24-10-90

SEMT One Day Meeting 24th Oct 1990

WELCOME TO THE SEMT ANNUAL ONE-DAY MEETING

INDEX

The SEMT wish to express their special thanks to:-

The Charing Cross hospital Post Graduate Centre.

Bio-Rad Microscience Ltd.

All the companies attending the Trade Exhibition and who have generously sponsored us during the past year.

EO Kennard for the catering.

Copyprint Bureau Ltd. for producing this programme.

We hope you will find the meeting interesting and informative and that you will attend our future FREE afternoon meetings held at the ICRF, Lincolns Inn Fields.

Next meeting Friday 7th December 1990

"The use of Computers in Electron Microscopy"

where we hope to have representatives of the major electron microscope manufacturers discussing their latest developments.

All members are invited to an informal sherry reception after today's meeting which will be followed by the Annual General Meeting.

An Introduction to

THE SOCIETY OF ELECTRON MICROSCOPE TECHNOLOGY

The society was formed in 1970 to promote the exchange of information and ideas in the field of electron microscopy. Membership is unrestricted and although mainly London based we have many members from further afield. By holding regular meetings with invited speakers and trade stands we aim to facilitate contact between all our members including instrument manufacturers and users. The current format includes a series of afternoon meetings, each concentrating on a specialist topic and an annual one day meeting with a broader based content. Our meetings are always well supported by the trade and information on the latest technology is usually on display. forthcoming events are advertised in the Proceeding of the Royal Microscopical Society (to whom we are affiliated) and various technical publications.

Membership of the SEMT has many advantages. For a newcomer to the field it provides a point of contact with others who can give support and encouragement as well as practical assistance. For those with more experience, co-operation and sharing of ideas is as important as keeping abreast of the latest developments in techniques and equipment. For the trade, it provides a unique opportunity to advertize to a keenly interested audience and to obtain customer-reaction directly.

Prospective members should contact Jill Lewis (Secretary), Electron Microscope Unit, St. Bartholomews Medical College, Charterhouse Square, London EC1M 6BQ, for a membership form. An annual subscription, to cover postage and stationery costs, (£5) is due on the 31st October and cheques should be made payable to "SEMT". Current committee members available for further information are listed below.

Mrs Pauline Barber	(Chairman) 071 837 6411x2042
Mr Chris Walker	(Vice-Chairman) 071 889 7607
Dr Jill Lewis	(Secretary) 071 982 6160
Ms Susan Barnes	(Treasurer) 071 938 9348
Mr Clive Wells	071 601 8888x8552
Miss Anne Drewe	071 846 1234x3602
Mr D Gunner	071 928 9292x3306
Dr J Plummer	071 387 2898x354
Dr R Blackburn	071 316 9966
Mr JP Cassella	071 928 9292x3306

PROGRAMME

9.30	Registration & coffee
10.30	Welcome by the Dean of the Medical School
10.40	THE APPLICATION OF MICROWAVES IN E.M. Dr David Hopwood (Ninewells Hospital, Dundee)
11.20	BIOLOGICAL APPLICATIONS OF TUNNELLING MICROSCOPY Dr Martyn Davies (University to Nottingham)
12.00	LOW TEMPERATURE S.E.M.: ADVANTAGES & APPLICATIONS Dr John Sargent (Islip, Oxford)
12.40	POSTER DISPLAY & TRADE EXHIBITION
	BUFFET LUNCH
2.00	HIGH PRESSURE S.E.M. - A REVOLUTION? Dr Jitu Shah (University of Bristol)
2.40	THE ROLE OF E.M. IN STUDYING THE DAWN OF ANIMAL LIFE Dr Simon Conway Morris FRS (University of Cambridge)
3.10	TEA
3.30	Discussion of Poster Communications
3.45	Sherry

ANNUAL GENERAL MEETING

APPLICATION OF MICROWAVES TO ELECTRON MICROSCOPY

David Hopwood. University of Dundee

The use of microwaves in Light Microscopy is well established in the fields of tissue stabilisation, processing, staining and immunohistochemistry. In electron microscopy, the resulting morphology is a more sensitive indication of the control of the various steps in the tissue processing.

