SYNOPSIS OF PAPERS OF MICROBIOLOGICAL INTEREST

SYMPOSIUM: PCR IN THE DIAGNOSIS OF INFECTIOUS DISEASE
Chairman: G. French

PCR: A BACTERIOLOGIST'S DREAM OR NIGHTMARE?

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The polymerase chain reaction has produced a potentially powerful new approach to bacteriology. This technique has advantages and disadvantages and offers many benefits if applied carefully. Advantages include its rapidity, specificity and sensitivity. It allows the identification of pathogens from amongst many other bacteria and also the presence of toxins or antibiotic resistance carriage. The ability to synthesise your own primers from published sequences removes the difficulty of obtaining probes from other workers. The disadvantages are important and must be recognised prior to interpretation of a result. False positives, non-pathogenic numbers of bacteria, presence of inhibitors of amplification, the requirement to identify a specific pathogen and the necessity for specific sequence information are all problems. These are not insurmountable but they must be considered.

A range of bacterial PCRs have been described in the literature. We have concentrated our work on Helicobacter pylori (Hp) and mycobacteria. A 16s rRNA PCR for Hp has been developed and early investigations of its value in gastric disease and other situations were described. Selected examples of the other uses of bacterial PCRs were discussed including the use of universal primers and toxin detection.

CAN PCR IMPROVE OUR DIAGNOSIS OF MYCOBACTERIAL INFECTIONS?

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Through public health and therapeutic initiatives, the incidence of tuberculosis has declined rapidly in the UK in the last 40 years. Sporadic cases and clusters of infection do still occur in both the indigenous and immigrant population with a much higher incidence in the immunosuppressed. Laboratory diagnosis relies heavily on direct microscopy of stained specimens and culture which takes up to 8 weeks. Commercial DNA probes for 16s rRNA sequences of mycobacteria are available but they are complicated to use and lack sensitivity. The specificity combined with extreme sensitivity of PCR-based amplification techniques makes them interesting candidates for the non-cultural identification of mycobacteria. Over the last 2 years a large number of PCR-based protocols for detecting mycobacteria has been published. Some of the nucleic acid targets that have been used are: (i) amplification of genes or parts of those genes encoding antigens such as the 65-kDa heat shock protein, MPB64 protein and antigen b; (ii) amplification of 16S rRNA and cDNA encoding it; (iii) amplification of repeated sequences. The results of using these various systems with clinical specimens were discussed. As expected, all investigators have been able to rapidly detect nucleic acid derived from mycobacteria in samples which are negative by microscopy and culture. By using appropriate primers and sometimes oligonucleotide probes to confirm the identity of the amplified product, identification of species is also possible. It is clear that great care is required to prevent contamination of samples. A control for the presence of inhibitors of amplification is used by the more careful workers and usually takes the form of DNA derived from a cloning vector such as pUC 19 together with primers to amplify a suitable sequence, this is added to the sample. Standardisation of the DNA extraction technique is important as some groups have had trouble with false-negative results due to poor bacterial lysis. At least two independent pairs of primers should be used as some specimen/primer combinations fail to amplify. Although the technique is technically sophisticated and requires an investment in equipment and expertise, it offers an exciting advance in the diagnosis of tuberculosis disease, particularly in meningitis.

THE APPLICATION OF PCR TO THE DIAGNOSIS AND MANAGEMENT OF TOXOPLASMOSIS

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Toxoplasmosis is a life-threatening opportunistic infection of the immunocompromised. In this group of patients, serological investigation is unreliable and parasite isolation may not produce results with sufficient rapidity. Consequently, a DNA-based assay was developed for the detection of Toxoplasma gondii. The assay involves sequential amplification of part of the P30 gene with nested primers. Following gel electrophoresis, the amplified DNA fragment can be detected either directly on the gel or by Southern hybridisation with radioactive or non-radioactive DNA probes. In-vitro assessment showed the assay could detect as little as 0.05 pg of toxoplasma DNA, which corresponds to a single organism, both in the presence and absence of contaminating human DNA. DNA of other protozoan species was not amplified, indicating the specificity of the assay. Toxoplasma DNA was detected in brain tissue of laboratory-infected mice. PCR has been used to diagnose toxoplasmosis in bone marrow graft recipients and AIDS patients and the assay has been applied to the investigation of toxoplasmosis associated with pregnancy.

PCR represent a method of potential value for the investigation and management of toxoplasma infection of
the immunocompromised. Further studies are required to define the relative merits of this technique in comparison with established diagnostic methods.

**RAPID DIAGNOSIS OF HERPES ENCEPHALITIS BY PCR**

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Controlled studies have shown that prompt acyclovir treatment leads to significant reduction in the mortality and morbidity of severe herpes simplex encephalitis (HSE). Because acyclovir is perceived as being a compound of low morbidity of severe herpes simplex encephalitis (HSE), has, therefore, had to develop “non-invasive” strategies in treatment leads to significant reduction in the mortality and morbidity of severe herpes simplex encephalitis (HSE). Because acyclovir is perceived as being a compound of low toxicity there has, in recent years, been a reluctance to perform brain biopsy in pursuit of diagnosis. The virologist has, therefore, had to develop “non-invasive” strategies in an attempt to provide early diagnosis of HSE.

Using the polymerase chain reaction (PCR) to amplify Herpesvirus simplex genetic material (a conserved region within the thymidine kinase gene) we have developed a method which promises early, non-invasive, diagnosis of HSE. In a retrospective study, we examined 39 samples of CSF taken during the first 10 days of illness (35 patients with virologically confirmed HSE): 36 CSF samples were PCR positive, three were PCR negative. Two of the negative samples were taken on the day of onset of neurological illness, one was taken on day 2; 109 non-HSE CSF samples gave negative results.

To determine the true value of PCR as a routine diagnostic procedure, we initiated a prospective study: 150 acute CSF samples from 126 patients with suspected HSE have been examined during a 9-month period. Twelve samples (11 patients) were PCR-positive, 138 were negative. Amongst the negative results we know of no “false negatives” and among positive results we have detected two probable false positives. Detailed follow up of all results was presented. PCR appears to offer a valuable non-invasive method for acute stage diagnosis of herpes encephalitis and an important aid in the differential diagnosis of conditions which mimic herpes encephalitis.

**PCR AS AN AID TO DIAGNOSIS OF CYTOMEegalovirus INFECTION**

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Increasing numbers of organ transplants and AIDS patients, and specific chemo- and immunotherapy for CMV has resulted in pressure to produce rapid, sensitive and specific identification of CMV infections. If PCR is to be accepted for routine diagnostic use it must be reliably better than other methods currently available.

An in-situ hybridisation (ISH) method with photobiotin-labelled CMV DNA had a sensitivity of 10–40 CMV copies/cell and a processing time of 5 h. PCR used the technique of nested CMV IE primers, with two rounds (40 and 30 cycles) giving a sensitivity of three CMV copies/sample.

PCR detected CMV sequences in a range of clinical specimens, even when diluted 1000-fold, in samples shown to be infected by ISH. There are reports of the detection by PCR of latent CMV in lymphocytes of CMV seropositive healthy individuals. This makes the clinical significance of CMV detection in blood samples unclear at present.

There are problems common to all PCRs—failure of amplification, contaminating sequences, accurate quantification of the initial CMV copy number and determination of cell types involved—which can pose considerable problems in the clinical interpretation of PCR results.

**PCR IN THE DIAGNOSIS OF Pneumocystis carinii pneumonia**

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The opportunistic fungus Pneumocystis carinii is the major cause of fatal pneumonia in immunosuppressed individuals, particularly AIDS sufferers. We have cloned the DNA from P. carinii, confirmed by characteristic in-situ hybridisation patterns. Using the sequence of the gene coding for the large subunit of the mitochondrial ribosomal RNA, we have designed primers which efficiently amplify both rat-derived and human-derived P. carinii. We have tested these primers using PCR with DNA from other pulmonary pathogens and over 100 closely related organisms and demonstrated that the primers are entirely specific to P. carinii. We have increased the sensitivity of detection of the products of DNA amplification by oligoblotting, with an internal oligonucleotide as a probe.

We have applied these methods to the detection of P. carinii in bronchoalveolar lavage samples and in non-invasive induced sputum samples from over 100 episodes of acute respiratory illness. No P. carinii was detected in samples from immunocompetent individuals. In the immunosuppressed, a strong DNA amplification signal correlated with clinical P. carinii pneumonia, whereas a weak signal was an indication of low, sub-clinical levels of infection.

