ANNUAL PROGRESS REPORT

FISCAL YEAR 1983
(1 October 1982 - 30 September 1983)

UNITED STATES ARMY INSTITUTE OF DENTAL RESEARCH
WALTER REED ARMY MEDICAL CENTER WASHINGTON, D.C., 20307

APPROVED FOR PUBLIC RELEASE: DISTRIBUTION UNLIMITED
UNITED STATES ARMY INSTITUTE OF DENTAL RESEARCH  
WALTER REED ARMY MEDICAL CENTER  
WASHINGTON, DC 20307-5300  

ANNUAL REPORT  
(1 October 1982-30 September 1983)  

Thomas P. Sweeney, COL, DC  

October 1983  

Supported by  
US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701-5012  

DOD DISTRIBUTION STATEMENT  

Approved for public release; distribution unlimited  

The findings in this report are not to be construed  
as an official Department of the Army position unless  
so designated by other authorized documents.
U.S. ARMY INSTITUTE OF DENTAL RESEARCH
WALTER REED ARMY MEDICAL CENTER
WASHINGTON, DC 20307-5300

ANNUAL PROGRESS REPORT
1 October 1982 - 30 September 1983

<table>
<thead>
<tr>
<th>DA Project</th>
<th>Task/Project</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A161101A91C</td>
<td>00</td>
<td>In-House Laboratory Research</td>
</tr>
<tr>
<td>3M161102B510</td>
<td>Task DA</td>
<td>Management of Dental Injury &amp; Combat Dentistry</td>
</tr>
<tr>
<td>3S162775A825</td>
<td>Task AA, AB, AC, AD</td>
<td>Combat Maxillofacial Injury</td>
</tr>
<tr>
<td>3M162734A875</td>
<td>Task AQ</td>
<td>Medical Defense Against Chemical Agents</td>
</tr>
</tbody>
</table>
1. REPORT NUMBER
MEDH-288-R1

2. REPORT PREPARATION DATE
22 Nov 83

3. TITLE AND SUBTITLE
U.S. Army Institute of Dental Research
Annual Progress Report FY 83

4. DATES COVERED
Annual 1 Oct 82 - 30 Sep 83

5. PROJECT NUMBER

6. RESEARCH ANG OR PROGRAM ELEMENT NAME AND NUMBER
U.S. Army Institute of Dental Research

7. CONTRACT OR GRANT NUMBER

8. MONITORING AGENCY NAME AND ADDRESS
U.S. Army Institute of Dental Research

9. NAMING STATEMENT OF REASON WHY RECLASSIFICATION IS NECESSARY

10. SECURITY CLASS. (of this report)
UNCLASSIFIED

11. SECURITY CLASS. (of this page)
UNCLASSIFIED

12. DISTRIBUTION STATEMENT (of this report)
Approved for Public Release: Distribution Unlimited

13. DISTRIBUTION STATEMENT (of this report)

14. SUPPLEMENTARY NOTES
None

15. KEY WORDS
(U) Ancillary Personnel; (U) Antimicrobial Agents; (U) Aphthous Stomatitis; (U) Base Metal Alloys; (U) Biocompatible; (U) Biodegradable; (U) Biodegradable Copolymer; (U) Biopolymers; (U) Bonding Agents; (U) Bone; (U) Bone Stimulating; (U) Castig Accuracy; (U) CD-1 Mice; (U) Cellulose Triacetate;

16. ABSTRACT
DA Project 3M1611028510 Management of Dental Injury and Combat Dentistry - Task DA

The objectives are to obtain information by the techniques of clinical and basic research on injuries and diseases, except communicable diseases, commonly seen in soldiers, especially in field operations and overseas. The work is divided according to the major dental specialties. Emphasis is placed on diseases and injuries that are receiving little or no study by --- OVER ---

DD FORM 1473 EDITION OF 1 NOV 85 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)
BLOCK 19. Continued:
(U) Ceramic; (U) Ceramic Block; (U) Cidex; (U) Citric Acid; (U) Cold Weather Survey; (U) Composite Restoratives; (U) Controlled Release; (U) Copolymer; (U) Copolymer Bandage; (U) Corrosion of Alloys; (U) Cranio-Mandibulofacial Complex; (U) Crevicular Fluid; (U) Cyclic AMP; (U) Dental Cutting Instrument; (U) Dental Emergencies; (U) Dental Identification; (U) Dental Materials; (U) Dental Porcelain; (U) Dental Radiology; (U) Dental X-ray; (U) Diphosphonoside-Lysozyme; (U) Dogs; (U) DOT-ELISA; (U) Foreign Bodies; (U) Gentamicin; (U) Gingival Exudates; (U) Gingival Trauma; (U) Granular Tricalcium Phosphate; (U) Guinea Pigs; (U) Health Services Research; (U) Herpetic Lesions; (U) Hydroxyproline; (U) Immunochemistry; (U) Immunopathology; (U) Implant; (U) Impression Materials; (U) Indomethacin; (U) Infection Control; (U) Inflammation; (U) Inhibition; (U) Inhibition of Bone Resorption; (U) Interceptive Methods; (U) Intermamillary Fixation; (U) Investment Techniques; (U) Isobutyl 2-Cyanoacrylate; (U) Kreb's Cycle Derivatives; (U) Laboratory Animal; (U) Laser Reflectance; (U) Lidocaine; (U) Lip Pathology; (U) Liver Surgery; (U) Logistic Models; (U) Lymphocytes; (U) Lymphokine; (U) Marginal Leakage; (U) Material; (U) Medical Materials; (U) Mice; (U) Microbiology; (U) Microencapsulation; (U) Microencapsulated Antibiotics; (U) Monoclonal Antibodies; (U) Nerve Agent; (U) New Bone Formation; (U) Opaque Porcelain; (U) Operations Research; (U) Oral Health Status; (U) Organ Culture; (U) Osseous; (U) PGE; (U) PLA/PGA Covering; (U) Polypeptide; (U) Porcelain-Metal Bond; (U) Post and Core Restorations; (U) Predictive Methods; (U) Prostaglandin; (U) Rabbits; (U) Radioisotope; (U) Radionuclide X-ray System; (U) RAM IV; (U) Rapid Detection; (U) Rats; (U) Recurrent Aphthous Stomatitis; (U) Recurrent Oral Ulceration; (U) Resin Restorative; (U) Rhineus Monkey; (U) Salivary Amylase; (U) Salivary Enzyme; (U) Salivary Physiology; (U) Segmental Mandibular Defects; (U) Serrated; (U) Shell-Like; (U) Soldering Base Metals; (U) Sporicidin; (U) Storage Stability; (U) Subtraction Radiography; (U) Sustained Release Antibiotic; (U) Tissue Adhesive; (U) Tooth; (U) Tracheal Grafts; (U) Tricalcium Phosphate; (U) Tumorigenicity Study; (U) Ultrasound; (U) Volunteers; (U) Wound Dressing; (U) Wound Exudate; (U) Wound Healing; (U) Wound Infection; (U) Xerocheiliosis; (U) X-ray Portable

BLOCK 20 Continued:

civilian research groups, and the work is aimed at providing better preventive measures as well as treatment.

DA Project 3M162775A827 Combat Maxillofacial Injury.

Task AA, AB

The objectives are to develop simplified procedures for the care of complex maxillofacial wounds and injuries which require long time-consuming procedures for reconstruction; to achieve minimal morbidity rates from oral emergencies, preventable oral disease, and restorative failures; and to develop more efficient, simplified, effective clinical and laboratory techniques which will result in better utilization of manpower and a saving in time and materiel.

DA Project 3M162734A875 Medical Defense Against Chemical Agents.

Task AQ

The objectives and purposes are the development of the basic scientific data required for systems of soldier CW agent antidotes, soldier/patient decontamination; and medical management of CW casualties.
<table>
<thead>
<tr>
<th>ID</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A161101A91C00</td>
<td>IN-HOUSE LABORATORY INDEPENDENT RESEARCH</td>
<td></td>
</tr>
<tr>
<td>DA 302937</td>
<td>Controlled Release of Antigens for One-Dose Immunization</td>
<td>1</td>
</tr>
<tr>
<td>DA 302938</td>
<td>Laser Transillumination and Ultrasound of Tissue</td>
<td>2</td>
</tr>
<tr>
<td>3H161102BS10</td>
<td>MANAGEMENT OF DENTAL INJURY AND COMBAT DENTISTRY</td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>Dentistry</td>
<td></td>
</tr>
<tr>
<td>DA OB 6037</td>
<td>Acceleration of Wound Healing</td>
<td>3</td>
</tr>
<tr>
<td>DA OD 6021</td>
<td>Problems Involved in Military Oral Health Care Delivery Related to Therapeutic Agents and Materials</td>
<td>12</td>
</tr>
<tr>
<td>DA 302908</td>
<td>Identification of Leukocyte Populations Responsible for Production of Osteoclast Activating Factor and Their Role in Bone Resorption</td>
<td>22</td>
</tr>
<tr>
<td>DA 302911</td>
<td>A Comparison of Hydroxyproline Levels of Gingival Tissue Exudates to Clinically Graded Levels of Inflammation</td>
<td>23</td>
</tr>
<tr>
<td>DA 302925</td>
<td>In Vivo Evaluation of a Wound Dressing Containing Poly-L(−)Lactide and Various Medicaments</td>
<td>24</td>
</tr>
<tr>
<td>DA 302926</td>
<td>Monoclonal Antibodies for the Isolation and Identification of Osteoclast Activating Factor and a Recently Identified Inhibitor of Osteolysis</td>
<td>25</td>
</tr>
<tr>
<td>DA 302927</td>
<td>Production and Utilization of Monoclonal Antibodies in the Rapid Identification of Anaerobic Microorganisms Associated with Maxillofacial Infections</td>
<td>26</td>
</tr>
<tr>
<td>DA 302928</td>
<td>A Study on 50:50 PLA:PGA Plus Diphosphoinositide-Lysozyme (DPI-L) for the Promotion of Calcification in Osseous Defects</td>
<td>27</td>
</tr>
</tbody>
</table>
**USAIDR PROJECTS, TASKS, AND WORK UNITS**

<table>
<thead>
<tr>
<th>Project Number</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA 302930</td>
<td>A Study of the Behavior of Bone in Organ Culture with Special Emphasis on PGE₂ and Indomethacin</td>
<td>28</td>
</tr>
<tr>
<td>DA 302933</td>
<td>A Study to Evaluate Copolymer of PLA:PGA and DPI-L for Bridging and Surgically Prepared Bone Discontinuity Defects in Dogs</td>
<td>29</td>
</tr>
<tr>
<td>DA 302935</td>
<td>In Vivo Evaluation of Ulramicroporous Cellulose Triacetate Wound Dressings as Slow-Release System for Antibiotics</td>
<td>30</td>
</tr>
<tr>
<td>DA 302936</td>
<td>Biological Activity Verification of Specified Microencapsulated Antibiotics In Vivo</td>
<td>31</td>
</tr>
<tr>
<td>DA 302967</td>
<td>A Study on the Immunogenicity of the Copolymer PLA:PGA Using a DOT-ELISA Method</td>
<td>32</td>
</tr>
<tr>
<td>DA 302968</td>
<td>A Study to Evaluate a Synthetically Produced Polypentapeptide to Induce Osteogenesis</td>
<td>33</td>
</tr>
<tr>
<td>3S162775A825</td>
<td>COMBAT MAXILLOFACIAL INJURY</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AB, AA, AC, AD Oral and Maxillofacial Sciences</td>
<td></td>
</tr>
<tr>
<td>DA OE 6022</td>
<td>Preventive Dentistry Measures of Military Significance</td>
<td>34</td>
</tr>
<tr>
<td>DA OG 6033</td>
<td>Development and Evaluation of Dental Materials and Material for Army Use</td>
<td>37</td>
</tr>
<tr>
<td>DA OG 6034</td>
<td>Development and Improvement of Metallic Restorative Materials</td>
<td>40</td>
</tr>
<tr>
<td>DA OG 6679</td>
<td>The Initial Treatment of Combat Wounds</td>
<td>41</td>
</tr>
<tr>
<td>DA OG 8670</td>
<td>Development and Evaluation of Dental Material for Field Use</td>
<td>43</td>
</tr>
<tr>
<td>DA OG 8672</td>
<td>Epidemiological Investigation of Dental Emergencies</td>
<td>46</td>
</tr>
<tr>
<td>DA OR 6030</td>
<td>Natural History of Oral Lesions Encountered in the Soldier</td>
<td>49</td>
</tr>
<tr>
<td>USAIDR PROJECTS, TASKS, AND WORK UNITS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA OH 6037</td>
<td>New and Improved Techniques for Grafts and Bone Regeneration in Traumatic Wounds</td>
<td>51</td>
</tr>
<tr>
<td>DA OK 6020</td>
<td>Biodegradable Materials for the Treatment of Fractures and Soft Tissue Wounds in the Military Situation</td>
<td>52</td>
</tr>
<tr>
<td>DA 301858</td>
<td>Tumorigenicity Study of Isobutyl 2-Cyanoacrylate</td>
<td>54</td>
</tr>
<tr>
<td>DA 302718</td>
<td>The Effectiveness of the Dental Record in the Identification of Combat Casualties</td>
<td>55</td>
</tr>
<tr>
<td>DA 302889</td>
<td>Storage Stability of Medical and Dental Materials</td>
<td>56</td>
</tr>
<tr>
<td>DA 302900</td>
<td>Statistically Based Method for Predicting Dental Emergencies</td>
<td>57</td>
</tr>
<tr>
<td>DA 302901</td>
<td>Design and Evaluation of a Combat Field X-ray Unit Using a Radiographic Source</td>
<td>58</td>
</tr>
<tr>
<td>DA 302902</td>
<td>Histologic Evaluation of Bony Defects Filled with Tricalcium Phosphate and Covered with PLA and PGA</td>
<td>59</td>
</tr>
<tr>
<td>DA 302903</td>
<td>A Method of Fixation for the Reduction of Maxillomandibular Fractures</td>
<td>60</td>
</tr>
<tr>
<td>DA 302904</td>
<td>Clinical, Radiographic, and Histologic Evaluation of Serrated Ceramic Tooth Implants</td>
<td>61</td>
</tr>
<tr>
<td>DA 302906</td>
<td>Surface Phenomenon of Opaque Porcelain Suspensions on Oxidized Metal</td>
<td>62</td>
</tr>
<tr>
<td>3M162734A875</td>
<td>MEDICAL DEFENSE AGAINST CHEMICAL AGENTS</td>
<td></td>
</tr>
<tr>
<td>AQ</td>
<td>Pathophysiology of Chemical Agent Poisoning</td>
<td></td>
</tr>
<tr>
<td>DA OG 0717</td>
<td>Study of Saliva as a Diagnostic Tool for Presence of Lethal Agents</td>
<td>63</td>
</tr>
<tr>
<td>USAIDR Publications - Articles and Abstracts</td>
<td>66-68</td>
<td></td>
</tr>
<tr>
<td>Distribution List</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>
FOREWORD

IN CONDUCTING THE RESEARCH DESCRIBED IN THIS REPORT, THE INVESTIGATORS ADHERED TO THE "GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS" AS PREPARED BY THE COMMITTEE ON CARE AND USE OF LABORATORY ANIMALS OF THE INSTITUTE OF LABORATORY ANIMAL RESOURCES, NATIONAL RESEARCH COUNCIL.
CONTROLLED RELEASE OF ANTIGENS FOR ONE-DOSE IMMUNIZATION

OBJECTIVE: (U) IT IS THE TECHNICAL OBJECTIVE OF THIS WORK TO DETERMINE THE FEASIBILITY OF APPLYING CONTROLLED RELEASE OF MULTIPLE VACCINE ANTIGENS IN A PREPROGRAMMED MANNER SO THAT A HIGH TITER ANTIBODY RESPONSE IS ELICITED IN THE HOST. SUCCESSFUL DEVELOPMENT OF THE UNIVERSAL VACCINE WOULD RESULT IN A ONE-DOSE, ONE-STEP IMMUNIZATION PROCEDURE FOR SERVICE PERSONNEL.

