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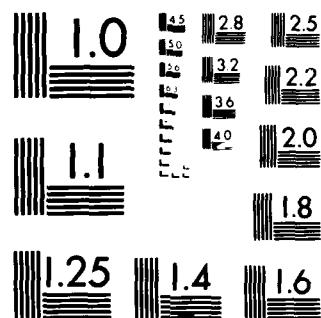
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RESEARCH ABSTRACTS OF 1981

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D. W. TURNER
Captain, DC, USN
Commanding Officer

International Association for Dental Research, 59th General Session, Chicago, Illinois, March 19-22, 1981.

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8. E. J. MUELLER* and M. R. WIRTHLIN - "Inhibition of Specific Periodontopathic Bacteria by Surface Active Agents" (Abstract #79)
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12. R. G. WALTER* and R. K. JOHNSON - "A Longitudinal Study of Caries Development in Initially Caries-Free Naval Recruits" (Abstract #898)
13. M. R. WIRTHLIN* and E. B. HANCOCK - "Regeneration After Biologic Treatment of Root Surfaces in Monkeys" (Abstract #101)

*Author presenting paper.

Fifth Annual Conference on Foods, Nutrition, and Dental Health, American Dental Association, Chicago, Illinois, September 23-25, 1981.

1. R. G. WALTER and I. L. SHKLAIR - "The Effect of a Low Molecular Weight Dextran on Dental Caries and Plaque in Rats and Hamsters"

Effects of a Marine Environment on Dental Instruments and Supplies.
F. AKER*, A. SEROWSKI and G. BAILEY. Naval Dental Research Institute,
Great Lakes, IL 60088

Dental materials and instruments stored or transported by Naval and Marine Corps units in fleet and field environments may be subject to adverse atmospheric conditions. Selected dental instruments and supplies were placed in two high-humidity chambers for 30 days; one chamber represented the marine (salt water) environment and the other a fresh water environment. Each item was categorized by manufacturer, metal(s), packaging and test environment, and was examined macroscopically and microscopically weekly for four weeks. The percent of area affected and a description of the deterioration were recorded. The metallic instruments and unprotected supplies exposed to the marine environment demonstrated marked and deleterious corrosive effects while those exposed to the fresh water environment showed rather minor corrosive effects. Chemical and biological deterioration of the non-metallic supplies and organic materials, except for plastics, were also greater in the salt water environment. Most of the instruments and supplies were considered unusable, after exposure to the marine environment. These observations indicate that unprotected metallic dental instruments and supplies quickly became unusable in a marine, high-humidity environment due primarily to corrosion. Recommendations for the prevention of deleterious effects on dental supplies while in storage and transit are being formulated.

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Salivary *S. mutans* and Lactobacillus Levels Related to Adult Caries Incidence. J. C. CECIL* and I. L. SHKLAIR. Naval Dental Research Institute, Great Lakes, IL 60088

The number of cariogenic bacteria present in dental plaque has been used to identify children with a high caries risk. The purpose of this study was to identify adults with a propensity for developing clinically detectable caries within six months using baseline enumerations of salivary *S. mutans* and lactobacilli as predictors. Unstimulated, morning saliva samples were obtained and a caries examination (using the criteria of Radike) was completed on 61 male, naval recruits, age 18 - 27 years. Selective media were used for the isolations and enumeration of *S. mutans* (MSB agar) and lactobacilli (Rogosa) contained in samples of saliva. A re-examination was conducted after a period of six months. Baseline counts (CFU/ml of saliva) of each organism were related to caries increment (tooth surfaces lost to caries) over the six-month period. Diagnostic reversals occurred in three percent of the subjects and were not excluded from the analysis. The counts for those subjects who exhibited a sound-tooth-surface decrement were: *S. mutans* 61.89×10^3 and lactobacilli 21.5×10^3 CFU/ml saliva (N=40), and for subjects who lost no surfaces to decay in six months the counts were: *S. mutans* 49.33×10^3 and lactobacilli 9.65×10^3 CFU/ml saliva (N=21). Although the results were not significant at the 0.05 level there was a tendency for those who developed caries in six months to have elevated initial counts of both organisms. The salivary counts of *S. mutans* and lactobacilli by themselves were not significant predictors in identifying adults at high risk for caries in six months.

