511 M ANDERSON, H WARDLE, R GRAY, D BRICKER and D DENNING (University of Manchester, Hopo Hospital, Salford): Characterisation of oral bacteria of known and unknown species by RAPD

The Random Amplification of Polynucleotide DNA (RAPD) technique has been used to differentiate closely related species of many different bacterial genera and to type different strains of the same species. The potential for using RAPD to differentiate and characterise Treponema species was investigated. Genomic DNA was extracted from four different Treponema species (T. CVD, T. pallidum, T. denticola, and T. denticola) using standard procedures. Five different primers (17, 19, 20, and 20/20) previously used with other spirochaetes and oral bacteria, were used to amplify polymorphic regions of the genomes. The four different species gave obviously different patterns of amplified fragments after agarose gel electrophoresis with only a few bands in common. Several treponemal isolates of unknown species were cultured and purified from dental plaque samples (vide Wardle et al. postcr.): These isolates were used to characterise whether the five primers used were scored for maxilla and common with known species. The matrix was used to show how similar the unknowns were to the known species.

Preliminary work shows that four of the unknown isolates from different patients all produce very similar RAPD patterns to each other.

512 MI NEAL, R GOODACRE, S BROM, AJ WEIGHTMAN and GW WADE (Biological Sciences, Aberystwyth, Dental School, Bristol and PABLO, UWCC, Cardiff): Identification of oral Eubacterium spp. by pyrolysis mass spectrometry.

Pyrolysis mass spectrometry (PyMS) has been shown to be a valuable technique for use in bacterial systematics and, specifically, identification. The aim of this study was to evaluate PyMS with neural network analysis for the identification of oral saccharolytic Eubacterium spp. Core-point pyrolysie mass spectra of 10 strains of Eubacterium were compared with the spectra of 10 known species and un-named taxa, together with 6 abscess isolates provisionally identified as P. helicobacteriidentes. Artificial neural networks (ANNs) were then trained by supervised learning (with a backpropagation algorithm) to identify isolates from their pyrolysis mass spectra. Analysis of the spectra generated clustering of strains consistent with that obtained from previous phenotypic and genotypic studies. The neural network correctly identified the known Eubacterium strains. The abscess isolates were identified as un-named Eubacterium taxon C.

This study has demonstrated that the combination of pyrolysis mass spectrometry and ANNs provides a rapid and accurate identification technique for oral Eubacterium spp.

513 MJ WILSON, GW WADE and AJ WEIGHTMAN (Dental School, Cardiff, UK; Dental School, Bristol, UK and Proud and Applied Biology, UWCC, Cardiff, UK): The design and validation of DNA probes against un-culturable bacteria.

Culture-independent analysis of the subgingival microflora by 16S ribosomal RNA sequencing has revealed the presence of previously uncultured bacteria. The aim of this study was to design specific oligonucleotide probes against these bacteria and use the probes to access samples of subgingival plaque. Areas of greatest sequence variability were chosen as targets for probe construction. Probes were labelled with biotin 16S rRNA and biotinylated DNA probes were hybridised with the Oligo package. Total nucleic acid was extracted from 46 samples of plaque from both healthy and periodontally diseased sites and hybridised to each of the three probes under stringent hybridisation conditions. Three probes (B4, A18 and A19), each exhibiting low self-complementarity and low tendency towards dimer formation, were used. No cross-reactivity was detected against 20 other oral bacteria but a strong positive signal resulted with the recombinant plaque cloned DNA as positive controls. The target bacterial sequences were not detected in any of the 46 subgingival plaque samples.

In conclusion, DNA probes to unculturable bacteria were successfully constructed. However, the presence of the target bacteria in clinical specimens was not detected.

514 J PARRY, R HOLT, H N SHAP, M WILSON (Deps. of Microbiology and Children's Dentistry, Barts Dental Institute, London): Use of impedance measurements for the detection of mycoplasmas in saliva samples.

Bacterial growth can result in changes in the electrical conductivity (impedance) of the medium and measurement of such changes can be used to detect the presence of bacteria, and their growth rate. The aim of this study was to determine whether mycoplasma growth is detectable in saliva by measuring changes in impedance (using the Rapid Bacterial Impedance Technique - RBIT) and to determine whether these media would be the most suitable for use with this system. Saliva samples were taken from 10 adult volunteers and a portion of each was plated onto a medium selective for mycoplasmas (Mycoplasma Experience Medium - MEM). These were incubated for 10 days and examined for characteristic mycoplasmal colonies. One colony was added to 5 ml of the following selective mycoplasma media: Hayflick's (H), Oxford's (O) and MEM. The electrical conductivity of the cultures was monitored every 9 mins over a 72 hr period. Mycoplasma colonies were cultured on agar plates from 7 of the 10 samples. Growth was detected by impedance measurements in 3 of the samples in MEM medium, 5 of the samples in H medium and 2 of the samples in O medium.

In conclusion, impedance measurements were less sensitive at detecting mycoplasmas than traditional culturing methods. However, the convenience of the impedance technique and its usefulness in clinical and experimental studies, justify further work on developing a medium suitable for use with the technique.


