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1. CLARK*, G. E., ANDERSON, D. M. and MINTEN, M. - "Purification of Permeability Factor from Carious Dentin Extracts." (Abstract #546).

2. GALICH*, J. W. - "Evaluation of Methods for Detection of Incipient Carious Lesions." (Abstract #1193).

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5. LAMBERTS*, B. L., SIMONSON, L. G., PEDERSON, E. D. and GAUGLER, R. W. - "A New Method to Assay Enzymes That Can Degrade Streptococcal Limit Glucans." (Abstract #47).

6. LEONARD*, E. P., REESE, W. V. and MANDEL, E. J. - "The Effect of Diphosphonates on Alveolar Bone Loss in the Rice Rat." (Abstract #563).

7. LEONARD, E. P., REESE, W. V. and MANDEL*, E. J. - "The Effect of Anti-Inflammatory Drugs on the Loss of Alveolar Bone in the Rice Rat." (Abstract #886).

8. LEONE*, S. A., YEAGER, J. E., WOODRUFF, L. L. and CLARK, G. E. - "An In Vitro Evaluation of the Cytotoxicity of Dental Implant Materials." (Abstract #893).

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10. SIMONSON*, L. G., LAMBERTS, B. L. and JACKOLA, D. R. - "Effects of Dextranases and Other Proteins on Adherence of S. mutans to Hydroxyapatite." (Abstract #441).

11. WIRTHLIN*, M. R., HANCOCK, E. B., CLARK, G. E., LEONE, S. A. and HOEFS, S. - "The Treatment of Diseased Root Surfaces to Remove Cementum-Bound Endotoxin." (Abstract #586).

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*Author presenting paper.

Purification of Permeability Factor from Carious Dentin Extracts.
G. E. CLARK*, D. M. ANDERSON, and M. MINTEN. Naval Dental Research Institute, Great Lakes, Illinois

The presence of permeability factor (PF) activity in extracts prepared from carious dentin has been reported by this Institute. The permeability activity was not evident in sound dentin extracts. The purification of PF from carious dentin was undertaken as an initial step in determining if PF contributes to the onset of inflammatory changes in the dental pulp tissue in advance of caries microorganisms. Carious dentin was obtained by excavation from deep lesions in teeth which were isolated by rubber dam. In preparation of extracts, carious dentin was homogenized by grinding in phosphate-buffered saline, pH 7.2, (PBS), centrifuged, filtered by 0.22 micron filter, concentrated by a Diaflo UM-2 membrane, dialyzed vs. deionized water and freeze-dried. Sound dentin extracts from unerupted 3rd molars were prepared in the same manner. The presence of PF in the extract materials was detected by the development of blue wheal reations at the intradermal injection sites of rabbits which had received I.V. injections of Evans Blue dye. PF, detected in carious dentin extracts, was purified by a sequential treatment consisting of anion exchange, molecular filtration, and hydroxylapatite adsorption chromatographies. The molecular weight of the PF molecule appeared to be about 35,000 daltons, and its isoelectric pH was about 4.5.

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Evaluation of Methods for Detection of Incipient Carious Lesions.
J. W. GALICH*. Naval Dental Research Institute, Great Lakes, Illinois

Little clinical attention is given to incipient carious lesions because they are difficult to detect and do not demand immediate treatment. The purpose of this study is to evaluate methods for the detection of incipient carious lesions. Evaluation of sixteen dyes, with aqueous and ethanol bases, using white and ultraviolet light has been conducted in vitro and in vivo. Naturally occurring lesions not normally visible on radiographs were studied on formalin fixed and freshly extracted human teeth. Additionally, 93 human subjects are involved in the clinical phase of the study. The absorption of sodium fluorescein, 8-hydroxyquinoline, and alizarin red-S indicates that incipient lesions possess increased porosity and will take up various marking materials. Sodium fluorescein (2%) in a water/ethanol base, viewed with long wave (365 nm) ultraviolet light provided the most consistent detection of lesions with gross subsurface softening. Those lesions which do not absorb the dye do not possess the subsurface softening, but they do exhibit the ultraviolet light "quenching" effect. Thus the absorption of selected dyes enables the differentiation of lesions with subsurface softening. Ultraviolet light transillumination was also evaluated and found to be effective in the anterior regions of the mouth due to the narrow facial/lingual dimensions of the teeth and proximal contacts. Comparative scanning electron microscopy is being conducted on lesions to correlate dye absorption with structural differences.

supported by NMRDC Project No. MR0000101.0017.