APPROACH: (U) A BIODEGRADABLE POLYMER POLY(DL-LACTIDE-CO-GLYCOLIDE) WILL BE USED TO COAT DISPERSED PARTICLES OF DP: VACCINE. MICROSHEPRES GREATER THAN 250 M WILL BE FORMULATED SINCE THEY ARE INJECTABLE THROUGH A CONVENTIONAL HYPODERMIC NEEDLE. THE RATE OF ANTIGEN RELEASE WILL BE CONTROLLED BY PROPER CHOICE OF POLYMER AND SYSTEM DESIGN. THE IMMUNE RESPONSE TO THE ENCAPSULATED ANTIGENS WILL BE MONITORED AND COMPARED TO CONVENTIONAL IMMUNIZED CONTROLS. A SUCCESSFUL IMMUNE RESPONSE TO THE DP (DIPHTHERIA, PERTUSSIS, TETANUS) ANTIGENS WOULD ENCOURAGE INCORPORATION OF ADDITIONAL VACCINE ANTIGENS. IT IS ANTICIPATED THAT THE QUANTITY OF ANTIGEN NEEDED TO STIMULATE THE DESIRED IMMUNE RESPONSE WILL BE CONSIDERABLY LOWER THAN THAT REQUIRED WHEN ADMINISTERED CONVENTIONALLY. IT IS POSSIBLE THAT THE POLYMER WILL HAVE AN ADJUVANT EFFECT THAT MAY ENHANCE THE IMMUNE RESPONSE.

PROGRESS: (U) none.
**Objective:**

The objective is to develop a technique and apparatus for looking through gingival tissues to quickly identify all embedded foreign bodies and produce a useful image of teeth and bone. This technique would be used to minimize exploratory trauma to the soldier. The goal is the selective replacement of X-ray usage and the need for laying of periodontal flaps when searching for foreign objects in the gingiva.

**Approach:**

(Laser transillumination and ultrasound techniques will be explored. Various frequencies of laser light will be evaluated to determine if laser light has the ability to pass through gingival tissue with minimum dispersion. Ultrasound techniques will be identified concurrently. Ultrasound offers a similar ability to look through tissue using sound waves. Ultra-high-frequency sound equipment with a resolution of 0.1 mm will also be assessed.

**Progress:**

None.
23. (U) Recent studies show that 10-12% of combat wounds involved the maxillofacial apparatus. Furthermore, 7% of noncombat injuries requiring hospital care involve the maxillofacial region. This results in the loss of approximately 1,000,000 man-hours per year.

24. (U) Studies on the effects of biochemical and physical factors to include chelate complexes, cyclic AMP, prostaglandins, and in vivo growth factors on the rate of healing in soft tissue and bone will be done. The mechanism of any beneficial alteration in healing effects will be investigated and pursued to human usage.

25. (U) Exogenous PGE was found to increase the rate of bone resorption in mouse calvarias while indomethacin inhibited spontaneous bone resorption in vitro. Fifty-fifty PLA:PGA plus DPI-L was found to promote cossous healing to a greater extent than 50:50 PLA:PGA. Diphenylhydantoin-sodium in conjuction with 50:50 PLA:PGA did not produce a statistical difference. A powerful inhibitor of osteolysis with a molecular weight of 6000 daltons has been identified. Hybrdromes are ready for assay to determine the extent to which Osteoclast Activating Factor (OAF) can be inhibited. Citric acid application significantly enhanced wound repair, indicating this treatment offers substantial benefit. Adhesion of tissue allowed nearly complete repair of exposed root surfaces to be accomplished, unlike control surfaces where repair was unsatisfactory.
A Study on the Behavior of Bone in Organ Culture with Special Emphasis on PGE$_2$ and Indomethacin

**PROBLEM:** It has been demonstrated that PGE$_2$ administered to bone implants results in an increase in osteoclasts and decrease in total bone volume. Cyclic AMP (cAMP) has been described in relation to prostaglandins (PG) and bone resorption (Yu et al., 1979). Cyclic GMP (cGMP) has been mentioned as a possible antagonist of cAMP (Elattar, 1978). It is fundamentally important to understand the principles of bone resorptive processes and agents that control or buffer those activities so that osteolysis can be controlled and bone morbidity can be decreased.

**APPROACH:** Calvaria were removed aseptically from CD-1, 5-7 day old neonatal mice and were placed into tissue culture wells containing enriched BGJ medium. Calvaria were divided equally into control and treatment groups of 4 calvaria per group. The treatment groups had either a known or putative PGE$_2$ inhibitor added to the medium (i.e., indomethacin, phenylbutazone). All calvaria were incubated at 37°C in 5% CO$_2$ and air. Specimens were retrieved at 24, 48, and 72 hours and were assayed by $^{125}$I-RIA for PGE$_2$, c-AMP, and c-GMP; and by atomic absorption spectroscopy for Ca++ concentration.

**RESULTS:** The per cent PGE$_2$ and Ca++ differences between treatment and control groups were used as bench marks for comparisons. The indomethacin (100ng/ml) displayed several levels of prostaglandin E$_2$ inhibition during the 72 hour test period. Maximum levels for indomethacin and niflumic acid were 98% and 80%, respectively. Phenylbutazone levels must be reconfirmed, as they were variable. Cyclic AMP levels of experimental calvaria were greater than control. The greatest increase (5 times control) occurred in PGE$_2$ supplemental media. The smallest increase (1 1/2 times control) took place in the niflumic acid supplemental media. Cyclic GMP levels, however, varied in their relation to control and experimental. The ratio of c-AMP/c-GMP ranged from 6/1 (24 hours) to 67/1 (72 hours) for PGE$_2$ medium. Other experimentally supplemented media exhibited temporal variations of c-AMP/c-GMP which were less drastic. Cyclic AMP increased in approximately a parabolic fashion and c-GMP decreased in approximately linear fashion in the PEG$_2$ medium. Relationships between c-AMP and c-GMP displayed an approximately non-sloping, linear relationship with all other experimental media. Results may be summarized by stating that known and probable inhibitors that were evaluated affected PGE$_2$ synthesis in vitro; however, certain inhibitors (i.e., indomethacin) caused almost complete PGE$_2$ inhibition and may, therefore, prove to be useful in controlling osteolysis in vivo. Furthermore, in vitro the presence of PGE$_2$ is associated positively.
with c-AMP and inversely with c-GMP. Similar associations between cAMP, cGMP, and PG inhibitors were not demonstrated.
A Study on 50:50 PLA:PGA Plus Diphostoinositide-Lysozyme (DPI-L) for the Promotion of Calcification in Osseous Healing

**Problem:** To determine if a biodegradable, biocompatible copolymer of PLA and PGA in combination with a diphosphoinositide inositol-lysozyme complex could induce osteogenesis in experimental wounds created in endochondral and intramembranous bones.

**Approach:** A material was formulated that consisted of a combination of a proteolipid and biodegradable, biocompatible copolymer. The proteolipid was prepared by combining a lysozyme and phosphatidyl inositol 4,5-diphosphate in a 1:1 weight ratio. A raw polymer of 50:50 poly (L(-)-lactide-co-glycolide) was solubilized in methylene chloride, reprecipitated with anhydrous methanol, and blended with the proteolipid at a 12 w/v ratio of proteolipid to copolymer. The combined material was formed into cylindrical implant plugs (1.95 mm x 2.05 mm) in a teflon mold and placed into a lyophilizer chamber for 48 hours at 40°C. The implants recovered were then sterilized with ethylene oxide for 6 hours and degassed. Implants were also prepared that consisted of only a plain copolymer of 50:50 poly (L(-)-lactide-co-glycolide), and these were managed in a fashion identical to the copolymer plus proteolipid. One hundred and eighty rats were randomly divided into three groups and wound sites were prepared in the tibias using a bone trephine (1.95 mm O.D.). One group (A) received copolymer-proteolipid implants; the second group (B) received plain copolymer; the third group (C) served as controls. At 3, 7, 14, 21, 28, and 42 days animals from each group were sacrificed and implant sites were retrieved, processed for plastic embedding, stained by Goldner-trichrome, and 3.5 µm sections were evaluated histomorphometrically using an image analysis system. In addition to the tissue histomorphometry, serum and bone alkaline and acid phosphatases were assayed; protein and hydroxyproline determinations were performed; and atomic absorption spectrophotometric evaluations were done to quantify calcium and phosphate molar ratios in host bone. Isoelectric focusing was also applied for isoenzyme identification.

**Results:** The copolymer-proteolipid implant group demonstrated overall increases in the total volumetric density of bone formation, trabecular diameter, osteoid thickness, and number of osteoblasts that exceeded the healing response in wounds of groups B and C. Similarly, the plain copolymer group results exceeded those of the control group. Overall trends between treatment groups displayed an ascending, predominantly linear difference over time; however, towards the latter stages a quadratic component of that trend was evident. Based solely upon histomorphometric data, therefore, it appears that the copolymer-proteolipid implant may be useful
for stimulating the important early phase of bone repair. Biochemical enzyme assays were an unpredictable indicator of osseous repair. In contrast, histochemical assays were considerably more representative and essentially the data on these analyses ran parallel to the histomorphometric variables. Atomic absorption spectrophotometry and isoelectric focusing proved to be of only marginal value in evaluating bone repair.
Enhanced Healing of Soft Tissue Wounds Using Diphenylhydantoin Sodium
Incorporated into a Meshwork of Biodegradable Copolymer
(50:50 poly L-(-) lactide-co-glycolide)

PROBLEM: To develop an agent that could be applied topically to a skin wound to hasten primary closure. To develop an agent that could "bulk-up" atrophic soft tissue morphology.

APPROACH: A fibrous mesh consisting of 50:50 poly L-(-) lactide-co-glycolide incorporating diphenylhydantoin-sodium (DPH) was prepared. An unembellished mesh (pure copolymer) was also prepared. Excisional wounds 3x5cm were made to the panniculus carnosus in backs of rats. Treatment consisted of either one or the other types of fibrous mesh dressing, IP - DPH, or no treatment. Wound sites were evaluated histologically, histomorphometrically, by total protein and hydroxyproline assays, by tensile testing, and by RIA (125I) for DPH.

RESULTS: The 1-28 day evaluation period analyses revealed little or no difference between treatments for the excisional wounds. Subtle differences were occasionally displayed by animals receiving IP-DPH. This group displayed a greater amount of collagen and a higher density of fibroblasts than the other treatment groups. The primary reason for the lack of a positive healing response may be explained as a consequence of the "wicking" action that was engendered by the fibrous nature of the copolymer mesh. This condition militated against an intimate tissue-dressing interface, obviating suitable DPH dissemination into the healing wound bed. A second generation "film-type" dressing has been prepared; however, continuation of this project can no longer be anticipated.
Identification of Leukocyte Populations Responsible for Production of OAF and Their Role in Bone Resorption

**PROBLEM:** Studies have shown 10-12% of all combat wounds involve the maxillofacial apparatus. Many of these result in contaminated osseous wounds. The lymphokine, Osteoclast Activating Factor (OAF), is one of the mediators of the delayed bone healing seen in contaminated osseous wounds. OAF is produced by white blood cells in response to bacterial products and stimulates local osteoclasts leading to bone resorption instead of bone growth. A better understanding of the mechanisms of action of OAF and of its chemical nature could lead to an abrogation of the detrimental effects of OAF and thus an acceleration of wound healing.

**APPROACH:**

1. **Production of OAF:** OAF is produced in vitro by stimulating human small lymphocytes with the mitogen phytohemagglutinin (PHA) in large volume cell culture. The culture supernatants are filter sterilized then passed through an ultrafiltration membrane with a 10,000 molecular weight cut-off. The retentate is made 1 molar with NaCl and filter dialyzed with phosphate buffered saline (PBS) on the same membrane. The ultrafiltrate and dialysate are combined and subjected to a second ultrafiltration on a 1,000 molecular weight cut-off membrane followed by filter dialysis with PBS. The retentate is fractioned on a Sephadex G-25 packed column with PBS as the mobile phase.

2. **Bioassays:**
   - (a) The presence of osteolytic activity in gel filtration fractions, culture supernatants, ultrafiltration retentates and filtrates is measured by the standard bone resorption bioassay. Briefly, rats in the 18th day of gestation are injected i.p. with $^{45}$Ca. The following day, the fetuses are removed aseptically and the radial and ulnar bones of each fetus are dissected free of muscle, connective tissue and cartilagenous epiphyses and placed individually in 4 wells of a 24-well culture plate containing 0.23ml BGJ, in each well. After an 18 hr preculture, the culture medium is removed and replaced with 0.125ml test solution and the other pair receives control solution. The bones are incubated for 120 hrs. at 37°C in 5% CO$_2$ and 100% humidity. The bones and culture supernatants are separated and placed in individual scintillation vials. The bones are decalcified with 5% CCl$_4$COOH and then the amount of $^{45}$Ca in both bones and culture supernatants is determined. The per cent Ca++ released from experimental and control bones is computed. A test/control (T/C) ratio is computed. T/C ratios >1 indicate increased bone resorption. However, ratios <1 are ambiguous since they could indicate either decreased bone resorption, increased calcium uptake or toxicity of the test solution to the bone culture. (b) To resolve the ambiguity seen when ratios >1 are encountered, we have developed the following modification of the standard
bioassay. The bones are not prelabeled with $^{45}$Ca prior to dissection. Instead, an equal amount of $^{45}$Ca is added to every well at the same time the test and control solutions are added. The incubation time is shortened to 6 hrs. The percent of calcium uptake is computed and a T/C per cent uptake determined. Thus, this modification is essentially a reversal of the standard bone resorption assay.

