted by DIP; and the data collected on these patients were analyzed separately.

Data collected on each patient at each visit are listed in Table 1. In addition, serum potassium, creatinine and digoxin levels were determined.

Each patient was followed for at least 2 weekly visits and most for 3 or more to achieve "steady state" measurement changes following alterations in digoxin dosages. Besides the clinical evidence of toxicity, an EKG was obtained on each patient visit; and the criteria of Beller et al(4) were used to determine electrocardiographic evidence of toxicity.

For comparing the MD and DIP level predictions, the measured serum digoxin level was extrapolated to a "mean measured serum digoxin level" according to known serum level-time relationships as modified by any decrease in renal function in the given patient. Besides the above, the MD was asked to record a "desired serum digoxin level" as well as

his "predicted serum digoxin level." Since physicians were accustomed to thinking in terms of digoxin dosage rather than serum level of digoxin, guidelines for setting "desired" and "predicted" levels were offered as Tables 2 and 3 respectively.

RESULTS AND DISCUSSION OF RESULTS

Forty-two patients completed the study on whom 142 serum digoxin levels were determined. There were 12 Group I patients, 7 Group II, 18 Group III, and 5 Group IV. No significant differences between control and experimental groups can be identified as regards sex, age, height, weight, serum creatinine and initial CHF Index (Table 4).

The mean measured mean serum digoxin level was .92± .60ng/ml whereas the mean MD prediction was .96±.33ng/ml and for DIP it was 1.05±.36ng/ml. The mean prediction errors calculated for the entire group are -.04±.64ng/ml for the MD and -.14±.56ng/ml for DIP ($p<.05$). Both indicate a systematic "over-prediction" by both the MD and DIP, which in the case of DIP is statistically significant.

Generation of dosage schemes: The coefficient of correlation between desired levels and levels attained are as follows: Control = .22, DA = .44, DR = .09. Only the DA was statistically signigicant ($p<.05$). The amounts of "miss" (i.e. mean non-achievement of desired levels), quantitated by subtracting the desired level from the mean measured level, are as follows: Control = -.03±.64ng/ml, DA = -.09±.54ng/ml, DR = -.22±.44ng/ml. Only the DR value was significantly different from zero ($p<.05$). The root mean square achievement errors were not significantly different among the three groups: Control = .64±.09ng/ml, DA = .54±.09ng/ml, DR = .49±.13ng/ml.

Outcome of therapeutic decisions - Toxicity and Efficacy: No clinical or EKG evidence of digoxin toxicity was encountered in this study.

Correlating the initial to final CHF-Index with corresponding

serum digoxin levels by individual patient revealed a mean decrease in the Index of .26±.48 (22±32%) accompanied by a mean decrease in serum digoxin level of .21±.71ng/ml (.56 ($p<.05$)). Similarly, the visit-to-visit variations in CHF-Index correlated significantly with serum digoxin levels as follows: mean increase in serum digoxin level = .08±.48ng/ml; mean decrease in CHF-Index per visit intervals = .10±32ng/ml (9±27%). [r.31 ($p<.05$)].

Analysis of the prediction data reveals that DIP predicted slightly but significantly ($p < .05$) better than the primary physician, both by correlation coefficient and by absolute prediction error. However, one may ask, does an increase in predictive accuracy of .05ng/ml justify using the computer program? The answer must be no. The root mean square errors suggest that both the MD and DIP are capable only of predicting within .65ng/ml (68% confid. interval) or 1.2ng/ml (95% confid. interval). Since the probably efficacious level of serum digoxin is around 1ng/ml and the probably toxic level is around 2ng/ml, the .05ng/ml improvement by the program compared to the size of the 95% confidence interval is non-consequential. We conclude that DIP is slightly superior to the MD in predicting serum digoxin levels but the difference is not important.

In terms of attaining desired serum digoxin levels a statistically significant positive correlation coefficient resulted for the DIGDOS-Assisted cases, whereas Controls and DIGDOS-refused cases did not demonstrate this. The average amount of "miss" was significant only for the DR cases. Although DIP appears to generate suggestions of digoxin dosages schemes which come closer to attaining desired serum digoxin levels, the differences are unimportant in view of the wide range of error. This is further substantiated by the efficacy studies, which showed no demonstrable advantage in improvement in symptoms or signs of CHF from use of DA.

The Index is not a perfect measure of digoxin effect nor was attainment of desired levels by the Experimental Group markedly superior to that of Controls. Since no toxicity was encountered by the clinical and electrocardiographic methods employed in this study, no conclusions can be drawn regarding any difference in ability to avoid toxicity between DA and non-assisted cases. If the usual toxicity

rate approaches 20%, its lack in this study deserves explanation. Possibly with such careful follow-up, patients are less likely to err on the side of excess digoxin ingestion. Since only Burroughs-Wellcome Lanoxin was used, perhaps the low variation in digoxin content in each tablet accounts for consistency in digoxin intake not attained in previous studies(10). Also the general level of serum digoxin attained in the patients of this study was below the toxic range since the mean of .92ng/ml for the group is lower than that for a group of non-toxic patients studied by Beller and Smith (4). The range of error in predictive capacity is uncomfortably large for both DIP and the MD, since the 95% confidence interval for predictions in the therapeutic range overlaps the toxic range.

PLANS

The study has confirmed a baseline level of performance for the computer program comparable to well-trained internists. Further development of a useful clinical method of measuring pharmacologic effect of digoxin therapy in ambulatory outpatients suffering from congestive heart failure is needed to answer the following questions:

1. What combination of weighted symptoms and signs of CHF best reflect variations in serum digoxin level?

2. Once this "weighted" Index is devised, what is its magnitude of change per unit of change of serum digoxin level?

3. Is "maintenance" digoxin therapy a justifiable therapeutic maneuver beyond initial improvement created by digitalization?

The DIGDOS-II Program is under development utilizing the feature of a "feed-back" system: measured serum digoxin levels will be utilized to modify the Program predictions for individuals in order to make all subsequent predictions more accurate.

