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American Association for Dental Research, 58th Annual Session, Los Angeles, California, March 20-23, 1980.

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*Author presenting paper.

Criteria for the Assessment of Dental Pulp Health Beneath Deep Caries.
D. M. ANDERSON*, K. LANGE LAND, G. E. CLARK and S. L. ORR. Naval Dental
Research Institute, Great Lakes, Illinois

The purpose was to determine the validity of various clinical criteria in history, examination and tests for diagnosing the healing potential of the dental pulp which has been affected by deep caries. Comprehensive clinical data was compared with the histologic evaluations for 176 permanent teeth of young male naval recruits. Disease was considered to be irreversible with histologic evidence of necrosis or obliteration of normal morphology by masses of chronic inflammatory cells. Significant indicators of irreversible pulp disease were; enlargement of the apical periodontal ligament space on radiographs, a history of long-duration severe pain, a history of pain due to pressure, and lack of response to two or more hot/cold/electrical vitality tests. In no instance did caries cause a severe pulpitis or necrosis until the decay had proceeded through three-fourths the dentin thickness by radiographic measurement. Of 47 pulp exposures observed histologically, 70% were correctly interpreted as perforations from radiographs. Necrosis occurred in only six teeth where actual pulp exposure could not be seen histologically. Bacteria had penetrated into the pulp tissue in most cases of irreversible disease. Although irritation dentin was generally thicker with greater disease and deeper caries penetration, large deposits were observed in cases with little or no inflammation of the dental pulp. Analysis of individual criteria indicated that it is possible to predict the status of dental pulp disease with diagnostic methods in current usage.

Supported by NMRDC Project No. M0095PN003.3008.

Incidence of Dental Caries After Six Months Among a Sample of Naval
Recruits. J. C. CECIL*, M. R. WIRTHLIN and E. J. MANDEL. Naval
Dental Research Institute, Great Lakes, Illinois

The purpose of this study was to identify naval recruits who would be at highest risk for new carious surfaces in the first six months of service. Overall caries attack rates (CAR) and posterior proximal tooth surfaces caries attack rates (PPCAR) were computed for 354 subjects completing six-months technical training at Great Lakes, IL. Both CAR and PPCAR were based on the decrement of sound surfaces at-risk between the initial and six-month examinations. Carious lesions were graded as to depth within dentin using bitewing radiographs (i.e. B=1/4, C=1/4 to 3/4, U=3/4 distance dentinoenamel junction to pulp). Pearsonian correlations were found to be significant ($p < 0.05$) between CAR and PPCAR and DS, MS, FS, DMFS at the initial examination. In addition, significant correlations were observed between CAR and PPCAR and the number of B, C, and U lesions, Navy Plaque Index scores, and the Armed Forces Qualification Test scores (AFQTS). Further analyses, using one-way ANOVA, indicated that the CAR and PPCAR at six months were significantly related ($p < 0.05$) to the initial number of both B-lesions and U-lesions. Those subjects with the lowest AFQTS had significantly ($p < 0.05$) higher DS, DMFS, and more severe lesions (i.e. U-lesions) at the initial examination and higher CAR and PPCAR at six months. It was concluded that initial dental caries measures (DS, DMFS, depth of lesions) as well as AFQTS might be used in identifying those with the highest risk for new carious surfaces in the first six months of enlistment.

Supported by NMRDC Work Unit ZF58524012.0006.

Fluoride Concentration in Plaque of Caries-Free and Caries-Active Naval Recruits. R. W. GAUGLER* and W. F. BRUTON. Naval Dental Research Institute, Great Lakes, Illinois

Dental plaque is known to contain 5-10 ppm of fluoride (F), but little is known of the role this F may play in the prevention of dental disease. Samples of posterior proximal dental plaque from 165 recruits -- 47 caries-free (CF) (DMFT=0), 57 caries-active (CA) (DT>5), and 61 with low caries prevalence (LC) (MFT=0, 0<DT<5) -- were examined for the total F concentration, how tightly the ion was bound to the plaque, and the ability of the plaque to absorb additional F from 1 ppm solutions. Plaque samples from another 97 recruits, 27 CF, 38 CA, and 32 LC, were assayed for the ability to absorb F from 225 ppm solutions. The mean F concentration in plaque of CF recruits was significantly greater than that in CA plaque, 8.2 ± 10.6 to 4.7 ± 3.9 ($p < 0.05$). The plaque of the LC group had a F content of 6.2 ± 4.7 ppm, which did not statistically differ from either CF or CA plaque. The F was bound to a similar degree in all 3 caries groups. Absorption of the ion from 1 ppm solutions was significantly greater for CA plaque than CF plaque ($p < 0.05$) with LC plaque again having an intermediate value not different from either group, 1.54 ± 1.07 , 0.97 ± 1.15 and 1.32 ± 1.44 ng F absorbed/mg plaque, respectively. Adsorption of F from 225 ppm solutions did not differ among the groups with 236, 220, and 231 ng F/mg plaque, for the CF, CA and LC groups. Higher fluoride levels in plaque were associated with lower caries prevalence, but there was no difference in how tightly it was held, or in the absorption of the ion from 0.05% F solutions.