RESULTS: We have reported identifying a substance in the PHA stimulated human mononuclear cell culture supernatants which causes a highly significant inhibition of osteolysis in the bone resorption bioassay. Further studies in the past year have shown that this substance, in nanogram quantities can block the activity of the known osteolytic agents prostaglandin (PGE$_2$) and OAF. Using the new modification of the bone assay, we have found that this substance causes a significant ($p<.0001$) increase in calcium uptake. This accounts for the antosteolytic activity of the substance and suggests that it may be a stimulator of osteogenesis.
PROJECT: 3M1611028810

WORK UNIT TITLE: (U) Acceleration of Wound Healing

PRINCIPAL INVESTIGATOR: COL Stephen G. Woodyard

Citric Acid Enhancement of Oral Soft Tissue Healing

**PROBLEM:** Citric acid application to uncontaminated root surfaces apparently enhances soft tissue healing. Soft tissue pedicle flaps do not heal satisfactorily when placed against contaminated tooth root surfaces as evidenced by a failure of such tissues to reattach. This investigation was designed to determine if citric acid applications might enhance soft tissue healing (adhesions) to contaminated root surfaces as might occur following oral wounding.

**APPROACH:** Single surface root recession defects were created in six monkeys followed by six weeks of exposure to oral fluids. Surgical repair utilizing soft tissue flaps placed against citric acid treated and untreated root surfaces followed. Histologic evidence of repair was evaluated.

**RESULTS:** Citric acid application significantly enhanced wound repair, indicating this treatment offers substantial benefit. Adhesion of tissue allowed nearly complete repair of exposed root surfaces to be accomplished, unlike control surfaces where repair was unsatisfactory.
# Related to Therapeutic Agents and Materials

### 01600 Pharmacology - 00200 Biochemistry - 01000 Microbiology

<table>
<thead>
<tr>
<th>U.S. Army Institute of Dental Research</th>
<th>USA Institute of Dental Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washington, DC 20307</td>
<td>Washington, DC 20307</td>
</tr>
</tbody>
</table>

### Project Description

- **Objective**: Evaluate the special military problems of drug storage, heat susceptibility, long-term drug potency, sterility of bulk items, lack of refrigeration in combat zones, and delivery to the patient. To investigate drug hazards. To investigate the use of biodegradable polymers for the long-term, slow release of drugs.

- **Results**: Improved means of drug delivery in the field using microencapsulated medications will be studied. The hazards in the use of various drugs and the use of biodegradable, biocompatible materials for surgical repair of combat wounds will be studied.

- **Future Work**: 5209-8309 Amoxicillin anhydrate microcapsules have been formulated using a 50:50 PL-PLGA excipient. The goal was achieved when highly loaded microcapsules with desired in vivo release kinetics were obtained in very good yields. The microcapsules were effective in vivo. Problems remain in the formulation of gentamicin sulfate microcapsules. Although release kinetics are not optional, the drug has been encapsulated in 50:50 DL-PLG.
Problem: Combat wounds are characterized by a high incidence of infection primarily because of the inevitable presence of devitalized tissue and foreign body contaminants from missile fragments that carry dirt and debris into the wound. During evacuation, the wound may be exposed to further contamination and delay before initial treatment. Wound healing in the combat casualty, therefore, must overcome adversities not seen in the highway victim or civilian counterpart. Among soldiers, infections have remained a major cause of morbidity that results in lengthened hospitalization and combat ineffectiveness.

Approach: Improved methods to deliver antibiotics to contaminated tissue following traumatic injury are needed in order that sustained and effective tissue levels of antibiotics can be maintained at the wound site despite the inadequate perfusion of blood resulting from shock or the destruction of blood vessels to devitalized areas. The improved method should be easily applied in a single dose to the wound site as soon as possible after injury when infection is most likely to be suppressed. Such a novel antibiotic delivery system is being developed in which ampicillin anhydrate and gentamicin sulfate are being incorporated individually into microspheres of biocompatible, biodegradable, copolymer that are formulated to slowly release the drug over a sustained period (14 days). These microspheres, which will completely biodegrade once all drug is released, exist as a free-flowing powder that can be easily dusted onto wounds under field conditions.

Results: Experiments were performed to evaluate the efficacy of prototype microcapsules in artificially induced infections. Wounds 2.5-3.0 cm long and 1 cm deep were made in the thigh muscle of albino rats. The muscles were traumatized by uniformly pinching with tissue forceps, and inoculated with known quantities of Staphylococcus aureus and Streptococcus pyogenes. Sterile dirt was placed in each wound to serve as an infection potentiating factor. The wounds were then treated within one hour by sprinkling sterile, preweighed amounts of microencapsulated antibiotic directly in the wound. Control groups consisted of animals with wounds receiving no therapy, unloaded microcapsules, or topically applied, free ampicillin anhydrate. All
wounds were sutured closed with 3-0 black silk.

The ampicillin anhydrate microcapsules effectively reduced bacterial counts in the contaminated wounds. S. pyogenes was present in 90% of the untreated wounds at 14 days, but was eliminated from microcapsule treated wounds within 48 hours. Although S. aureus remained in all microcapsule treated wounds at 7 days, compared with untreated controls, the bacterial count decreased (>2 log 10/gram of tissue) between day 2 and 7. This reduction was not observed in untreated controls. Wounds treated with unloaded DL-PGL microcapsules, or topically applied free ampicillin anhydrate remained infected at 14 days with >10^7 organisms per gram of tissue. Whereas, 60% of the wounds treated with microencapsulated ampicillin anhydrate were sterile.

Successful controlled release of bioactive ampicillin anhydrate was achieved in vitro and in vivo. The system developed provides a successful model that encourages efforts to encapsulate additional antibiotics. Often, it may be desirable for broad spectrum control to combine two or more antibiotics in treating wounds. It is anticipated that mixtures of different antibiotic-containing microcapsules may be blended and packaged together to increase the versatility of the product.
RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

(U) Identification and Control of Oral Infections

23. (U) To investigate the source and treatment of orofacial infections encountered in field conditions, foreign countries and diverse climates. To evaluate the special agents, instruments and chemicals necessary under military conditions.

24. (U) Orofacial infections of significance in the diverse military environment will be studied by microbiological, immunological, and electron microscopy methods. Possible sources of oral infections will be evaluated and the effectiveness of commercially available as well as in-house designs will be studied for their ability to control or prevent oral infections.

25. (U) Several in vivo evaluations of wound dressings containing povidone iodine and benzalkonium chloride have been performed. To date, all experiments have suggested that while these antimicrobial agents can decrease somewhat the bacterial counts in the wound, they do not reach an acceptable level at 72 hours. An in vitro evaluation of the effectiveness of nitrofurazone resulted in similar findings. At present, steps are underway to evaluate clindamycin, ampicillin, and chlorhexidine diphosphate in the same wound dressing material. Studies have begun on the production and use of monoclonal antibodies for the rapid identification of maxillofacial infections. The similarity of cellular protein patterns has been determined by polyacrylamide gel electrophoresis. Hybridoma cells producing antibody reactive with Fusobacterium nucleatum have been cloned and antibody produced and purified from mouse ascites fluid. Steps are underway to accomplish this for the five other anaerobic microorganisms selected for study.

Available in restricted form and subject to annual review.

DD FORM 1498

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE
ALL COPY READ BEFORE ARMY USE AND DISPOSAL
PROJECT: 3M161102BS10

WORK UNIT TITLE: (U) Identification and Control of Oral Infections

PRINCIPAL INVESTIGATOR: COL Jack W. Vincent, DC

**In Vivo Evaluation of a Dressing Containing Poly-L(-)**

**Lactide and Povidone Iodine**

**PROBLEM:** In a battlefield environment, the feasibility of immediate evacuation of a patient may not exist. Wounds which are contaminated or which run the risk of contamination will require an effective means of therapy for extended periods. Antimicrobial agents presently must be administered on a continuing basis which may not be advantageous in a hostile environment. If wound infection is not prevented or controlled, the detrimental effects which hinder the healing process may become life threatening or, at the least prolong recovery time before the soldier can return to duty.

**APPROACH:** A wound dressing of a non-woven poly-L(-) lactide has been developed. This material has been shown to be able to incorporate both povidone iodine and benzalkonium chloride and once incorporated, to release this material in a controlled fashion over a 72 hour period *in vitro*. This determination involved release into a reservoir of buffer. This study was designed to evaluate these materials *in vivo* to determine if the release kinetics were the same and, if so, were these preparations effective in treating an infected wound. The guinea pigs were selected as the animal model due to the anatomical similarity of the subcutaneous tissue of the paravertebral area in relation to that of a human. A controlled size full thickness wound created in this area would receive a known quantity of a known pathogen. This area would then be covered with a wound dressing containing the antimicrobial agent to be tested. An identical wound receiving the same inoculum will be covered with the same wound dressing without the antimicrobial agent and thus serve as a control. Following application, the wound dressing will be secured in place with tissue adhesive. After 72 hours all wounds will be assayed for viable micro-organisms from the initial inoculum. Tissue biopsies can be removed, homogenised and resulting supernatant assayed for cfu/mg tissue weight. An alternate means of assay will be to isolate the wound surface with a sterile wash-basin and scrubbing the surface with a mild detergent solution. This solution can then be evaluated by serially diluting and plating on culture medium to determine cfu/ml which can be converted to cfu/surface area. Bacterial counts of $10^5$ cfu (or greater)/cm$^2$ is indicative of an infected wound.

**RESULTS:** During this period, several *in vivo* experiments have been accomplished in order to evaluate these antimicrobial agents. Initially thirty guinea pigs (twenty experimental and ten control) were wounded by full thickness dissection in the paravertebral area inoculated with
approximately $10^9$ of *Staphylococcus aureus* following which they were covered with the appropriate wound dressings: ten controls, ten containing povidone iodine and ten containing benzalkonium chloride. Following 72 hours incubation, tissue biopsies were removed, homogenized and assayed. Counts from both experimental groups were low (0-10$^7$ cfu/gm) however, counts were also low in the control group animals (0-10$^7$ cfu/gm). The possibility of absorption of the inoculum into the non-woven dressing was a distinct possibility which although possibly a good characteristic for a wound dressing did little to allow evaluation of the efficacy of the antimicrobial agent involved. This experiment was repeated and at the time of assay, the wound dressing material was also evaluated for viable S. aureus. Both the experimental and control dressing demonstrated high counts of S. aureus indicating the inability of povidone iodine to control this organism. Pure povidone iodine (22mg/cm$^2$ wound) also proved ineffective in the management of this wound model. At this time by a standard tube dilution method for its ability to inhibit the growth of S. aureus in vitro. A concentration of 6.2 g/ml inhibited visible growth of S. aureus however, when this material was plated, high counts were obtained even from samples containing 50 g/ml of nitrofurazone. These results suggest that nitrofurazone would not be effective in this model system. Efforts are presently being directed toward characterization of non-woven dressing powders and microcapsules containing clindamycin, ampicillin, and chlorhexidine diphosphonilate.
Production and Utilization of Monoclonal Antibodies in the
Rapid Identification of Anaerobic Microorganisms Associated With
Maxillofacial Infections

PROBLEM: There is currently no rapid method for the identification of microorganisms present in a wound exudate. Conventional techniques require approximately 2 to 3 days for this procedure. Such a delay in evaluating contaminated maxillofacial wounds and in selecting appropriate antibiotic therapy could result in severe sequelae. A method of rapid detection would appear to be ideal for the prevention of such a delay and may serve as a model for the detection of biological agents which might be utilized in a biological warfare environment.

APPROACH: The technique of monoclonal antibodies will be used to develop a system for the rapid identification of anaerobic microorganisms. A 2% suspension of whole cells of bacteria will be used to immunize BALB/c BYJ mice by an intraperitoneal injection (0.25ml) weekly for five weeks. At three days before the cell fusion a final injection will be given to each mouse. After anesthesia, the spleen will be removed aseptically and minced to a single cell suspension and residual red blood cells lysed by the addition of 0.17N ammonium chloride. Approximately $10^8$ splenocytes will be combined with either $10^7$ P-3 mouse myeloma cells or $10^7$ P-3 Ag 8.653 mouse myeloma cells in the presence of 35% polyethylene glycol to allow for fusion and then plated in a selective medium which will inhibit the growth of all but successfully fused hybrid cells. These hybrids will be screened by serological methods so as to identify clones of cells which carry the genetic information enabling synthesis and secretion of monoclonal antibody and, also, the ability to survive in cell culture. Appropriate hybrid clones will be isolated by limiting dilution and grown in sufficient volume so as to be preserved in cryoprotective media. Following isolation, these clones can be injected (10 viable cells) intraperitoneally into BALB/c mice which have been previously primed with pristane. The ascites fluid produced by the resulting tumors should contain 25-75mg/ml of antibody. When isolated this monoclonal antibody will be used to develop a system for rapid (2-3 hours) detection of wound contaminants. Such a system (most probably the ELISA) must display extreme sensitivity and specificity which should be provided by these techniques.

RESULTS: BALB/c BYJ mice have been immunized with the following anaerobic microorganisms: Fusobacterium nucleatum, Bacteroides gingivalis, Bacteroides fragilis, Peptococcus magnus, Peptostreptococcus micros and Peptostreptococcus anaerobius. Both the cell lines P-3 and P-3 Ag 8.653 have
been used for fusion procedures. To date several fusion attempts have been destroyed by fungal growth or by failure of the selective medium to inhibit the growth of unfused cells. To date, a hybrid cell line containing nuclear material from splenocytes sensitized to *F. nucleatum* and P-3 mouse myeloma cells has been cloned and shown to be positive for anti-*F. nucleatum* antibody by ELISA. Additional fusions have been performed using other splenocytes and P-3 Ag 8,653 mouse myeloma cells. Preliminary results suggest hybrid cells have resulted which are producing antibody reactive with *Peptococcus magnus* and *Bacteroides fragilis*. Work is presently ongoing to accomplish these same procedures with the remaining anaerobic microorganisms selected for study.
Use of Monoclonal Antibodies for the Isolation and Identification of Osteoclast Activating Factor (OAF) and a Recently Identified Inhibitor of Osteolysis

**PROBLEM:** The study of OAF presently requires a prolonged procedure in order to obtain the material from the culture supernatant of stimulated human leukocyte. Once obtained in the form of 105, 5ml fractions, each fraction must be tested in a bone biossey system in order to identify OAF activity. A maximum of 15 fractions/week can be tested. In addition, there is presently no technique which can be used to identify OAF in tissue specimens or to determine its site of activity. When available, monoclonal antibodies could be utilized in vivo to block OAF activity much in the same way as they are presently used to block graft vs host reactions in bone marrow transplants.