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TABLE I

SEMI-QUANTITATIVE GRADING OF MEASUREMENT PARAMETERS

	GRADING			
	ABSENT=0	MILD= 1+	MODERATE= 2+	SEVERE= 3+
Historical				
H_1: Orthopnea	None	2 pillows	3 pillows	Must sit up
H_2: PND	None	< 1/wk	>1, <7/wk	>7/wk
H_3: Exer. tole: Stairs	Unlimited	SOB or stop @ 2 flights	>½, >2 flights	<½ flight
H_4: Blocks	Unlimited	Unlimited except when carrying pkg. or at rapid pace	> 1 block, flat	< 1 block, flat
Physical				
0_1: Wt $X=\frac{\text{Wt-usual Wt}}{\text{usual Wt}}$	.03	X = 0.03-.06	X = .06-.1	X = >.10
0_2: HR	< 100	> 100, <115	> 115, <130	> 130
0_3: S_3	Absent	—	—	Present
0_4: Est. CVP	0	0-2 cm above clav. at 90°	> 2cm, < angle of jaw	To angle of jaw
0_5: Edema	0	1+	2+	3+
Toxicity:				
Nausea	–	.....		Present
Vomiting	–	.....		Present
Visual changes	–	.....		Present

$$\text{CHF Index} = \frac{H_1+H_2+H_3+H_4+0_1+0_2+0_3+0_4+0_5}{9}$$

TABLE II

GUIDELINE FOR "DESIRED SERUM DIGOXIN LEVEL" (DDL)

DDL	Usual dose to achieve desired "amount" of effect in avg. patient with normal renal function	Corresponding mean digoxin blood level at steady state
"very low"	.125 mg/d	.2 - .6 ng/ml
"low"	.25 mg/d	.6 - 1.0 ng/ml
"medium"	.375 mg/d	1.0 - 1.4 ng/ml
"high"	.5 mg/d	1.4 - 1.8 ng/ml

TABLE III

GUIDELINE FOR PREDICTION OF SERUM DIGOXIN LEVEL (PDL)

PDL	Usual dose to achieve observed "amount" of effect in avg. patient with normal renal function	Digoxin blood level
absent	—	.2 ng/ml
"very low"	.125 mg/d	.2 - .6 ng/ml
"low"	.25 mg/d	.6 - 1.0 ng/ml
"medium"	.375 mg/d	1.0 - 1.4 ng/ml
"high"	.5 mg/d	1.4-1.8 ng/ml
"very high"	>.5 mg/d	>1.8 ng/ml

SENSORY CHARACTERISTICS OF AUTISTIC CHILDREN

Ernest Giraldi, Major, MC

PROBLEM

To identify and define communicative characteristics of autistic children by direct observation of facial expressions of these children when presented with sensory stimuli. To differentiate on a five-point aversion scale communicative characteristics of autistic children.

APPROACH

Design: Simple comparative design

T-1 = Autistic children T-2 = Normal controls

The design procedures are principally for gathering data which will afford opportunity to definitively examine communicative characteristics of treatment groups. They will be accomplished through filming 14,440 sensory situational responses for later analysis. Each stimulus will be highly specific and concentrated in order for us to better evaluate the responses. Each response will be recorded on film by four cameras operating at the same time from four different angles, focusing on the face.

RESULTS AND DISCUSSION OF RESULTS

Phase I and II have been completed.

Phase I - Orientation, preparation phase in which subjects were introduced to the test facility and procedures.

Phase II - The overall data collection which was conducted at the test facility. This consisted of filmed testing, 24 sets of tests in each of the five sensory modalities.

Phase III - To be completed at Brooke General Hospital after 21 September 1972.

Phase III - Data analysis whereby the investigator will retrieve filmed data and analyze according to a stimulus code.

FUTURE PLANS

Completion of Phase III over the next two years.

Completion of project paper within next two years.

Several articles will be written based on film analyses.

LECITHIN/SPHINGOMYELIN RATIO IN AMNIOTIC FLUID AND ITS CLINICAL APPLICATION

Donald L. Snyder, Colonel, MC

PROBLEM

Phospholipids, particularly lecithin, that appear in amniotic fluid originate principally from the fetal lung. Since lechithin is the major component of the surface active alveolar-lining layer, its biosynthesis appears to reflect the status of alveolar stability. Gluck et al have reported in 1971 that the changes during pregnancy in the amniotic fluid phospholipids reflect those in fetal lung. Therefore, it is proposed that the lecithin-sphingomyelin ratio may be used clinically to predict with a degree of accuracy maturity or immaturity of the fetal lung and thus which fetus is mature and will or will not develop Respiratory Distress Syndrome (RDS) if delivered at a specific time.

The aim of this study is to: 1) Establish a laboratory technique to measure lecithin and sphingomyelin in amniotic fluid and apply its measurement to clinical obstetrical management. 2) Correlate the L/S ratio in amniotic fluid as an indicator of intrauterine fetal pulmonary maturity. 3) Correlate the L/S ratio in amniotic fluid and the development of respiratory distress syndrome (RDS) in the newborn. 4) Correlate the L/S ratio in amniotic fluid with other currently employed procedures for predicting fetal maturity.

APPROACH

Lecithin/sphingomyelin ratios are measured by the technique described by Borer and Gluck. The technique is done as follows: The total lipids in previously centrifuged amniotic fluid are extracted with methanol and chloroform. The surface active phospholipids in the chloroform extract are isolated by adding ice cold acetone which causes them to precipitate. The surface-active phospholipids are separated by thin layer chromatography. Detection is by staining with

Bromothymol blue detector, heat and NaOH vapors. The ratio is obtained by dividing the product of the height times width of the lecithin by the sphingomyelin spots. If the L/S ratio is 1.5 or less the fetal lung is immature, if it is 1.5 to 1.8 the fetal lung is transitional in development and if it is 1.8 or above the fetal lung should be mature and thus should not develop RDS if delivered.

Collection of fluid: Five (5cc) of amniotic fluid, placed in a clean culture tube will be collected at the time of every abdominal amniocentesis. A sample will also be collected via the vagina in cases of established premature labor prior to indicated amniotomy. Each sample will be frozen until analysis is done.