Supported by NMRDC Project No. MR0000101.0016.

Histologic Assessment of Probing in the Presence of Periodontitis.
E. B. HANCOCK* and M. R. WIRTHLIN. Naval Dental Research Institute,
Great Lakes, Illinois

In normal gingiva and in areas with gingivitis the tip of the periodontal probe rests within epithelium coronal to the CEJ. The purpose of this study was to determine the location of the probe tip in areas affected by periodontitis. Amalgam restorations were placed on the facial surface of maxillary incisors in 7 adult Rhesus monkeys. Doubled, 1/8", medium force orthodontic elastics were placed around the teeth and changed every 2 weeks for 18 weeks. Two weeks after band removal the pocket depths were recorded using gold-coated acrylic replicas of periodontal probes. Pressures of probing were under 35 gm. The probes were luted to the tooth and block sections removed. The specimens were decalcified and processed for viewing by light microscopy. Clinically the marginal and attached gingivae were reddened and edematous. Bleeding on probing occurred routinely. Pocket depths were moderate (2.3 ± 1.0 mm). Histologically there was a moderate to dense inflammatory infiltrate from the gingival margin to the connective tissue attachment. The tip of the probe lay in a pouch of extremely thinned and flattened epithelium and distorted connective tissue. The epithelium was discontinuous in some areas at the tip and just lateral to the tip of the probe. The tip of the probe stopped at the level of the connective tissue attachment. It was concluded that in periodontitis the probe tip sometimes penetrated epithelium and probing depth estimates indicated the level of a healthy dentogingival junction.

Supported by NMRDC Project No. M0095.PN003.3010.

Salivary pH-Rise Profiles of Oral Organisms in Relation to Caries
Experience. B. L. LAMBERTS*, I. L. SHKLAIR and E. D. PEDERSON.
Naval Dental Research Institute, Great Lakes, Illinois

Certain salivary components enhance the metabolic activity of oral flora to produce salivary pH-rise effects. The purpose of this study was to compare the pH-rise responses of pure strains of oral organisms to salivary sediment organisms, in tests of whole saliva samples from caries-free and caries-active subjects. Paraffin-stimulated whole saliva was collected under ice from 24 caries-free and 23 caries-active naval recruits, and pH-rise profiles were determined over 6-hour periods, using the sediment/supernatant/2.8 mM glucose assay system of Kleinberg (1973). The pH-rise profiles for the selected strains were obtained with washed cells of S. salivarius, S. sanguis 10556, S. mutans 10449, A. viscosus, and L. casei, using each strain in place of the sediments in the assay mixtures. All of the mean pH-rise profiles for the caries-free subjects were higher and showed higher minima than those for the caries-active subjects. The L. casei and S. sanguis strains showed sharp pH-rise responses respectively after 15 and 30 minutes; the sediment response occurred after 2 hours. In contrast, the other strains showed very slight pH-rise responses. The pH profiles of the S. mutans and S. salivarius strains dropped sharply, remaining below pH 5.0 after the initial 45 minutes. The marked variations in pH-rise response observed among common oral strains indicated that sensitive strains can be used in place of salivary sediments for assessing pH-rise and buffering properties of salivas from subjects of differing caries experience.

Supported by NMRDC Project No. ZF58524012-0026.

An Evaluation of Porosity of Aluminum Oxide Implants. S. A. LEONE*,
J. E. YEAGER and E. S. PEPPER. Naval Dental Research Institute,
Great Lakes, Illinois

Earlier studies of porous aluminum oxide in primates done at this Institute resulted in failure of all implants. In order to determine whether this was due to the density of the material rather than the lack of biocompatibility, an in vitro and in vivo evaluation was conducted. In an in vitro study, disks of three different densities of aluminum oxide were placed in contact with gingival fibroblasts. After two days, the cells were stained with a vital dye. There was no evidence of toxicity. In an in vivo study, cylinders of aluminum oxide and titanium and ticonium metals were surgically placed into the edentulous ridges of primates. At ten weeks, the implants were surgically exposed to the oral cavity and at six months were removed in block section and studied histologically. Chi square analyses revealed a significantly lower retention rate for the porous implants compared to the more dense ones. However those porous implants that remained in the bone with subsequent covering by the gingiva showed no signs of inflammation histologically. In order to determine if saliva penetration of the porous implants could be contributing to their failure, dye penetration studies were conducted. These trials showed that various dyes would completely penetrate the porous aluminum oxide implants. Porous implants are highly biocompatible but their porous structure may allow penetration of saliva leading to their failure. Therefore, the use of porous aluminum oxide should be confined to sub-periosteal areas.