S. mutans Glucan Production and Proximal Caries Activity in Rats.

R. W. GAUGLER*, I. L. SHKLAIR, E. P. LEONARD and W. F. BRUTON.
Naval Dental Research Institute, Great Lakes, Illinois

Extracellular glucan production is believed to be a significant factor in the cariogenicity of *S. mutans*. The purpose of this study was to assess the relationship of the extracellular glucan production from 14 human isolates of *S. mutans* (serotype c) to the caries activity of these organisms in animals. The organisms were grown in 5 ml of a chemically defined medium with 5% sucrose, and the amounts of soluble and insoluble glucan (expressed as mg of glucose equivalents per mg of DNA) were determined. Each organism was implanted into eight weanling antibiotic treated rats and the animals were maintained on the cariogenic diet 2000. The animals were killed 60 days after implantation and their caries scores determined by the procedure of Keyes. The proximal caries scores ranged from 2.2 to 15.5 and fell into two distinct groups. The first group contained 6 of the *S. mutans* strains and had proximal caries scores between 2.2 and 7.5. The remaining 8 organisms had proximal caries scores from 11.5 to 15.5. Glucan production in the low caries groups was 49.8 $\pm$ 32.3 mg of soluble glucan and 58.5 $\pm$ 24.1 mg of insoluble glucan. The high caries groups produced 40.4 $\pm$ 37.1 mg of soluble glucans and 130.5 $\pm$ 77.5 mg of insoluble glucan. The difference in the production of insoluble glucan by these groups was significant ($p<0.05$). The results indicate a relationship between proximal caries activity and insoluble glucan production by these organisms.

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

In normal gingiva the tip of the periodontal probe rests within epithelium, at or slightly apical to the coronal extent of the junctional epithelium. The purpose of this study was to determine the location of the probe tip in the presence of gingivitis. Amalgam restorations were placed on the facial surface of mandibular incisors in 8 adult Rhesus monkeys. Doubled 1/8", medium force orthodontic elastics were placed around the teeth and changed every two weeks for 18 weeks. Two weeks following band removal the pocket depths were recorded using gold-coated acrylic replicas of periodontal probes. The probes were luted to the tooth with cyanoacrylate; and the tooth, probe, attached gingiva, and alveolar crest were removed in block section, decalcified, and processed histologically. Clinically the marginal gingiva was reddened, edematous and bled easily on probing. Pocket depths were shallow (1.1 $\pm$ 0.32 mm) and some recession had occurred (1.4 $\pm$ 0.69 mm). Histologically there was a moderate to dense inflammatory infiltrate in the marginal gingiva. The junctional epithelium did not extend apical to the CEJ. The tip of the probe displaced, but did not penetrate the crevicular epithelium. The probe tip was contained within a shallow epithelial pouch 1.3 $\pm$ 0.65 mm coronal to the CEJ. Even in the presence of marginal inflammation, the junctional epithelium resisted displacement from the enamel and penetration by the probe tip.

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

A New Method to Assay Enzymes That Can Degrade Streptococcal Limit Glucans. B. L. LAMBERTS*, L. G. SIMONSON, E. D. PEDERSON, and R. W. GAUGLER. Naval Dental Research Institute, Great Lakes, Illinois

Water-insoluble glucans from Streptococcus mutans can be degraded by Penicillium sp. dextranases to "limit glucans" that may contain 90% or more α-1,3 linkages. This paper reports a sensitive technique to monitor in liquid culture media the activities of enzymes effective against the limit glucans. The method is based on the complexing of limit glucan with Cibacron Blue dye. The enzymatic degradation of this complex releases soluble blue products which can be assessed spectrophotometrically. Limit glucan, prepared from Strep. mutans strains OMZ 176 or K-1R, was complexed with Cibacron Blue F3GA by the method of Bohme et al. (1972). Excess dye was removed by washing the insoluble product successively with water and cold 2:1 (v/v) ethanol:water mixtures, and the residue was lyophilized. For determinations of glucanase activity, the procedure of Koh and Khouw (1970) for dextranases was modified by use of the blue limit glucan instead of Blue Dextran as a substrate. Tests of about 40 soil or sludge samples showed glucanase-producing organisms present in about two-thirds of the samples. Paper chromatography of degradation products from the limit glucan revealed that actual hydrolysis of glucosidic linkages occurred rather than simple release of the dye moiety from the glucan. The method shows considerable promise as a means to determine enzyme characteristics and the optimal conditions for culturing glucanase-producing organisms.

Supported by NMR&DC Project No. 62758NZF1524012.0022.

