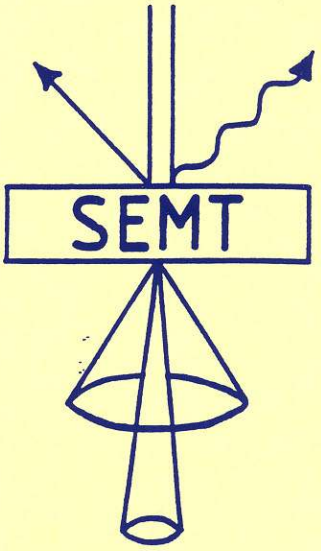
SEMT

One Day Meeting, 1993

Friday, 22nd October, 1993

Institute of Dental Surgery
Gray's Inn Road
London WC1



THE SOCIETY OF ELECTRON MICROSCOPE TECHNOLOGY

Prospective members should contact Jill Lewis (the Secretary), 19, Bellfield Avenue, Harrow Weald, Middlesex, HA3 6ST for an application form. Current committee members are listed below, and are available for further information.

The SEMT wishes to express their special thanks to:

The Institute of Dental Surgery, as hosts, **Stuart Cabeldu Ltd**, as caterers, **Fisons Instruments**, for supplying the folders and drinks and to the following companies for attending the trade exhibition (in alphabetical order):

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The SEMT hopes that you find the this One Day Meeting interesting and informative, and that you will be able to attend our forthcoming afternoon meetings.

The next SEMT meeting will be the *Annual Award for Young Researchers*, and will take place at 1400 on Friday, 3rd December, 1993 at the Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2.

WELCOME TO THE ONE DAY MEETING

Wednesday, 22nd October, 1993

Conference Centre
Institute of Dental Surgery
Gray's Inn Road
London WC1

PROGRAMME

0930	Registration and Coffee
1015	Introduction by the Chairwoman *Sue Barnes (Natural History Museum)*
1030	Plaque, Teeth and Gums *Pauline Barber (Institute of Dental Surgery)*
1100	The SEMT's Connection With Romania *Jenny Anne Drewe (Charing Cross and Westminster Medical School)*
	The EM Laboratory in METAV-S.A., Bucharest *Carmen Bunescu (S.C. METAV-S.A., Bucharest, Romania)*
1130	Application of Confocal and Electron Microscopy to the Study of Cardiac Intercellular Communication Junctions *Robert Gourdie (University College)*
1200	Applications of Environmental Scanning Electron Microscopy *Christopher Gilpin (University of Manchester)*
1230	Lunch
1400	Poster presenters in attendance at their work
1430	The Use of EM in Unravelling the History of Life on Land *Margaret Collinson (Royal Holloway College, Egham)*
1500	After the Fire - an EM analysis of Inhaled Smoke Particles *Brian Michelson (RAF Institute of Pathology, Halton)*
1530	Bugs in The Sticks - EM at Porton Down *Barry Dowsett (Public Health Laboratory Service, Porton Down)*
1600	Tea and Close

ABSTRACTS OF SPEAKER'S PAPERS

Plaque, teeth and gums

Pauline Barber (Institute of Dental Surgery)

Chronic inflammatory periodontal disease (CIPD) is one of the most widespread diseases of mankind and one of the major causes of tooth loss. Many previous studies have shown that differences in the prevalence of the disease may be related to age, sex, race, geographical areas, local and systemic factors and oral hygiene levels. However, earlier studies also indicate that when the disease is directly related to oral hygiene all these differences disappear. Although current thinking suggests that CIPD is of microbial origin and caused by a build up of plaque on the tooth surface, its precise aetiology and pathogenesis remains unclear.

The clinical features of CIPD are bleeding upon probing, the formation of deep pockets and loosening of the teeth. The main histological feature is a high level of soft tissue destruction. At this Institute we have examined at the ultrastructural level the root surfaces of periodontitis-affected teeth, the soft tissues which form the pockets and plaque, from patients suffering from CIPD. Changes to the host response cells, soft tissue morphology and plaque morphotype at the advancing front of the lesion will be reported. We will also discuss the significance of our findings and their possible relationship to the aetiology and pathogenesis of this disease.