Supported by NMRDC Project No. M0095PN003.3011.

Inhibition of *Treponema oralis* by Surface Active Compounds. E. J. MUELLER* and M. R. WIRTHLIN. Naval Dental Research Institute, Great Lakes, Illinois

Spirochetes have been associated with necrotizing gingivitis. This study was carried out to evaluate surface active agents for their antitreponemal properties. Test strains used were six isolates of *Treponema oralis* obtained from patients with necrotizing gingivitis. Specimens obtained from healthy patients yielded no spirochetes. Primary cultures and broth dilutions of the surfactants were made in veal infusion broth which was supplemented with 400 ug/ml nicotinamide, 150 ug/ml spermine, 20 ug/ml sodium isobutyrate, 5 ug/ml cocarboxylase and 0.5 g/l cysteine. Anionic, cationic, nonionic surfactants and EDTA were diluted in supplemented veal infusion broth. Serial dilutions of benzalkonium chloride, cetylpyridinium chloride, Tween 80, Tween 60, Tween 40, sodium lauryl sulfate, Teepol and EDTA were prepared and inoculated with 3 drops of an actively growing culture of treponemes. All dilutions were tested with each of the 6 strains. The cultures were incubated anaerobically at 36°C for 10 days. After incubation, each dilution was examined by darkfield microscopy. Those compound concentrations which prevented growth, when compared to the control cultures, were considered inhibitory. All of the compounds tested prevented spirochetal growth at concentrations of 6.25 ug/ml. The low human toxicity of these chemicals in the concentrations necessary to inhibit spirochetes suggests that they may be of value in the treatment or prevention of necrotizing gingivitis.

Supported by NMRDC Project No. MR0000101.0018.

Glucan Synthesis by *S. mutans* from Caries-Active and Caries-Free Naval Recruits. I. L. SHKLAIR*, R. W. GAUGLER, W. F. BRUTON and A. E. DEMPSEY. Naval Dental Research Institute, Great Lakes, Illinois

The purpose of this study was to compare the ability of *S. mutans*, from caries-active (CA) and caries-free (CF) naval recruits, to synthesize soluble and insoluble glucans, and relate the amount of glucans synthesized to the individual's caries experience. Two interproximal plaque samples were collected from 25 CA (DT>5) and 25 CF (DMFT=0) males, 17-22 years of age. The samples were placed in 2 ml of a holding medium, dispersed by sonication, and diluted 1:10, 1:100, and 1:1000. Each dilution was filtered through a 0.45 μ Millipore membrane filter. The filters were placed on Mitis-Salivarius-bacitracin media to which 0.02 μ Ci/ml of [glucose-U-¹⁴C]-sucrose had been added. After 48 hours of anaerobic incubation, the organisms and the soluble and insoluble glucans were separately recovered from the filters. The incorporated radioactivity of the glucans was determined and related to the number of organisms present, as determined by DNA analysis. The *S. mutans* from the CA group synthesized significantly more insoluble glucans, 1666 $\pm$ 1114 CPM/mg DNA than the *S. mutans* isolated from CF recruits, 1012 $\pm$ 677 CPM/mg DNA ($p < 0.025$). There were no significant differences in the amount of soluble glucans recovered from the CA group, 283 $\pm$ 393 CPM/mg DNA, when compared with the CF group, 158 $\pm$ 339 CPM/mg DNA. The insoluble glucan synthesizing ability of *S. mutans* isolated from CA and CF individuals can be related, on a group basis, to their caries experience. The results offer further evidence that insoluble glucans are important in the caries process.

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International Association for Dental Research, 58th General Session,
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1. CLARK*, G. E., ANDERSON, D. M. and MINTEN, M. L. - "The Effect of Vascular Permeability Factor on Primate Dental Pulp." (Abstract #413)
2. SIMONSON*, L. G., LAMBERTS, B. L. and REIHER, D. - "Isolation and Characterization of a New Bacterial α -1, 3-Glucanase from Pseudomonas sp." (Abstract #292)
3. WALTER*, R. G., SHKLAIR, I. L. and GAUGLER, R. W. - "The Prevention of Tooth Destruction by a Low Molecular Weight Dextran." (Abstract #20)

*Author presenting paper.