Supported by NMRDC 62758N MF58.524.012-0030.

Characteristics of Responders and Non-Responders to a Dental Recall Notice. J. C. CECIL, M. E. COHEN*, T. P. McCARTHY. Naval Dental Research Institute, Great Lakes, IL

The purpose of this study was to examine characteristics of naval personnel, which might predict nonresponse to a dental recall notice. The values of sixteen independent variables, all accessible by inspection of dental treatment records, were recorded for each subject. These variables can be grouped into four categories: oral condition (N=7), clinical attendance (N=4), personal characteristics (N=4), and record availability (N=1). Multiple linear regression indicated that two binary variables were able to account for most of the explained variation. Response to recall was more likely if the individual had completed all needed dental care at the time of his last examination (78% of the responders, 35% of the non-responders for whom adequate records were available; $\chi^2(1)=10.60$, $p<.01$) and if the dental clinic was in possession of the individual's original dental record completed during recruit training (76% of the responders, 45% of the non-responders; $\chi^2(1)=8.55$, $p<.01$). Although the mechanism for the latter relationship is unclear, one may speculate that those less concerned about their dental health might be less likely to insure the safe transport and storage of their dental records. The multiple correlation between the two predictors and the dependent variable, response/nonresponse, was 0.51 $F(2/100)=17.44$, $p<.01$. As a result of this evaluation, a second followup notice is being sent to nonresponders to encourage preventive behavior.

Supported by NMRDC 62758N MF58.524.012-0030.

Diagnosis and Treatment of Deep Carious Lesions in Naval Recruits.
G. E. CLARK,* D. M. ANDERSON, and K. LANGE LAND. Naval Dental
Research Institute, Great Lakes, IL

A two part study in naval recruits is in progress to establish predictable and reliable methods of conservative treatment for deep carious lesions. Teeth with caries which had penetrated at least 3/4 through the dentin thickness are included in both parts of the study. Clinical criteria such as history of symptoms, periapical radiographs, and diagnostic electric, cold, percussion, and heat tests are recorded for all teeth prior to treatment. In the diagnostic part of the study, 171 teeth were extracted for reasons other than caries. Histologic examination of the pulp tissue in these teeth were related to the clinical criteria prior to extraction. Clinical criteria associated with irreversible pulpitis were: presence of sclerosis and/or enlargement of periodontal ligament space; pain duration greater than one hour and spontaneous or severe pain; and no response to a diagnostic test. In the treatment part of the study, 148 teeth were treated by conservative means and the treatment materials and methods were recorded. Pulpitis criteria were used to determine success/failure of treatment at yearly recall intervals. Success for recall years one through four was 80% or greater for the following treatment groups: all caries removed - no pulp exposure, indirect pulp cap, and direct pulp cap, all with permanent restorations. The establishment of criteria for determining irreversible pulp disease in teeth with deep carious lesions should permit success at a rate greater than the 80% success rate found in the treatment study.

Supported by NMRDC Project No. M0095PN003.3008.

Interproximal Plaque S. mutans and Caries Experience in Young Adults.
R. G. ESQUIRE*, I. L. SHKLAIR, J. C. CECIL, and R. W. GAUGLER.
Naval Dental Research Institute, Great Lakes, IL 60088