The SEMT's Connection with Romania

Jenny Anne Drewe (Charing Cross & Westminster Medical School)

Despite the easing of restrictions since the revolution of 1989, Romanian scientists are still having problems at work as well as in everyday life - shortage of books and journals, and thus of contact with colleagues in other countries, and shortage of equipment, spare parts and consumables. The SEMT can do a little to help our Romanian colleagues by making contact with people working in similar areas, and by passing on surplus equipment and other items and reprints. These items will be carefully matched to the places needing them. Some equipment has already been sent. We have to make a contribution to the cost of sending things out, so fund-raisers are needed.

The EM Laboratory in METAV-S.A., Bucharest

Carmen Bunescu (S.C. METAV-S.A., Bucharest, Romania)

The Research Department of METAV-S.A. Company was built 13 years ago to develop melting, casting, forging and non-conventional technologies for the aircraft industry. The structural analysis laboratory plays an important role within the Department and includes two

SEMs; a JEOL JSM-50A and a Philips SEM 515 with EDS and WDS and a STEM type Philips CM30 with EDS and EELS.

The investigations performed in the EM laboratory are microstructural, microanalytical and fractographical studies especially on materials employed in the aircraft industry. For a more complete characterization of the materials, a metallographical optical microscope, microhardness tester are used, and an X-ray diffractometer and static and dynamic mechanical testing machines are also available.

The EM laboratory also performs other types of analysis, for example on geological samples, fossils, etc. for outside customers.

Application of Confocal and Electron Microscopy to the Study of Cardiac Intercellular Junctions

Robert Gourdie (University College)

Gap junctions are aggregates of intercellular membrane channels, that in the heart electrically couple myocytes and enable conduction and propagation of action potential throughout cardiac muscle. We have used scanning laser confocal microscopy in conjunction with electron microscopy to study the 3-dimensional organization of cardiac gap junctions. This work has involved the raising of antibodies with specificity against gap-junctional sub-unit proteins (connexins) and devising single and double-label protocols for immunohistochemical and immunocytochemical localisation of these proteins. We have also developed methods for the digital quantification of junctional area from confocal images and validated our methods by comparative measurements taken on the electron microscope. This work has provided new and important information on the mechanisms governing the electrophysiological behaviour of the embryological, adult and diseased heart.

Applications of Environmental Scanning Electron Microscopy

Christopher Gilpin (university of Manchester)

The introduction of a commercially available environmental scanning EM (Electroscan ESEM) has allowed the imaging of a range of samples previously thought difficult if not impossible. The ESEM differs from conventional SEMs in that it uses a unique low voltage secondary electron detector rather than an Everhart-Thornley photomultiplier for signal collection. One benefit of such a low voltage detector is that the microscope specimen chamber does not have to be maintained at high vacuum. Indeed the environment inside the specimen chamber is fully under operator control. The mechanisms of vacuum maintenance, signal production, amplification and environmental control will be discussed.

Broad use of the potential of the ESEM has been made in this laboratory and results will be presented from selected applications. For example, the use of artificial prostheses in vascular surgery depends on the ability of such devices to present a "natural" surface to circulating blood. Attempts are being made to grow endothelial cells on the surface of a variety of

vascular grafts and to assess the quality of coverage by EM. Results obtained from the grafts will be presented comparing observations made using conventional SEM and ESEM which illustrates the advantage of using wet material.

As samples can be viewed without conductive coating, liquids can be imaged without difficulty. Furthermore, with the protection of a differential pumping system and a nickel plated chamber, old enemies of the electron microscopist such as oil can be viewed directly. Results obtained from a study of oil-bearing minerals will be presented which amply demonstrate the ease with which almost anything can be imaged in the ESEM. Brief anecdotal results from a range of other studies will also be presented.

The use of Electron Microscopy in Unravelling the History of Life on Land

Margaret Collinson (Department of Geology, Royal Holloway College, Egham, Surrey)

Ancient life is preserved, as fossils, in rocks up to almost 3,000 million years old. However, multicellular organisms only appear over the last 1,000 million years and for over 500 million years of their early history, these were restricted to the oceans. The fossil history of land organisms, the subject of this presentation, can only be traced over the most recent 420 million years of earth history.