We find that DNA amplification for P. carinii offers great sensitivity and specificity and that the results can be correlated with clinical disease.

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**14th C. L. Oakley Lecture**

**CANDIDA HSP 90: LINK BETWEEN PROTECTIVE AND AUTOIMMUNITY**

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Heat shock proteins are thought to play a role in the aetiology of autoimmune diseases, but are also common targets for the immune response to many infections. Patients recovering from systemic candidosis produce antibodies to Candida albicans HSP 90, both to species-specific epitopes and, more commonly, epitopes shared with human HSP 90. One such autoreactive antibody was protective in a mouse model of systemic candidosis.


**Amplification and Characterisation of Gene Fragments Encoding Global Regulatory Proteins from *Listeria monocytogenes, Yersinia enterocolitica* and *Yersinia pseudotuberculosis***

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In order to potentiate disease, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Y. pseudotuberculosis* have to survive a range of extracellular and intracellular environments. Recently, it has been demonstrated that many bacterial factors are controlled by a limited number of conserved two-component global regulatory proteins which respond to environmental stimuli. Often the global regulatory protein co-ordinately controls the transcription of a number of genes directly involved in virulence. Recent studies have shown the regulatory protein PhoP to be essential for the survival of *Salmonella typhimurium* in macrophage cells and the related regulatory proteins OmpR to be associated with the invasiveness of *Shigella flexneri*. Furthermore, *S. typhimurium* strains bearing mutations in the *phoP* and *ompR* genes have been found to be highly attenuated in BALB/c mice. The aim of this study was to amplify, sequence and mutate the *phoP*, *ompR* and other global regulatory gene equivalents from *L. monocytogenes*, *Y. enterocolitica* and *Y. pseudotuberculosis*.

Using published sequence data from the *S. typhimurium* and *Escherichia coli* *phoP* and *ompR* genes, degenerate primers were synthesised and used in the PCR to amplify the equivalent gene fragments from *L. monocytogenes*, *Y. enterocolitica* and *Y. pseudotuberculosis*. The expected 341-bp fragment was amplified from the genomic DNA of all three species. Sequence analysis of the cloned fragments revealed that several independent members of the family global regulatory genes had been amplified. These included three clones each from *L. monocytogenes* and *Y. enterocolitica*, and five clones from *Y. pseudotuberculosis*. The derived amino-acid sequences from these clones revealed 26–77% homology to *PhoP* and 28–52% homology to *OmpR*.

The genes encoding the global regulatory proteins will be mutated and the resultant mutants will be compared to the wild-type strain. The mutational analysis of such genes should provide a range of *L. monocytogenes* and *Yersinia* strains for pathogenicity, immunological, attenuation and vaccination studies.

**Rapid Identification of Toxigenic *Clostridium difficile* by Polymerase Chain Reaction with Toxin A and B-Specific Primers**

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*Clostridium difficile* is the aetiological agent of pseudomembranous colitis and antibiotic-associated diarrhoea in man. The pathogenicity of *C. difficile* is, in part, related to the production of at least two toxins, toxin A, an enterotoxin, and toxin B, a cytopathic toxin. Currently, diagnosis depends on the isolation and identification of the organism, which takes up to 72 h and/or the demonstration of toxins in faecal specimens of patients by tissue culture or ELISA techniques. More rapid diagnosis is essential to enable prompt treatment. The aim of this study was to develop a rapid, sensitive and specific method for the identification of toxigenic *C. difficile* strains by PCR with primers specific for toxins A and B.

Samples for PCR experiments consisted of simple boildates from characterised *C. difficile* strains which were tested for toxigenicity by tissue culture. Standardised PCR conditions were used in all experiments, with 30 cycles and an annealing temperature of 45°C. Amplified PCR products were electrophoresed for 1 h on agarose 1% gels and visualised under UV light. The total time for the procedure, including sample preparation, was 3–4 h.

Results with the toxin A primers revealed that 201 out of 202 toxigenic strains and none of the 35 non-toxigenic strains were PCR positive. Results with the toxin B primers revealed that all 202 toxigenic strains produced the expected 591-bp product by PCR analysis which was absent for all 35 non-toxigenic strains. These results suggest that the presence of either toxin gene is a good indicator of toxin expression. Analyses of 45 other bacterial species, including 14 clostridial species, were PCR negative with the exception of three toxigenic *S. sordellii* strains which were positive with the toxin A primers. The PCR appeared three times more sensitive with the toxin A primers compared with the toxin B primers and was successful when as few as 20 toxigenic bacteria were present. The PCR method could detect this level of toxigenic *C. difficile* even in the presence of 10⁶ other bacteria.

The PCR-based method, which does not require hybridisation technology or the use of radioactivity, should be useful for the rapid identification of toxigenic *C. difficile* strains from pure and mixed culture and could be applied to cultured specimens.

**Application of PCR with Mixed Oligonucleotide Primers to Amplify, Clone and Sequence *htrA* Gene Fragments from *Yersinia enterocolitica* and *Campylobacter jejuni***

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In response to stress, all organisms apparently upregulate the synthesis of specific groups of proteins which maintain the integrity of the cell. Many of these so-called heat shock proteins are highly conserved among prokaryotic and eukaryotic organisms, reflecting their importance for the well-being of the cell. There is a wealth of recent evidence to suggest that heat shock proteins also play an important role in infections of a range of invasive bacteria. In particular, HtrA, a serine protease, has been found to be important in *Salmonella typhimurium* virulence. *S. typhimurium* HtrA negative mutants have a decreased ability to survive oxidative stress which may be indicative of the inability of these mutants to survive the oxidative bursts produced by macrophages in response to invasion by foreign organisms. Investigation of the *Yersinia enterocolitica* and *Campylobacter jejuni* htrA gene equivalents should provide a better understanding of the survival mechanisms of these organisms in the host and of host immune responses.

We have developed an adaptation of the basic PCR, with mixed oligonucleotide primers (PCRMOP), for the convenient amplification of conserved gene fragments from a range of organisms. The application of PCRMOP allows the detection of genes in one species with sequence data from a homologous gene of protein from another species. Using HtrA amino-acid sequence data derived from *Escherichia coli* and *S. typhimurium*, degenerate primers were...
designed and used in the PCR with 0.1 μg of genomic DNA from Y. enterocolitica and C. jejuni. The expected PCR products of 476 bp were amplified for both organisms and subsequently cloned into pUC19 and sequenced. The amplified Y. enterocolitica and C. jejuni gene fragments revealed 88 and 75% homology with the derived amino acid from S. typhimurium HtrA, respectively. Y. enterocolitica and C. jejuni htrA mutants will be constructed which will be useful for pathogenicity, immunological, attenuation and vaccination studies.

The PCRMOP method described has several advantages over conventional methods employing amino-acid sequence data to study genes. It is quicker and easier than the use of degenerate oligonucleotide probes and filter hybridisation methods and can tolerate far greater degeneracies than these techniques. The PCRMOP technique is rapid, convenient and makes it possible to specifically study determinants involved in virulence and gene regulation.

PCR AND THE EPIDEMIOLOGY OF BORRELIA BURGDORFERI IN SCOTLAND

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Lyme disease is caused by the spirochaete Borrelia burgdorferi and transmitted to animals and man by the bite of infected ixodid ticks. Much of the work on Lyme disease has been conducted in the USA where the white footed mouse has proved to be the main animal reservoir for the spirochaete. Although Lyme disease, as it is now known, was first described in Europe in 1909, the role as reservoirs, of our indigenous mammal population has not been fully investigated.

The Isle of Rum on the west coast of Scotland was chosen as an enclosed study site, having an abundance of ticks and a large deer population. Initially, ticks were collected and assayed by PCR, to check for the presence of B. burgdorferi. When this was confirmed, deer samples including spleen, kidney, liver, ear punch, urinary bladder and synovial tissue were collected and subjected to a similar assay. By refining our assay we were able to determine the incidence of B. burgdorferi infection in both adult and nymphal ticks, and locate the infected tissues in the deer tested for the first time. Having shown PCR to be an effective tool for investigation, we have broadened our study in the hope of discovering the true story of Lyme disease in Scotland.

RAPID DETECTION OF ALTERED PENICILLIN BINDING PROTEINS IN NEISSERIA MENINGITIDIS BY THE POLYMERASE CHAIN REACTION

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Meningococcal resistance to penicillin has been a relatively rare phenomenon. Recently, however, there has been an increase in the number of reported cases of penicillin resistance in these bacteria. Resistance can be due to either the production of a β-lactamase or alteration of penicillin binding protein 2 (PBP-2) which has a decreased affinity for penicillin. We have been developing PCR as a tool for the rapid identification of the altered PBP-2 and penicillin resistance.