The purpose of this study was to examine the relationship of caries experience to number of S. mutans colony forming units (CFU) within the total plaque flora sampled. Interproximal plaque was collected from the posterior dentition of 100 male naval recruits, 17 to 29 (mean 19.0 ± 2.1 S.D.) years of age. Dental caries experience was reflected in DMFS scores which varied from 0 to 60 (mean 15.6 ± 14.4) surfaces. Plaque specimens, obtained using dental floss, were assayed for S. mutans and total organisms on MSB and blood agar. The mean count of S. mutans was $5.91 \pm 10.58 \times 10^4$ CFU/mg ($\pm$ S.D.) and the mean of total numbers of plaque organisms was $2.70 \pm 2.43 \times 10^6$ CFU/mg. The mean percent of S. mutans was $3.32 \pm 6.16\%$ of the total numbers of plaque organisms. The means of S. mutans CFU/mg plaque for the DMFS ranges were: DMFS 0-5 = $2.75 \pm 6.61 \times 10^4$ CFU/mg; DMFS 6-15 = $5.39 \pm 11.43 \times 10^4$ CFU/mg; DMFS 16+ = $8.84 \pm 11.79 \times 10^4$ CFU/mg. The mean percentages of S. mutans in the total numbers of plaque organisms were: DMFS 0-5 = $2.39 \pm 5.63\%$; DMFS 6-15 = $2.81 \pm 5.52\%$; DMFS 16+ = $4.37 \pm 7.00\%$. No trend was apparent in mean total organism CFU/mg when related to the same DMFS ranges. These data suggest that the mean number of S. mutans CFU/mg of posterior interproximal plaque in young adults is associated with caries experience as expressed in mean DMFS scores.

Supported by NMRDC 62758N MF58.524.012.0028.

Salivary pH-Rise Profiles of Caries-Free and Caries-Active Naval
Recruits. B. L. LAMBERTS* and E. D. PEDERSON. Naval Dental Research
Institute, Great Lakes, IL 60088

The aim of this study was to compare salivary pH-rise responses of caries-free (CF) and caries-active (CA) naval recruits to determine whether pH-rise profiles differed according to caries experience. Paraffin-stimulated whole saliva was collected in chilled containers from the recruits and was separated into supernatant and sediment fractions by centrifugation. The pH-rise measurements were made over 5-hour periods on CF/CA samples by the sediment/supernatant/glucose assay system of Kleinberg (1973). Data were accumulated for 104 subjects, using 0.28 and 2.80 mM glucose respectively in assays for two successive sets of 26 CF and 26 CA subjects. Concomitant assays were conducted for each subject in which arginine (3.33 mM) and water replaced the supernatants in the assay mixtures. The mean CF pH-rise profiles consistently appeared at higher pH, with higher minima, than the mean CA profiles for the supernatants and arginine. This CF/CA contrast was also observed for the water profiles, even though they showed no pH rise. We reported similar profile relationships earlier (AADR, 1980), when CF and CA salivary supernatants were tested with selected oral bacterial strains in place of salivary sediments. It was concluded that such pH-rise profile relationships reflect differences in microbial composition of sediments as well as in buffering and/or pH-rise-enhancing components of supernatants from CF and CA saliva samples.

Supported by NMRDC 62758N MF58.524.012-0026.

Decreased Alveolar Bone Resorption in Rice Rats Treated with Chlorhexidine and Stannous Fluoride. E. P. LEONARD,* W. V. REESE, J. C. CECIL and A. J. HORTON. Naval Dental Research Institute, Great Lakes, IL

The purpose of this study was to compare the effect of oral swabbing with chlorhexidine gluconate (CH), stannous fluoride (F) and combinations thereof, on the loss of alveolar bone in the rice rat (Oryzomys palustris). Eight; eight male weanlings were divided into eight groups of eleven animals each. Four groups were swabbed once each day, five times per week, with either A 0.2% CH; B 2% CH; C 0.2% CH + 10% F in combination; or D deionized water. The other four groups were swabbed twice, once at the start of the experiment and once at day 30. These groups were swabbed with either E 0.2% CH; F 10% F; G 0.2% CH + 10% F; or H deionized water. All animals were fed diet L-2000 ad libitum. The animals were killed at 60 $\pm$ 2 days after initiation of the experiment. The mandibular quadrants were removed, defleshed and alveolar bone loss was quantitated along the buccal and lingual surfaces by direct measurement with a micrometer eyepiece. The mean scores for each group were as follows: A 15.9 $\pm$ 4.9; B 20.7 $\pm$ 7.9; C 23.5 $\pm$ 5.5; D 31.7 $\pm$ 12.8; E 44.8 $\pm$ 12.5; F 29.8 $\pm$ 15.2; G 33.2 $\pm$ 8.6; H 44.2 $\pm$ 16.1. Following an analysis of variance, the means were compared using either Student's t-test or Scheffe's multiple comparisons of the means, as appropriate. Significant reductions in bone loss were seen in those groups receiving either 0.2% or 2% chlorhexidine at five times per week or 10% SnF₂ twice only. The more frequent applications appeared to exert a significant beneficial effect as compared to less frequent swabbing.