Most organisms undergo some transport and/or decomposition prior to incorporation into sediments. The potential fossils are therefore often only fragmentary remains. In addition, fossilization processes often alter the original state of the material so that techniques applied in investigations of living organisms cannot always be applied to fossils. In order to study the evolution of life on land it is necessary to reconstruct the organisms and determine their characteristics and their interactions with other organisms. The degree of success with which this can be achieved often depends upon observation of small and superficially unimportant details which provide clues to systematic relationships and functional biology.

The scanning electron microscope has provided much of the answer to this challenge. The SEM is often adopted as the routine microscope for the study of small, fragmentary and often structurally altered fossils. The use of SEM has made information available from fossils which would previously have been ignored or treated as too poorly preserved to be worthy of investigation. Furthermore, the details which can be seen and the three-dimensional nature of the images have made a significant contribution to our understanding of ancient organisms. The transmission electron microscope is less frequently used, partly because of problems associated with embedding and sectioning fossil material. TEM is most frequently applied in studies of plant pollen and spores where ultrastructure within the wall is of fundamental biological significance.

This presentation will concentrate on the role of electron microscopy in elucidating the history of life on land over the last 100 million years during which time communities dominated by flowering plants and mammals (like those of the present day) superseded those dominated by dinosaurs, extinct seed plants and ferns. Major topics covered will include the origin and evolution of flowering plants; the early history of wetland floras and the evolution of interactions between mammals and flowering plants (involving diet and dispersal).

After the Fire - an Electron Microscopical Analysis of Inhaled Smoke Particles

Brian Michelson (RAF Institute of Pathology, Halton)

This work started initially as a retrospective study of lung epithelial ultrastructure following smoke inhalation associated deaths. However, the structural integrity of the tissues was too poor for any meaningful work, but the smoke particles themselves showed surprising diversity at high magnification. Further examination using TEM with electron spectroscopic imaging (ESI) and electron energy loss spectroscopy (EELS) revealed a diverse structure and composition to these particles. Further EM analytical techniques were used to give a more detailed view of the physical and chemical nature of the particles. This revealed their complex nature and may in turn provide a greater understanding of the long term respiratory problems found in some survivors of smoke inhalation injuries.

Bugs in the Sticks - Electron Microscopy at Porton Down

A. Barry Dowsett (CAMR, Porton Down, Salisbury)

In the minds of the press and indeed many people, the name Porton Down is synonymous with "germ warfare" and highly pathogenic microorganisms. However, since the management of the Centre passed from the Ministry of Defence to the Public Health Laboratory Service in 1979, its role has been to conduct research into infectious disease which presents an actual or possible threat to public health, leading to the development and production of vaccines and therapeutic products. This presentation will endeavour to provide an overview of the role of the CAMR EM Unit in the work of the Centre by illustrating the contribution that transmission and scanning EM have made to various programmes of research.

Plaque, Teeth and Gums - Pauline Barber

Periodontal disease has been known from ancient times; it is basically inflammation of the gums, giving rise to spontaneous bleeding on probing, loosening of the teeth; the gingiva recedes to give the appearance of "long in the tooth". Non-systemic causes are related to bad oral hygiene. In these, improvement is obtained by root planing and scaling, antibiotics, and improved oral hygiene.

Biopsies are obtained from bad cases; the material is bloody and friable. Destruction of soft tissue and cartilage is seen. The polymorphonucleocytes may have reduced phagocytic capacity and response, and may become destructive when lysed into the tissues because of the release of enzyme granules. There are probably several populations of PMNs, and some may already be degenerate when they reach the site of the trouble.

There are more than 325 different organisms in human dental plaque (including Treponemes); fewer at the advancing edge of the lesions. Some bacteria have a capsule of surface-associated material which resorbs bone etc.

50% of the organisms in plaque cannot be grown.

For immunocytochemical studies, fix in ½% glutaraldehyde (but the glycocalyx cannot be seen without osmium as well). Embed in araldite: then decalcify when in the araldite, with EDTA: then re-embed.