Strains of Neisseria meningitidis from a number of geographical sources were examined by PCR with primers specific for PBP-2. Samples included NCTC control strains and clinical samples with a range of MICs to penicillin of 0.01 μg/ml (fully sensitive) to 0.64 μg/ml (relatively insensitive). Strains which were fully sensitive to penicillin produced almost identical results independent of serogroup and area of isolation. In contrast, strains with a reduced penicillin sensitivity showed a variety of patterns (including no amplification at all) with primers derived from the N. meningitidis gene. Several isolates were amplified with primers derived from N. flavescens as well as N. meningitidis. These results give further support to the idea of multiscalar penicillin resistance and are in accord with the notion of a mosaic gene structure for the penicillin resistant PBP-2.

DETECTION OF STAPHYLOCOCCAL ENTEROTOXINS BY THE POLYMERASE CHAIN REACTION

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Staphylococcal enterotoxins have long been recognised for their emetic and food poisoning abilities. They have recently been shown to have a powerful mitogenic effect on cells involved in the immune response. This effect is due to their ability to bind to the T-cell receptor and to HLA class 2 antigens on the surface of these cells inducing a proliferation response. Until recently, methods for the detection of the enterotoxins were based on growth of bacteria and immunological techniques for measuring toxin production. Five major serological groups have been described, labelled A-E. In this study, clinical and non-clinical isolates of staphylococcal species including the coagulase-negative staphylococci and positive controls were examined by the polymerase chain reaction for the presence of the enterotoxin genes.

Primers specific for each of the five enterotoxin genes were synthesised based on previously published sequences. Using restriction endonuclease digestion, each set of primers was shown to be specific for the enterotoxin gene in question. Initial studies have shown that the presence of the enterotoxin genes in any particular isolate can be determined from a single colony on a plate. There is a wide degree of diversity as to the presence of the various enterotoxin genes. We are currently investigating the different enterotoxin profiles of clinical isolates and also among the staphylococcal population of the normal body flora.

PCR OF BORRELIA BURGDORFERI IN HUMAN SAMPLES AS A VALID TOOL FOR THE DIAGNOSIS OF LYME DISEASE

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Lyme disease is a recently recognised disease affecting both animals and man. It is caused by the spirochaete Borrelia burgdorferi and transmitted by the bite of ixodid ticks. On initial infection, a characteristic rash known as Erythema Chronicum Migrans (ECM) appears around the tick bite. A flu-like illness generally accompanies this rash. Weeks or months later a second stage of the disease including meningitis and carditis may develop. Late stages of the disease, represented by an arthritis not unlike rheumatoid arthritis, may occur many years after the initial infection.

Precise diagnosis based on clinical observation is difficult as symptoms often mimic those of other neurological and rheumatological disorders. Culture of the spirochaete is slow
and ineffective, and serodiagnosis is problematic, giving both false positives and false negatives.

Considering the success of antibiotic treatment following correct diagnosis, and the severity of untreated cases, it is highly desirable that early and accurate detection of the spirochaete be achieved on a routine basis. PCR has been used successfully to detect B. burgdorferi in both cerebrospinal and synovial fluids. Using this approach, we have screened blood samples sent to Ninewells Hospital for routine screening of Lyme disease. The primers selected were used to amplify specific DNA regions of the B. burgdorferi outer surface protein B (OspB) located on its 49-kb linear plasmid. This set of primers has been shown to discriminate North American or European isolates of B. burgdorferi from related bacterial species, thus providing an effective screening tool for our study.

**Incorporated Detection of Chlamydia trachomatis from Males with Non-gonococcal Urethritis by the Polymerase Chain Reaction**

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The acceptance of the polymerase chain reaction (PCR) as a routine procedure, when compared with established detection methods, will depend to some extent on the widespread use of the technique in conjunction with the clinical follow-up of discrepant results.

The detection of Chlamydia trachomatis from patients with non-gonococcal urethritis (NGU) in Sheffield has been approximately 35–40%, although a higher prevalence is suspected clinically and epidemiologically. Use of PCR, therefore, offered the potential to increase the detection of C. trachomatis, and allowed us to investigate these cases. A total of 95 urethral specimens from males with NGU was tested for C. trachomatis by enzyme immunoassay (EIA-Novodiagnost™) and PCR at different laboratories in a blind trial. Of 32 specimens positive by EIA, two were found to be negative by PCR; however, PCR amplified chlamydia DNA in a further 16 samples. For those patients who were positive for C. trachomatis by PCR only, 14 had symptoms as well as signs of infection, with 10 displaying large numbers of pus cells on microscopy. In this group, contact history data was unavailable in the majority of cases where casual partners could not be traced. However, four patients were found to have chlamydia-positive partners.

**Typing of Enterococcus faecalis from Faeces and Urine**

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Enterococcus faecalis is a common cause of urinary tract infection (UTI) in hospitals and is also a common member of the normal faecal flora. An investigation was carried out into endogenous infection as a cause of E. faecalis UTI.

The first stage was to identify the number of E. faecalis strains carried by normal individuals. Up to 12 such organisms from each of 26 faecal samples were typed by antibiogram and biochemical methods. Sets of organisms from five individuals were also typed by a DNA restriction method.

The results showed that 21 (80.8%) of 26 people carried only one E. faecalis type. Four (15.4%) carried two and only one (3.8%) had three strains. DNA results were always consistent with antibiogram and biochemistry.

The second stage is to investigate if one of the strains present in the faecal flora is the same as that causing infection in cases of E. faecalis UTI. In the one case so far investigated, the organisms from the urine and faeces had identical antibiograms and biochemistry. DNA studies will be used to confirm the identity of these strains.

**A Standard Protocol for Analysis of Electrophoretic Band Patterns**

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Further uses for electrophoretic techniques continue to be found in microbiology despite well-recognised difficulties concerning reproducibility and pattern recognition. A formula has been devised to translate band position into a figure representing percentage migration within a user-defined segment (defined by extrapolation of marker bands across the entire width of a gel). Results generated by using this formula on samples run on more than one gel may then be treated as if they had been run on a single “virtual gel” thereby improving both intra- and inter-gel reproducibility.

The formula states:

\[ R_{SB} = \frac{(D_{SB} - D_{MB})}{(D_{MB} - D_{MB})} \times 100 \]

Where

- \( R_{SB} \) is the relative migration of a band \( x \) produced by a sample within a particular segment (expressed as a percentage).
- \( D_{SB} \) is the distance travelled by band \( x \).
- \( D_{MB} \) is the distance travelled by the marker band defining the start of...
the segment (i.e., nearer the origin) and $D_{MB}$ is the distance travelled by the marker band defining the end of the segment (i.e., further from the origin), all distances being measured from the baseline (a line representing the position of the sample well).

**Serodiagnosis of Apparently Culture-Negative Endocarditis**
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Serum from 72 cases of apparently culture-negative endocarditis were immunoblotted against pressates of two enterococcal (Enterococcus faecalis, E. faecium), six streptococcal (Streptococcus bovis, S. sanguis, S. mitis, S. mutans, S. agalactiae, S. pneumoniae) species and Candida albicans. Immunoblotting identified the aetiological pathogen in 47% of cases; in 43%, this was species specific. In 32% of cases with apparently culture-negative endocarditis where there was a negative serodiagnosis, a different clinical outcome was found. In only 21% of cases did a clinical diagnosis of culture-negative endocarditis remain, with the pathogen being completely evasive in only 4%.

The use of immunoblotting greatly improved the clinical diagnosis of culture-negative endocarditis, identifying the causative organism in 47% of which 91% were streptococcal/enterococcal in origin.

**Detection of Chlamydia trachomatis in Patients with Acute Epididymitis**
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Infection with Chlamydia trachomatis is the most frequent cause of acute epididymitis in sexually active men under 45 years of age. However, due to the relative insensitivity of laboratory methods the detection rate does not confirm the importance of this organism when assessed by serological techniques. Furthermore, it is known that prostatic fluid is toxic to McCoy cells and is unsuitable for testing by tissue culture.

In an initial study, 11 patients presenting with acute epididymitis were examined for C. trachomatis by growth in tissue culture and antigen detection (EIA), and for other micro-organisms by conventional microbiological tests. In addition, the polymerase chain reaction (PCR) was applied to detect any residual C. trachomatis DNA in urethral and expressed prostatic secretions. Conventional tissue culture and EIA were able to identify C. trachomatis infection in four patients; PCR confirmed these infections and detected C. trachomatis in a further five cases. Gardnerella vaginalis was isolated from one patient in the absence of evidence in infection by any other organism, and in one patient no organisms were isolated.