Patients to collect fluid on: 1) All elective or indicated amniocenteses for other reasons. 2) All elective repeat Cesarean sections. 3) All primary Cesarean sections where technically feasible. 4) All patients in active labor with bulging membranes, when the membranes can be needled via the vagina. 5) All patients between 16-20 weeks gestation at the time saline amniocentesis for voluntary interruption of pregnancy.

RESULTS AND DISCUSSION

Data on over two hundred (200) sample determinations has revealed good correlation of the ratio of these two phospholipids and the length of the gestation of the intrauterine pregnancy from which the amniotic fluid was collected with other reported studies. In all cases when the L/S ratio was greater than 1.8 regardless of length of gestation, the baby did well in the nursery and did not develop respiratory distress syndrome indicating fetal lung maturity. During the period of study there were no infants delivered who developed RDS so there is no comparison of cases of RDS and the L/S ratio.

Data is presently being analyzed to correlate the L/S ratio results with the other available laboratory and clinical parameters in regards to reliability as a diagnostic test for prediction of fetal maturity.

FUTURE PLANS

Data appears to indicate the value of the test as a useful parameter as regards predicting fetal maturity, but more samples need to be collected especially in the critical range of 32-35 weeks gestation before definitive conclusions can be made.

A simplified Shake Test as a diagnostic test for the presence of surface active material in amniotic fluid has recently been described. A comparison study of the Shake Test versus the L/S ratio is presently being done.

Data is to be presented at the Armed Forces District Meeting of the American College of Obstetrics and Gynecology to be held at Seattle, Washington, October 1972.

IN VITRO STUDY OF THE PRAUSNITZ-KUSTNER REACTION

Joseph L. McGerity, Colonel, MC
Edward Spitz, Major, MC

PROBLEM

The Prausnitz-Kustner reaction has for many years been a useful method for diagnostic study of atopic patients whose skin is not suitable for direct skin tests. This includes patients with atopic eczema or other skin disorders. This in-vivo technique was more recently used in the isolation of reagin (gE) and in the study of "blocking antibody" produced by hyposensitization therapy. In the research milieu of today, use of human subjects in such studies is questionable and a reasonably accurate and available in-vitro test to replace the in-vivo technique is desirable. Several techniques utilizing monkey or human lung, human WBC's, rat mast cells, etc., have been developed. They have had the disadvantage of technical difficulty or limited availability of experimental material. In addition it would appear advantageous to be able to study in-vitro on an organ that is so frequently involved in-vivo allergic reactions i.e., the human skin.

It would be useful to be able to use surgical specimens of human skin for the in-vitro study of the biochemistry of anaphylaxis and the disease postulated to result from the interaction of skin sensitizing antibody and antigens. However, previous attempts to use such material have not been successful.

APPROACH

A bioassay system for histamine using the electronically amplified and recorded contraction of guinea pig ilieum has been established. Passive sensitization of human lung and release of histamine from the lung on challenge with appropriate antigen or anti-IgE has been accomplished. Using these established techniques serums of atopic patients that are capable of sensitizing in-vitro human tissue has been and continues to be collected and used first in vitro

sensitization of human lung and then applied in the study of in vitro sensitization of human skin.

RESULTS AND DISCUSSION

Human skin samples obtained incidental to routine surgical procedures has been successfully sensitized with atopic serum in vitro and such tissue has been found to release histamine on appropriate challenge in our prelininary studies.

FUTURE PLANS

1. The initially positive results are to be confirmed with repeated experiments including the use of furhter controls.

2. The ideal parameters of the time, temperature, and chemical medium for maximum sensitization and antigen release of histamine is to be found.

3. A comparison of the sensitivity of in vitro technique and in vivo techniques should be made.

4. The use of this technique to establish the release of SRS-A (slow reacting substance of anaphylaxis) from human skin in-vitro is contemplated.

5. Histologic study of the localization of the skin sensitizing antibody in-vivo and in-vitro is to be attempted using an adaption of the peroxidase-labelled antibody method of Nakane. In particular the presence or absence of localization of IgE to mast cells in the skin is to be studied before and after the in-vitro sensitization and challenge.

6. Application of this laboratory technique to the clinical problem is to be attempted.

   a. We will attempt to develop an in-vitro method for the determination of "blocking antibodies" using as clinical model patient's hyposensitized with grass pollen extract.

b. This in-vitro technique will be used in a continuing study of the cross reactivity of Cedar and Cypress pollen with particular application to the cross reactivity of Sugi (Japanese Cedar), Mt. Cedar, and other members of the Cedar-Cypress family present in world wide distribution. Preliminary animal studies here indicate cross reactivity of precipitating antibodies of many of the Cedar-Cypress and suggest that these antigens have potential as significant allergens to personnel in many areas of the world.

c. With establishment of the reliability of the technique measurement of both sensitizing and blocking antibodies in individuals infected with Trichophyton is proposed.

ANTIBIOTIC THERAPY OF ACUTE OTITIS MEDIA IN INFANTS AND YOUNG CHILDREN. INFECTIONS CAUSED BY HEMOPHILUS INFLUENZAE

James L. Stewart, Jr., Lieutenant Colonel, MC

PROBLEM

The management of otitis media is a major aspect of child health care. Although the common pathogens (pneumococci and Hemophilus influenzae) are well established and a variety of treatment regimens are available, no one regimen is agreed to be superior. Even though erythromycin is often used if the patient is allergic to penicillin, there is no good data as to its effectiveness in eradicating Hemophilus influenzae from the middle ear. The purpose of this study is to make a comparison of the effectiveness of ampicillin, erythromycin ethyl succinate and erythromycin ethyl succinate with triple sulfonamides in the therapy of acute bacterial otitis media with particular reference to Hemophilus influenzae.

APPROACH

This study has been designed by the Department of Clinical Development of Abbott Laboratories to be carried out in several medical centers.