Application of Confocal and Electron Microscopy to the Study of Cardiac Intercellular Junctions - Robert Gourdie

The gap junction is the bridge between cells; the largest gap junctions are on the lateral edges of the myocytes. The smaller junctions are below the resolution of the light microscope, but freeze-fracture shows that there are large numbers of small gap junctions. Tight junctions are found only in developing cells, not in adult ones.

The maximum thickness of sections which can be examined in the confocal microscope is 50 um; more than this, and the signal disappears. This depends on the objective, the laser, and the clarity of the tissue.

Applications of Environmental Scanning EM - Christopher Gilpin

In the ElectroScan, the pressure at the specimen region is 5 torr; at the detector, 10^{-5}, and at the gun 10^{-6} torr. There is a 20 um aperture at the top of the detector, and a $\frac{1}{2}$ um aperture at the bottom, and these act as differential pumping apertures. Gas can be allowed into the chamber in a controlled manner, usually as water vapour. The electrons of the bean collide with the water vapour to produce more secondary electron, so the water acts as an amplifier. Control of the water vapour pressure determines how wet the specimen will be.

The detector is not a photomultiplier, so a light and a hot stage can be situated inside the EM.

Most of the morphological change in biological material occurs during drying. Fully hydrated cells and gelatin on dacron weave were shown. Oil-containing rocks, with a fairly light oil in pores and channels in the rock, could have the oil evaporated while imaging, by control of temperature and pressure; the pores must be open if the oil is to be extracted.

Hydrated contact lenses - laser holes were made partly through to improve gas permeability, but provided hiding place for bacteria. Paint and cartilage are examined. On cell cultures, you may just see the smooth glycocalyx.

XRMA can be a problem because the electron must travel 13 mm through the chamber to the specimen; this is difficult with a hydrated specimen because there is so much scatter; oxygen is seen in the spectrum, from the chamber.

Beam skirting - things outside the area imaged will contribute to the spectra. It is not necessary to coat the specimens, because thre is no build-up of charge. Live specimens can be viewed. On biological specimens, the specimen resolution runs out before the EM res. does.

EM in Unravelling the History of Life on Land - Margaret Collinson

Body-fossils are of the whole animal; trace-fossils of burrows, bite-marks etc. Pollen grains 120 million years old have been found. Charcoalified specimens look totally black by light microscopy, but detail can be seen in the SEM. EM can give understanding of structure, eg a spore has irridescence because of small packed spherical particles, similar to the silica in precious opal; this pattern is produced by self-assembly.

Well-preserved rodents 50 million years old, still have fragmented seeds in the belly; the pattern of chewing can be seen.
The patterns of wear on ancient and modern teeth can be compared.

After the Fire - Brian Michelson

Smoke is an aerosol of gas and soot. Smoke particles can be found in the lung, about 3mm from the surface. Aromatic polyamides, found in many bits of furniture, burn to produce many chemicals; the levels of carbon monoxide and cyanide in the blood can bemvery high. Solid particles can also be complex. If metallic particles are filtered out onto activated charcoal, they retain their toxicity; in the body, these are absorbed onto the soot particles in the lungs, so may retain toxicity in survivors. Particles may be found in blood vessels, as they can be forced in by holding the breath; usually there is a strong gradient against them going in. These particles are much more toxic t than the gases; if they are filtered out, the gases are ten times less toxic !!

Smoke hoods on aircraft cost about 50p each. Sprinkle systems cost about £150,000 per plane, and are less good for people with breathing difficulties.

Bugs in the Sticks - Barry Dowsett

Use Stereoscan S2A and Philips 410.

Lipid-producing yeast - by freeze-etching, the lipid vacuoles have the typical onion-skin appearance.

Phage can be used as genetic vectors; one is used in the fermentation of salami; we don't want them in our cultures.

Ebola virus is huge and haemorrhagic.

Legionella, Salmonella, Meningococcus.

Aids virus causes the cells to fuse to form syncytia; the virus is found in the Golgi vesicles, then the vesicles migrate to the outside.

Pneumocystis carinii is found in the alveoli.

In a condition where the eyes are permanently almost closed, botulinum toxin is used as a treatment; the patient can then still blink a bit.

De-waxeds sections in the SEM, can be very useful for screening to find the right area.