The Effect of Diphosphonates on Alveolar Bone Loss in the Rice Rat.
E. P. LEONARD*, W. V. REESE, and E. J. MANDEL. Naval Dental Research Institute, Great Lakes, Illinois

Research on diphosphonates suggest the possible application of these compounds as inhibitors of bone resorption. The purpose of this study was to measure the effects of diphosphonate administration on alveolar bone resorption in the rice rat. Thirty-six male rice rats (Oryzomys palustris) were placed on diet L-2000 at weaning. After 34 days on the diet the animals were divided into groups of 12 animals each. The next day the animals of each group were injected with either (a) 4.0 mg/kg body weight of ethane-1-hydroxy-1, 1-diphosphonate (EHDP); (b) 4.0 mg/kg of dichloromethylene diphosphonate (Cl_2MDP); or (c) 0.9% sodium chloride (control). Each animal received one subcutaneous injection per day for 10 days (in a volume of 0.1 ml). Fifteen days after the last injection (day 60 after weaning) the animals were killed by CO_2 inhalation. The upper jaws were fixed, decalcified and sectioned for histologic examination. The lower jaws defleshed and dried and alveolar bone loss was quantitated by direct measurements along buccal and lingual surfaces with the aid of a micrometer eyepiece. The mean socres for the three groups were as follows: Cl_2MDP, 6.22 $\pm$ 1.99; EHDP 7.98 $\pm$ 2.74; saline control 8.58 $\pm$ 3.39. Comparison of values were made by Welch's t-test. The results of analysis showed a significant reduction in alveolar bone loss in the Cl_2MDP group as compared to both the control group ($p<0.01$) and the EHDP recipient group ($p<0.05$). Although mean scores were less in the EHDP group than the controls, this difference was not statistically significant.

Supported by NMR&DC Project No. MR0412002.0480.

The Effect of Anti-Inflammatory Drugs on the Loss of Alveolar Bone in the Rice Rat. E. P. LEONARD, W. V. REESE and E. J. MANDEL*. Naval Dental Research Institute, Great Lakes, Illinois

Tissue culture studies indicate that aspirin-like drugs inhibit the activity of certain bone-resorption stimulating factors. The purpose of this study was to determine the relative effects of two non-steriod anti-inflammatory drugs (indomethacin and salicylate) on the loss of alveolar bone in the rice rat. Thirty-four male rice rats (Oryzomys palustris) were placed on diet L-2000 at weaning (21 days). At day 30, the animals were divided into groups. Two groups were offered diet L-2000 to which either sodium salicylate or indomethacin were added. A third group was offered only diet L-2000. Daily food consumption was monitored and the drug dilution was modified so as to provide the animals with a daily drug intake of 2 mg. Sixty days after initiation of the experiment the animals were killed by CO_2 inhalation. 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: A (salicylate) 7.55 $\pm$ 2.17; B (indomethacin) 6.43 $\pm$ 1.70; control 6.47 $\pm$ 2.45. A comparison of values using students' t-test revealed no significant differences between groups. These results suggest that the alveolar bone loss occurring in the rice rat is not responsive to the non-steriod anti-inflammatory drugs as used in this study.

Supported by NMR&DC Project No. MR0412002.0408.

An In Vitro Evaluation of the Cytotoxicity of Dental Implant Materials. S. A. LEONE*, J. E. YEAGER, L. L. WOODRUFF, and G. E. CLARK. Naval Dental Research Institute, Great Lakes, Illinois

Aluminum oxide, ticonium, titanium, vitreous carbon, and acrylic are currently used as dental implant materials. The purpose of this study was to determine the cytotoxicity of these materials in tissue culture. Human gingival fibroblasts obtained from a 34 year old male were grown on a petri dish until the late log phase of growth. At that time, a thin layer of 1.5% methylcellulose in media was placed over the cells. Once the cells reached confluency, discs of the test materials were placed into the methylcellulose allowing for close adaptation of the material to the cells. Discs of known toxic and non-toxic controls were used in each plate. After 48 hours, the cells were stained with 3% neutral red in an agar-media overlay. Clear zones of non-vital cells when noted and confirmed microscopically, were indicative of cytotoxicity. Preliminary results have shown that none of the materials tested have toxic effects on the fibroblasts. The technique of using a methylcellulose overlay allows for very close adaptation of the test material to the cells while maintaining a stable position of the material. This provides a mechanism for in vitro testing of dental materials.

Supported by NMRDC Project No. MR0000101.0021.

The Bacteroides fragilis Group of Organisms and Periodontal Pockets.