These results confirm the diagnostic value of the PCR and the importance of C. trachomatis in epididymitis.

**Analyses of Skin Surface Lipid in Three Microbial Skin Diseases**
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The total casual skin surface lipid, the free fatty acids and the fatty acids of triglycerides in three groups of patients have been analysed by thin-layer and gas-chromatography. Two groups of patients had diseases associated with lipophilic organisms; acne vulgaris is associated with Propionibacterium acnes whilst pityriasis versicolor and seborrhoeic dermatitis are associated with Malassezia (Pityrosoporum) spp. The remaining patients had atopic dermatitis in which the skin is almost invariably colonised by Staphylococcus aureus. Discriminant analysis showed that all three groups of patients could be separated on the basis of skin lipid composition suggesting that lipid may be involved in predisposition to certain diseases. Differences in skin lipid pre- and post-treatment may reflect the changes in microbial flora during therapy or more directly reflect host changes in the skin ecosystem. Manipulation of skin lipid composition might prove an effective way of controlling some disease, perhaps by its effect on the skin flora.

**Aerobic Non-Lipophilic Coryneforms from Human Feet**
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Aerobic, non-lipophilic coryneform bacteria from the toe webs of coal-miners with tinea pedis have been studied by analysis of cell-wall sugars, lipids, and amino acids and by phenotype tests using the API CORYNE kit. Many of the isolates were assignable to the genus Brevibacterium, probably B. epidermidis, none was assignable to Dermabacter. Two apparently new taxa were recovered. One is probably close to Brevibacterium in that isolates may produce methane thiol and degrade tyrosine though not xanthine but they do not possess meso-DAP in the cell wall and colonies are yellow pigmented. The other is orange pigmented, does not produce methane thiol, degrade tyrosine or xanthine or possess meso-DAP. It also differs substantially in its lipid composition and is sensitive to mupirocin, a characteristic not shared by other coryneform genera from human skin. This brings to four the taxa of aerobic, non-lipophilic coryneform from human skin.

**A Taxonomic Study for the Genus Prevotella**
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Gram-negative anaerobic bacilli, previously known as the melanogenicus-oralis group of Bacteroides, have recently been assigned to a new genus, Prevotella.

Several approaches were used to characterise 22 reference strains of Prevotella (Bacteroides) spp. and 96 clinical isolates from 21 adult patients with periodontal disease. Strains were examined in tests for: conventional test reaction patterns (CTRP); fluorescence under UV light; gas-liquid chromatography (GLC); pre-formed enzyme tests; SDS-PAGE and pyrolysis mass spectrometry (PMS).

Species were distinguished by CTRP, GLC and pre-formed enzyme tests and an identification scheme was designed for non-pigmented and pigmented Prevotella spp.
Twelve groups (A-L) were obtained for 96 clinical isolates; 63 strains gave results identical with those of reference strains and 26 showed only minor variations. The commonest species were *P. buccae* (42), *P. veraldis* (15) and *P. oralis* (8). PMS and CTRP classifications were compared with a classification based on visual analysis of SDS-PAGE patterns. There was close correlation between CTRP, SDS-PAGE and PMS. Clustering membership in the PMS and SDS-PAGE classification was particularly good; both gave a larger number of clusters than the CTRP classification. Furthermore, SDS-PAGE and PMS divided two species into sub-groups: three in *P. buccae*, and five in *P. veraldis*.

**RECOGNITION OF MALASSEZIA FURFUR ISOLATES BY PULSE FIELD GEL ELECTROPHORESIS**

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The lipophilic yeast *Malassezia furfur* is a common commensal of post-pubertal individuals which has also been associated with the superficial skin diseases dandruff, seborrhoeic dermatitis and pityriasis versicolor. In recent years, *M. furfur* has also been reported as a cause of fungaemia in neonates receiving intravenous lipid emulsions.

There are no reported methods of strain recognition for *M. furfur* although various morphological groups can be identified by microscopy, SDS-PAGE and serological techniques. Karyotyping by pulsed field gel electrophoresis has successfully differentiated strains of *Candida albicans* (2) and serotypes of *Cryptococcus neoformans* (4). This technique was therefore applied to a collection of *M. furfur* isolates obtained from the skin of inpatients and nurses of obstetrics and paediatrics wards and the range of karyotypes was compared. The results demonstrated close agreement with the morphological groups, but little strain variation was observed.

**ESTABLISHMENT OF INTERPRETATIVE STANDARD FOR SUSCEPTIBILITY TESTING OF METRONIDAZOLE AGAINST HELICOBACTER PYLORI**


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Susceptibility testing of *Helicobacter pylori* to metronidazole (MTZ) has an important role in the management of *H. pylori* infection as clinical isolates with a minimum inhibitory concentration (MIC) of 16 mg/L or more may not respond to therapy. Simpler and more precise methods of susceptibility testing are required for routine use. The aims of this study were (i) to determine the distribution of MIC of clinical isolates of *H. pylori* by a plate dilution method (PDM); and (ii) using the MIC values obtained to establish a breakpoint system by correlating the zone diameters of inhibition given with a 5-mg MTZ-disk diffusion test (DDT). The susceptibilities of 121 clinical isolates were determined by both the PDM and the DDT in duplicate during 3, 4, 5, 6 and 7 days of incubation. Correlation of MICs and zone diameters was calculated by logarithmic regression, and the equation X1S2 + X2S1/(S1 + S2) was used to establish the minimum zone diameter indicating sensitivity of the isolate. The distribution of MICs of MTZ against *H. pylori* exhibited a bimodal distribution among sensitive and resistant isolates. The highest percentage of isolates among the sensitive isolates was those with MIC of 1 mg/L and among resistant isolates were those with MIC of 32 mg/L. The best correlation between MICs and zone diameters was obtained after incubation for 4 days (r = 0.9960) followed by incubation for 3 days (r = 0.9819). Using the above equation, a zone diameter of 12 mm was found to be the most useful in determining sensitivity. After incubation for 3 days a 3% false reading for both sensitive and resistant isolates occurred. In conclusion, distribution of MIC of MTZ against *H. pylori* was bimodal. Results from PDM and the DDT showed a good correlation. A zone of inhibition more than 12 mm with the DDT indicated sensitivity of the isolates.

**FAILURE OF ANTIREACTIVATION VACCINES IN A DORMANCY MODEL OF MURINE EXPERIMENTAL TUBERCULOSIS**

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Since most people in developing countries are infected with tubercle bacilli when young, "antireactivation" vaccines would have a much greater effect than "preventive" vaccines of the BCG type. We described the results with putative antireactivation vaccines in a dormancy model in the mouse. Our BALB/c mice were infected intravenously with *Mycobacterium tuberculosis*, H37Rv and the disease allowed to progress for 2 weeks to reach 6-49 log_{10} cfu/spleen. Treatment was given with isoniazid 20 mg/kg and pyrazinamide 1000 mg/kg in the diet for 14 weeks. At the end of treatment, only one spleen from the organs of 20 mice was positive. The mice then received: A, a heat-killed *M. vaccae* vaccine into footpads; B, isoniazid-resistant BCG divided between three subcutaneous sites, repeated after 2 weeks; or C, saline into footpads. Three months later, the entire spleens and lungs were inoculated into liquid medium to measure the relapse rate to a culturable infection. The proportions of positive cultures from spleens were 32 of 45 in group A, 28 of 45 in B and 39 of 49 in C and from lungs 16 of 45, 16 of 45 and 18 of 49, respectively. The vaccines had no influence on the relapse rate.

**THE ROLE OF PCR IN THE DIAGNOSIS OF TUBERCULOSIS**

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Recently several large clinical trials have shown that, although PCR is more sensitive than laboratory culture for the detection of *Mycobacterium tuberculosis*, there may be problems with interpretation of PCR positive, culture negative specimens.

To evaluate the role of PCR more accurately, we compared laboratory culture of pleural fluid with PCR of the pleural fluid. Culture and histology of the pleural biopsy as well as clinical evidence were used to confirm PCR results.