Dr. Tony Brain
E.M. Unit, King's College
Manresa Road, SW3 6LX
071-333 4924

Chris Jones, Natural History Museum - invitation to visit EM unit.

There may be a lapidiary mill available for Romania.

ABSTRACTS OF POSTERS

Ultrastructure of a Cyst-like Structure in Intestinal Spirochaetes From a Gulf Arab Population

Pauline Barber and S.P.Barrett (Inst. of Dental Surgery and St Mary's Hospital Medical School)

Cyst-like structures (CLS) have been demonstrated in Reiter treponemes, oral treponemes and the intestinal spirochaete *Brachyspira salborgi*. They have a highly complex ultrastructure and it is unclear whether they represent a degenerate or a germinative form of the organism. In the present study, we have identified these structures in cultures of isolates of a large unnamed intestinal spirochaete. Seven isolates of spirochaetes obtained from the culture of stools collected in the Sultanate of Oman were grown anaerobically in broth cultures for four and eight days and either routinely processed for TEM or examined unfixed and snap frozen in the SEM. Mature spirochaetes were seen in close association with large granules and within CLS in all the isolates examined by both methods. The CLS were large membrane-bound profiles with a highly varied intracellular content which included mature spirochaetes. Degenerative organisms were rare and the age of the culture did not appear to be relevant.

The abundance and organised intracellular content of these "multispirochaetal" profiles and lack of degenerate organisms would seem to indicate that CLS are more likely to form part of a complicated cyclical germinative rather than degenerative process.

Cadmium Insult - Pathological Effects on Glomerular Morphology and Their Alleviation by Chelating Agents

Julie Bennett, Don Prashad, & [1]Richard Blackburn (School of Biological & Chemical Sciences, University of Greenwich, London, SE18 6PF and [1]School of Environmental Sciences, University of Greenwich, London SE8 3BW)

Toxic insult by cadmium is known to cause thickening of the glomerular basement membrane. This study was concerned with whether this effect could be alleviated by the two chelating agents FeEDTA and MgEDTA (ferric and magnesium salts of EDTA).

21 day old chicks were used ($n=36$; 223.0 ± 8.0g) using a standard injection regime (im, 0.5ml, every 48h) as follows. Controls (group 1) were given isotonic NaCl up to age 61d and group 2 birds 0.2mg Cd^{2+} (in saline) up to the same age. In groups 3 and 4, Cd^{2+} was administered as above up to age 41d; then either 1% FeEDTA or 1% MgEDTA respectively was given up to age 61d. Birds in all groups were sacrificed at ages 41, 51 or 61d and kidney tissue excised for TEM.

GBM thickness (197.0 ± 5.3nm) in group 2 birds was significantly ($p<0.001$) increased compared with controls (161.0 ± 1.9nm). FeEDTA treatment after Cd^{2+} insult (group 3), resulted in similar GBM thickness (165.0 ± 3.7nm) to controls, suggesting that FeEDTA could partly alleviate the effects of Cd^{2+}. MgEDTA therapy significantly ($p<0.001$) reduced

GBM thickness (151.0 ± 3.0nm) compared to controls; this was significantly ($p < 0.01$) more than birds treated with FeEDTA after Cd^{2+}.

The findings suggest that GBM thickening associated with Cd^{2+} toxicity in birds may be reduced by either chelating agent. Alterations in membrane thickness may result from collagen and glycoprotein synthesis (Rao et al., 1989): whether FeEDTA and MgEDTA act directly or indirectly to facilitate excretion of the Cd^{2+} or whether inhibition of synthesis in the GBM occurs remains unresolved.

Rao, P.V.V., Jordan, S.A., Bhatnagar, M.K. (1989) *J. Environ. Pathol. Toxicol. Oncol.* 9, 19-44.

Astroglial association with nodes of Ranvier in the mouse optic nerve: a morphological study of single dye-filled cells in the intact nerve

A.M. Butt, A. Duncan, K. Colquhoun and M. Berry (UMDS, London)

Astrocytes are implicated in the physiology of nodes of Ranvier because their perinodal processes are intimately associated with axonal membranes at nodes. Previously, it was suggested that in the optic nerve these processes emanated from a specialised perinodal astrocyte, namely the type-2 astrocyte (ffrench-Constant & Raff, 1986; Miller *et al.*, 1989). the aim of this study was to investigate the true nature of the astroglial association with nodes in the mouse optic nerve, by correlating confocal microscopy and light microscopic analysis with electron microscopic observations of single dye-filled astrocytes.