Tissues may be stabilised rather than fixed by microwaves and require secondary fixation before processing for transmission electron microscopy. Tissues are very sensitive to the temperature achieved in the microwave oven. In humans and mammals, this is up to about 55°C, giving about 10°C range above which cells are disabled. The effects of microwaves are different to those of ethanol. The virtue of microwave treatment is that the complete tissue block is affected and stabilised simultaneously, rather than having the gradient effect from cell to cell seen in chemical fixation. Microwaves can also be used in the preparation of tissue for scanning electron microscopy. Again, the same temperature range is pertinent. In the gut, above 55°C, enterocytes are shed from the basement membrane, revealing the underlying fenestrated basement membrane. At higher temperatures more severe changes occur.

Microwaves have also been used with the immunohistochemical techniques demonstrating an extensive list of antigens at light and ultrastructural level. The use of proteases, common after formaldehyde fixation is often unnecessary after microwave stabilisation. Microwaves also speed the reaction between antigen and antibody and the washing of tissues with physiological saline. The use of colloidal gold has allowed quantitative studies of tissue antigens. The process of immunostaining at the electron microscopic level can be considerably speeded up. The individual components of the immunohistochemical reaction have been dissected and analysed using an ELISA technique.

The tissue is stabilised at about 45'C; damaged above 55'C. It must then be post-fixed, or will disintegrate during subsequent processing. The mechanism of action of the microwaves is probably heat alone; good control of time and temperature is necessary. The specimen may be no more than 1 cm thick; it is better if it can be raised off the floor of the oven by a bridge.

With tissues in a bath of liquid, we have the problem of heat diffusing slowly through the tissues.

BIOLOGICAL APPLICATIONS OF SCANNING TUNNELING MICROSCOPY: INTEGRATION WITH COMPUTATIONAL CHEMISTRY

DE Jackson, MC Davies, CD Melia, SJB Tendler and PM Williams
VG STM Laboratory for Biological Applications
Department of Pharmaceutical Sciences, University of Nottingham.

The ability of scanning tunneling microscioy to provide fundamental information on biological molecules is now being realized with several studies reporting good image data on a range of molecule types. The potential for topographical information at near-atomic resolution on single surface-adsobed biomolecules indicates that STM is emerging as a new and very important complementary biophysical technique for the structural analysis of biomolecules. However, in order to establish STM in this biophysical role, initial data sets need to be interpreted in the light of structural parameters derived by other techniques (eg. X-ray crystallography, high-field nmr, computational chemistry). We believe that the most effective way to integrate such comlementary 3-dimensional data sets is through the development of appropriate software tools for use on high performance computer graphics systems.

This presentation will describe the ongoing programme of work in our laboratories to establish STM as a valuable tool for the structural elucidation of biomolecules. Initial results from the analysis of several biological samples including DNA and proteins will be presented. New software algorithms will be described for the manipulation, interrogation and display of STM data sets. In addition image analysis techniques utilizing Fast Fourier Transforms for convolutions will be presented.

With a scanning tunnelling probe, the piezo tip is just above the surface of the specimen, and a raster passes it across the surface. The current rises as the tip gets closer to the surface, and the apparatus must be adjusted to constant current mode. Information can be taken from the STM and processed elsewhere.

Work was described of individual carbon atoms on a graphite surface; calf thymus DNA strands; molecules air-dried onto a graphite substrate.

LOW TEMPERATURE SEM - ADVANTAGES AND APPLICATIONS

John Sargent
Electron Microscopy Consultant

At ambient temperature low melting point materials melt under the electron beam and hydrated specimens lose water in the high vacuum of the SEM. These problems can be avoided by examining specimens at a temperature close to that of liquid nitrogen. Chemical fixatives and solvents are avoided and specimen components are immobilized prior to X-ray microanalysis. Examples will be shown of the application of the technique to examine a wide range of 'difficult' specimens.