Eighty-four adult patients with pleural effusions were entered into a prospective study. On the basis of the results of conventional detection techniques as well as an evaluation of the clinical data, 53 patients were considered to have active tuberculosis. Analysis of the pleural fluid confirmed a sensitivity for PCR of 81%. The sensitivity of pleural fluid culture, culture of pleural biopsy, and histology of biopsy was 52.8%, 69.8% and 77.3% respectively. There were, however, seven PCR-positive results within the control group; six of these were in-patients with malignant effusions. We conclude that for the diagnosis of *M. tuberculosis*, PCR is more sensitive than laboratory culture as determined by
the analysis of pleural fluids. Positive PCR results among patients with malignant effusions may be false positives or the result of latent tuberculous infections.

**BCL-2 expression in Epstein-Barr virus (EBV) associated post-graft B lymphoproliferative disease**

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Over-expression of the B cell lymphoma-2 gene protein (bcl-2) appears to provide a selective growth advantage to B cells in vitro and protect them from programmed cell death/apoptosis. Recent DNA transfection studies have shown that enhanced survival of EBV-infected human B cells in vitro is conferred by the EBV latency-associated membrane protein, LMP, through LMP-induced upregulation of bcl-2.

This immunocytochemical study examined bcl-2 expression in EBV-related post-graft B cell tumours in which most of the tumour cells displayed the full repertoire of latent viral antigens usually associated with EBV-induced immortalsation of normal B cells in vitro. The latent viral gene products include EB nuclear antigens (EBNAs) 1-6 and LMP, of which EBNA2 and LMP appear to have essential roles in maintaining continued cell proliferation. Positive bcl-2 staining was found in about 50% of tumours. Double labelling studies revealed selective bcl-2+/EBNA2+ and bcl-2+/LMP+ expression on only a proportion of neoplastic cells. This phenotypic heterogeneity suggests that EBNA2 as well as LMP may serve in bcl-2 regulation and that bcl-2 prolonged cell survival may contribute to the outgrowth of neoplastic B cells in some but not all cases of post-graft B lymphoproliferative disease.

**B cell leukaemia/lymphoma (BCL)-2 gene expression in nasopharyngeal carcinoma and normal oronasopharyngeal epithelium**

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Bcl-2 gene expression is associated with inhibition of programmed cell death in B cells. In recent DNA transfection studies, the prolonged survival of Epstein Barr virus (EBV) infected B cells relates to expression of the latent viral gene product, latent membrane protein (LMP)-1 and LMP-1 induces upregulation of bcl-2. LMP-1 also has tumourigenic properties, and in EBV associated undifferentiated nasopharyngeal carcinoma (uNPC), LMP-1 forms part of the restricted latent viral antigen profile displayed by the tumour cells.

This study examined the relationship between bcl-2 and LMP-1 expression in frozen and routinely processed samples of EBV-associated uNPC. Immunocytochemical demonstration of bcl-2+ tumour cells in 12 (86%) of 14 samples of uNPC was confirmed by immunoblotting in six cases. Since bcl-2 expression in uNPC has not been reported previously, further studies showed that bcl-2 was present in 5 (62%) of eight histological variants of NPC which had no evidence of EBV involvement, and was constitutively expressed in some basal cells of normal (EBV−) oral and nasopharyngeal squamous epithelium. By contrast, bcl-2 was not expressed in oral hairy leukoplakia which indicates the presence of replicating EBV. These findings indicate that bcl-2 is not exclusively dependent on EBV latent gene expression in NPC and may be involved in growth regulation of some normal squamous epithelium.

**Epstein Barr virus (EBV) associated T cell lymphoma in AIDS**

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The strictly B lympho-epitheliotropic nature of Epstein Barr virus (EBV) has been challenged by recent evidence that normal T cells and thymocytes support short-term EBV infection in vitro. Furthermore, EBV involvement has been clinically documented in small numbers of patients with T cell disorders ranging from reactive hyperplasia to high grade malignant lymphoma.

Here we report oral mucosal-associated T cell lymphoma in three HIV-seropositive individuals in whom the tumour cells were found to contain EBV DNA and express latent viral gene products. The T cell nature of the lesions was established by immunophenotyping, and by gene re-arrangement studies with the polymerase chain reaction. AIDS-related T cell lymphoma may be a new clinical entity and EBV involvement in these lesions has not been described previously. An important implication of these findings is that severe immunosuppression induced by HIV (and possibly other as yet undefined causes) may increase the range of host cells susceptible to EBV infection and sustain neoplastic transformation in these cells.

**ORAL PRESENTATIONS**

**The rapid detection and typing of Neisseria meningitidis by polymerase chain reaction**

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Neisseria meningitidis remains one of the most common causes of bacterial meningitis in the UK. Whilst its diagnosis is usually straightforward by examination of cerebrospinal fluid (CSF), this can be a problem when the number of organisms are low, or the infection has been partially treated by antibiotics. Due to its sensitivity and the ability to detect non-viable bacteria, we have investigated the use of the polymerase chain reaction (PCR) for the detection of *N. meningitidis.*

Over 100 isolates have been examined by PCR with primers designed from the outer membrane protein 1 (OMP1) sequence, using dilute suspensions of bacteria within sterile CSF, or CSF taken from patients with bacterial
mucococcal meningitis. As well as NCTC meningococcal and non-mucococcal controls, clinical isolates included a number of serogroups and serotypes from a wide range of geographical sources.

Bacteria could be detected by PCR in samples of CSF from clinical cases of meningococcal meningitis. Overall, a variety of patterns was seen following PCR, both with respect to slight variation in the size of the amplified segment and more obvious variation in restriction endonuclease (RE) profile. In some cases serogroups could be distinguished by RE digest pattern, often with further subdivision possible, even though OMP1 is not responsible for serogroup or serotype specificity. PCR examination of samples of CSF represents a promising tool for the rapid diagnosis of meningococcal meningitis.

**PCR in the investigation of 4-quinolone resistance**

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Resistance to the 4-quinolone antibiotics is a gradually increasing phenomenon. In clinical isolates the cause of this resistance is primarily due to a mutation in the gyrA gene, which codes for GyrA subunit of DNA gyrase, the enzyme target of the 4-quinolones. In *Escherichia coli* and *Staphylococcus*, a single coding change towards the 5' end of the gyrA gene results in a quinolone-resistant gyrase enzyme. This change, due to one base substitution, can be detected at the molecular level because it removes a HincII restriction site. We have amplified the relevant region from the chromosomal DNA of strains of *E. coli* and *Staphylococcus epidermidis* by PCR and shown that quinolone-resistant strains have altered restriction fragment profiles compared to sensitive strains.

Sequencing of the PCR products showed a C to T transition resulting in an amino acid change from serine to threonine. As well as NCTC meningococcal and non-mucococcal meningococcal controls, clinical isolates included a number of serogroups and serotypes from a wide range of geographical sources.

**Susceptibility to ciprofloxacin in Neisseria gonorrhoeae**

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Ciprofloxacin is now recommended as a first line therapy for gonorrhoea. However, there is little information regarding the relationship between minimum inhibitory concentration (MIC), treatment failure and dosage. To monitor the susceptibility of *Neisseria gonorrhoeae* to ciprofloxacin and to identify potentially resistant isolates we have used a breakpoint agar dilution method. Media containing ciprofloxacin at 0.008, 0.03 and 0.12 mg/L were used. To date we have tested 1163 consecutive isolates of *N. gonorrhoeae* from patients attending St Mary's Hospital, London and have found the majority, 1101 (94.6%), to be sensitive to ≤0.008 mg/L. Of the remainder, 59 (5.1%) had a MIC of 0.015–0.03 mg/L and three (0.3%) had a MIC of >0.03 mg/L. These three isolates had MICs of 0.12 mg/L (two strains) and 0.5 mg/L to ciprofloxacin and of 256 mg/L to nalidixic acid.

In-vitro spontaneous mutation experiments of clinical isolates to ciprofloxacin resistance have shown the rate to be between 10⁻⁷ and 10⁻⁹. Two classes of mutants were obtained—those exhibiting 2–4-fold increase to ciprofloxacin with little or no concomitant increase to nalidixic acid and those showing 64-256-fold increase to ciprofloxacin with associated 128-fold increases to nalidixic acid. We have experienced no clinical failures during the last 2 years with a 500 mg stat dose for gonorrhoea but would recommend caution in the use of low therapeutic doses to prevent selection of these mutants in vivo.

**The activity of ciprofloxacin alone, and of ciprofloxacin with rifampicin against Mycobacterium tuberculosis**

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The in-vitro susceptibility to ciprofloxacin of *Mycobacterium tuberculosis* has been studied by a number of methods. Using the BACTEC radiometric growth detection system, we have demonstrated that inoculum size is an important determinant of the apparent level of resistance as determined by this system. Drug resistance is not entirely masked, however, even with an inoculum substantially smaller than would normally be used.