Mature mice (aged between 30 and 60 days) were killed and their optic nerves removed and placed in a brain slice chamber saturated with oxygen. The cells in the optic nerve were maintained viable in this incubation. Glial cells were impaled with glass microelectrodes and dye filled using iontophoretic injection. Cells were filled with either lysinated rhodamine dextran for confocal imaging, or with horse radish peroxidase for light and electron microscopic analysis.

Over 200 individual astrocytes in the mouse optic nerve were examined by light and confocal microscopy. All had primary processes ending at the subpial glia-limitans and to blood vessels (contributing to the blood brain-barrier). Small collateral branches ending in the nerve were often given off by the primary processes. Analysis of the branching pattern of their processes indicated that these cells belong to a single population with a normal distribution of morphologies.

Seven HRP filled astrocytes representative of this population (identified and drawn by light microscopy prior to osmication) were studies electron microscopically be serial sectioning. Any electron dense HRP filled processes in section were thus known to be from the identified cell. The presence of pial and vascular end feet was confirmed at the EM level, and additionally all the cells were found to have fine processes closely apposed to the axonal membrane at nodes of Ranvier. These perinodal processes were formed by discrete side branches from primary processes. From this observation it is proposed that the small offshoots from primary processes, seen by light and confocal microscopy, are in many (if

not all) cases forming association with nodes of Ranvier.

This study gives a new insight into the astroglial association with nodes of Ranvier. It appears that there is a single species of astrocyte in the mouse optic nerve. According to their morphology each cell appears to be capable of fulfilling all the proposed astroglial functions, including a role in the formation or function of nodes of Ranvier. Our results do not support the contention that specialised astrocytes serving nodal function exist in the optic nerve.

ffrench-Constant, C. and Raff, M.C. (1986) *Dev. Brain Res.* **3**, 371-386.
Miller, R.H., Fulton, B.P. and Raff, M.C. (1989) *Eur. J. Neurosci.* **1** 172-180.

Some Aspects of Romania

Jenny Anne Drewe (Charing Cross & Westminster Medical School)

A few glimpses of the towns, the countryside, the geography, the problems .....

RAISING INTRALUMINAL PRESSURE INCREASES THE LABELLING OF ENDOTHELIAL CELL VESICLES WITH NATIVE FERRITIN IN SINGLE PERFUSED MICROVESSELS.

MOFFITT, H., CLOUGH, G.F. AND MICHEL, C.C.

Depart. of Physiology and Biophysics, St. Mary's Hospital Medical School, Imperial College, London W2 1PG, U.K.

The path by which macromolecules are transported across microvessel walls is a controversial subject. There is evidence to suggest that the endothelial cell vesicular system could constitute a possible route, but there is also evidence to suggest that occasional large discontinuities in the intercellular clefts could account for macromolecular transport.

One argument which has been levelled against vesicle transport is that, macromolecular transport has been demonstrated to be convectively coupled to fluid movement across the vessel wall and therefore dependent on the microvascular pressure. There was no evidence to suggest that vesicle transport was pressure sensitive, and the aim of our study is to investigate the effects of raising intraluminal pressure on the vesicular transport of the macromolecular tracer ferritin across the walls of single frog mesenteric microvessels 15 - 40μm in diameter.The vessels were perfused with Ringer solution containing native ferritin (6.7g/100ml) and bovine serum albumin (1.0g/100ml), occluded downstream of the cannulation site and the pressure in the closed segment held constant for 2-3 minutes. The tissue was fixed in situ and prepared for TEM. Vesicle labelling was quantitatively assessed from ultrathin transverse sections of the vessels.

A positive correlation was found between the fraction of vesicles labelled and the intraluminal pressure. No correlation was found either between the fraction of luminal vesicles labelled, or the number of molecules per luminal vesicle and the luminal ferritin concentration. The data suggest that entry of ferritin into the vesicular system is pressure sensitive and raises the possibility of convective transport of macromolecules via this route.