515 HS SHIU, H KHAM, D ANDREA, K SUGAWARA and S GUH (Dep. Microbiology & Genesetion, Barts Dental Institute, London): Biofilm energy assimilation by Peptostreptococcus spp. and Peptostreptococcus micros as a model for studies of microbial interactions in the oral cavity.

The role of the oral cavity biofilm in the formation of microbial species that are well adapted for growth in such an existing ecosystem. Growth and survival of bacteria at these sites must ultimately depend on nutrient availability and is likely to vary with the clinical condition. In the present study we utilised measurements of conductometric changes to study energy assimilation by Peptostreptococcus micros and Peptostreptococcus spp. to determine the identity of the species in response to amino acid utilization by P. endosporum and P. micros. In former, amino, lyase and histidine were utilized after the first 5 hours of incubation. While methionine utilization was started after 1 hour. This was reflected by an increase in the electrical conductivity of the medium which was increased on incubation media. The amino acid uptake of amino acids was considerably faster in the former and followed a linear response except for asparagine which was utilized within the first hour. Result in the reduction in the conductivity of the culture medium for 14 hours followed by a peak in biofilm conductivity. The results of this study suggest that the uptake amino acids represent an important source of nutrients for P. endosporum and P. micros and, by inference, may also be an important substrate for other species infecting the root canal. This in vivo and in vitro study P. micros may be a source of amino acids more efficiently than the former species. The slower diffusion of amino acids result in a depression of activity showed by the outside of the biofilm.

516 K A YOUNG, R P ALLAKER, M H HARDIE and R A WHELIE (Department of Oral Microbiology, London Hospital Medical College): Pathogenicity mechanisms in mixed infections with Eubacterium corrodens and Streptococcus milleri-group' organisms.

'Streptococcus milleri-group' organisms (S. intermedius, S. constellatus and S. anginosus) and E. corrodens together produce localised suppurative infections at oral and other body sites. Congregation, enhancement of hydrolytic enzyme activities and growth stimulation were investigated with combinations of these organisms. Congregation reactions occurred frequently between S. constellatus (18%) and streptococci (17%), S. intermedius (16%) and E. corrodens, but were infrequent between S. intermedius and E. corrodens (28%). No enhancement of enzyme activities against lipids, phospholipids, proteins and saccharide substrates were detected with combinations of oral microorganisms to the species selected. Exponential growth of S. constellatus and S. intermedius, in mixed culture with E. corrodens, occurred within 6h post inoculation, in comparison to 23h without E. corrodens. No growth stimulation of S. constellatus was observed.

It is concluded that both congregation and growth stimulation occur between E. corrodens and S. milleri-group organisms, and may be important mechanisms in the establishment of mixed infections involving these bacteria.

517 V S LUCAS, D BEIGHTON and G J ROBERTS (1 Great Ormond Street Hosp, Dept of Dental Science, King College Hosp, London): Oral Syndrome Changes Following Total Body Irradiation / Chemotherapy for Bone Marrow Transplantation.

Objectives: Evidence implicating oral streptococci as opportunistic organisms in systemic infections in bone marrow transplantation (BMT) patients. For several years we have been following Oral Syndrome Changes Following Total Body Irradiation / Chemotherapy for Bone Marrow Transplantation (Oral Surg Sept 64 711-176). In this study we are studying the effects of oral flora in children following total body irradiation (TBI) and chemotherapy have been investigated. From the results it appeared that patients who were matched with normal children who showed an improvement in oral health post transplantation had an increase in neutrophil granulocytes. The neutrophil count was 0.8 x 10^9/1 and 1.2 x 10^9/1 in the patients with severe neutropenia. Conclusions: Oral flora changes following TBI and chemotherapy may increase the risk of systemic infection from S. mitis and S. sanguis.

This work was supported by the Sir Jules Thorn Charitable Trust.

518 P TOWNES, M A WILFC and A J COTT (Department of Oral Surgery, Medicine and Pathology UWCM, Cardiff, UK): Repid Auto-antibody System (RAAS) provided by an oral microbiology unit in a dental teaching hospital.

The aim of this study was to provide information on the source, specimen type, disorder and culture results for material submitted to an oral microbiology unit that provides a bacteriology and mycology service. A total of 1,433 consecutive request forms received in the oral microbiology unit between January 1992 and November 1994 were examined. Samples were received predominantly from the oral medicine clinic (69%), examination and emergency unit (18%) and oral surgery unit (7%). The conditions investigated most frequently were oral lesions (40%) and carious lesions (24%), followed by acute pain (10%) and dental abscesses (13%). The majority (80%) of requests originated from mucosal conditions with 20% coming from dentulous patients. Samples from suspected cases comprised of 44% oral infections (OR), 41% of which yielded candida, and 42% of which yielded streptococcus (17%). Mycoplasma corrodens (6%) were isolated with about 6% of all infections (24%). Culture of these specimens revealed strict anaerobes alone in 30%, facultative anaerobes alone 2% and a mixture in 49%. Scarcity or no growth occurred in 18% of samples. In contrast, when streptococcus yielded only strict anaerobes in 11% of cases and facultative anaerobes alone in 13%. The greatest study has confirmed that microbiological investigations play a role in the clinical management of a variety of oral conditions. However, in some disorders ensuring the appropriate specimen may increase the value of the subsequent microbiological report.