Four hundred and fifty children over six months of age and less than 5 years of age, weighing between 10 and 60 pounds with clinically diagnosed otitis media as a primary diagnosis will be enrolled and randomly assigned to an initial antimicrobial therapy schedule. Bacteriological data will be obtained from culture of middle ear aspirate. Children with chronic otitis media, receipt of antibiotics during previous 2 weeks, or allergy to penicillins, erythromycin, or sulfonamides will be excluded. All medication is dispensed by a person other than the treating physician who is blinded as to which regimen is being given. All groups

receive the same analgesics and decongestants. Scheduled follow-up visits will be made on days 3, 5, 14 and 31 for the physician to assess the disease course and adverse reactions to medications.

At each visit the parent brings all remaining antibiotics for measurement in order to verify amount of drug actually used. Clinical progress will be assessed on the basis of fever data, otoscopic appearances, and the presence or absence of malaise. Specimens of the middle ear aspirates are immediately inoculated in broth and sent to Dr. Sarah Sell, Vanderbilt University School of Medicine and Dr. Walton Grundy at Abbott Laboratories. No clinical improvement within 48 hours or the persistence of pain, fever, otoscopic appearance on days 5 and 14 will be considered a therapeutic failure.

RESULTS AND DISCUSSION

From the several hospitals contributing patients to the study, over 100 cases have been entered so far. Seven cases of proven bacterial otitis media have been entered from LGH. The study continues to be an important study to provide worthwhile medical information and enhance the training program by allowing the residents to participate in clinical research.

FUTURE PLANS

The study will continue under the guidance of Abbott Laboratories. All necessary equipment, drugs, bacteriological studies and statistical analysis are provided by Abbott Laboratories.

THE STUDY OF ADRIAMYCIN NSC-123127 FOR DISSEMINATED MALIGNANCY

Michael P. Corder, Major, MC
Neil W. Culp, Lieutenant Colonel, MC
James L. Stewart, Lieutenant Colonel, MC

PROBLEM

There are several varieties of disseminated malignancy for which there is no efficacious therapy of proven value and the clinical situation often occurs in those tumors for which there is efficacious primary therapy of refractoriness to said therapy. For this reason, new chemotherapeutic agents are continually being screened in animal systems and then evaluated in man for both toxicity and clinical efficacy. Phase II clinical trials attempt to identify the antitumor spectrum of drugs which have been shown to be active in animal systems and for which the clinical spectrum of toxicity has been determined. Adriamycin (NSC-123127) is an antitumor antibiotic obtained from Streptomyces peucetius (variety caesius). It is closely related to Daunorubicin (NSC-82151) with Adriamycin having an improved therapeutic index over Daunomycin (1.21 vs 0.67). Adriamycin appears to act by inhibiting DNA synthesis and by intercalation between base pairs and to inhibit RNA synthesis by template disordering and steric obstruction.

In preliminary trials in man antitumor activity has been noted in ALL, AUL, CML, Wilm's tumor, Ewing's sarcoma, neuroblastoma, and some anaplastic sacromas. There have also been responses in transitional cell carcinomas of the bladder, reticulum cell sarcomas, lymphosarcoma, Hodgkin's disease, carcinoma of the breast, colon, testicle, chorio-epithelioma of the uterus, nasopharynx, soft tissue sarcomas and Ewing's sarcoma in adults.

The experience to date is limited with all of these tumor

types and many tumor classes have had less than five evaluable cases treated. It is the objective of this study to determine the antitumor efficacy in a variety of disseminated malignancies.

APPROACH

Adriamycin is given to patients for whom there is a microscopically proven diagnosis of cancer, and unavailability of or resistance to therapy of proven value. The patients are given Adriamycin for three days IV consecutively and then a rest period until recovery of the counts and/or an additional 18 days has elapsed. Measureable tumor parameters are followed and a minimum of two courses is given prior to removal from study. The patients are evaluated weekly for toxicity and/or therapeutic response.

The patients who are previously untreated with myelosuppressive agents are given the drug 25 mg/m^2 per day for three days and previously treated patients with myelosuppressive therapies are given 20 mg/m^2 per day for three days. The drug dosage is modified for toxicity according to the protocol 71-2.

It is anticipated that results will be pooled with those of Major Nicholas DiBella, MC, at Fitzsimons General Hospital at the completion of the study.

RESULTS

To date, six patients have been treated and four are evaluable for response. The two unevaluable patients were both patients with mesiothelioma and one patient expired on day nine after initiation of therapy due to peritonitis, not related to therapy. The other patient had his therapy discontinued on day three due to an impression of cardiotoxicity which subsequently was demonstrated to be bacterial endocarditis.

One colon carcinoma metastatic to the liver was treated for two cycles and had progression in disease, one

carcinoma of the larynx with local recurrence was treated for two cycles and had progression in disease, one carcinoma of the nasopharynx with metastases to liver, bone, and epidural space had a greater than 50 percent reduction in tumor size by day 18 of the first course and the duration of the response was two months. The patient received four courses of Adriamycin. One patient with liposarcoma had no response after two courses of therapy.

FUTURE PLANS

Additional numbers of patients are required before any conclusions can be drawn. Patients will continue to be entered in the study.

TECHNIQUES OF VITREOUS SURGERY

Alexander R. Irvine, Major, MC
Floyd L. Wergeland, Jr., Lieutenant Colonel, MC
John P. Shock, Jr., Lieutenant Colonel, MC

PROBLEM

Massive vitreous hemorrhage is an all too common problem in the military. In young eyes, following ocular injury, vitreous hemorrhage often leads to strand formation and vitreous contraction, in turn producing inoperable retinal detachment. Recent methods of vitreous removal and replacement, as described by Dr. Machemer (1), offer a possible solution to the problem of vitreous opacification by hemorrhage. The present study was designed to determine the best method and time for such removal of vitreous hemorrhage.

APPROACH

Massive intraocular hemorrhage was simulated in rabbits by the intravitreal injection of 0.3 cc of vitreous blood. At varying times after injection, the opacified vitreous was removed and replaced with a physiologic salt solution using a Machemer type vitreous cutter. Control eyes included eyes with blood injection which were not operated upon and eyes which were operated upon without prior blood injection.