Twenty-five isolates of *M. tuberculosis* complex (including five resistant to more than one first line agent) were studied. Eleven isolates were further studied by subculture from drug-containing to drug-free vials after incubation for 7 days. Twenty-four isolates were inhibited by ciprofloxacin at a concentration of 1 mg/L, one isolate was inhibited at a concentration of 2 mg/L. All five isolates resistant to the primary antituberculous agents were sensitive to ciprofloxacin at the level of 1 mg/L. From all 11 isolates subcultured after incubation for 7 days in ciprofloxacin-containing vials, mycobacteria were recovered.

In view of the reported antagonism of rifampicin and ofloxacin for *M. fortuitum*, the combination of rifampicin and ciprofloxacin for *M. tuberculosis* was studied. Antagonism was not detected. Ciprofloxacin has substantial activity against organisms of the *M. tuberculosis* complex. However, further study of the effect of combinations of ciprofloxacin with other agents together with clinical studies are required to clarify its role.

**Media effects on the susceptibility of Xanthomonas maltophilia to β-lactam antibiotics**

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*Xanthomonas maltophilia* is an opportunist pathogen in seriously ill patients and is resistant to many antibiotics, including β-lactams. It appears more resistant to many penicillins and cephalosporins on Mueller Hinton (MH) agar than on Diagnostic Sensitivity Test or IsoSensitest (IST) agars. We showed, additionally, that its susceptibility varied according to the dilution at which these media were prepared. MIC and disk tests were performed on solidified MH and IST media prepared at 0·1, 0·3, 1 and 3 x the nutrient concentrations recommended by the manufacturer, but with a constant agar concentration; nine of 10 *X. maltophilia* strains showed increased susceptibility to meropenem, cefotaxime, piperacillin, cefoperazone and latamoxef as the nutrient concentrations of the media were
increased. A single NCTC strain (10257) showed the opposite behaviour on MHA and behaved inconsistently on ISTA. Medium-dependent susceptibility was noted in laboratory mutants which constitutively hyperproduced, or were deficient for, the two chromosomal β-lactamases which are normally inducible in *X. maltophilia*. This observation belies the possibility that the medium effect could relate to the efficiency of β-lactamase induction. Altered permeability, therefore, seems a likely cause of the phenomenon, and we noted that growth in richer media led to the induction of additional outer membrane proteins with mol. wts of 22–25 kDa in *X. maltophilia*. These may affect antibiotic uptake, but this hypothesis remains to be tested. Irrespective of their precise basis, these medium effects suggest that clinical laboratory susceptibility results for *X. maltophilia* should be interpreted with the utmost scepticism.

**Pathogenicity of antimicrobial multi-resistant Escherichia coli isolated from urinary tract infection**

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During the period August 1989 to July 1990, 94 isolates of *Escherichia coli* resistant to amoxycillin, chloramphenicol, streptomycin, sulphonamides, tetracycline and trimethoprim were cultured from specimens of urine submitted for routine culture at St Thomas' Hospital from patients with urinary tract infection.

Clinical information was obtained from 64 patients enabling the infection to be classified as significant (upper tract infection and cystitis) or symptomless colonisation. Data were also obtained on underlying conditions predisposing to urinary tract infection and the patients were classified as compromised or uncompromised; 44 (69%) had significant infection and 20 (31%) had symptomless colonisation, 28 (44%) patients had underlying conditions predisposing to infection and 36 (56%) were uncompromised. These results indicate that antimicrobial multi-resistant strains are as likely to infect an uncompromised patient as a compromised patient, and once established more likely to cause a significant infection than symptomless colonisation. These findings are evidence against the widely held belief that antimicrobial multi-resistant organisms have little or no pathogenic potential.

**Cytochemical demonstration of bacterial enzyme activity: a new approach to rapid presumptive identification**

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Light microscopic cytochemical techniques offer a direct means of rapidly assigning specific activities to individual microbial cells without an intervening period of in-vitro culture. They should, therefore, allow determination of identity and activity at a stage in processing when organisms present in a clinical specimen are detectable by microscopy.

Establishment of a procedure for immobilising viable bacteria on optical quality glass has enabled us to develop cytochemical techniques which demonstrate β-galactosidase and cytochrome oxidase in unfixed bacterial cells. This report documented our first steps in examining the potential clinical application of these procedures. β-Galactosidase demonstration was achieved with indoxyl (100 min), indoxyl-tetrazolium (20 min), and fluorescein digalactoside (FDG) (8 min) based techniques. A 10-min diaminobenzidine (DAB) technique demonstrated oxidase activity. FDG and DAB techniques were applied to over 40 recent blood culture isolates of gram-negative bacilli. No discrepancies from the conventional macroscopic lactose fermentation and oxidase tests were observed. Preliminary studies on preparations made directly from blood cultures indicate that the presence of blood-cell debris does not affect the reliability of the FDG and DAB tests.

Cytochemical tests offer a promising new approach to the rapid provision of information relevant to the management of acute infections.

**Symposium: Microbiology and the Mouth**

(in collaboration with the Oral Microbiology and Immunology Group)

Chairman: I. Phillips

**Oral manifestations of HIV infection**

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Oral manifestations of HIV infection are not only an integral part of the diagnosis and management of HIV infection, they are frequently the earliest signs of HIV infection and conversion to the full AIDS syndrome. To date, over 40 oral signs of HIV infection have been reported including eight types of fungal infection, 11 types of bacterial infection, nine types of viral infection and various lesions not of secondary infective origin. Recently these lesions have been classified into three groups: I, those strongly associated with HIV infection; II, those commonly associated with HIV infection; and III, those possibly associated with HIV infection. Category I contains lesions including hairy leukoplakia and pseudo-membranous candidiasis which are almost pathognomonic of HIV infection as well as necrotising ulcerative gingivitis, HIV-associated periodontal disease and HIV gingivitis, oral Kaposi's sarcoma and non-Hodgkin's lymphoma. Up to 40% of those with HIV and 80% of those with AIDS may present with candida infection. Hairy leukoplakia can be found in up to 14% of HIV-positive and may be a prognostic marker for early conversion to AIDS. A similar range and prevalence of oral lesions has been reported with HIV-2 infection.

Recognition of the oral manifestations of HIV infection is an essential part of management. The oral cavity provides an accessible mucosal site not only for the diagnosis but also for investigation of the effect of HIV infection on mucosal immunity.

**Oral viral infections**

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Viruses may cause disease by establishing acute infections, persistent infections (including those exhibiting latent or oncogenic potential) and by stimulating autoimmunity.
are relevant to oral disease. Acute viral infections of the mouth include herpetic gingivostomatitis, herpangina and hand, foot and mouth disease. Other systemic viral infections, e.g., infectious mononucleosis, may also have oral manifestations. Latent infections are epitomised by herpes viruses, and reactivation of both herpes simplex virus (HSV) and varicella zoster virus may affect the territory of the trigeminal nerve. Asymptomatic shedding of herpes viruses into saliva requires further investigation. Epstein-Barr virus (EBV) is causally linked with both Burkitt’s lymphoma and nasopharyngeal carcinoma, and is consistently associated with oral hairy leukoplakia. Some human papilloma viruses are strongly implicated in the aetiology of oral squamous cell papillomas. Oral diseases with an immune pathogenesis may be triggered by viruses. Latent EBV is present in salivary glands and the vigorous immune response in Sjögren’s syndrome may result from breakdown of the mechanism for viral persistence. Circumstantial evidence suggests a potential role for HSV in the aetiology of aphthous ulceration and Behcet’s disease, possibly by impairing T cell immunoregulation. In summary, viruses may be responsible for many oral diseases. The scope of their involvement is likely to widen with application of molecular biological techniques.

ACTIVE AND PASSIVE IMMUNISATION IN THE PREVENTION OF DENTAL CARRIES

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Streptococcus mutans is the principal organism in the development of dental caries. A streptococcal antigen (SAI/II) with a M, of 185 kDa and a cross-reactive SA with a M, of 3800 have been extensively investigated. They are expressed on the cell surface of S. mutans and the 185-kDa SA may function as an adhesin and has been cloned and sequenced. Both, high and low M, SA elicit natural serum and salivary antibodies and T cell responses in man. The antigens and a synthetic peptide of 17 residues are immunogenic in non-human primates in which active immunisation elicits a protective immune response against colonisation with S. mutans and the development of dental caries.