E. J. MUELLER*, I. L. SHKLAIR, E. B. HANCOCK, M. R. WIRTHLIN and C. V. MAYO. Naval Dental Research Institute, Great Lakes, Illinois

Since the Bacteroides fragilis group has been implicated in abscess formation and tissue necrosis, its relationship to periodontal pockets was investigated. Specimens were obtained from maxillary and mandibular molar areas in twenty-seven male naval recruits, ages 17-19, with chronic inflammatory periodontal disease. A sterile Williams probe was used to measure pocket depth and obtain debris for culture. Depth of pockets varied from 2-6 mm (mean:3.4 mm). The integrity of the contact, presence of calculus, amount of plaque, GI score, and presence of carious lesions were recorded. The material collected was placed directly onto kanamycin-esculin bile agar (KEB) and the plates were stored anaerobically for transport to the laboratory. Plates were transferred to and streaked in an anaerobic chamber. After 48 hours incubation at 35° C, the plates were examined and, if no growth was observed, incubated for an additional 5 days. Although slight blackening of the KEB medium was observed at the point of initial specimen inoculation, no bacterial colonies were obtained from any of the specimens. The blackening of the medium is thought to be due to materials present in periodontal pocket debris. This sampling regimen failed to demonstrate the B. fragilis group in chronic periodontal pockets and indicated that it was probably not involved in this stage of chronic inflammatory periodontal disease.

Supported by NMRDC Work Unit MR0000101.0021.

Effects of Dextranases and Other Proteins on Adherence of S. mutans to Hydroxyapatite. L. G. SIMONSON*, B. L. LAMBERTS, and D. R. JACKOLA. Naval Dental Research Institute, Great Lakes, Illinois

The ability of Streptococcus mutans to adhere and aggregate on human tooth surfaces may be a prime virulence factor in dental caries initiation. The purpose of this investigation was to study factors that affect the initial adherence of S. mutans to hydroxyapatite. We used an in vitro assay method similar to that described by Clark and Gibbons (1977). Hydroxyapatite (HA) discs were pretreated with either phosphate-buffered saline (PBS), a Pencillium dextranase (PD) with a low affinity for hydroxyapatite, or a Fusarium dextranase (FD) with a high affinity for hydroxyapatite. The pretreated discs were incubated with ^{3}H-labeled S. mutans cells (strains NCTC 10449 or OMZ 176) in 0.01 M phosphate buffer, pH 7.0, with and without sucrose for 24 hr. Only the FD significantly ($p<0.05$) reduced bacterial adherence. Sucrose had no apparent affect on initial attachment; hence, the FD adherence inhibition was attributed to its strong affinity for HA binding sites. Similar inhibitory effects were observed when the discs were pretreated with mucin or a phosphoprotein. The effects of pretreating isotopically-labelled cells of the two strains of S. mutans with PD, FD, papain, trypsin, and hyaluronidase were also determined. The dextranases and hyaluronidase had no effect on bacterial adherence whereas trypsin and, to a lesser extent, papain greatly reduced the adherence. We concluded that S. mutans initial adherence is due, at least in part, to a protease-susceptible cell component.

The Treatment of Diseased Root Surfaces to Remove Cementum-Bound Endotoxin. M. R. WIRTHLIN*, E. B. HANCOCK, G. E. CLARK, S. A. LEONE, and S. HOEFS. Naval Dental Research Institute, Great Lakes, Illinois

Untreated periodontally involved teeth contain cementum-bound endotoxin, that may prevent periodontal new attachment during healing. The purpose of this study was to restore biocompatibility to diseased root surfaces by a non-invasive treatment compatible with surgical therapy. Untreated human teeth, removed for severe chronic periodontal disease, were frozen after forceps extraction. The teeth were split buccal-lingually, the level of of the connective tissue attachment was scribed, and the specimens cleaned of plaque and visible calculus. Cementum removal was not attempted. The roots were rinsed, autoclaved, and assigned random numbers. Four roots were treated by sterile saline (control) and 5 with 2% sodium deoxycholate solution on a sterile cotton pellet for one minute. Roots were incubated in a suspension of fibroblasts for 48 hours, then rinsed with sterile phosphate buffered saline, fixed in methanol-ether, and stained with 0.5% trypan blue. When an ocular grid was used at 20x, each grid square enclosed an area 0.25 x 0.25 mm. Counts were made of the number of attached cells, 0.5 mm coronal to the connective tissue attachment. The code was broken, and by Welch's t-test there was a significant ($p<0.001$) difference between the control and experimental counts. Root surface treatment with 2% sodium deoxycholate improves the attachment of connective tissue cells to debrided diseased root surfaces, in vitro.

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These abstracts are a synopsis of research reported in 1979 by the scientific staff of the Naval Dental Research Institute and collaborators from other research activities.

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