The Effect of Vascular Permeability Factor on Primate Dental Pulp.
G. E. CLARK*, D. M. ANDERSON and M. L. MINTEN. Naval Dental Research
Institute, Great Lakes, Illinois

Studies are underway to determine the agent(s) of the dental caries process which induce pulp disease. Vascular permeability factor (PF), isolated from carious dentin extracts, was tested in primate teeth for its effect on the dental pulp. Extracts were prepared by solubilizing carious dentin in phosphate buffered saline, pH 7.2 (PBS), filtering the caries-PBS to remove microorganisms, and freeze-drying the filtrate. The presence of PF in the extract was demonstrated by a characteristic wheal reaction in rabbit skin. The PF was isolated from the caries extract by anion exchange, molecular filtration, and hydroxyapatite adsorption chromatographies. Buccal cavities were prepared to about 1 mm from the pulp of monkey molar teeth. Then PF in PBS was applied to the pulpal floor of the preparations on filter paper disks. The PF-disks were sealed against the pulpal floor by teflon disks and spherical amalgam. Control molar teeth were treated in the same manner using PBS without PF. After 24 hours the teeth were removed, fixed in formalin and decalcified.

Microscopically, minimal disruption of the pulp odontoblast layer was seen under the cavity preparations in both control and PF-treated teeth. However, vessels engorged with red blood cells and polymorphonuclear leukocytes and an infiltration of plump plasma cells with scattered Russell's bodies could be seen throughout the pulp tissue of the PF-treated teeth. These observations indicated caries PF was capable of causing adverse inflammatory changes in the dental pulp.

Supported by NMRDC Project No. M0095PN003.3008.

Isolation and Characterization of a New Bacterial α -1, 3-Glucanase
from Pseudomonas sp. L. G. SIMONSON*, B. L. LAMBERTS, and D. REIHER.
Naval Dental Research Institute, Great Lakes, Illinois

Recent evidence illustrates the importance of water-insoluble streptococcal glucans rich in α -1, 3-linkages as a prime virulence factor in dental caries initiation. Enzymatic hydrolysis of these water-insoluble glucans could result in the reduction or control of these oral bacteria. We have discovered a new bacterial source of α -1, 3-glucanase which is elaborated extracellularly by a species of Pseudomonas. The enzyme was found to be inducible on α -1, 3 "limit glucan" (Lamberts, IADR Abstracts, 1979). The highest yield of enzyme was obtained when the initial culture pH was 8.0. Incubation beyond 3 days did not appreciably increase enzyme production. Purification of the cell-free activity was accomplished by ultrafiltration, ammonium sulfate precipitation, and gel permeation chromatography. The temperature optimum was found to be near 56°C and the pH optimum (at 37°) was near 5.0. Paper chromatography of enzymatic end products, fractionated by Sephadex G-15 gel filtration, indicated an endohydrolase mechanism of enzyme activity. The enzyme was shown to be a true α -1, 3-glucanase by proton N.M.R. and by substrate specificity studies. Gel column chromatography with BioGel A-15M and Ultrogel AcA34 resolved two distinct peaks of α -1, 3-glucanase activity. The molecular weights of these two fractions were determined to be 279,000 and 67,400 by interpolating from the linear regression of the elution volumes of m.w. standards. The two activity peaks may represent the aggregated enzyme separated from its subunits.

Supported by NMRDC Project No. ZF58524012.0027.

The Prevention of Tooth Destruction by a Low Molecular Weight Dextran.
R. G. WALTER*, I. L. SHKLAIR and R. W. GAUGLER. Naval Dental Research
Institute, Great Lakes, Illinois

The role of insoluble glucan in the development of smooth surface decay has been described, but little information is known on methods to interfere with its synthesis. This paper describes the effect of a low molecular weight dextran, T-10, on in vitro glucosyltransferase activity and caries development in rats and hamsters. T-10, in concentrations of 0.01, 0.1 and 1.0 percent, was added to [glucose-U-¹⁴C]-sucrose in the presence of Streptococcus mutans K1-R glucosyltransferase. The soluble and insoluble glucan formed in 2, 4, 8 and 16 hours were determined and compared to the controls. The anti-caries effect of T-10 was evaluated using weanling Osborne-Mandel derived rats and Golden hamsters. The animals were infected with Streptococcus mutans 6715, and maintained on one of two cariogenic diets, each supplemented with 1% T-10 in the experimental groups or unsupplemented in the controls. T-10 delayed the in vitro synthesis of insoluble glucan and increased the rate of soluble glucan synthesis. Increasing concentrations of T-10 prolonged the delay. When 1% T-10 was added to diet 2000, containing 56% sucrose, there was no significant reduction in decay in either rats or hamsters. However, when added to diet 78053, with 25% sucrose, T-10 reduced caries activity in hamsters by 88%; there was no reduction in rats. Thus, in hamsters, 1% T-10 was able to overcome the cariogenic challenge of low sucrose levels and S. mutans but unsuccessful with high sucrose levels. Rats were not protected in either case. It appears that insoluble glucan synthesis was related more to smooth surface caries activity in hamsters than in rats.

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