**APPROACH:** The technology of monoclonal antibodies can be utilized due to their specificity or ability to recognize and react with a single antigenic determinant when present in a vast mixture of substances. Mice which have been immunized with the antigen of interest provide sensitized lymphoblasts which, in the presence of polyethylene glycol, can be fused with an established mouse myeloma cell line. Resulting hybrid cell lines carry nuclear material from both cells thus are coded for synthesis and secretion of monospecific antibodies and the capability of survival in continuous culture. Although OAF is a small molecule (molecular weight 1500 daltons) for an effective immunogen, other small molecules can be detected by antibody reactions. If it is possible to develop monoclonal antibodies to OAF, such antibodies could be used to extract OAF from crude culture supernatants by affinity chromatography. Such a capability could be accomplished in days rather than the months now required to fractionate and test culture supernatants acquired from phytohemagglutinin (PHA) stimulated human leukocyte populations. These antibodies could also be used to identify the specific active site of OAF in tissue specimens. Lastly, as anti-T-cell monoclonal antibodies vs host responses with bone marrow transplants in immuno-deficient hosts, anti-OAF antibodies could be used to control or eliminate OAF activity in vivo. An additional substance whose biological activity appears to be one of inducing osteogenesis has also been identified in these same culture supernatants of stimulated human leukocytes. The activity of this substance is such that it can apparently block OAF activity and the activity of other known osteolytic agents when evaluated with a molecular weight of 6000 daltons requires the identical, lengthy procedures for isolation and identification as does OAF. Monoclonal antibodies reactive with this substance would provide the same advantages as
those previously described for OAF.

RESULTS: A crude culture supernatant from a PHA stimulated human leukocyte culture was tested to verify both OAF and the osteolytic inhibitor activity. The crude form was utilized in the hopes that these substances might be present in a complex form which might prove to be more immunogenic. An additional sample of culture supernatant was mixed with bovine serum albumin (BSA) (1mg/ml) because of the known tendency of OAF to complex with BSA and thus take advantage of a more complex and more immunogenic substance. On a weekly basis for five weeks two BALB/c BYJ mice were infected intraperitoneally with 0.25ml of this complex. Fusion of splenocytes obtained from these mice was performed three days following the final immunization. Approximately 10⁶ splenocytes and 10⁷ P-3 mouse myeloma cells were exposed to 35% polyethylene glycol and then plated in a selective medium which would inhibit the growth of all but hybrid cells. Following twenty-one days of incubation, twenty-four hybrid clones were detected and transferred to larger volume culture. When viability counting identified the presence of at least 5x10⁶ cells, the clones were transferred to a cryoprotective medium and frozen for preservation in liquid nitrogen at a concentration of 5x10⁶ cells per ml. Testing for specificity was accomplished by an inhibition assay combined with the bone bioassay. Due to technical problems, only samples of the osteolytic inhibitors were available. This 6000 molecular weight fraction would also contain some OAF as this 1500 molecular weight substance is known to exist in complexed forms in the mol. wt. range of 1500 to 18,000 daltons. The inhibition assay involved incubation of the osteolytic inhibitors with the culture supernatant from hybrid clones prior to the bone bioassay with the same osteolytic inhibitors incubated with uninoculated culture medium served as appropriate controls. Of the hybrids tested, none appeared to inhibit the activity of the osteolytic inhibitor but four hybrid clones appeared to enhance the osteogenic effect. It is speculated that this phenomenon may represent an inactivation of residual OAF in this fraction. These hybrid clones are preserved and available for testing against OAF at this time.
RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

U.S. DEPARTMENT OF THE ARMY
OFFICE OF THE DEPUTY CHIEF OF STAFF
WARRIOR CARE AND REHABILITATION

PROGRAM (LINE) NUMBER 002300
PROJECT NUMBER 002800
TASK AREA NUMBER 010200
WORK UNIT NUMBER 102

( U ) IDENTIFICATION OF LEUKOCYTE POPULATIONS RESPONSIBLE FOR PRODUCTION OF OSTEOCLAST ACTIVATING FACTOR AND THEIR ROLE IN BONE RESORPTION

MOHICAN INSTITUTE OF DENTAL RESEARCH USAIDR
WASHINGTON DC 20307

SWEENEY, T. P.
MD 20

APPROACH: ( U ) OAF IS PRODUCED BY STIMULATING HUMAN LEUKOCYTES WITH THE NITROGEN PHYTOHEMAGGLUTININ (PHA) IN LARGE VOLUME CELL CULTURE. THE CULTURE SUPERNATANTS, WHICH CONTAIN OAF IN NUDEGRAM QUANTITIES, ARE FIRST SUBJECTED TO AN ULTRAFTILATION PROTOCOL WHICH CONCENTRATES AND ISOLATES COMPOUNDS WITH MOLECULAR WEIGHTS BETWEEN 1,000 AND 10,000 DALTONS. THIS IS FOLLOWED BY GELFILTRATION ON A STANDARDIZED COLUMN. OAF IS DETECTED IN CULTURE SUPERNATANTS AND PURIFICATION FRACTIONS BY A STANDARD BONE RESORPTION BIOASSAY WHICH INVOLVES THE RELEASE OF SUPERSCRIPT 45Ca FROM FETAL RAT LONG BONES IN VITRO. THIS PROJECT WAS STARTED AS DA00B007.

PROGRESS: ( U ) NONE.
(U) A COMPARISON OF HYDROXYPROLINE LEVELS OF GINGIVAL TISSUE
EXUDATES TO CLINICALLY GRADED LEVELS OF INFLAMMATION

OBJECTIVE: (U) HYDROXYPROLINE (HYP) IS A UNIQUE COMPONENT OF CONNECTIVE TISSUE. ITS PRESENCE IN TISSUE EXUDATES INDICATES SOME DEGREE OF COLLAGEN DESTRUCTION. EXUDATES ORIGINATING IN INFECTED WOUNDS MIGHT REASONABLY BE EXPECTED TO DEMONSTRATE INCREASING LEVELS OF INFECTION AND COLLAGEN DEGRADATION, WHILE THE OPPOSITE WOULD OCCUR IN HEALING WOUNDS, USING PERIODONTAL POCKETS AS A CONVENIENT MODEL REPRESENTING CHRONIC INTRADRelial INFECTIONS. EXUDATES ARE TO BE COLLECTED, EVALUATED FOR HYP AND COMPARISONS MADE BETWEEN SUCH LEVELS AND CLINICALLY GRADED LEVELS OF INFLAMMATION. THE GOAL IS TO DEVELOP AN OBJECTIVE, NONINVASIVE MEANS FOR EVALUATING THE EFFECTS OF THERAPY AND DEGREE OF INFLAMMATION EXISTING IN ORAL WOUNDS AND INFECTIONS.

APPROACH: (U) FOLLOWING CLINICAL GRADING OF TISSUE INFLAMMATION, SAMPLES OF TISSUE EXUDATE ARE COLLECTED. SAMPLES ARE HYDROLYZED AND PROCESSED TO ALLOW MEASUREMENT OF HYP CONTENT USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY. CONVENTIONAL MEANS OF TREATMENT WHICH REDUCE OR ELIMINATE INFLAMMATION AND INFECTION FOLLOW, WITH SUBSEQUENT TISSUE EXUDATES BEING COLLECTED. HYP LEVELS SHOULD DECREASE IF THIS IS TO BE A USEFUL INDICATOR. THIS PROJECT WAS STARTED UNDER DA09P8034.

PROGRESS: (U) NONE.
**Wound Infections**

**Objective:** The ability to control or prevent wound infections in a field environment is crucial for the early return of a soldier to effective duty. The concept of controlled release of antimicrobial agents provides the technology of supplying an effective level of agent to a given site for a prolonged period of time without requiring multiple applications. The concept of topical application would allow an effective dose to be maintained locally without obtaining high levels systematically which might result in toxicity or other detrimental effects.

**Approach:** *In vitro* evaluations of an antimicrobial agent or biologicals have been done with results indicating controlled release of antimicrobial agents. This study provides a method whereby the efficacy of a dressing containing a known inoculum of *Staphylococcus aureus* and containing the wound dressing containing the antimicrobial agent. After 72 hours, the wound is assayed to determine the ability of the dressing to control or preferably, eliminate the microorganisms. This project was started under DA0786024.

**Progress:** None.
MONOCLONAL ANTIBODIES FOR THE ISOLATION AND IDENTIFICATION OF OSTEOCLAST ACTIVATING FACTOR (OAF) A RECENTLY IDENTIFIED INHIBITOR OF OSTEOLYSIS

OBJECTIVE: (U) CONTAMINATION OF AN OSSOS WOUND OF THE MAXILLOFACIAL COMPLEX WOULD RESULT IN HISTOCENIC STIMULATION OF THE HOST'S IMMUNE SYSTEM. ONE OF THE RESULTANT BIOLOGICALLY ACTIVE SUBSTANCES PRODUCED WOULD BE OAF WHOSE ACTION RESULTS IN ENHANCED OSTEOCLASTIC ACTIVITY AND INCREASED OSSOS RESORPTION. THE UNDERSTANDING OF THE MODE OF ACTIVITY AND SITE OF ACTION OF OAF WILL GREATLY ENHANCE THE ABILITY TO CONTROL BONE LOSS ASSOCIATED WITH SUCH WOUNDS.

APPROACH: (U) MONOCLONAL ANTIBODIES ARE RECOGNIZED FOR THEIR EXQUISITE SPECIFICITY IN THE ABILITY TO RECOGNIZE A SINGLE ANTIGENIC DETERMINANT. THIS TECHNOLOGY WILL BE UTILIZED TO DEVELOP A SYSTEM WHICH CAN BE USED TO EXTRACT PURE OAF FROM CULTURE SUPERNATANTS. SUCH MONOCLONAL ANTIBODIES COULD BE USED TO IDENTIFY THE PRESENCE OF OAF IN TISSUE SPECIMENS AND IDENTIFY THE ACTIVE SITE. BY REACTING OAF WITH MONOCLONAL ANTIBODIES IT CAN BE EXPECTED THAT OAF ACTIVITY COULD BE BLOCK IN VIVO. THIS PROJECT WAS STARTED UNDER DA088037.

PROGRESS: (U) NONE.
RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

A. NEW
B. มว. K
C. ลก.
D. ของ
E. ของ
F. ของ
G. ของ
H. ของ
I. ของ
J. ของ
K. ของ
L. ของ
M. ของ
N. ของ
O. ของ
P. ของ
Q. ของ
R. ของ
S. ของ
T. ของ
U. ของ
V. ของ
W. ของ
X. ของ
Y. ของ
Z. ของ

(U) PRODUCTION AND UTILIZATION OF MONOCLONAL ANTIBODIES IN THE RAPID IDENTIFICATION OF ANAEROBIC MICROORGANISMS ASSOCIATED WITH MAXILLOFACIAL INFECTIONS

OBJECTIVE: (U) MICROBIAL CONTAMINATION OF TRAUMATIC INJURIES TO THE MAXILLOFACIAL COMPLEX RESULTS IN DELAYED HEALING AND OTHER SEQUELAE WHICH HINDER RETURN OF THE PATIENT TO DUTY. A METHOD WHICH WILL RAPIDLY AND SPECIFICALLY IDENTIFY THE OFFENDING MICROORGANISMS WOULD BE MILITARILY RELEVANT IN ENHANCING WOUND HEALING IN MAXILLOFACIAL WAR WOUNDS. SUCH A SYSTEM COULD SERVE AS A USABLE MODEL IN THE RAPID DETECTION OF BIOLOGICAL WARFARE AGENTS.

APPROACH: (U) THE REMARKABLE SPECIFICITY AND AVAILABILITY OF MONOCLONAL ANTIBODIES TO SELECTED MICROORGANISMS WILL BE UTILIZED TO DEVELOP A RAPID ASSAY SYSTEM ONCE THE ANTIBODIES CAN BE PRODUCED AND ISOLATED. THE SELECTION OF AN ASSAY SYSTEM WILL BE DEPENDENT ON SIMPLICITY, SPECIFICITY, SENSITIVITY, AND FIELD ADAPTABILITY. THIS PROJECT WAS STARTED UNDER DA056024.

PROGRESS: (U) NONE.
(U) A STUDY ON BO:BO PLA:PGA PLUS DIPHOSPHOINOSITIDE-LYSOZYME

(OPT) FOR THE PROMOTION OF CALCIIFICATION IN OBESOUS DECIENCES

002000 BIOCHEMISTRY

012000 PHYSIOLOGY

OCT 83

CENT

DA

C. IN-HOUSE

028870

028870

HODC INSTITUTE OF DENTAL RESEARCH USAID

WASHINGTON DC 20307

SWEENEY, T P

MD 20

202-876-3444

FILE NO.

F.I.C.A.

MILITARY

(U) LAB ANIMAL; (U) RATS; (U) DIPHOSPHOINOSITIDE-LYSOZYME

(U) BIODEGRADABLE; (U) BIODEGRADABLE; (U) IMPLANT; (U) BONE STIMULATING; (U) RAM IV;

OBJECTIVE: (U) THE TECHNICAL OBJECTIVE WAS TO DETERMINE IF A BIODEGRADABLE, BIODEGRADABLE CO-POLYMER OF POLYACTIC ACID AND POLYGLYCOLIC ACID, IN COMBINATION WITH A DIPHOSPHOINOSITIDE INOSITOL-LYSOZYME COMPLEX, COULD INDUCE OSTEOGENESIS IN EXPERIMENTAL WOUNDS CREATED IN ENDOCHONDRAL AND INTRANOSAL BONES. THE APPLICATIONS OF SUCH A MATERIAL WILL BE FOR FIXATION DEVICES AND AS IMPLANTS FOR OBESOUS INDUCTION IN THE MAXILLOMANDIBULOFacial COMPLEX WHEN BONE IS LOST DUE TO TRAUMA, PATHOLOGY, OR ABLATION, BECAUSE APPROXIMATELY 10 PERCENT OF ALL COMBAT INJURIES ARE IN THE MAXILLOMANDIBULOFacial REGION, DEVELOPMENT OF A BONE INDUCING AGENT IS HIGHLY IMPORTANT.

APPROACH: (U) AFTER INSERTION OF THE IMPLANT MATERIALS, TISSUE COMPATIBILITY AND BONE INDUCTION TESTS WERE PERFORMED IN RABBITS AND RATS. SERUM AND HYDROXYLASE PROTEIN AND HYDROXYLPROLINE ASSAYS WERE DONE, AND ANOMIC ABSORPTION SPECTROPHOTOMETRY WAS USED TO DETERMINE CALCIUM TO PHOSPHATE MOLAR RATIOS IN HOST BONE. ISOELECTRIC FOCUSING WAS ALSO FOR ISOENZYME IDENTIFICATION. THIS PROJECT WAS STARTED UNDER DA0838037.