Limitations of ambient-temperature SEM include:

- melting; liquids cannot be examined
- dessiccation of hydrated material
- toxic fixatives
- shrinkage and distortion during fixation
- solvent extraction of components
- loss or movement of compounds
- small organisms or particles are difficult to collect and orientate.

Below -130°C, the specimen remains hydrated. Therefore:

- fixation is not necessary
- solvents are not neseccary
- motile organisms are immobilised; aquatic ones can be collected on a filter.

This work is mostly done at 25 kV.

HIGH PRESSURE SEM

JS Shah
HH Wills Physics Laboratory
University of Bristol

Normally, SEM cannot not be performed at high pressures because in any electron microscope a well defined beam of high energy electrons, nescessary for imaging, has to be produced and kept in a high vacuum environment. The beam cannot travel long distances in a high pressure gaseous environment without being scattered by gas atoms or molecules and losing its energy. Recent developments have made it possible to look at specimens kept at higher pressure in the range of 1 torr to 20 torr. To mark these developments, the term HIGH PRESSURE SCANNING ELECTRON MICROSCOPY (HPSEM) is coined. It actually refers to a family of SEM techniques performed at a pressure above 1 torr (133 Pa or 1.33 mbar) and includes techniques such as Moist Environment Ambient Temperature Scanning Electron Microscopy (MEATSEM), Environmental Scanning Electron Microscopy (ESEM), WET-SEM, Atmospheric Scanning Electron Microscopy (ASEM).

Fundamental principles relating to these developments will be discussed. In particular, considerations behind the actual design of the apparatus for keeping the specimens in high pressure and the electron beam column at high vacuum will be explained. It is proposed to discuss also the consequences of electron scattering within the experimental pressure range, expounding on the effects of ionization of amient gases and the manner in which the the SE and BSE signals are recovered.

HPSEM offers a variety of novel uses:

(1) Morphological and analytical examination can be made of materials of biological origin, virtually without any preparation or treatment. Therefore it is possible to view them in a state as close as possible to their natural state without subjecting them to any volume and compositional changes.

(2) Materials which have inherently high vapour pressure can be examined without loss of volatile constituents. e.g. shale rocks, aromatic substances.

(3) Dynamic processes which occur solely in high pressure environment can be studied.

(4) Examination of poorly conducting materials (e.g. plastics, wax, uncoated embedded tissue sections, semiconductors, glass, ceramic, wood, cement etc.) without any coating.

These applications will be discussed with practical examples and illustrations to point out advantages and disadvantages of HPSEM.

THE ROLE OF EM IN STUDYING THE DAWN OF ANIMAL LIFE

Simon Conway Morris. University of Cambrige

Electron microscopy is routinely used by palaeontologists, and has revolutionized understanding of such groups as the coccolithophorids and foraminifera. These groups possess skeletons of calcium carbonate, and it is a curious fact that such skeletal material only began to be secreted by animals and plants near the base of the Cambrian, about 550 million years ago. The widespread onset of biomineralization, after about 85 per cent Earth history had elapsed, is marked by an extraordinary array of skeletal forms, especially in animals. Many are of millimetric to submillimetric size, and so lend themselves to study by scanning electron microscopy. Here I will review how detailed examination of these skeletal fragments tells us much about their organization and the types of biomineral employed. Use of polished and etched sections can be particularly informative, but even if the original skeletal material has been destroyed imprints of the skeleton may still be exquisitely preserved in the sediment that infilled the cavity of the shell. Such studies using the electron microscope reveal both the complexity of early skeletal formation and the inter-relationships of groups during this major evolutionary event.

CHANGES IN THE GLOMERULAR BASEMENT MEMBRANE ULTRASTRUCTURE IN RESPONSE TO CADMIUM INTOXICATION

J Bennett, D Prashad, RO Blackburn

School of Biol. and Chemical Sciences, Thames Polytechnic, London.