RESULTS AND DISCUSSION

The opacified vitreous could be successfully removed at any time from one day to one month following blood injection. The incidence of operative complications was approximately the same at all times after blood injection. After one month in rabbits, the hemorrhage tended to clear spontaneously with some strand formation but without the severe contraction and retinal detachment seen so often in young

humans.

A few other probelms became apparent. Our present version of the Machemer instrument requires refining so as to insure a constant sharp cutting edge. When the edge becomes dulled from use, vitreous is pulled into the instrument and does not cut well, and hence excessive traction is put on the retina. A modification yielding a self-sharpening blade and a spring to hold it against the cutting edge is needed and planned. The second problem is that the relatively large lens and small vitreous cavity of the rabbit make it extremely difficult to work without touching the lens and producing a post-operative cataract. Evaluation of post-operative results must then be done on formalin fixed enucleated eyes rather than in vivo, since the cataract precludes viewing the retina in vivo.

The above problems, however, are relatively minor, and the technique has developed to the point where our first patient was operated upon in late March 1972. He has had a successful result, with return of useful vision in an eye that had only been able to perceive light for 18 months previously.

Two other projects have developed as offshoots of the original vitreous surgery. A method of coagulating abnormal vessels in the vitreous under direct microscopic control has been tested in rabbits and used successfully in one human eye which was being enucleated for other reasons. This procedure may have extremely important applications in diabetic retinopathy. Secondly, an inexpensive and simple technique has been developed for removing cataracts in animals or humans through a small incision by fragmenting the lens with ultrasound. This technique may have a very important effect on routine cataract extraction, by alleviating the problem of the large incision needed for traditional intracapsular lens extraction while avoiding the extreme expense and complexity of the ultrasound cataract instrument recently developed by Dr. Kelman (2).

FUTURE PLANS

Future work is planned along three lines. Our present vitreous suction cutter needs modification to insure a constantly sharper cutting surface. It is also possible that a fiber optic light source may be added to better illuminate the field deep in a hemorrhagic vitreous. Secondly, our method

of trans-vitreal diathermy coagulation needs further testing and refinement. A bipolar system may give a more localized tissue effect, an important advantage when one is treating abnormal vessels emanating from the optic nerve and must avoid damaging the latter. Finally, the ultrasound probe we are presently using for cataract work needs testing and refinement. Dr. Irvine will be leaving the Army to join the full time staff in the Ophthalmology Department of the University of California in San Francisco. Drs. Shock and Wergeland will carry on this research and Dr. Irvine plans to work in close cooperation with them and also continue this work at the University of California.

REFERENCES

1. Machemer, R., et al. Amer. J. Ophthal. 73:1, 1972.

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EVALUATION OF THE RENIN ANGIOTENSIN I SYSTEM IN LABOR

Carl M. Hoffman, Major, MC
Alex Karu, Ph. D.
Jai Dev, B.S.M.S.

INTRODUCTION

The etiology of pre-eclampsia has long been sought. With recent advances in radioimmunoassays the possible role of the Renin-Angiotensin-Aldosterone system has been studied. Many problems are inherent in s dying this system but several recent studies have established differences between normal, hypertensive and pre-eclamptic pregnancies.

Most studies have involved isolated blood samples not taken during labor. In order to demonstrate a difference in these groups and to investigate a possible uterine contribution to the system a serial study of Renin activity during labor was undertaken.

PATIENTS AND METHODS

Fourteen patients were included in the study. All patients were followed from 8 - 14 weeks gestation through term and 6 weeks post partum. There were 4 normal controls, 5 pre-existing hypertensives and 5 pre-eclamptics. The normal controls had normal blood pressure (mean arterial pressure 90) throughout pregnancy and in labor no blood pressure change of mean arterial pressure greater than 10. The pre-existing hypertensives all had initial M.A.P. greater than 90 and pressures during labor of M.A.P. greater than 10 from pre-labor M.A.P., one of these patients also had super imposed severe pre-eclampsia. The pre-eclamptic group had M.A.P. less than 90 until 37-40 weeks when M.A.P. was greater than 90 with a change of M.A.P. greater than 10 over normal, 2+ proteinuria or greater and hyper-reflexia; all returned to normal post partum.

All patients were evaluated during labor when cervix was

dilated 4-6 cm. All patients had Corometric internal fetal monitoring and blood pressures recorded by the Arteriosonde (except one patient in each group), anesthesia consisting of Epidural block was in effect in one patient in each group without change in blood pressure.

The Arteriosonde uses a doppler principle to measure systole and diastole by flow changes in the brachial artery and used a ultrasound system to accomplish this. It has been shown to be as accurate as intra-arterial catheters in other studies.

The Corometric monitor is routinely used on high risk patients to detect fetal distress and was used in determining time of blood sample collections in addition to fetal monitoring.

Venous blood was drawn in 7 ml vaccutainers containing 1 ml of solution (pH 7.5) of 0.15 MEDIA, .9% NACE and 0.015 M BAL (2,3 dimercapto 1-propanol) as Renin preservative. The sample was immediately placed on ice and spun at 4° C 1500 R.P.M., plasma was separated and quick frozen for 1-2 weeks and run in groups of 10.

The samples were drawn with the patient in the lateral position from an antecubital vein with free flow of blood. Samples were collected prior to, upslope, peak, downslope, and post contraction.

METHOD OF ASSAY

Plasma Renin activity in all blood samples was measured by radioimmunoassay method. Labeled 125-I Angiotensin-I and antiserum (in guinea pigs) were produced at Biochemistry Lab at LAIR.

Material and Methods: Radiolabeling of Angiotensin-I with 125-I

Synthetic Angiotensin-I (ASP-1 - Ilau 5) was used for

radiolabeling and to immunize guinea pigs. (Supplied by Cal-Biochem).