Passive immunisation by monoclonal antibodies (MAb) to SAI/II decreases colonisation of teeth by S. mutans and the development of dental caries. Passive immunisation studies were then pursued in human subjects. Topical application of MAb to SAI/II in man prevented colonisation of both artificially implanted exogenous strains of S. mutans, as well as natural recolonisation by indigenous S. mutans. The properties of the protective MAb were then investigated and the epitope specificity within the SAI/II molecule was found to be essential but not the immunoglobulin class. The requirement for complement activating and the phyocytog binding sites of the Fc fragment of MAb was not essential, as the F(ab)2 fragment of the MAb was as protective as the intact IgG, but the Fab fragment failed to prevent colonisation of S. mutans in the human mouth. Prevention of recolonisation was specifically restricted to S. mutans, as the proportion of other organisms such as S. sanguis failed to show a significant change. The surprising feature of these experiments was that protection against colonisation of S. mutans lasted up to 2 years, although MAb was applied for only 3 weeks and functional MAb was detected on the teeth only up to 3 days following application of the MAb. The long-term protection could, therefore, not be accounted for by a persistence of MAb on the teeth but may be due to a shift in the balance of the oral flora which replaced the ecological niche occupied by S. mutans by another plaque organism.

CROSS-INFECTION IN DENTISTRY

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The medical microbiologist must be aware of the problems of cross-infection in dentistry in order to provide dentists with accurate information and realistic advice. Dentistry presents many potential cross-infection problems that are compounded by a rapid patient turn-over rate. The “high risk” patient is the main potential source of infection, principally for the dentist and surgery assistant but other personnel may also be considered. Significantly, dental personnel may also be a source of infection. The most important potential micro-organisms are Hepatitis B, Human Immunodeficiency Virus (HIV), Herpes simplex, antibiotic multi-resistant staphylococci and tuberculosis. “At risk” patients require special consideration and include some of the “high risk” groups and also the immunocompromised patient such as those with acute leukaemia who are especially vulnerable to a wide range of micro-organisms such as Pseudomonas spp., the common cold and influenza viruses. Dental units are frequently colonised by Pseudomonas spp. The prevention of cross-infection through dentistry is about awareness and common sense coupled with a sound microbiological knowledge of the nature of micro-organisms. In particular, correct instrument sterilisation procedures and routine disinfection of potentially contaminated areas must be strictly adhered to; hands and eyes must be protected; relevant immunisations must be kept up-to-date; and contaminated waste correctly disposed of.

PATHOGENESIS OF BACTERIAL ENDOCARDITIS

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Bacterial endocarditis remains an important cause of morbidity and mortality. It is characterised by the production of thrombotic vegetations at sites of endocardial damage, which become colonised by bacteria. Although many organisms can cause endocarditis, the oral streptococci are the most common, and among these Streptococcus sanguis and S. oralis predominate. The pathogenic mechanisms by which organisms give rise to endocarditis are poorly understood but dextran production, presence of lipoteichoic acid, interaction with fibronectin and aggregation of platelets have all been suggested as important in S. sanguis-mediated endocarditis. There is some controversy as to which of the above is the most important factor but some work implicates surface protein as responsible for bacterial adherence to vegetation. Also, the ability of S. sanguis to interact directly with platelets would explain its predilection for thrombotic vegetations and the majority of strains of this organisms cause platelets to aggregate. Recently, a 65 kDa protein has been suggested as the ligand responsible for platelet aggregation and we have evidence that a platelet-membrane glycoprotein (lb) is the target receptor.
Escherichia coli

mutlinucleation and giant cell formation in Vero cell

0 groups being 06, 032, 075 and 088. In addition

E. coli have been isolated from sheep and deer. The

commonest

monolayers. Since 1990, all

cells. Of the 235 bovine isolations,

cases, septicaemia. Some of the cattle strains produced a

mannose-resistant adhesin and others were associated with

isolates were associated with disease conditions ranging

705

from diarrhoea to acute haemorrhagic enteritis and, in some

particulars, in the tissues of diarrhoeic piglets was much smaller. The

EXPERIMENTAL INFECTION OF PIGLETS WITH

cytotoxic necrotising factor (CNF)-

producing Escherichia coli

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Two litters of 2-3-day-old piglets were infected orally with

CNF-producing Escherichia coli. Of the six piglets in the

litter challenged with 10^9 E. coli O32, two died the day after

challenge and three developed diarrhoea. Large numbers of

the challenge strain were isolated from the viscera of those

dying of septicaemia, whereas the number of organisms in

the tissues of diarrhoeic piglets was much smaller. The

pathological changes in the major organs were consistent

with diarrhoea and the changes included hepatitis, glomeru-

lonephrosis and limited cerebrocortical necrosis. An exuda-

tive broncho-interstitial pneumonia occurred suggesting that

entero-invasion may have occurred.

The second litter was infected orally with 10^9 E. coli O32

and the piglets were killed at 1, 3, 4, 5, 6 and 7 days after

challenge. Large numbers of the challenge strain were

recovered from the faeces and intestinal contents and two

piglets developed diarrhoea 2-3 days after challenge. The

enterocolitis was milder than that observed in the previous

litter but of a more persistent nature. Toxaemic changes in

internal organs and pulmonary lesions were detected. The

results suggest a pathological role for CNF toxin.

THE PATHOGENICITY OF HUMAN AND ANIMAL STRAINS OF Fusobacterium necrophorum

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Animal necrobacillosis is caused by Fusobacterium necro-

phorum (FN) biovar A, but other bacteria are often also

present. Though the minimum infective dose (MID) of FN

is high (>10^9 organisms), rapidly fatal necrobacillosis can

be produced in mice by subcutaneous inoculation with pure

cultures. Thus for example, in one experiment 14 x 10^9

organisms fatally infected 25 of 26 mice. The MID can,

however, be reduced to <10 organisms by suspending the

fusobacteria in sub-lethal doses of broth cultures of other

bacteria, e.g., Staphylococcus aureus.

Seven human strains of FN were studied. They originated

from a neck abscess (1), cerebrospinal fluid (1), and blood

cultures (5), but their history of laboratory subculture was

unknown. Six of the strains resembled biovar B strains in

being much less pathogenic than animal biovar A strains:

doses of 10 x 10^6 to 40 x 10^6 organisms given subcutaneously

to mice produced no more than mild ulcerating lesions that

healed rapidly, and infectivity was not appreciably enhanced by S. aureus. The seventh strain (A2433), however, in a dose

of < 60 x 10^6 organisms produced protracted infections with

severe lesions, and its infectivity was greatly enhanced by S.

aureus. The clinical signs and lesions produced in mice by

strain A2433 nonetheless differed strikingly from those produced by animal biovar A strains.

The results suggest that the term "necrobacillosis" (FN infection) as used by veterinary and medical microbiologists

refers to diseases that differ in important respects.

CAMPYLOBACTER JEJUNI AS AN AEROBE

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When isolated from the gut, Campylobacter jejuni requires

microaerobic conditions for successful isolation. However, in

the environment this organism undergoes a number of

fundamental changes. While it is not able to multiply outside

the gut, other than on laboratory media, in the environment

C. jejuni becomes adapted for prolonged survival. In

non-nutritive surroundings, e.g., natural waters, the organism

progressively becomes coecal, non-culturable but still viable,

and finally dies. In survival experiments when nutrition is

available, by making surface viable counts it can be

demonstrated that the organism adapts to an aerobic

metabolism and that survival is longer in air as opposed to

maintained microaerobic conditions. Surviving cells grow

on subculture in air or microaerobic conditions with equal

facility and the aerobic cultures can be subcultured appar-

ently indefinitely in air. The adoption of aerobic metabolism

is accompanied by changes in outer membrane protein

profiles on SDS-PAGE and by altered colonial morphology.

Serotype reactions and the ability to colonise mice are

unaltered.

Aerobic forms of C. jejuni are readily produced experimen-
tally but recently we have isolated such aerobic forms

directly from environmental water samples.

NUCLEOSIDE-DEPENDENT REPLICATION OF IFDO

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IFDO is a novel replicating agent of comparable size to

small viruses, which multiplies in cell-free medium and has

characteristics in common with prions. It differs from prions

in being inactivated by chemicals which alter nucleic acid

and by RNAase.

A chemically defined growth medium has been prepared

which contains Tween 80, Ca^2+, P_{2}O_{5}^-, NaCl, glutamic

acid, trypsin, haemoglobin, horse high density lipoprotein

(HDL) or human low density lipoprotein (LDL) and
nucleosides. Omission of any of the above ingredients except NaCl prevents replication of the agent.