Toxic insult by cadmium is known to cause considerable damage to renal function morphology. Previous investigations have indicated that cadmium will induce marked thickening of the glomerular basement membrane, which would influence the maintenance of renal homeostasis. The current study has shown that this thickening may be partly reduced by administration of certain compounds of EDTA Subsequent to cadmium intoxication.

DISTRIBUTION OF ANIONIC SITES ON THE ENDOTHELIAL CELLS OF DORSAL ROOT GANGLIONIC CAPILLARIES

MS Bush

Rita Lila Institute, Middlesex Hospital, London.

To determine the distribution of anionic sites on the cell membranes and basal laminae of endothelial cells, cationic tracers were administered by intravenous injection and perfusion and also by incubating tissue slices and LR White resin sections in tracer solutions. Diaphragms of caveolae and fenestrations are highly anionic but do not label with cationic colloidal gold at pH 2.0. Luminal membranes and basal laminae are moderately anionic and abluminal membranes weakly anionic. Tracers were not pinocytosed and do not penetrate fenestral diaphragms and inter-endothelial junctions.

PESENCE OF CONTRACTILE FIBROBLAST-LIKE CELLS, MYOFIBROBLASTS IN HUMAN 'PANNUS' TISSUE

CL Chander, I Appleton, G Lewis, DA Willoughby.

Department of Experimental Pathology, St. Bartholomew's Hospital Medical College, Charterhouse Square, London.

Myofibroblasts have been identified in many pathological tissues eg. neoplasia and hypertrophic scars. In this study we demonstrated that human 'pannus' tissue can contract in response to pharmacological agents and ultrastructural examination revealed microfilaments characteristic of myofibroblasts.

HEART ASSIST DEVICES: INDUCED HYPERTENSION AND ALTERATIONS IN VASCULAR MORPHOLOGY IN A CALF MODEL

JP cassella, S Cassella, J Hay

Department of Cell Pathology, Institute of Dermatology, St. Thomas's Medical School, Lambeth Palace Road, London.

Department of Experimental Surgery, The Royal London Hospital, Ashfield Street, London.

School of Pharmacy, Leicester Polytechnic, PO Box 143, Leicester.

Light and Electron microscopical examination was performed in calf aorta post-implantation of a prosthetic cardiac pump. LM revealed morphological changes to the tunica intima and tunica media of aorta taken from calves with transplanted cardiac replacement device. Ultrastructural changes, especially intimal thickening were suggestive of a hypertensive effect. In some instances, sloughing of the intima was observed. Degranulated connective tissue mast cells were observed in the region immediately underlying the intima. The precise pathogenesis of these tissue reactions remains obscure.

NEEDLE-BIOPSY OF PARAFFIN BLOCKS: NON-DISRUPTIVE METHOD OF OBTAINING TISSUE FOR TRANSMISSION ELECTRON MICROSCOPY

JP Cassella, J Hay, SA Cassella

St. Thomas's Hospital, London; Leicester Polytechnic, Leicester; Royal London Hospital, London.

Removal of small cores of tissue from an intact paraffin block using a modified Menghini renal biopsy needle (1.2mm internal diameter), permitted tissue to be obtained for re-processing into resin without disruption of the original block. The piece of tissue provided by the needle-biopsy technique is easier to handle than a section. Furthermore, the original block remains more or less intact.

HISTOMETRIC STUDY OF IMMUNOGOLD DEPOSITION ASSOCIATED WITH INTACT TOXOPLASMA TISSUE CYSTS IN MOUSE BRAIN

TA Sims, J Hay, C Poon

Leicester Royal Infirmary. Leicester Polytechnic, Leicester.

Histometric analysis of immunogold deposits within, and in the immediate vicinity of, intact Toxoplasma tissue cysts in the brains of mice with congenital toxoplasmosis, confirmed that Toxoplasma antigen was preferentially located in the intracystic matrix. There was no difference in antigen deposition between intracystic parasites and host brain components.