PROGRESS: (U) NONE.
OBJECTIVE: (U) THE TECHNICAL OBJECTIVE WAS TO DETERMINE IF A NONSTEROIDAL, ANTI-INFLAMMATORY AGENT, INDOMETHACIN, COULD INHIBIT PROSTAGLANDIN SYNTHESIS IN BONE ORGAN CULTURE. COMBAT INJURIES CAN PRODUCE MECHANICAL STRESS TO PERIOSTIUM AND ENDOSTEUM, CAUSING THE SYNTHESIS AND RELEASE OF PROSTAGLANDINS OF THE E SERIES. THE CONSEQUENCES ARE BONE LOSS AND BONE MORBIDITY. SURGICAL REPAIR AND HEALING OF OSSSEOUS WOUNDS WOULD BE MORE PREDICTABLE AND RELIABLE INCIDENCE OF REPAIR IF THE ENDOGENOUS PROSTAGLANDINS OF THE E SERIES COULD BE INHIBITED EFFECTIVELY.

APPROACH: (U) A TOTAL OF 182 NEONATAL MICE CALVARIA WERE DIVIDED INTO FOUR TREATMENT GROUPS: (1) CONTROL; NO EXOGENOUS AGENTS; (2) INDOMETHACIN: 200 NG/ML OF MEDIUM; (3) PGF2: 100 NG/ML OF MEDIUM; AND (4) COMBINATION: 200 NG/ML OF INDOMETHACIN PLUS 100 NG/ML OF PGF2. CALVARIA WERE HARVESTED AT 24, 48, AND 72 HOURS AND EVALUATED HISTOMORPHOMETRICALLY BY RIA'S FOR PGF2, COMP, CAMP, AND BY ATOMIC ABSORPTION FOR CALCIUM AND PHOSPHATE. THIS PROCEDURE WAS REPEATED ONCE EACH MONTH AND VARIABLES (3)-(4) WERE ALTERED. THIS PROJECT WAS STARTED UNDER DA088037.

PROGRESS: (U) NONE.
A STUDY TO EVALUATE COPOLYMER OF PLA:PGA & DISPHOSPHOINSITIDE-LYSOZYME FOR BRIDGING A SURGICALLY PREPARED BONE DISCONTINUITY DEFECT IN DOGS

OBJECTIVE: (U) The technical objective was to determine if a biocompatible, biodegradable copolymer of PLA and PGA, in combination with a disphosphoinositide-lysozyme complex, could induce bone formation in a mandibular discontinuity defect in the mandible of a dog. Such a material would be for mandibular fixation and an implant for digital induction when part of the mandible is missing. Development of bone stimulating substance for mandibular repair is militarily significant; approximately 10 percent of all combat injuries involve the maxillomandibular region.

APPROACH: (U) Initial preparation of the host bone will involve selective extraction of maxillary and mandibular teeth. Implant blocks will be prepared by solubilizing 80:20 poly (L- Lactide-co-Glycolide) in ethylene chlorides, reprecipitating in anhydrous methanol, and adding a complex consisting of OPM-L. Bone compatibility and induction test will be performed using prepared bone defects in the dog mandible. At selected time periods, experimental sites will be clinically evaluated and radiographed; serum and bone acid and alkaline phosphatases will be assayed; histochimistry and histomorphometry will be performed on plastic embedded undecalcified specimens. Protein and hydroxyproline assays will be accomplished; RIA's for cyclic nucleotides will be attempted; and atomic absorption spectrophotometry will be used to determine calcium to phosphate molar ratios. This project was started under DA-88037.

PROGRESS: (U) None.
RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

PROJECT TITLE: WOUND DRESSINGS AS SLOW-RELEASE SYSTEM FOR ANTIBIOTICS

UNDERWATER RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

PROJECT NUMBER: 61098

TASK AREA NUMBER: DA-34307

WORK UNIT NUMBER: 01 OCT 83

REPORT CONTROL SYMBOL: 01 OCT 83

OBJECTIVE: (U) UNTIL RECENTLY AVAILABLE ARTIFICIAL WOUND DRESSINGS WERE MADE OF COTTON GAUZE. THESE DRESSINGS PROTECT WOUNDS FROM THE EXTERNAL ENVIRONMENT, BUT LITTLE ELSE. AN IMPROVED WOUND DRESSING THAT CLINGS TO WOUNDS WITHOUT ADHERING, KEEPS THE WOUND MOIST WITHOUT ALLOWING FLUID ACCUMULATION, ALLOWS OXYGEN EXCHANGE, AND PREVENTS INFECTION IS REQUIRED. IT IS THE OBJECTIVE OF THIS WORK TO DEVELOP A MATERIAL WITH THESE CHARACTERISTICS THAT IS EASILY APPLICABLE, WILL PROTECT THE WOUND, DECREASE INFECTION, AND ENHANCE WOUND HEALING.

APPROACH: (U) AN ULTRAMICROPOROUS CELLULOSE TRIACETATE MATERIAL (POROPLASTIC) WHICH POSSESSES MANY INHERENT CHARACTERISTICS THAT ARE THEORETICALLY DESIRABLE IN A WOUND DRESSING IS BEING EVALUATED IN VIVO. EFFORTS ARE DIRECTED TOWARD DEVELOPING THE MATERIAL AS A CONTROLLED RELEASE VEHICLE FOR ANTIBIOTICS. THIS PROJECT WAS STARTED UNDER DA-34307.

PROGRESS: (U) NONE.

EVALUATION: (U) IN VIVO EVALUATION OF ULTRAMICROPOROUS CELLULOSE TRIACETATE WOUND DRESSINGS AS SLOW-RELEASE SYSTEM FOR ANTIBIOTICS.

OBSERVATING: DOOSOBI CLIN MEDICINE 010.10 MICROBIO

OBSERVATIONS: DA 34307

CONCLUSIONS: DA 34307

NAME: MOROC INSTITUTE OF DENTAL RESEARCH USAID

ADDRESS: WASHINGTON DC 20307

NAME: SWEENEY, T. P.

ADDRESS: 202-576-3484

NAME: SETTERSTROM, J. A.

ADDRESS: 202-576-3484

NAME: VINCENT, J. W.

U.S. GOVERNMENT PRINTING OFFICE: 1983

4TH PRINTING

30
**Research and Technology Work Unit Summary**

**Program Element:** Research and Technology Work Unit Summary

**Task Area:** Microbiology

**Work Unit Number:** 010100

**Project Area:** Microbiology

**Program:** Pharmacology

**Contractor:** 003300

**Investigator:** Clinic Med

**Contact:** F. C. U.

**Number:** 10

**Address:** Washington DC 20307

**Objectives:**

- **U.** Biological activity verification of specified microencapsulated antibiotics in vivo

**Approach:**

- **U.** Antibiotics are incorporated individually into microspheres of biodegradable, biocompatible polyethylene glycol copolymer.

**Progress:**

- **U.** None.

---

**Research and Technology Work Unit Summary**

**Program Element:** Research and Technology Work Unit Summary

**Task Area:** Microbiology

**Work Unit Number:** 010100

**Project Area:** Microbiology

**Program:** Pharmacology

**Contractor:** 003300

**Investigator:** Clinic Med

**Contact:** F. C. U.

**Number:** 10

**Address:** Washington DC 20307

**Objectives:**

- **U.** Lab animals

**Approach:**

- **U.** Amoxicillin amnhydroate and gentamicin sulfate are being incorporated individually into microspheres of biodegradable copolymer. The microspheres are being engineered to slowly release the antibiotic over a known period after which they will completely biodegrade within the wound site. This project was started under DACAMO021.

**Progress:**

- **U.** None.
OBJECTIVE: (U) STUDIES HAVE DEMONSTRATED THAT BOTH MONOPOLYMERS AND COPOLYMERS OF CERTAIN KREBS CYCLE DERIVATIVES CAN BE EFFECTIVELY EMPLOYED AS WOUND REPAIR MATERIALS. THESE MATERIALS HAVE BEEN SHOWN TO BE TISSUE TOLERANT, BASED UPON CELLULAR EVALUATION. A RECENT ADVANCE IN THE FIELD OF IMMUNOLOGY HAS MADE IT POSSIBLE TO DETERMINE IF THE KREBS CYCLE BIOPOLYMERS ARE AS INNOCUOUS HUMORALLY AS THEY HAVE BEEN SHOWN TO BE CELLULARLY.

APPROACH: (U) ANTISERUM REACTIVE WITH THE COPOLYMER PLA:PGA WILL BE PREPARED. THE COPOLYMER WILL ALSO BE PREPARED IN 1mg/ml AND 2mg/ml CONCENTRATIONS AND EMULSIFIED IN AN EQUAL VOLUME OF COMPLETE FREUND'S ADJUVANT. PLAIN COPOLYMER AND COPOLYMER PLUS ADJUVANT WILL SERVE AS THE IMMUNOGENS FOR IMMUNIZATION OF 2 EQUAL GROUPS OF RABBITS (5 ANIMALS/GROUP). THE DOT-ELISA METHOD WILL BE USED TO DETECT THE PRESENCE OF REACTIVE QUANTITIES (GREATER THAN NANOGRAM LEVELS) OF ANTIBODY IN THE RABBIT SERA RECOVERED AT WEEKLY INTERVALS FOR 8 WEEKS.

PROGRESS: (U) NONE.
A STUDY TO EVALUATE A SYNTHETICALLY PRODUCED POLYPEPTIDE TO INDUCE OSTEOGENESIS

OBJECTIVE: (U) NUMEROUS INVESTIGATIONS HAVE BEEN CONDUCTED USING A VARIETY OF IMPLANT MATERIALS TO SUPPORT AND PROMOTE OSSUS WOUND REPAIR OF THE CRANIO-MANDIBULAR COMPLEX. THE PURPOSE OF THE PROPOSED INVESTIGATION IS TO DETERMINE IF A SYNTHETICALLY DERIVED POLYPEPTIDE CAN INDUCE NEW BONE FORMATION IN ENDOCHONDRAL WOUNDS IN THE DIAPHYSSES OF THE TIBIAS OF RATS.

APPROACH: (U) THE AREA OVERLYING THE ANTERIOR MEDIAL ASPECTS OF THE RIGHT TIBIAS OF 70 ADULT WALTER REED RATS WILL BE PREPARED FOR INSERTION OF A POLYPEPTIDE IMPLANT. THE LEFT DIAPHYSIAL AREA WILL SERVE AS A CONTROL. AT 7, 14, 21, 28, AND 42 DAY INTERVALS TEN ANIMALS WILL BE EUTHANIZED AND BONE HOST-IMPLANT AND CONTROL SITES WILL BE RECOVERED AND TISSUES WILL BE PROCESSED FOR PLASTIC EMBEDDING, HISTOCHEMISTRY, AND HISTOMORPHOMETRY. THE HISTOTOPIC SITES FOR POLYPEPTIDE EVALUATION WILL BE THE RIGHT GLUTEAL MUSCLE MASS. AFTER INSERTION OF THE IMPLANT, SPECIMENS WILL BE RECOVERED AT 7, 21, AND 42 DAYS AND WILL BE PROCESSED FOR HISTOLOGIC EXAMINATION.

PROGRESS: (U) NONE.
**RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY**

<table>
<thead>
<tr>
<th>10. NO./CODE</th>
<th>PROGRAM ELEMENT</th>
<th>PROJECT NUMBER</th>
<th>TASK AREA NUMBER</th>
<th>WORK UNIT NUMBER</th>
<th>19. TITLE (Provide with Secret Classification Code)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. PRIMARY</td>
<td>62775A</td>
<td>36162775A825</td>
<td>AR</td>
<td>014</td>
<td>(U) Preventive Dentistry Measures of Military Significance</td>
</tr>
</tbody>
</table>

**15. SCIENTIFIC AND TECHNICAL AREAS**

<table>
<thead>
<tr>
<th>14. EPIC AREA</th>
<th>CURRENT</th>
<th>GROSS COMPLETION DATE</th>
<th>FUNDING AGENCY</th>
<th>PERFORMANCE METHOD</th>
<th>C. IN-HOUSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>012900</td>
<td>7101</td>
<td>CONT</td>
<td>DA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>002400</td>
<td>7101</td>
<td>CONT</td>
<td>DA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>003500</td>
<td>7101</td>
<td>CONT</td>
<td>DA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**16. CONTRACT/AGENCY**

| 16. TITLE: | 12100    | 7101      | 011000      | 012900 Phytoogy | 002400 Biomedical Engineering | 003500 Clinical Medicine |

**22. GENERAL USE**

**23. (U) Preventive Dentistry Measures of Military Significance**

| 23. (U) To develop new and simplified methods of preventing disease related dental emergencies in the field. To assess other methods of (1) improving the biologic management of militarily relevant oral conditions and (2) improving the cost-effectiveness factors of preventive dental therapy in the military. |

| 24. (U) Studies will be conducted which will (1) develop and evaluate improved methods of dental care which will prevent dental emergencies in the field; (2) develop more rapid and effective means of identifying and treating soldiers with at-risk-profiles for field dental emergencies. |

| 25. (U) (8210-8310) Records of emergency and routine visits to two of three dental clinics are being collected in order to determine factors in dental status that contribute to emergency visits. The data gathering phase is 75 complete. All data collection will be completed by 15 November. Final analysis of the data will begin as soon as the data base can be compiled. Study of a simplified method for restoring endodontically treated teeth using composite resins has appeared to be promising as a field expedient, but was terminated due to personnel losses. |

**26. TECHNICAL OBJECTIVE:** To develop and simplify methods of preventing disease related dental emergencies in the field. To assess new methods of (1) improving the biologic management of militarily relevant oral conditions and (2) improving the cost-effectiveness factors of preventive dental therapy in the military.

**PRINCIPAL INVESTIGATOR:** Sweeney, T. P.

**TELEPHONE:** (202) 576-3484

**SOCIAL SECURITY ACCOUNT NUMBER:** 089-76-4845
Problem: Marginal leakage, at the tooth/restoration interface, is a weakness shared by all restorations done with traditional restorative materials and operative techniques. Marginal leakage of restorations may cause or contribute to several undesirable consequences to include: recurrent caries, staining, postoperative pain, chronic hypersensitivity, and pulpal pathosis. Any one, or combination of these conditions, may lead to premature failure of the restoration and/or the production of a dental emergency on the battlefield, necessitating removal of the soldier from combat. The development of techniques to decrease or eliminate marginal leakage around restorations is thus highly desirable.

Approach: Extracted human teeth will be used in the study. Cavity preparations with traditional and experimental cavosurface margin designs will be prepared. The cavity preparations will be restored with several commercial dental resins and the teeth dissolved with nitric acid. The remaining resin restorations will be prepared for scanning electron microscopic evaluation. The scanning electron microscope will be used to evaluate the adaptation of the various resins to the traditional and experimental cavity designs. If the adaptation is good, then leakage studies will be done followed by clinical testing.