This study was supported under NMRDC Project No. MR041.20.02-0408.

Inhibition of Specific Periodontopathic Bacteria by Surface Active Agents. E. J. MUELLER* and M. R. WIRTHLIN. Naval Dental Research Institute, Great Lakes, IL 60088

Surfactants have demonstrated antibacterial and antiplaque activity. In this study, their antibacterial effects on specific anaerobes associated with periodontal diseases were examined. Surfactants tested were: benzalkonium chloride (BZC), cetylpyridinium chloride (CPC), sodium lauryl sulfate (SLS), Teepol 610, Tween 80 and Tween 60. Five fresh isolates each of Bacteroides melaninogenicus ss intermedius, B. ochraceus and Fusobacterium nucleatum, from periodontal patients who had received no therapy for 3 months prior to sampling, were identified with API 20A strips and gas/liquid chromatography. Two-fold dilutions of each surfactant were prepared in Lombard-Dowell broth and inoculated with 1 drop of a broth culture of the bacterial strain to be tested. The minimum inhibitory concentration was that which did not develop turbidity. The minimum bactericidal concentration was that which failed to yield viable bacteria upon subculture. CPC, BZC and SLS were inhibitory in concentrations of 50 ug/ml or less and bactericidal at less than 100 ug/ml in all cases. Teepol 610, Tween 80 and Tween 60 were not inhibitory in concentrations of 100 ug/ml.

Supported by NMRDC Work Unit 62758N MF58.524.012-0029.

The Effect of Alexidine Dihydrochloride on the Loss of Alveolar Bone
in the Rice Rat. W. V. REESE*, A. J. HORTON and E. P. LEONARD. Naval
Dental Research Institute, Great Lakes, IL 60088

Alexidine dihydrochloride is an effective plaque-reducing agent. The purpose of this study was to measure the effect of alexidine dihydrochloride on alveolar bone resorption in the rice rat. Thirty-three male rice rats (*Oryzomys palustris*) were placed on diet L-2000 at weaning. The animals were divided into three groups. One group of 12 animals was swabbed once each day, five times per week, with 0.035% alexidine in a 15% alcohol solution. One control group of 11 animals was swabbed with 15% alcohol in a similar manner. A second control group of 10 animals was not swabbed. The animals were killed by CO₂ inhalation at 60±2 experimental days. The maxillae were removed for histologic examination. The mandibles were defleshed and dried and alveolar bone loss was quantitated along the buccal and lingual surfaces by direct measurement with a micrometer eyepiece. The scores were expressed in linear units (1 unit = 0.7 mm). The mean scores for each group were as follows: alexidine 40.2±15.5; placebo 35.0±14.2; no swab 40.5±14.1. A comparison of values using either students t-test or Scheffe's multiple comparisons of the means revealed no significant differences between the groups. These results suggested that the alveolar bone loss occurring in the rice rat is not responsive to the daily topical application of alexidine dihydrochloride.

This study was supported under NMRDC 61153N MR041.20.02-0408.

The Inhibitory Effect of Various Compounds on S. mutans Glucosyltransferase Activity. I. I. SHKLAIR*, R. W. GAUGLER, and W. F. BRUTON. Naval Dental Research Institute, Great Lakes, IL 60088