THE USE OF PARAFORMALDEHYDE-FIXED, LOWICRYL-EMBEDDED KIDNEY BIOPSIES FOR ROUTINE ULRASTRUCTURAL EXAMINATION AND IMMUNO-ELECTRON MICROSCOPY

J Moss, I Shore, D Woodrow

Department of Histopathology, Charing Cross and Westminster Medical School, London.

We have embedded human kidney biopsies for electron microscopy in Lowicryl K4M, after fixation in 4% paraformaldehyde and ethanol dehydration at low temperature. This technique provides adequate preservation of glomerular basement membrane structure, electron-dense deposits and amyloid fibrils for routine ultr-structural examination. Using a post-embedding immunogold technique, we also localize many different antigens including immunoglobulins, complement (C3), amyloid P component, amyloid A, type IV collagen and fibronectin. In addition the simultaneous localization of two different antigens can be performed in the same section (Shore I, Moss J, Histochem. J. 1988, 20, 183-184.).

IMMUNOGOLD LABELLING OF APICAL BORDER PLAQUE

BA Watt, PM Barber, HN Newman
Eastman Dental Hospital

Periodontitis is one of the commonest diseases which affect mankind. It is of microbial origin, and if left untreated may result in the loss of the dentition. A very complex relationship exists between plaque and this disease. Although the oral flora comprises some 325 different species of bacteria, current thinking suggests that at the advancing front of the lesion, the number of morphotypes is greatly reduced and that the species involved are predominantly of the Gram-negative type. If we are to understand the pathogenesis and aetiology of this disease identification of organisms at the advancing front of the lesion is of prime importance.