It is concluded that IFDO replicates by a biological process, and that it is a self-replicating nucleic acid which utilises host-derived protein. Self-assembly of nucleic acid replicas of IFDO is plausible if IFDO is a RNA-ribonuclease of the type exemplified by *Escherichia coli* RNAase P. IFDO may be the first example of the hypothetical self-replicating RNA presumed to have been involved in the evolution of living agents.

**COMPARATIVE DIAGNOSIS OF PEPTIC ULCER DISEASE CAUSED BY *HELICOBACTER PYLORI***

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The increasing awareness that *Helicobacter pylori* is important in the development, treatment and recurrence of peptic ulceration has placed greater emphasis on the accuracy of identification of the organism's presence in the management of peptic ulcer disease. A range of tests [culture, histology and urease activity (CLO test) of gastric biopsy material] and serological measurement of specific antibodies were performed on 134 patients in whom peptic ulcer disease was suspected. The sensitivity, specificity and predictive values of histology and urease test compared with culture ranged between 90% and 97% whereas the corresponding values for the serological assays were lower (ELISA 61–75% and latex agglutination 61–67%). Concomitant treatment with non-steroidal anti-inflammatory agents (NSAIDs) further reduced the sensitivity and specificity of the serological tests. No difference was seen with the histology or urease test on gastric biopsies from patients on NSAIDs. These findings suggest that *H. pylori* is more reliably diagnosed by culture, histology and CLO-test than by the two serological tests especially in patients taking NSAIDs. However, consideration should be given to the potential benefits in that the latter tests are non-invasive and could be appropriately used as screening tests.

**EFFECT OF A REDOX AGENT ON THE SUBGINGIVAL MICROFLORA OF PATIENTS WITH CHRONIC PERIODONTITIS***

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Redox potentials as low as -300 mV exist subgingivally in patients with periodontitis enabling the survival of anaerobic periodontal pathogens. By raising the subgingival redox potential it should be possible to create an environment unsuitable for the growth of these bacteria. Therefore, a small-scale trial was carried out to evaluate the effect of a redox agent, methylene blue, on various microbiological and clinical indices of chronic periodontitis.

Methylene blue was applied subgingivally on each of 7 test sites in seven patients and the sites were evaluated microbiologically and clinically for 14 days; 25 control sites received water. The test sites showed statistically significant differences from the control sites in terms of changes in microbiological and clinical indices. In the test sites the proportions of anaerobes, gram-negative anaerobes, spirochaetes and motile bacteria decreased, as did the crevicular fluid flow, while the proportions of facultative anaerobes and cocci increased. These changes are indicative of a shift in the microflora towards one that is more compatible with periodontal health.

The encouraging results of this preliminary study suggest that the use of redox agents in the treatment of chronic periodontitis warrants further investigation.

**SURFACE-ASSOCIATED MATERIAL FROM PERIODONTOPATHOGENIC BACTERIA STIMULATE BONE RESORPTION BY MULTIPLE PATHWAYS***

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Anaerobic bacteria have been implicated in the alveolar bone loss of periodontitis but the mechanism(s) involved remain unclear. We have extracted the capsules from three periodontopathogens: *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Eikenella corrodens* by gentle stirring in saline. The extracted material, hereafter referred to as surface-associated material (SAM), was shown to stimulate dose-dependent resorption of murine calvarial bone in vitro with significant resorption being seen with SAM 10 ng/ml.

To determine the mechanism of resorption, bone cultures were stimulated with SAM in the presence of graded concentration of various inhibitors including: (i) indomethacin; (ii) interleukin-1 receptor antagonist (IRAP); (iii) a neutralising antibody to murine TNF-α and (iv) a neutralising antibody to murine IL-6. The bone resorbing activity of SAM from *P. gingivalis* was completely inhibited by indomethacin, IRAP and anti-TNF. SAM from *E. corrodens* was moderately inhibited by indomethacin and almost completely inhibited by anti-TNF. In contrast the SAM from *A. actinomycetemcomitans* was unaffected by all inhibitors. Neutralising IL-6 had no effect on the action of any SAM. Thus multiple pathways of resorption may produce alveolar bone loss in periodontitis.

**SERUM FROM PERIODONTITIS PATIENTS CONTAIN ANTIBODIES CapABLE OF INHIBITING BONE BREAKDOWN INDUCED BY SAM FROM PERIODONTOPATHOGENS***

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Solubilised surface-associated material (SAM) from a number of periodontopathogens has been shown to be a potent stimulator of bone resorption in the murine calvarial bone resorption assay. Patients with severe generalised periodontitis (SGP) and localised juvenile periodontitis (LJP) have serum antibodies to SAM from such bacteria. Addition of sera from SGP patients containing high titres of antibodies to SAM from *P. gingivalis* to calvarial cultures stimulated with SAM from this organism completely inhibited bone resorption. Sera from patients with low titres of antibody failed to inhibit resorption. High titre sera (containing antibodies to SAM from *A. actinomycetemcomitans*) from patients with LJP were added to calvaria stimulated with SAM from this organism. Of six high titre sera tested, only four inhibited bone breakdown. Low titre sera were inactive.

Thus, the antibody response to the SAM from periodontopathogens may serve to inhibit or slow alveolar bone resorption but this protective activity appears to be dependent on the idiotypic nature of the antibody response.
CLINICAL SIGNIFICANCE OF AUXOTROPHIC MUTANTS OF PSEUDOMONAS AERUGINOSA IN ADULTS WITH CYSTIC FIBROSIS

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We have reported previously that many sputum isolates of Pseudomonas aeruginosa from patients with cystic fibrosis (CF) are auxotrophic and that the majority of auxotrophs which depend on a single factor, require methionine for growth.

We compared (a) the percentage of auxotrophs in the total bacterial count in sputum from 14 clinically stable CF out-patients and 30 CF in-patients with acute exacerbations, and (b) the antimicrobial susceptibilities of auxotrophic and prototrophic isolates of P. aeruginosa retrieved from the same sputum sample of eight CF patients.

Auxotrophic mutants of P. aeruginosa were present in the sputum of 40 (91%) of 44 CF adults. The majority (>50%) of colonies were auxotrophic in 22 of 30 patients with acute exacerbations compared with six of 14 stable out-patients (p = 0.05).

MIC values of 12 anti-pseudomonal agents (four penicillins, three aminoglycosides, ciprofloxacin, colistin, cefazidime, aztreonam and imipenem) were established for 18 auxotrophs and 15 prototrophs from eight CF patients. For each patient and antimicrobial agent, the most resistant and most sensitive isolates were identified, giving a total of 192 possible results. For 110 of these 192, there was ≥2-fold difference in MIC values between auxotrophs and prototrophs. Of those isolates that were more sensitive, 40 were prototrophs and 18 auxotrophs, and of those most resistant, nine were prototrophs and 43 auxotrophs (p < 0.001). These differences in antimicrobial susceptibilities were observed for all antimicrobial agents tested.

We conclude that in CF the proportion of auxotrophic mutants in P. aeruginosa is increased in patients with acute exacerbations and that auxotrophic isolates of P. aeruginosa from CF adults are more resistant to anti-pseudomonal agents than prototrophs.

DIFFERENTIATION OF PATIENT ANTIBODY RESPONSE TO COMMON AND TYPE-SPECIFIC LIPOPOLYSACCHARIDE OF PSEUDOMONAS AERUGINOSA

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Two varieties of smooth lipopolysaccharide (LPS) have been identified in Pseudomonas aeruginosa. One LPS determines serotype specificity while the other is common to many serotypes. We have investigated the response of patients to each LPS form. Type-specific serum antibodies were detected by the formation of characteristic ladder bands with a single serotype reference strain in immunoblotting with a panel of 10 frequent serotypes. Antibodies to common LPS produced identical ladder profiles, in immunoblotting, against different serotypes. Sera from 35 patients (23 cystic fibrosis (CF), 11 osteomyelitis and bone infections, one septicaemia) were examined. Of these, 19 (54%) reacted with common LPS antigen and 14 (40%) with a type-specific LPS. Eight patients (23%) had antibodies to both forms of LPS. More CF patients (15 of 23, 65%) had antibody to common LPS than osteomyelitis patients (3 of 11, 27%). The results support the view that as P. aeruginosa strains in CF patients lose type specific antigens, the response to common LPS antigen becomes dominant. They also suggest that the antibody response to this antigen may be a useful marker of chronic infection with P. aeruginosa.