Reagents

0.1 IM Phosphate buffer with 0.85% NaCl of pH=7.5; INH+Cl; Kl - 0.01 mg/10 ml buffer Soln; Chloramine T - 25 mg/10 ml; Angiotensin -1 + 1 mg/ml in 0.02 M Acetic Acid; 125-1 = 2.0 mc. (All reagents should be freshly prepared and kept on ice)

Procedure

Add 1.5 ml of Kl Soln. to a silanized small beaker + 10 Angiotensin - 1 + 2.0 mc 125-1 carefully. Adjust N_2 gently to blow over surface of reaction mixture. Put electrodes in touch of reaction mixture (platinum-mercury electrode was used). Note initial e.m.f. on scale. Slowly add Chloramine T (50 units or more as required) to start reaction. Note slow increase in e.m.f. Stop the reaction with $Na_2S_2O_5$ when e.m.f. goes 60-70 mv higher than initial value. It will slowly come down to initial value. At this stage immediately add 0.5 ml of INHCL to stop the reaction. The whole reaction takes about 5-10 minutes.

Purification of Labeled Material

Dowex 1-X^4 and 50W-X^4 were used as ion exchanges. 125-1-Angiotensin-1 was eluted with 0.5NNH_4OH. Fractions were freeze dried and stored at -40°C (good enough for use for 30 days). It was diluted to give desired c.p.m. as needed from time to time. Silanized glassware was used to store this material.

Immunization of Guinea Pigs

Angiotensin-1 coupled with GPSA (guinea pig serum albumin 33% $(NH5)_2SO4$ fraction in presence of ECDI (1 Ethyl-(3 dimethylaminopropyl-) carbodiimide to make it immunizable.

Reaction Mixture

4.0 mg GPSA, 8.0 mg Angio-1 - dissolved in 0.25 ml of distilled H_20; 150 mg of ECDI dissolved in 0.25 ml of H_20 (ECDI Soln. was slowly added to Soln drop by drop

with gentle stirring at room temp. It was allowed to react 2-4 hours at room temp. (Generally formation of white ppt. is indication of reaction completion). Reaction mixture was dialysed at 4°C against H_2O overnight. It was mixed in 1:1 ratio with Freund's Adjuvant and Sonified).

Immunization Schedule

1st injection: was given in toe pads (100λ - 150λ) under anesthesia (Diabetol was used as anesthetic). 2nd: After 8 days 2nd injection was given in neck and thighs (intramuscular). 3rd: Same as above after 8 days. After 15 days 3rd injection blood was taken by heart puncture. Serum was separated and quick frozen and stored in a small aliquots at -40°C (temp. was never allowed to rise more than 4°C). It was tested for specificity and diluted as required for assays. (1:100 dilution was generally used).

Assay Method: Reagents:

0.1M Phosphate buffer with 0.5 mg/ml. Lysozyme (pH=7.5); Angiotensin-1 1 mg/ml in above buffer (stock A); 10λ of stock A + 0.99 ml buffer - (stock B); 10λ of stock B + 0.99 ml of buffer - (stock C). (Stock C can be frozen and stored. Conc. = 10-4 mg/ml).

Procedure: Standard Curve

Stock C can be diluted further as required to make Standard Angiotensin-1 Soln. (0.05 - 1.0 mg). Seven tubes (glass) were set up for Standard Angiotensin -1 Soln. as follows:

	d1	d2	d3	d4	d5	d6	d7
Stock C	10λ	20λ	40λ	60λ	100λ	140λ	200λ
Buffer (ml)	0.99	0.98	0.96	0.94	0.90	0.86	0.80
(Conc. of Angio-1 in 50λ aliquot in ngs)	0.05	0.10	0.20	0.30	0.50	0.70	1.0

50λ aliquots were taken from the above tubes to the seven (plastic) tubes numbered in the same manner containing 0.5 ml of phosphate buffer w/Lysozyme. 5,000 - 10,000 c.p.m. of 125 I-Angiotensin-1 were added to each tube.

Three more tubes were set up as controls and numbered as d0, d0c and d7c. d0 contains no cold Angio-1, d0c contains no cold Angio-1 and no antisera, and d7c contains only 1.0 ml cold Angio-1 but no antisera. Each of them contains same amount of buffer and c.p.m. 50λ of antisera (1:100) was added to each tube at the end except d0c and d7c and shaken well. These were incubated at 4°C for 24 hours and free and bound counts were separated with Dextran coated charcoal (0.5 ml Dextran coated charcoal was added and centriguged). Supernatant was transferred carefully to other tubes numbered in the same manner and counted on solid crystal well type Y-counter). Standard curves should be done in duplicate or triplicate to get maximum accuracy for each assay.

Procedure

1. Mark two sets of plastic tubes for 0 minute and 5 minute incubation time at 4°C and 37°C (each tube should contain the same amount of phosphate buffer and c.p.m. of 1-125-Angio-1 as tubes for standard curve). 2. Mark two sets of glass tubes for samples for assay (one set for 4°C incubation and other for 37°C incubation). 3. Add to each glass tube: (a) 1.3 ml of glass distrilled water; (b) 0.1 ml of 0.2M Phosphate buffer with .3 ml EDTA (pH=6.0); (c) 0.1 ml of Renin-Substrate (0.5 mg/ml in above buffer, Renin-Substrate was extracted from hog blood in the laboratory and used in all assays.) Make one tube as control for Renin-Substrate. Add 0.5 ml of water instead of plasma. 4. Add 0.5 ml plasma for assay to each tube (everything must be kept on ice). 5. Transfer 50λ aliquots immediately to the plastic tubes containing buffer and c.p.m. of 1-125-Angio-1 marked at 0 minute (from both sets marked at 4°C and 37°C. 6. Put one set in water bath maintained at 37° and set timer at 5 minutes. 7. After 5 minutes exactly transfer another 50 aliquots from each set of tubes to plastic tubes marked for 5 minutes as quick as possible. 8. Add 50λ of antisera to each plastic tube and shake them well. Incubate them at 4°C for 24 hours. Separate free and bound counts by adding 0.5 ml Dextran coated charcoal and transferring supernatant to other tubes marked in the same manner. Count them on λ counter.