Glucosyltransferases (GTF) from S. mutans synthesize water-insoluble glucans which have been implicated as a virulence factor in dental caries. The purpose of this study was to test a number of enzyme inhibitors, antibacterial and anti-carries agents for their ability to inhibit GTF activity in synthesizing water-insoluble glucans. The compounds were tested in vitro at concentrations ranging from 0.002 to 1.0 percent by the procedure of Germaine, et al. (J.D.R. 1974). The enzyme was recovered from culture supernates of S. mutans strains K-1R or NTCC #10449. The following compounds were evaluated: Tween 40, 60, and 80; Teepol 610; inorganic salts of, Ca++, Zn++, Mg++ and Mn++; thioglycollic acid; monolaurin; sodium lauryl sulfate (SLS); cetylpyridinium chloride (CPC); cetyltrimethyl ammonium bromide (CMB); benzathonium chloride (BC); methylbenzathonium chloride (MBC); and chlorhexidine (CH). There were little differences in the results whether the GTF was derived from either strain K-1R or 10449. The most effective compounds were: SLS, CPC, CMB, BC, MBC, and CH. With these compounds at concentrations of 0.1 percent or lower, at least 85 percent of the GTF's ability to synthesize insoluble glucans was inhibited. The tweens, inorganic salts, and Teepol 610 were not inhibitory at the concentrations used. Thioglycollic acid at 0.1 percent and monolaurin at 0.5 percent inhibited GTF activity 25 and 75 percent respectively.

Supported by NMRDC 61153N MR041.20.02-0441.

Prevention of Experimental Dental Caries in Hamsters by a Bacterial
 α -1, 3-Glucanase. L. SIMONSON*, B. LAMBERTS, D. REIHER, and R. WALTER.
Naval Dental Research Institute, Great Lakes, IL 60088

Many studies have shown water-insoluble streptococcal glucans rich in α -1, 3-linkages to be a prime virulence factor in dental caries. A new bacterial source of α -1, 3-glucanase was isolated from Pseudomonas sp. (IADR Abstract 292, 1980). Preliminary work has shown the enzyme to be capable of preventing ($p < 0.05$) sucrose-dependent accumulation of ^3H -thymidine-labeled Streptococcus mutans cells on hydroxyapatite surfaces. The enzyme was then tested in animals to evaluate its caries-preventive potential. Seventeen day-old male syrian hamsters were randomly assigned to three experimental groups, 12 animals/group. Group A consisted of uninfected controls while groups B and C were challenged with S. mutans 6715-13-wt. Group B, the positive controls, received no enzyme. Group C received the α -1, 3-glucanase ad libitum in their drinking water. All animals were fed diet 2000. No significant weight gain difference was noted among the groups at the end of the 47 day experimental period ($p > 0.05$). The total area affected by caries was significantly different ($p < 0.05$) among the groups: group A, 4.27 ± 2.15 (mean $\pm$ S.E.); group B, 40.85 ± 3.17 ; group C 28.58 ± 3.23 . Total caries scores were also different ($p < 0.05$) among the groups: group A, 9.09 ± 4.88 (mean $\pm$ S.E.); group B, 103.40 ± 8.49 ; group C, 69.50 ± 8.75 . No adverse effects were noted in any of the animals in the enzyme treated group. We concluded that the α -1, 3-glucanase was effective in reducing specific bacterial accumulation in vitro and in preventing dental caries in hamsters.

Supported by NMRDC 62758N MF58.524.012-0027.

A Longitudinal Study of Caries Development in Initially Caries-Free Naval Recruits. R. G. WALTER* and R. K. JOHNSON. Naval Dental Research Institute, Great Lakes, IL 60088

The purpose of this study was to describe the subsequent caries experience of a group of initially caries-free naval recruits. The initial caries status of each patient was determined independently by two examiners while subsequent caries status was established from dental records. Recall information of at least one year was obtained on 84 subjects. Recall information was available for 75, 62 and 47 subjects at 2, 3 and 4 years, respectively. Only 4 (5%) of 84 subjects developed caries within their first year of naval service. This result differed from an earlier survey (Carroll, JDR, 1967) which reported that 50% of the initially caries-free naval recruits developed caries within one year. In addition, caries was noted for the first time in 9 (12%), 6 (10%) and 7 (13%) of the available subjects at the end of 2, 3 and 4 year recalls. Four subjects developed caries in the four years but the dental record did not denote the date of incidence. Of the 30 subjects, 26 (87%) had been shown to harbor Streptococcus mutans in initial plaque samples grown in a selective colorimetric broth medium (Shklair, et al., IADR Abst. 241, 1976) and the remaining 4 (13%) did not. Overall, 65 (76%) of the 84 subjects were S. mutans carriers. A total of 79 carious lesions were detected in the 30 subjects; ninety percent of the lesions involved the occlusal surface, 8% involved the proximal surfaces and 2% were gingival lesions. The carious lesions occurred more frequently in maxillary than mandibular teeth (4:3) and second molars were the most frequently attacked.