Results: It was determined from scanning electron microscopic evaluation that all of the commercial dental resins adapted well to both the traditional and experimental cavosurface margin designs. Tooth harvest, literature review, and discussions are currently taking place so that the leakage studies can be done as the next planned step.
EVALUATION OF A NEW RESTORATIVE PROCEDURE FOR POST AND CORE RESTORATIONS DESIGNED TO BE MORE ECONOMICAL AND TO PREVENT DENTAL EMERGENCIES ON THE BATTLEFIELD

PROBLEM: Post and core restorations are involved in the restoration of teeth that have been endodontically treated. The traditional techniques used to fabricate post and core restorations are very time consuming and expensive as these techniques require substantial amounts of time from the doctor, the dental assistant, the laboratory technician, and the patient. In addition, relatively large amounts of precious metals (usually gold) are required. Also, the exacting various steps to be redone. In addition, trauma to anterior teeth that have been treated with traditional cast metal post and core restorations frequently results in a fracture of the root and subsequent loss of the tooth. These teeth must then be replaced requiring additional time (by the dentist, dental assistant, dental laboratory, and patient) and expense (precious metal framework for a fixed or removable prosthesis). The development of new, simplified, more economical techniques for post and core fabrication, that require less personnel time and do not utilize precious metals, is highly desirable. Composite resins adapt well to etched tooth surfaces and have reasonable compression strength. This material may be satisfactory for use in new, simplified, more economical techniques for post and core fabrication.

APPROACH: Extracted, single-rooted, human teeth will be used in the research. Routine endodontic therapy will be performed in their canals. The canals will then be etched with acid to remove the smeared layer produced by instrumentation. Next, the canals will be filled with a low viscosity resin. The teeth will be longitudinally split in half and processed for scanning electron microscopic evaluation. The scanning electron microscope will be used to evaluate how well the resin adapted to the dentinal tubules. If the resin adaption is good, then additional studies will be done to evaluate leakage and shear strength. If these studies are promising, then clinical testing of the technique is planned.

RESULTS: It was determined from scanning electron microscopic evaluation that the low viscosity resin reliably penetrated the dentinal tubules and adapted well to them. Tooth harvest, literature review and discussions are currently taking place so that the leakage and shear strength studies can be done as the next planned step.
(U) DEVELOPMENT AND EVALUATION OF DENTAL MATERIALS AND MATERIEL FOR ARMY USE.

(RNI) COMPOSITE RESTORATIVES
(RNI) IMPRESSION MATERIALS
(RNI) CORROSION OF ALLOYS
(RNI) PROCEILAIN-METAL BOND
(RNI) RAM IV

OBJECTIVE: (U) TO EVALUATE NEW MATERIALS AND MATERIEL OF SPECIAL INTEREST TO THE ARMY DENTISTRY. CRITERIA FOR SELECTION OF MATERIALS, DEVICES OR TECHNIQUES FOR EVALUATION ARE BASED ON ANTICIPATED HIGH POTENTIALS FOR: (1) SAVINGS OF FISCAL AND/OR MANPOWER RESOURCES; (2) WORK SIMPLIFICATION; (3) IMPROVED HEALTH CARE DELIVERY IN COMBAT AREAS; AND (4) ENHANCED SAFETY WITH RESPECT TO PROFESSIONAL AND ANGULAR PERSONNEL AS WELL AS TO THE PATIENT.

APPROACH: (U) NEW MATERIALS WILL BE EVALUATED ON THE BASIS OF THE FOLLOWING PARAMETERS: COMPOSITION, MICROSTRUCTURE, PHYSICAL AND MECHANICAL PROPERTIES, CYTOTOXICITY, AND CLINICAL PERFORMANCE. STORAGE STABILITY OF MATERIALS FOR FIELD USE WILL BE EVALUATED.

PROGRESS: (U) THE INTERACTION BETWEEN OPAQUE LIQUIDS AND CERAMIC METALS HAS BEEN DEMONSTRATED. VITA LIQUID DEMONSTRATED THE LOWEST CONTACT ANGLES FOLLOWED BY NEY, CERAMIC, WEL-CREAM, AND BIZ BOND LIQUIDS. SURFACE ROUGHNESS PREPARATION WAS FOUND TO HAVE STATISTICALLY SIGNIFICANT EFFECTS ON CONTACT ANGLE MEASUREMENTS ON OPTION, BAKE-ON N/P, AND TRIUMPH METALS. SITE VISITS TO THE SIXTH U.S. ARMY LOGISTICS COMMAND AND LETTERTMAN ARMY MEDICAL CENTER LOGISTICS DIVISION PROVIDED SUFFICIENT DATA ON WHICH TO BASE RECOMMENDATIONS FOR THE DELETION OF GROUPS OF SUPPLIES FROM CONSIDERATION FOR STORAGE STABILITY EVALUATION INASMUCH AS THEY ARE NOT PROJECTED FOR FIELD USE. ADDITIONAL WORK DONE UNDER AGENCY ACCESSION NUMBER DA302899 AND DA302890.
Surface Phenomenon of Opaque Porcelain on Oxidized Metal

PROBLEM: The ease of manipulation of porcelain and metal systems is of paramount importance in the modern military dental laboratory. Knowledge of handling characteristics of porcelain opaque suspensions on oxidized metal surfaces can improve the fabrication time of restorations and benefit the military dental laboratory in training and utilizing constantly rotating personnel.

APPROACH: The wetting mediums for five dental opaque porcelains were evaluated by sessile drop contact angle measurements technique on five dental ceramic alloys. Eight replications on both smooth and sandblasted metal surfaces were evaluated in a fully randomized design.

RESULTS: Statistically significant interactions of opaque liquids on specific ceramic metals were observed in this study, indicating that broad generalizations of the wetting characteristics of opaque liquids on oxidized metals cannot be made. Even though a trend did develop with Vita liquid demonstrating the lowest contact angles, followed by Ney, Ceramco, Will-Carem, and Biobond; the statistical significance of this ranking varied with each of the five metals and two surfaces evaluated. Surface roughness preparation was found to have statistically significant effects on contact angle measurements on Option, Bake-on N/P, and Triumph metals.
PROJECT: 38162775A825
WORK UNIT TITLE: (U) Development and Evaluation of Dental Materials and Material for Army Use

PRINCIPAL INVESTIGATOR: LTG G. D. Woolsey, DC

Storage Stability of Medical and Dental Material

PROBLEM: Dental and medical material currently used by the U.S. Army Medical Department is stored in 6 major depots (2-in CONUS, 2-in Asia, and 2-in Europe). The monitoring of the serviceability of these supplies is outlined in "Appendix M" of the "Quality Control Depot Serviceability Standards". The standards outlined in "Appendix M" are those derived from manufacturers and are usually tests of no more than 60°C for one week. Dental and Medical teams must be able to depend upon supplies which will function after prolonged storage in areas of varying temperature and humidity.

APPROACH: The initial phase of this work will be the collection of background information on the Army medical supply storage system, how materials and supplies are stored, types of material stored and the storage environments within the major medical supply depots. The basis of this investigation will be information within AR 40-61, site visits to Tracy Army Depot, the Defense Procurement Support Command (where some limited materials testing is conducted on materials in the Army supply system), and the U.S. Army Medical Material Agency (source of world-wide deployment of medical and dental supplies). The second phase of this study will be the laboratory testing of those dental and medical supplies found in medical depots and determined potentially sensitive to adverse environmental storage conditions. It is imperative that the temperature and humidity within each major storage facility be determined to properly design a laboratory study of storage stability. Knowledge of perishable supplies currently stored in the major depots (as outlined in Appendix "E" of AR 40-61) will yield a study design more relevant to the modern mobile Army. An environmental chamber model LR-366-C-MP will be used to evaluate environmental effects on dental and medical material.

RESULTS: The identification of major military depots where major and minor medical assemblages are stored has been accomplished through site visits to the Sixth U.S. Army Logistics Command and the Letterman Army Medical Center Logistics Division. The evaluation of "Appendix M" of "Quality Control Depot Serviceability Standards" and AR 40-61 have formed the reference basis for the initial work on this project. Through the initial phase of this work, groups of materials have been identified which are not stored and can be deleted from consideration for this study.
(U) Development and Improvement of Metallic Restorative Materials

(U) Metallurgy and Metallography; (U) Inorganic Chemistry; (U) Biomechanics

(U) Soldering Base Metals; (U) Investment Techniques

23. (U) Annual Army expenditures for precious metals utilized in the fabrication of fixed dental prostheses are in the vicinity of $1,000,000. The cost of an equal volume of base metal alloy is $30,000. Properties of base metal alloys indicate, however, that these alloys cannot be utilized for small castings without drastic metallurgical modifications. This work is therefore being conducted to (1) develop heat treatment methods for controlling properties of nickel-chromium based casting alloys; (2) evaluate nickel-chromium based alloys for use in operative dentistry.

24. (U) The properties of nickel-chromium based alloys will be studied in detail by various physical methods available in order to devise procedures which will optimize their usefulness. Any improvement obtained will be evaluated clinically.

25. (U) Loss of the principal investigator prevented significant progress on this work unit. Continued efforts to develop reliable techniques for soldering base metal alloys have not been successful.
The Initial Treatment of Combat Wounds

To develop a multipurpose wound dressing which will provide anesthetic, antisepsis and hemostasis so that, where appropriate, the result will be rapid return of the wounded soldier to duty as well as reduction of the morbidity occasioned by delayed definitive treatment and secondary complications.

Contract developed drug release systems will be evaluated in animal models developed specifically for that purpose. Various methods and materials for maintaining contact over a variety of maxillofacial contours will be evaluated for their utility of application and use in the combat situation.

Wounds infected with Staphylococcus aureus (≥ 10^5 organisms/cm^2) when covered with POROPLASTIC® impregnated with gentamicin (6.0 – 8.0% w/w) were free of infection. The POROPLASTIC® appears to provide an excellent vehicle for sustained topical delivery of antibiotics and shows promise as an improved wound dressing. In future studies additional antibiotics will be incorporated and efforts will be directed toward improving its ability to cling to the wound surface.
In Vivo Evaluation of POROPLASTIC® Wound Dressings

Problem: Until recently, available artificial wound dressings were made of cotton gauze. These dressings are unable to prevent wound surface dehydration and due to wound adherence, they are difficult to remove without disrupting the healing process. Research has confirmed that epithelialisation proceeds most rapidly in a moist environment. Easily applied wound dressings are needed which will provide a moist environment, are permeable to oxygen, resist bacterial permeation, are bactericidal, and promote wound healing.

Approach: POROPLASTIC®, a material made of cellulose triacetate, is available for development of a new wound dressing. It is ultramicro-porous and easily impregnated with drugs which release slowly into aqueous solutions. The material is supple, resilient, conformable to wound topography, and transparent, which allows for continuous observation of wounds. Moisture vapor transmission, oxygen permeability, biocompatibility, and toxicity tests must be performed to evaluate its applicability as an optimal wound dressing. Its ability to release drugs into tissue has been investigated. Gentamicin-impregnated POROPLASTIC® has been overlaid on wound infected with gentamicin-sensitive Staphylococcus aureus. Wash solutions obtained by repeated scrubbing of the wound surface were subjected to bacterial plate counts to ascertain the quantity of viable bacteria/cm². The bacterial counts obtained for treated and untreated wounds were compared.

Results: A comparison was made of the effectiveness of cellulose triacetate wound dressings which were either unloaded or loaded with gentamicin (4.0-6.4 wt%). All wound dressings had been applied to animal wounds infected with 4.5 X 10⁷ cfu of S. aureus. After three days, all control wounds yielded an average of 5.39 X 10⁷ S. aureus, while the gentamicin-treated group yielded an average of 7.21 X 10⁷. Those animals that had received a wound dressing of >6 wt% gentamicin (n=4) had sterile wounds. When wound dressings were placed on freshly reinfected, established wounds, control animals displayed 3.76 X 10⁷ S. aureus while the gentamicin-treated group (>6.2 wt%) yielded an average of 8.6 X 10⁷ S. aureus. Results to date indicate that the POROPLASTIC® impregnated with >6 wt% gentamicin provides an effective means of treatment for both acute and established infections of S. aureus.
(U) Development and Evaluation of Dental Material for Field Use

(U) Bioengineering; OI0300 Miscellaneous Materials; 008500 Isotopes

23. (U) To assist in the development of dental equipment capable of reliable performance and easy maintenance under all field operational conditions. Included are the development of concepts for field dental equipment which is miniaturized, lightweight, energy efficient and low cost.

24. (U) Conceptual and basic engineering requirements for a field dental x-ray system and a field dental cutting instrument will be studied. Current technology will be reviewed for its ability to produce the needed design criteria and advanced technology requirements will be identified. Experimental devices will be constructed.

25. (U) A prototype dental x-ray camera has been designed and fabricated which is the size of a pack of cigarettes, weighs eight ounces, requires no electrical power, and is nearly indestructible. This unit uses radioactive Gadolinium as its x-ray source with a half-life of nearly one year. Equipment allowing standardization of radiographs has been developed and a comparison between conventionally viewed and computer subtraction processes radiographs has been completed. Diagnostic accuracy is greatly enhanced using computer subtraction techniques. Very small changes in bone apposition following arrest of disease were successfully documented in the monkey, but histologic correlation has not been completed. No results are yet available from the implant studies.
PROBLEM: The present dental X-ray unit is a cumbersome, relatively delicate instrument. It is nothing more than an office X-ray unit that fits into a carrying case. It requires two men to transport it and needs 100 volts AC to power it. This unit also requires a darkroom and processing equipment which also tend to reduce the portability of the system.

APPROACH: In concert with the Dental Radiology Department, University of California at San Francisco, an extremely portable dental X-ray unit is being developed which will use a radioactive source to generate x-rays. The use of Polaroid film with image-intensifying screens is being explored to remove the need for a darkroom and developer.

RESULTS: A contract for the joint development and testing of the above system was drawn with the Dental Radiology Department, University of California at San Francisco. A prototype dental X-ray camera has been designed and fabricated. It is the size of a pack of cigarettes, weighs eight ounces, requires no electrical power, and is nearly indestructible. This unit uses radioactive Gadolinium as its x-ray source with a half-life of nearly one year. Preliminary data shows that x-ray exposure levels to the patient will be 200 times less than those taken with conventional dental X-ray units. Radiographs of the skull taken using this system show details with clarity at least as good as conventional Panorax radiographs.
PROJECT: 3S162775A825

WORK UNIT TITLE: (U) Development and Evaluation of Dental Material for Field Use

PRINCIPAL INVESTIGATOR: MAJ Michael P. Rethman, DC

Subtraction Radiography for the Diagnosis of Bone Lesions in Dogs

PROBLEM: A computer program has been developed at NIH to allow processing of sequential standardized radiographs by digital computer. Structured noise (unchanged imagery) is eliminated by subtraction, leaving only images of that which has changed. Such radiographs would be extremely useful for monitoring osseous healing in experimental animals if correlation could be made with demonstrated histological and established wound healing parameters. Equipment allowing standardization of radiographs in a live animal model was to be developed. Following development of such equipment, the usefulness of computer subtracted images for location of osseous wounds was to be determined. If preliminary data was promising, documentation of osseous wound healing was to be evaluated.

APPROACH: Radiographic cones were manufactured which keyed directly into custom splints which held the film in a reproducible relationship. Small intrasosseous wounds were placed in the mandibles of anesthetized dogs and sequential healing radiographs were secured. One monkey which had naturally progressive periodontal disease was documented following nonsurgical treatment designed to arrest that disease. Current implant studies were documented using the same radiographic equipment.