CALCULATIONS

1. For the control tubes of the standard curves, total the c.p.m. for the supernatant and the charcoal residue fraction. Calculate the correction factor as:
$Oc = F/B+F$ Where F = The C.P.M. of charcoal residue (free Angio-1)
and B = The C.P.M. of Supernatant (Angio-1 bound to antibody)

2. For each level of standard and for each clinical assay, total B and F. Calculate the corrected (Tc) total as:

$(B + F)\ Oc = Tc$

3. Calculate the corrected bound fraction, Bc: Tc-F=Bc

4. Calculate F/Bc

5. Plot F/Bc vs. ng/ml Angio -1 for standard curve.

6. For the clinical assays, determine ng for each F/Bc from the standard curve.

7. Renin activity is calculated as Angiotensin -1 released per ml per minute. Renin activity = (ng 37°C - ng 4°C) 20 = ng/ml/min.

RESULTS

All three groups showed Renin activity during contractions. The activity tended to increase and peak after peak of the contraction. The most marked increase occurred in the pre-existing hypertensive group. The normal group showed an increase in M.A.P. with the contraction and an increase in Renin activity double the pre-contraction level. In the pre-eclamptic M.A.P. with an elevated pre-contraction level and increase during contraction, the Renin activity showed a marked rise. In the pre-existing hypertensive M.A.P. elevated, the increase during a contraction was marked and the Renin activity showed a marked increase during contractions. In comparing the three, the normal patients maintained Renin activity throughout contractions and between, while the other two groups had much lower activity between contractions and marked increase with contractions. M.A.P. in all groups increased with contractions to the same degree with the hypertensive groups

reaching M. A. P. 100 and the normal group below 100 at peak contractions.

DISCUSSION

The Renin Angiotensin system has been studied in normal, hypertensive and pre-eclamptic patients in many studies. During pregnancy the plasma of normal patients shows a(1) Rise in Renin Activity and in (2) substrate of Angiogensin 1. Although the Renin increases and more vasoactive Angiotensin 11 is activated the blood pressure remains stable. The normal pregnancy produces a decreased (3) sensitivity to Angiotensin 11. Recent work (4 & 5) has shown a possible feedback control between Angiotensin 11 and Aldosterone as the Angiotensin 11 level increases the Aldosterone level increases. Aldosterone (6) levels are increased during pregnancy and reach a peak at 34-36 weeks(7). The Renin Substrate Angiotensinogen is increased by estrogen (8). Progesterone causes Na loss by acting as an Aldosterone antagonist. This increases both Aldosterone production and Renin release from the juxtaglomeruro cells. This yields increased (9) Angiotensin 11 levels and activates both Aldosterone release from the adrenal gland and Norepinephrine release from sympathetic nerves.

Patients with pre-eclampsia and/or pre-existing hypertension in pregnancy tend to have lower Renin levels during a pregnancy, although there is a great overlap between the groups (19 & 11). The highest Renin levels have been found in somen with molar pregnancy and Rh sensitized erythroblastotic fetus. The pre-eclamptic patient has been shown to be more sensitive to Antiotensin 11 infusion and require 1/2 as much Angiotensin 11 to give the same blood pressure response as normotensive pregnancies. Their response is similar to normal non-pregnant patients.

Angiotensinase activity has been shown to be increased in normal pregnancy (13) and decreased (14) in pre-eclamptic and hypertensive pregnancies reutrning to normal post partum. The (15) enzyme L-Aspartyl-B Naphthylamide Hydrolase inactivates Angiotensin 11. This enzyme has

been shown to be increased by estrogens but not by progesterone.

The source of Renin has long been felt to be solely from the juxtaglomeruluo cells in the kidney and the previous studies have assumed this source as the only one. Recent work in both tissue culture, amniotic fluid and uterine muscle analysis suggest a possible fetoplacental source (16). Tissue culture of human myometrium, chorion, amnion, placenda decidua and chorion plate showed both chorion and myometrium to produce renin in utero. The myometrium of humans does not produce Renin in vivo but the rabbit's myometrium (17) does have significant Renin activity. The Renin produced by the human myometrium did not appear until 12-14 days after culture and might reflect a normal inhibition of enzyme activator which was lost during culture. The chorion however showed production of significant Renin activity within 24 hours of culture. Other workers have confirmed a nigh Renin activity in chorion and fetal surface of the placenta (18 & 19).

The possibility of the Renin being the same as the "hysterotonin" found by Doctors Hunter and Howard (20) in placenta of patients with pre-eclampsia is possible. Renin has been isolated in the amniotic fluid and showed markedly higher concentration than Renin in the venous blood of the women (21). The Renin activity was even found at 11 weeks gestation. Other vasoactive polypeptides possibly Angiotensin or an analogue have recently been studied in amniotic fluid (22).

The presence of a source of production and actual production of high concentrations of Renin in the utero placental complex raises speculation as t. its role in maternal-fetal hemostasis. The placenta has been shown to produce many hormones with fetal contributions which maintain homeostasis although their total function is not known. Among these are Human Chorionic Gonadotropin, Human Placental Lactinogen, Thyropotropin and now Renin.

It is possible that the Renin is used to help maintain the blood flow to the placenta by increased release during times of decreased blood flow in order to prevent fetal anoxia.

The uterine blood flow is known to be decreased during labor (23). Studies of the rhesus monkey have shown reduction of uterine blood flow during contraction and the greater the contraction pressure the less the placental flow. Renal blood flow slightly increased as did other visceral organ blood flow.

In our experiments we showa 2-3 fold increase in Renin activity during contractions with return to normal prior to next contraction. The source of this Renin could be renal or uterine. Since renal blood flow does not decrease with contractions no stimulus for sudden release is operating. The uterus has a decreased blood flow and could be releasing the Renin in order to elevate blood pressure to increase its blood flow (8). Renin activates Angiotensinogen-1 rapidly which is converted to Angiotensin 11 by a converting enzyme. Blood pressure response to Angiotensin 11 occurs in 14-16 seconds with return to normal in 3 1/2 minutes. The possibility of Norerinephrine or Epinephrine contributing to the overall elevation during labor is possible since Angiotensin 11 causes release of Norephinephrine but takes 15-20 minutes to occur.