Supported by NMRDC 61152N MR000.01.01-0026

Regeneration After Biologic Treatment of Root Surfaces in Monkeys.
M. R. WIRTHLIN* and E. B. HANCOCK. Naval Dental Research Institute,
Great Lakes, IL 60088

The purpose was to evaluate gingival healing after use of biological products which could detoxify endotoxin adsorbed on diseased roots. In 31 M. mulatta (mean age 90 months) periodontal pockets were created surgically about upper incisors and maintained by plastic bands for approx. 50 days. Samples of pockets disclosed Gram - and + microorganisms. Teeth were assigned to experimental (EXP) and control groups and there was no significant difference in pocket depth, width of attached gingiva, or recession of gingival margin. Two weeks after band removal, pedicle flaps 3 mm wide were raised on midfacial surface, and pocket tissue was removed to the depth of probing (Wirthlin, et al., 1980). No root planing was done to remove cementum. Control teeth were treated by rubbing exposed roots with saline on cotton pellets for 1 min. EXP teeth were treated with 2% sodium desoxycholate, followed by 5% human plasma fraction Cohn IV₁ (Wirthlin and Hancock, 1980). Flaps were replaced and animals put on soft diet. Evaluation was done at 0, 2, 7, 14 and 21 days on EXP, control, and unwounded (UW) specimens by rupture strength, collagen analysis (Mailman, 1979) and histologic examination. At 21 days: rupture strength was EXP 180g $\pm$ 193 (S.D.), control 37g $\pm$ 44, UW 125 $\pm$ 56 (N=4 in each group); Collagen production was EXP 10.4% $\pm$ 1.8, Control 8.9% $\pm$ 2.1, UW 8.8% $\pm$ 1.0 (N=4 in each group); all histologic control (N=3) had long junctional epithelium to the nick in root marking depth of surgery. The EXP had 2 specimens with epithelial new attachment and 4 with epithelial and connective tissue new attachments coronal to the nick.

Supported by NMRDC 63067N M0095-PN.003-3010.

The Effect of a Low Molecular Weight Dextran on Dental Caries and Plaque in Rats and Hamsters. ROBERT G. WALTER, DDS and IRVING L. SHKLAIR, PhD

The purpose of this investigation was to determine the effect of a low molecular weight dextran, T-10, on caries development and plaque accumulation in Osborne-Mendel derived rats and Golden hamsters using various feeding regimens. The animals were inoculated with Streptococcus mutans 6715 and maintained on one of two cariogenic diets: (1) diet 2000 (Teklad), containing 56% sucrose; or (2) diet 78053, containing 25% sucrose. An appropriate number of experimental groups of each species received one of the cariogenic diets supplemented with 1% dextran. The feeding frequencies were varied so that some experimental groups and the associated controls were fed the test diet on a daily basis, every other day or on alternating weeks. The daily feeding of diet 2000 plus dextran to both rats and hamsters resulted in no reduction in the development of dental caries during the 60-day experimental period. However, when diet 78053 plus dextran was utilized daily the mean caries scores and standard deviations for the experimental hamsters compared to the controls were: molars affected (0.64 ± 1.3 vs 5.3 ± 1.5), number of cavities (0.83 ± 1.3 vs 5.9 ± 1.9), areas affected (0.89 ± 0.9 vs 6.6 ± 2.9) and total caries scores (2.1 ± 4.4 vs 18.0 ± 8.9). Using Welch's "t" test, all comparisons demonstrated a statistically significant reduction ($p < .001$) in the incidence of dental caries in hamsters. In no case was there a reduction in dental caries of rats. Addition of the low molecular weight dextran to the diet in order to influence the accumulation of plaque in hamsters produced equivocal results. In the hamster model T-10 dextran was able to overcome the cariogenic potential of the 25% sucrose content of diet 78053 but not the 56% sucrose concentration of diet 2000.

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