RESULTS: The equipment allowing standardization of radiographs has been developed and a comparison between conventionally viewed and computer subtraction processed radiographs has been completed. Diagnostic accuracy is greatly enhanced using computer subtraction techniques. Very small changes in bone apposition following arrest of disease were successfully documented in the monkey, but histologic correlation has not been completed. No results are yet available from the implant studies.
(U) Epidemiological Investigation of Dental Emergencies

012900 Physiology; 012600 Pharmacology; 003500 Clinical Medicine

23. (U) To determine the causes of dental emergencies in a population of soldiers receiving regular dental care and to determine "at-risk-profiles" for those soldiers in critical occupation specialties so as to minimize problems with dental casualties during deployment.

24. (U) Studies will be conducted among soldier populations to pinpoint the causes of dental emergencies, their frequency and diagnostic strategies which will permit the prediction of the potential of each soldier for such emergencies. The goal is to select out the "at-risk" group for dental treatment and thus minimize dental casualties during deployment.

25. (U) (8210 - 8310) Analysis of data from the cold weather survey indicates that while actinic exposure is a risk factor in acute lip injury, relative humidity is even more significant. The modifying effects of complexion were also significant; in cold weather dark complexion is a risk factor, while it is a protective factor in hot weather. Based on these findings, the use of emollient preparations is recommended to protect the lips from dessication in a dry climate.
The Prevalence of Lip Injury During U. S. Army Cold Weather Exercises

PROBLEM: It is commonly assumed that extended outdoor exposure to extreme climates is a contributory cause of lip pathology, since the facial area is poorly protected from the environment. Soldiers on military operations are exposed to adverse climates to a greater degree than are civilians living in similar environments. They are often subjected to extreme physical exertion or long periods of minimal activity while exposed to an extremely cold environment. In cold weather for example, civilians spend, on the average, less than 5 to 10% of the day outdoors, while soldiers in the field spend 30% or more of the day outdoors. While acute lip problems are not medical emergencies, they are a morale problem for the troops. The prevalence and nature of cold weather lip damage has never been studied in a systematic manner. It was the purpose of this study to observe active duty soldiers engaged in cold-weather training and to document the prevalence of acute lip injury.

APPROACH: The studies were conducted at Fort Drum, New York. The first study occurred in January, 1980 during the "Empire Glacier" exercise. Participants were 763 personnel from Fort Bragg, North Carolina. The second study was conducted in January, 1982 during the "Snow Eagle" exercise. The participants were 659 personnel from Fort Campbell, Kentucky. Each survey was conducted during the third week of a four-week exercise. The subjects were interviewed and examined while they were waiting in mess hall lines. Each examination/interview for the purposes of this study took approximately 10-15 seconds. If lesions were found, a more thorough examination was performed. Data on the percentage of time devoted to outdoor duties were obtained by interview and were categorized as (a) more than 50% of time outdoors, (b) less than 50% outdoors, or (c) equal time outdoors and indoors. Age by decade and lip protectant use (Army issue; commercial, none) were elicited from the subjects. The presence of acute lip damage and type of complexion were also recorded during the examination. All examination data were agreed upon by both the examiner and the recorder.

RESULTS: Fifteen subjects (1.1%) exhibited severe acute lip damage; 743 (52.3%) exhibited moderate changes, and 664 (46.7%) had normal lips. Herpetic lesions were found in 32 (2.4%) of the 1331 soldiers included in the survey. The data on the frequency of acute lip injury during two field exercises were analyzed by age, use of lip protectant, complexion, amount of exposure, and weather. The association
between acute lip damage and age was not statistically significant in the sample. Eighty-five per cent of the study population was in the
17–29 age range. Dealing with a relatively small age range and record-
ing age by decades, however, may have reduced the sensitivity of our
study with regard to age as a risk factor. The hot-weather study
dealt with a similar population. The prevalence of chronic lip damage
was found to increase with age, but no information on age and fre-
quency of acute lip damage was presented. The small number of females
in our survey population (1.8%) did not justify stratification of the
variables by sex. The association of acute lip injury with complexion
was significant. Higher rates of acute lip damage were found in
darker-complexioned individuals. This finding was in conflict with the
hot-weather survey. In dealing with a similar complexion distribu-
tion, both acute and chronic lip damage were found to vary signifi-
cantly with complexion; with darker complexions having lower preva-
lence rates. A possible explanation for the conflicting results is
that during the cold-weather surveys, the amount of actinic exposure
was much less than that during the hot-weather survey. The amount of
duty time spent outdoors was not significantly associated with the
prevalence of lip injury. When the frequencies of acute lip damage in
the two cold-weather surveys were compared (23% in 1982 and 12% in
1980), the differences were found to be significant. The weather dur-
ing the second survey was colder, had less sun exposure, and resulted
in an increased problem of lip damage. The relative humidity did not
vary substantially during the two surveys. The modifying effects of
complexion were significant; in cold weather, dark complexion is a
risk factor, while it is a protective factor in hot weather. Age and
amount of time spent outdoors are not significant risk factors in
acute cold-weather lip injury.
(U) NATURAL HISTORY OF ORAL LESIONS ENCOUNTERED IN THE SOLDIER

OBJECTIVE: (U) TO RECOGNIZE, CHARACTERIZE AND DEVELOP EFFECTIVE THERAPEUTIC MEASURES FOR THOSE LESIONS AND CONDITIONS WHICH EFFECT THE SOLDIER DUE TO MILITARY DUTY. THE RECOGNITION OF ENVIRONMENTAL AND OTHER FACTORS WHICH PARTICIPATE IN THE ETIOLOGY OF LESIONS AND CONDITIONS UNIQUE TO THE MILITARY OR ARE CASUALLY RELATED TO MILITARY DUTY WILL ENABLE THE DEVELOPMENT OF INTERVENTIONAL OR THERAPEUTIC MEASURES.

APPROACH: (U) TO DETECT THROUGH CLINICAL AND/OR MICROSCOPIC OBSERVATION ORAL LESIONS OR A CONDITION UNIQUE TO THE MILITARY POPULATION, TO IDENTIFY ORAL LESIONS OR CONDITIONS WHICH, THOUGH NOT UNIQUE TO THE SOLDIER, ARE ETIOLOGICALLY RELATED TO THE PERFORMANCE OF DUTY. ONCE IDENTIFIED THE NATURAL HISTORY INCLUDING ETIOLOGY, THERAPY, AND PROGNOSIS WILL BE ESTABLISHED UTILIZING APPROPRIATE METHODS SUCH AS SURVEYS, ANIMAL, AND HUMAN INVESTIGATIONS.

PROGRESS: (U) 8210-8310. WITH APPROXIMATELY HALF OF THE NECESSARY DATA GATHERED, THE FOLLOWING TRENDS ARE APPARENT: (1) CYTOTOXIC ACTIVITY OF FRESH RAS SERA EXCEEDS THAT OF MATCHED CONTROL SERA; (2) CYTOTOXIC ACTIVITY OF RAS MONONUCLEAR LEUKOCYTES IS NOT SIGNIFICANTLY GREATER THAN MATCHED CONTROLS; (2) IN SOME CASES, HEAT-ACTIVATED RAS SERA SHOW SIGNIFICANTLY GREATER CYTOTOXIC ACTIVITY WHEN COMBINED WITH RAS MONONUCLEAR LEUKOCYTES (DURING EARLY STAGE OF ACTIVE DISEASE, ONLY). BOTH COMPLEMENT-MEDIATED AND CELL-MEDIATED ANTIBODY-DEPENDENT CYTOTOXICITY ARE IMPLICATED IN THIS SYSTEM.
PROBLEM: Recurrent aphthous stomatitis (RAS), a stress-related condition prevalent in military populations, significantly impairs performance of duty of duty (in its more severe forms) and reportedly retards oral soft tissue wound healing. The objective of this research is to elucidate pathophysiological mechanisms responsible for the destruction of tissue and prolongation of healing associated with RAS.

APPROACH: A radioisotope-release assay utilizing trypsinized, $^{3}H$C-labeled, allogeneic, nonkeratinizing oral epithelial cells has been developed to quantify immune cytotoxic activity in the serum and mononuclear leukocytes of RAS patients.

RESULTS: With approximately half of the necessary data gathered, the following trends are apparent:

1. Cytotoxic activity of fresh RAS sera exceeds that of matched control sera.

2. Cytotoxic activity of RAS mononuclear leukocytes is not significantly greater than matched controls.

3. In some cases, heat-inactivated RAS sera show significantly greater cytotoxic activity when combined with RAS (autologous) mononuclear leukocytes than with control leukocytes (during early stage of active disease, only).

Both complement-mediated and cell-mediated antibody-dependent cytotoxicity are implicated in this system.
Regeneration in Traumatic Wounds

012900 Physiology: 002400 Bioengineering; 002600 Biology

19. (U) New and Improved Techniques for Grafts and Bone

20. (U) Tricalcium Phosphate; (U) Ceramic Block; (U) Segmental Mandibular Defects; (U) Granular Tricalcium Phosphate; (U) Laboratory Animals

21. (U) Current methodologies for managing combat maxillofacial wounds and preventing/treating dental emergencies in the field will be extremely difficult to apply under the condition anticipated in future wars. New methods are required which will permit more rapid definitive care, reduce morbidity and decrease logistic load. Thus the objective of this work unit is to develop simple, rapid methods for soft tissue or bone grating utilizable by the dental specialist in the field.

22. (U) The fate, metabolism, osteogenic potential and tissue compatibility of ceramic and copolymer materials will be studied alone and in combination. The application of these and other materials to avulsor type wounds in both animals and humans will be pursued.

23. (U) Due to loss of principal investigator and difficulties in the production of biodegradable, unidirectional porosity Tricalcium Phosphates (TCP), no significant progress has been made. A third generation TCP is in production and will be evaluated in experimental animals as soon as it becomes available. Implants of 50:50 Polylactic-Polylactide Acid (PLA/PGA) have been surgically inserted in five dogs. Two dogs have been sacrificed. The remaining surgery will be completed by Dec 1983. The remaining animals will be sacrificed by Dec 1983. Histologic preparation of the specimens and evaluation of results should be completed by June 1984.
Biodegradable Materials for the Treatment of Fractures and Soft Tissue Wounds in the Military Situation

23. (U) To develop rapid and improved methods of treating combat injuries of the head and neck in the field using biodegradable materials. To develop premedicated biodegradable tissue fixation devices.

24. (U) Biodegradable polylactic acid, polyglycolic acid and various combinations of these polymers as well as other polymers being developed will be applied in the development of surgical procedures for a variety of hard tissue, soft tissue and hollow organ injuries in animals and extended to man where appropriate.

25. (U) Implant blocks have been inserted and evaluated in 8 dogs. By 7 months, implant-treated defects were completely healed. Implants for an additional 25 dogs were synthesized. Preparatory surgery has been performed on 3 animals. Biochemical and histochemical assays demonstrated suitable host response to the implant.
A Study to Evaluate Copolymer of PLA:PGA and Diphosphoinositide-Lysosome for Bridging a Surgically Prepared Bone Discontinuity Defect in Dogs

PROBLEM: Materials such as bone grafts and implants, collagen gels, ceramics, bone derivatives, and biopolymers are some of the many agents which have been employed for initiating osseous repair or for replacing bone. Failure rates ranging from 13% to greater than 30% have lead to a renewed interest in the development of more predictable compounds. A material was formulated, therefore, that consisted of the biopolymers PLA:PGA combined with a proteolipid (mucoprotein-N-acetylmuramolyhydro-lase:phosphatidyl inositol 4,5-diphosphate).

APPROACH: Two series of experiments were performed on a group of eight and a group of 25 adult mongrel dogs (mixed sexes, weighing 45-55 lbs). Selective, surgical extraction of teeth in ipsilateral arches was accomplished, and this preceded preparation of the host bed for receiving the implant. The implant was made by dissolving 50:50 PLA:PGA into methylene chloride and reprecipitating with anhydrous methanol. The proteolipid was added to this mixture which was placed into a Teflon mold. The mold was inserted into a vacuum oven at 50°C, 5 millitorr for 48 hours, followed by ethylene oxide sterilization. Host sites in the dogs' mandibles were prepared in the following manner: 1. Following 8 weeks of post-extraction healing, a 20mm segment was ablated, producing a complete discontinuity. This procedure was done on both sides of the mandibular arch. 2. A block of the implant of identical geometry to the defect was fabricated and was inserted into one defect. Stabilization and fixation were accomplished using ligature wire, a stainless steel plate, and stainless steel screws. 3. The contralateral defect was left untreated to serve as a control. All dogs were evaluated clinically at periodic intervals and lateral mandibular radiographs were taken.

RESULTS: The discontinuity defects in the mandibles of the group of 8 dogs that had been treated with the PLA:PGA-proteolipid implant developed an osseous union after six months. Bilateral palpation and manipulation of the mandible following removal of the internal fixation revealed complete stability. Radiographically, host-implant sites appeared within normal limits. The group of 25 dogs displayed results parallel to the group of 8 animals. Presently, histomorphometric and microdensitometric analyses of bone enzymes are underway. In both groups, complete tissue tolerance to the implant was demonstrated. Initial indications suggest that the PLA:PGA proteolipid may be useful for stimulating bony repair at intramembranous wound sites.
U) TUMORIGENICITY STUDY OF ISOBUTYL 2-CYANOACRYLATE

OBJECTIVE: (U) ISOBUTYL 2-CYANOACRYLATE (IBC) HAS BEEN DEMONSTRATED TO BE AN EFFECTIVE TISSUE ADHESIVE FOR USE IN SUTURELESS WOUND CLOSURE. THE USE OF THIS MODALITY COULD PROVIDE A TIME SAVING AND SOMETIMES LIFE-SAVING DIMENSION TO THE MANAGEMENT OF COMBAT WOUNDS. THE PURPOSE OF THE PRESENT STUDY IS TO DETERMINE IF ANY TUMORIGENIC EFFECTS ARE PRODUCED BY THE LONG-TERM IMPLANTATION OF IBC.


PROGRESS: (U) NONE.
The effectiveness of the dental record in the identification of combat casualties.

Objective: To determine the reliability of the military dental record in postmortem identification of a combat casualty.

Approach: Denial identification of combat casualties will be simulated using at least 200 active duty Army patients presenting for routine examinations at Walter Reed Dental Clinic. Simulated antemortem records (dental records and radiographs) will be duplicated and assigned a unique number. A simulated postmortem record will be prepared subsequent to the examination. It will consist of routine dental charting and duplicates of any radiographs ordered by the examining officer. The record will also be assigned a unique number. A panel of dental officers will attempt to match the simulated antemortem and postmortem records. A logistic model will be constructed to predict the probability of a correct identification as a function of the quantity and quality of the information in the simulated antemortem record.