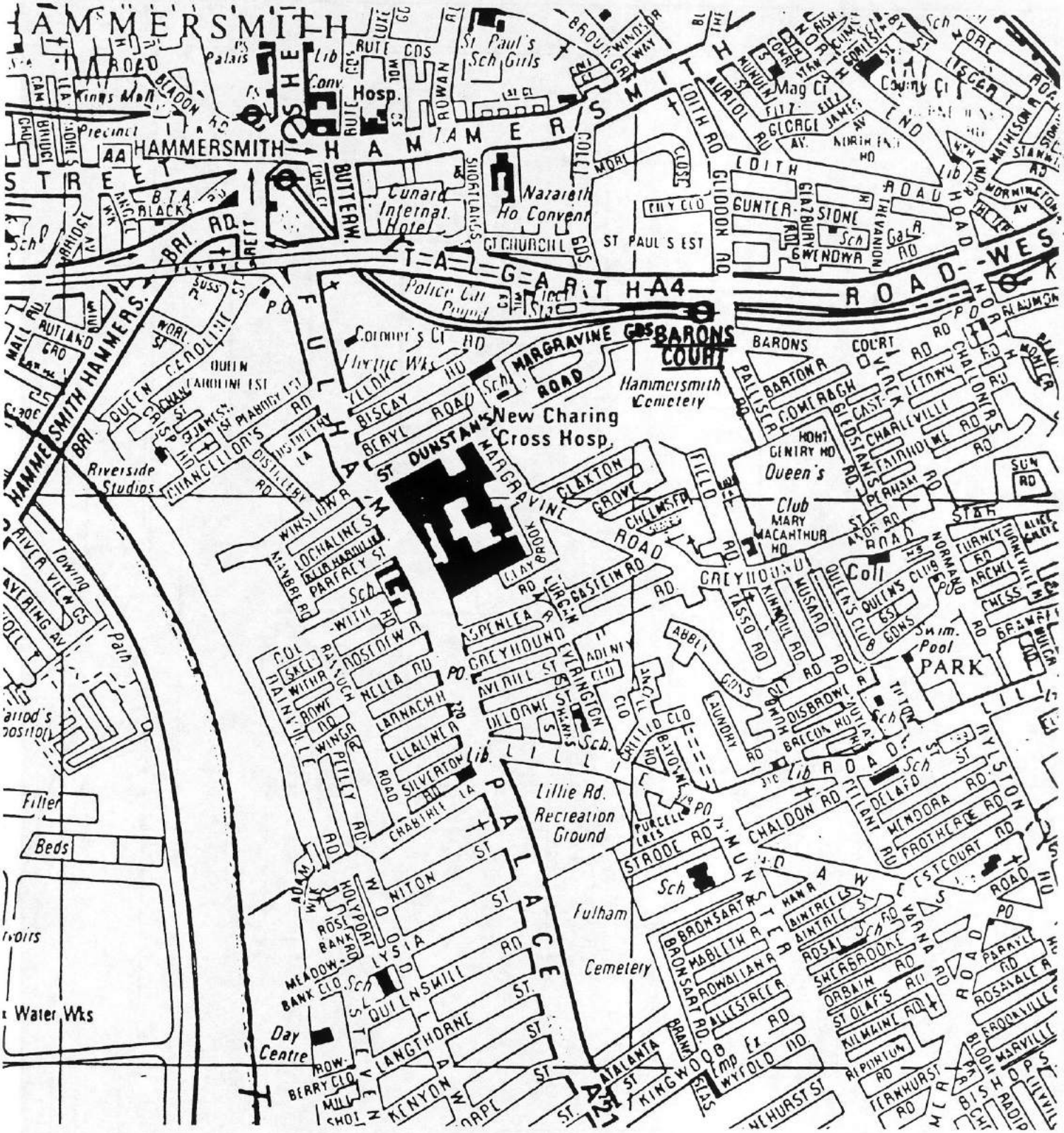
By conventional transmission electron microscopy Gram-negative organisms are ultrastructurally similar and identification is difficult. Attempts at culturing plaque isolates are reasonably sucessful, but up to 50% of the species may be lost due to culturing difficulties. In this study we have developed primary antibodies to Porphyromonas gingivalis, a Gram-negative plaque species currently considered a major periodontal pathogen. We have applied post-embedding immunogold labelling (IGL) to sections of natural human periodontitis associated apical border plaque, and to pure culture samples as a method of identifying bacteria in situ. The plaque specimens showed selective labelling fo some Gram-negative organisms and 100% labelling was achieved in our pure culture sections. This study suggests that immunogold labelling may provide a suitable methodology for the identification of organisms within the periodontal lesion in situ.

VASCULAR IMPAIRMENT IN THE BRAINS OF MICE WITH CONGENITAL TOXOPLASMA INFECTION: AN ULTRASTRUCTURAL STUDY

TA Sims, J Hay, IC Talbot

Leicester Royal Infirmary; Leicester Polytechnic, Leicester; St. Mark's Hospital, London.

Lymphocytes were found adhering to the outer endothelium of small vessels in the brains of mice congenitally infected with Toxoplasma. Basement membrane of the endothelium was thickened. Perivascular cuffing was apparent. In many cases, the inflammatory exudate contained lymphocytes and plasma cells; cell lysis was present. Tissue cysts in the vicinity of these vessels sometimes had lymphocytes in close apposition to their outer wall. Lymphocytes may be attracted towards intact tissue cysts from inflammatory foci around affected vessels.



TRAVEL TO CHARING CROSS HOSPITAL

Buses 11, 220, 283, and 295 to/from Hammersmith Broadway stop outside the Hospital.

The Hospital is 10 minutes walk from both Hammersmith and Baron's Court stations. Owing to the rebuilding works in Hammersmith Broadway the journey on foot is easier from Baron's Court.

Hammersmith and Baron's Court are on the District and Piccadilly lines; Hammersmith is also served by the Hammersmith & City (Metropolitan) line, and by buses 9, 10, 27, 33, 72, 73, 91, 266, 267, 290, R69, and Green Line 715.

COMPANIES ATTENDING TRADE EXHIBITION

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