In the normal patient with decreased arteriolar responsiveness to Angiotensin 11, the blood pressure stays within "normal" levels although does increase in response to the increased Renin and the blood pressure can reach dangerous levels. Studies of Angiotensinase activity may explain the decreased reaction to Angiotensin 11 if Angiotensinase increases the Angiotensin 11 is destroyed more rapidly with lower levels. Angiotensinase increases in Angiotensin 11 could have much more vasoactivity. The vasospasm produced is marked at maternal arteriolar beds throughout the body and easily seen in the eye grounds. The vasospasm cause anoxic changes at these areas in the kidney glomerulous, liver, CNS and myoneural function (24). The renal lesion specific for pre-eclampsia is swelling of the endothelial cell cytoplasm in the glomerulous capillary wall causing narrowing of the lumina. The other findings such as thickening of basement membrane and fibrinoid deposits are not universal and are probably secondary to fibrin deposits in

response to the cell wall changes. The swelling of arteriolar walls is secondary to edema and reversible when biopsied post delivery. Lung changes showing decreased perfusion have (25) been found associated with mild intravascular coagulation in pre-eclampsia and eclampsia. The liver shows periportal damage mainly but extension to the center of the lobule occurs. Hemorrhage and fibrin deposit in the sinusoids is common. The brain shows edema and occasional hemorrhage but all specimens are at atopsy. The heart shows generalized arteriolar involvement and edema. The adrenals show petechial hemorrhage, thrombi and necrosis again post mortem.

THEORY

The primary lesion is arteriolar wall damage secondary to spasm with mild to moderate disseminated intravascular coagulation through clotting activation at the site of cell wall damage. The initiating factor is increased Renin release in a patient with decreased Angiotensinase activity.

With the onset of labor the uterus releases increased amounts of Renin with each contraction causing marked blood pressure changes. In the normal patient with "decreased arteriolar responsiveness" secondary to increased Angiotensinase activity, the blood pressure stays within "normal" levels although does increase in response to the increased Renin. The hypertensive and pre-eclamptic have marked arteriolar reaction to the sudden increased Renin and decreased Angiotensinase ability to inactivate Angiotensin 11 and the blood pressure can reach dangerous levels.

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DOCUMENTATION OF HAND FUNCTION: A Comparative Analysis of Direct Electrostatic Systems with Standard Clinical Procedures

PROBLEM

Evaluation of hand disability requires the use of objective, reliable and reproducible methods for measuring joint motion, muscle action, coordination, sensory loss, dimensional change and surface appearance. Serial goniometric measurements, voluntary muscle tests, and functional evaluations are the tools conventionally used by the therapist to describe a hand disability or deformity. The results of these tests appear as a numerical description and written summary which are frequently lengthy columns of figures and ambiguously worded narrative descriptions. They require much time to prepare as well as interpret.

It has been stated that photographic methods provide the most accurate means of recording deformity and disability. The elaborate equipment, cost of supplies, technical skill and time delay in developing the film have prevented use of this technique in general practice. Polaroid photography eliminates the time factor in film development, however, this process is expensive, the prints produced are small and they are not easily reproduced.

Several years ago Regenos and Chyatte conducted a limited study with a Xerox copier to record deformity and the effects of treatment on the rheumatoid hand. The present study was initiated to evaluate this approach with other copying equipment and in other evaluation areas. It was hypothesized that this technique would be an improved method for evaluating, recording and documenting physical disability and/or deformity; the number of manhours spent in evaluating and documenting would be reduced; and the associated costs would be lowered.

APPROACH

All patients referred to the Physical Medicine Service, Occupational Therapy Section and Physical Therapy Section

at Letterman General Hospital for evaluation of a hand disability automatically became a part of the study. The final sample size was 109 patients. Data collected included name, age, sex, hand dominance, diagnosis and date of injury or onset of disease. Their diagnoses included peripheral nerve injuries, lacerations, rheumatoid disease, amputations, Dupuytren's contractures, hemiplegia, dermatomyositis, brachial plexitis and hemangioma. Some patients had multiple diagnoses. The following routine evaluation procedures were recorded by conventional methods: range of motion, voluntary muscle tests, sensory and functional tests. After all evaluations were completed patients were referred for documentation of hand function with the use of the office copier (Dennison Copymaster). The time required to perform the various evaluations was recorded by the individual therapists.

RESULTS AND DISCUSSION

Office copying equipment can be used as a practical method to accurately document hand function, appearance, sensory involvement and range of motion. Initial evaluation of the raw data indicates the photocopy technique requires one-sixth the time standard evaluation procedures require. Looking at the photocopy provides an immediate picture of the problem and eliminates the necessity of interpreting columns of numbers or grades as well as the writing and reading of detailed narrative descriptions.

The photocopies provide immediate information to the referring physician when placed in the patient's chart and they also provide a serial record which can be used to evaluate treatment and the use of modalities, exercises, splinting, bracing and medication. They are invaluable documents for those persons who must determine disability ratings and/or insurance compensation. They can also be used for clinical education and research.

If range of motion measurements are necessary, they can be made directly on the photocopies with a ruler and protractor since the image of the hand on the photocopy is the exact size of the patient's hand. This permits the therapist to make the measurements at a convenient time or they can be made by a well-trained assistant. The range of motion, muscle test strengths, sensory evaluation and grip strengths

can be recorded directly on the photocopy thus eliminating several extra reports from the patient's chart.

Ten professional individuals from the hospital staff (not involved with the project) were provided with the photocopies and standard evaluation forms of five subjects to make a comparative analysis of the two techniques. Evaluation of the raw data extracted from the questionnaires answered by these persons indicates a general preference for the photocopies. They also felt that there would be times when the standard evaluation procedures would be necessary, i.e. - for surgical considerations and present army regulations governing disability ratings. Time required to evaluate and understand the patients problem was approximately one-half that needed for the standard forms. The therapists involved in the study stated that they would prefer to use the photocopier instead of the standard procedures because of the time element, patient interest and permanency of the records.

Perhaps the most positive result of the study is the fact that the Physical Medicine Service has continued the contract for the photocopier for documenting hand function.

FUTURE PLANS

This study is now essentially complete, however statistical analysis of the raw data is still required for inclusion in the technical report presently being prepared.

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