Progress: None.
<table>
<thead>
<tr>
<th>RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY</th>
<th>PROGRAM ELEMENT</th>
<th>PROJECT NUMBER</th>
<th>TASK AREA NUMBER</th>
<th>WORK UNIT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. NEW U</td>
<td>6278A</td>
<td>231627843A28</td>
<td>AB</td>
<td>11</td>
</tr>
</tbody>
</table>

**STORAGE STABILITY OF MEDICAL AND DENTAL MATERIALS**

<table>
<thead>
<tr>
<th>OCT 83</th>
<th>CONT</th>
<th>DA</th>
<th>C. IN-HOUSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F. HUM/TOT</th>
<th>1.0</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* MONARC INSTITUTE OF DENTAL RESEARCH USAIDR
  WASHINGTON DC 20307

**MILITARY**

**STORAGE STABILITY ; SHELF-LIFE ; MEDICAL MATERIAL ; DENTAL MATERIALS ; RAM IV**

OBJECTIVE: The military logistic system is stored in six major depots worldwide. The extremes of temperature and humidity can potentially render some of these supplies ineffective. Accurate shelf-life data on potentially perishable medical and dental materials is vital to ensure the viability of supplies after periods of storage and transportation.

APPROACH: Background information on the military logistic system will be studied to identify potentially perishable supplies. Determine methods currently used for storage and identify specifics of environmental factors to which medical and dental supplies are exposed. The second phase of this work will be the physical testing of identified material and the determination of accurate shelf-life data after exposure to an environmental testing chamber. This project was initiated as DAAGB035.

PROGRESS: (U) NONE.
<table>
<thead>
<tr>
<th>RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA-08S-0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INSTITUTION</th>
<th>PROGRAM ELEMENT</th>
<th>PROJECT TITLE</th>
<th>TASK AREA NUMBER</th>
<th>WORK UNIT NUMBER</th>
<th>WORK UNIT NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORDIC INSTITUTE OF DENTAL RESEARCH</td>
<td>HUMAN PACT</td>
<td>ODESSO CLIN MEDICINE</td>
<td>DENTAL EMERGENCIES</td>
<td>PREDICTIVE METHOD</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OCT 83</th>
<th>NO.</th>
<th>C. IN-HOUSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>1984</td>
<td>0.4</td>
<td>$50</td>
</tr>
</tbody>
</table>

**MIAD INSTITUTE**

**WASHINGTON DC 20307**

**SWEENEY, T.P.**

**202-510-3131**

**GROVER, P.**

**415-991-3162**

**MILITARY**

**OBJECTIVE:**

(U) EMERGENCY VISITS TO THE DENTAL CLINIC DEPLETE THE FIGHTING STRENGTH OF A COMBAT UNIT. CAUSES AND TREATMENT OPTIONS FOR THE EMERGENCY PATIENTS OF REPRESENTATIVE TROOP UNITS WILL BE EXAMINED AND TABULATED. THE OBJECTIVE IS TO WEIGH CASUAL FACTORS AND DEVELOP PREDICTIVE AND/OR INTERCEPTIVE METHODS TO DECREASE UNPLANNED DENTAL VISITS AND THEREFORE INCREASE THE TIME THE SOLDIER CAN BE PRESENT FOR DUTY.

**APPROACH:**

(U) RECORDS OF EMERGENCY AND ROUTINE VISITS TO THE TROOP DENTAL CLINICS AT THE DIFFERENT MILITARY POSTS WILL BE ANALYZED. STATISTICAL TECHNIQUES WILL BE USED TO ESTABLISH THE FACTORS IN THE DENTAL STATUS THAT CONTRIBUTE TO THE EMERGENCY VISITS. IF POSSIBLE AN INDEX WILL BE CONSTRUCTED THAT WILL ALLOW THE PREDICTION OF FUTURE DENTAL EMERGENCY VISITS FROM PATIENT HISTORY AND PRESENT DENTAL HEALTH. THIS PROJECT WAS STARTED UNDER DAE8023.

**PROGRESS:**

(U) NONE.
**Title:** POSTER AND EVALUATION OF A COMBAT FIELD X-RAY UNIT USING A RADIOGRAPHIC SOURCE

**Project:** DOD96083

**Author:** Sweeney, T.P.

**Location:** Washington, DC 20307

**Abstract:**

The present dental X-ray unit is a cumbersome, relatively delicate instrument. It is nothing more than an office X-ray unit that fits into a carrying case. It requires two men to transport it and needs 110 volts of AC to power it. This unit also requires a darkroom and processing equipment which also tend to reduce the portability of the system.

**Approach:** In concert with the dental radiology department of the University of California at San Francisco, an extremely portable dental X-ray unit is being developed which will use a radioactive source to generate X-rays. The use of Polaroid film with image intensifying screens is being explored to remove the need for a darkroom and developer. This project was started under DAAD8670.

**Progress:** None.
OBJECTIVE: The technical objective of this study was twofold: (1) to determine clinically if a 50:50 mixture of PLA and PGA can be used to contain TCP in osseous defects, (2) to determine histologically if this PLA/PGA covering will act as a barrier to epithelial migration in surgically created lesions adjacent to teeth.

APPROACH: Seven dogs were used. The anatomy of the mandible of each dog allowed for the creation of four experimental and two control sites per quadrant. Defects were made 2mm wide and 4mm deep adjacent to a tooth. Two defects were filled with TCP and two defects were filled with TCP and covered with the mixture of PLA/PGA. Animals were sacrificed to obtain specimens at 1, 2, 4, 6, 8, 12, and 16 weeks. The specimens will be prepared for histologic evaluation. This project was started under DAHC-037.

PROGRESS: None.
A METHOD OF FIXATION FOR THE RESECTION OF MAXILLOMANDIBLE FRACTURES

APPROACH:

(1) DESIGN OF A SURE PROTOTYPE WHICH WILL BE SUITABLE TO MEET THE CRITERIA AS ESTABLISHED IN THE PROTOCOL.

DEVELOPMENT OF AN INTERIM FIXATION TECHNIQUE THAT WILL SIMPLIFY AND EXPEDITE THE TREATMENT OF CASUALTIES WHO SUSTAIN MAXILLOMANDIBLE FRACTURES. IN THIS MANNER, THE PROCEDURE WILL BECOME MORE EFFICIENT IN THE HAND OF THE CLINICAL OFFICER SO THAT HE MIGHT BE AVAILABLE TO TREAT TISSUE INJURIES OF GREATER IMPORTANCE.

(2) IN VITRO INVESTIGATION WILL BE CONDUCTED TO DETERMINE THE BEST AGENT AVAILABLE.

IN VITRO TESTS WILL BE PERFORMED TO DETERMINE THE SUITABILITY AND EFFECTIVENESS OF VARIOUS BONDING AGENTS. CYANOACRYLATES AND GLASSES OF THE RESPECTIVE CHARACTERISTICS WILL BE TESTED.

IN VITRO INVESTIGATION WILL BE CARRIED OUT ON THE HOPE DEVELOPED CYANOACRYLATES.
(U) CLINICAL, RADIOGRAPHIC, AND HISTOLOGIC EVALUATION OF SERRATED CERAMIC TOOTH IMPLANTS

(RAW TEXT)

MILITARY

(RAW TEXT)

(U) SERRATED; (U) CERAMIC; (U) TOOTH; (U) IMPLANTS; (U)

Ram IV: (U) LAB ANIMALS: (U) DOGS; (U) MONKEYS

Objectives: (U) The purpose of this research proposal is fourfold. First, it is to examine the early healing at the tissue-implant interface through histologic evaluation. Second, to determine if early healing can be enhanced with placement of tricalcium phosphate in any voids between the osseous socket and the implant. Third, to determine the usefulness of a PLA:PGA BANDAGE over the implant was also evaluated. Finally, to determine, using histologic data, the earliest time the serrated tooth root can be placed into function.

Approach: (U) Four alumina ceramic tooth implants were placed in each of four Macaca mulatta monkeys in fresh extraction sockets. The four implants in each monkey were placed in a similar manner but each implant was treated in the following manner: (a) A plain root implant, (b) A root implant with top filling all serrations and voids, (c) A root implant with PLA:PGA BANDAGE. Implants were placed so that each animal would represent a 30, 45, 60, and 90 specimen. This project was started under DAA030874.

Progress: (U) None.
(U) SURFACE PHOTOPHONON OF OPAQUE PORCELAIN SUSPENSIONS ON OXIDIZED METAL

MORAD INSTITUTE OF DENTAL RESEARCH USAID
WASHINGTON DC 20307

Sweeney, T. P.
202-373-2414

MILITARY
(U) OPAQUE PORCELAIN ; (U) DENTAL MATERIALS ; (U) DENTAL PORCELAIN

OBJECTIVE: (U) THE EASE OF MANIPULATION OF PORCELAIN AND METAL SYSTEMS IS OF PARAMOUNT
IMPORTANCE IN THE MODERN MILITARY DENTAL LABORATORY. KNOWLEDGE OF THE HANDLING
CHARACTERISTICS OF PORCELAIN OPAQUE SUSPENSIONS ON OXIDIZED METAL SURFACES CAN HASTEN THE
FABRICATION TIME OF RESTORATIONS AND BENEFIT THE MILITARY DENTAL LABORATORY IN TRAINING AND
UTILIZATION OF CONSTANTLY ROTATING PERSONNEL.

APPROACH: (U) FIVE PORCELAIN OPAQUE LIQUIDS WERE EVALUATED PHOTOGRAPHICALLY BE SENSIBLE DROP
CONTACT ANGLE MEASUREMENTS ON FIVE BRANDS OF METAL. CONTACT ANGLE MEASUREMENTS WERE MADE ON
BOTH SMOOTH AND SAND BLOWED METAL SURFACES AFTER AN EQUILIBRIUM PERIOD OF ONE MINUTE. THIS
PROJECT WAS STARTED UNDER DA009033.

PROGRESS: (U) NONE.
(U) Study of Saliva as a Diagnostic Tool for Presence of Lethal Agents

11. NEGATIVE AND TECHNICAL AREA

002300 Biochemistry
012600 Pharmacology - 012900 Physiology

23. (U) To determine if saliva can be used as a diagnostic tool in evaluating the exposure of combat troops to chemical agents. To determine if constituents in saliva can be used to monitor the progress of therapy for chemical agent exposure. Develop a rapid, simplified field technique for diagnosis of chemical agent exposure in the combat soldier.

24. (U) Changes in saliva produced by chemical agents and prophylactic antidotes will be evaluated. The particular areas of study will be enzyme, nucleotide, and protein components. Possible methodology developed will be evaluated in the field and at the hospital level.

25. (U) (8210-8310) Previous work had suggested that variations in salivary amylase/total protein ratios may serve as the basis for the diagnosis of chemical agent exposure in the field. A new micro technique for the analysis of salivary cholinesterase has been developed. Salivary cholinesterase levels were determined for baseline and experimental saliva collections following exposure to the chemical agents and one of the prophylactic pretreatments. Work to date suggests that salivary cholinesterase may be useful for the diagnosis of nerve gas poisoning because organophosphates may be differentiated from the prophylactic levels of carbamates. Future work will examine enzyme levels of other agents and other carbamates as well as various combinations of each.
Study of Saliva as a Diagnostic Tool for Presence of Lethal Agents

PROBLEM: One of the major concerns of the U. S. Army Medical Department is the survival of combat soldiers who have been exposed to chemical agents. In order to provide proper medical therapy, rapid diagnosis of nerve agents must be made. Presently, clinical signs are the basis of diagnosis of chemical agents in the field. The objective of this study was to determine if a biological parameter could be identified in saliva which could serve as the basis of a non-invasive method for the diagnosis of nerve gas poisoning in the combat soldier.

APPROACH: Biological parameters will be identified by monitoring changes in salivary composition produced by the organophosphate DFP (diisopropylfluorophosphate) and the prophylactic carbamates pyridostigmine, physostigmine and Mobam. Rhesus monkeys will be exposed to the anticholinesterase because of the biochemical similarity of its saliva to that of man. The particular parameters of study will be salivary flow rate, protein and the enzymes: cholinesterase, kallikrein, lysozyme, and amylase.

RESULTS: Work to date indicates that variations in salivary cholinesterase may serve as the basis for the diagnosis of chemical agent exposure. A radio-metric technique has been developed for the analysis of salivary cholinesterase. Salivary cholinesterase levels were determined for baseline and experimental collections following exposure to DFP and physostigmine. The results indicate that cholinesterase levels in saliva reflect blood cholinesterase levels following exposure to the organophosphate DFP. On the other hand, salivary cholinesterase levels were increased rather than inhibited like the blood cholinesterase levels following exposure to the carbamate physostigmine. Both physostigmine and DFP decreased salivary levels. They also increased the salivary levels of total protein, amylase, and lysozyme, but not significantly. The cholinergic drug physostigmine increased salivary flow rate. The organophosphate (DFP) caused a decrease in the rate of salivation which is in disagreement with previously reported signs of organophosphate poisoning.

Implications for Medical Defense Against Chemical Agents.

One of the characteristic clinical signs of nerve gas poisoning is increased salivation; however, this was not evident following exposure to 3/5 LD50 dose of DFP. In fact, a decrease in flow rate was observed. This direct disagreement with previously reported characteristic signs of
organophosphate intoxication indirectly questions other reported signs of CW agent poisoning. Blood cholinesterase is the current clinical diagnostic procedure for organophosphate exposure. Findings to date suggest that using salivary cholinesterase for diagnosis of CW agent exposure is superior to the existing procedure because it can be used to differentiate organophosphate agents from the prophylactic carbamate pretreatment. In addition, a non-invasive technique can possibly be developed into a test strip for the soldier in the field.


G.L. Henley*, J.R. Wynkoop, and J.C. Baumgartner: Cytochemical Localization of Histamine and Dopamine in Injured Dental Pulps.


J.R. Wynkoop, L. Kazyak*, R.A. Miller, and J.S. Harrington: Determination of Circulating Lidocaine Levels in Rabbits by GC/MS/DS.


J.O. Hollinger, J.J. Tamura, Jr.*, and S.A. Gee: Integration of Bone Histomorphometry and Histochemical Analyses.


DISTRIBUTION LIST

5 copies to:
US Army Medical Research and Development Command
Fort Detrick, Frederick, MD 21701

12 copies to:
Defense Technical Information Center
ATTN: DDC-DDA
Alexandria, VA 22314

1 copy to:
Superintendent
Academy of Health Sciences, US Army
ATTN: AHS-COM
Fort Sam Houston, TX 78234

1 copy to:
Director
Biological & Medical Sciences Division
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217

1 copy to:
Commander
Naval Medical R&D Command
National Naval Medical Center
Bethesda, MD 20014

1 copy to:
HQ APMSC/SCPA
Brooks AFB, TX 78235

1 copy to:
Director of Defense Research and Engineering
ATTN: Asst Director (Environmental and Life Sciences)
Washington, DC 20301