SEMT

One Day Meeting, 1991

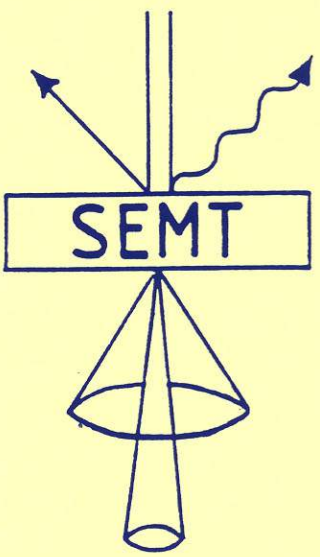
Wednesday, 23rd October, 1991

Postgraduate Centre

Charing Cross and Westminster Medical School

Fulham Palace Road

London W6



THE SOCIETY OF ELECTRON MICROSCOPE TECHNOLOGY

Prospective members should contact Jill Lewis (the Secretary), Electron Microscope Unit, St Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ for an application form. Current committee members are listed below, and are available for further information.

Officers

Chair	Pauline Barber
Secretary	Jill Lewis
Treasurer	Sue Barnes

Committee

Richard Blackburn
John-Paul Cassella
Ann Drewe
Nicola Mordan
Jenny Plummer
Chris Walker

The SEMT wishes to express special thanks to:

The Charing Cross Hospital Postgraduate Centre, as the hosts;

Hitachi Scientific Instruments, for supplying the folders and the drinks;

E.O. Kennard for the catering;

and to the following companies for attending the trade exhibition

(in alphabetical order)

Agar Scientific
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The SEMT hopes that you will find the One Day Meeting interesting and informative, and that you will be able to attend our forthcoming afternoon meetings. The next SEMT meeting is entitled "Alternatives to Gold Labelling", and will be held at 1400 on Friday, 6th December, 1991 at the Imperial Cancer Research Fund, Lincoln's Inn Fields, London.

WELCOME TO THE ONE DAY MEETING

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PROGRAMME

0930	Registration and Coffee
1030	Introduction by Don Claugher
1040	Scanning Probe Microscopies Mark Welland (University of Cambridge)
1120	The Use of EM in Veterinary Science Tony Scott (MAFF, Weybridge)
1200	Ultrastructure and Freeze-Fracture of Airway Epithelium Robert Godfrey (NHLI, Royal Brompton Hospital)
1240	Poster Display and Trade Exhibition Buffet lunch
1400	All in a Day's Work - From Bugs to Bran Mary Parker (Food Research Institute, Norwich)
1440	The Forensic SEM Robin Keeley (Metropolitan Police Laboratory)
1510	Tea
1530	Discussion of Poster Communications
1545	Sherry, followed by **The Annual General Meeting**

ABSTRACTS OF SPEAKERS' PAPERS

Scanned Probe Microscopies

M.E. Welland (University of Cambridge)

Since the invention of the scanning tunnelling microscope 10 years ago a number of related scanned probe microscopes have been developed. All use the principle that if a "sharp" tip is brought up to a surface so that some interaction exists between the two, then by scanning the tip over the surface an image may be produced. Clearly, the image obtained, and the resolution within the image, depend upon the nature of the interaction between the tip and surface and its dependence on tip-sample distance.

The STM is the most widely used probe microscope and relies on the tunnelling current produced when two conducting surfaces are brought to within 1nm of each other with a small applied voltage bias. Images obtained are truly atomic in detail and both the geometric and electronic structure of the surface are measurable. The great limitation of the STM is its inability to image insulating surfaces and it is here that the atomic force microscope is important. The interaction here results from the close proximity of tip and surface producing a small atomic force between the two. Images with a resolution < 1nm have been achieved on a variety of surfaces including single crystals of sodium chloride and sausage skin casings.

Other related microscopes include optical, electrochemical, noise, magnetic and electron transmission.

The Use of EM in Veterinary Science

T. Scott (Central Veterinary Laboratory)

The electron microscope unit at the Central Veterinary Laboratory (CVL) was first set up in 1965. Its present equipment includes one scanning and two transmission electron microscopes and its purpose is to provide consultancy, research and services to promote animal health and welfare. this specialised support is available throughout the whole of England and Wales via a network of Veterinary Investigation Centres.

One of the unit's earliest functions was to develop and offer a rapid diagnostic service for animal virus infections. This simple negative stain technique is particularly useful for integumentary and enteric samples.

In addition to this diagnostic service a number of specialised research and development projects are undertaken. These projects are multidisciplinary and a wide range of methods are used to examine specimens from species that vary from exotics to red squirrels and hedgehogs.

The investigation of new and emerging diseases of animals is an area where the electron microscope can play an important role. Bovine spongiform encephalopathy (BSE) is one such disease.

The electron microscope unit at the CVL played a major part in the initial confirmation of this disease by demonstrating the presence of abnormal fibrils in brain extracts from the first reported cases. Further studies have improved the extraction technique and shown it to be specific for both BSE in cattle and scrapie in sheep. they have also provided useful information on the distribution of the

fibrils within bovine and ovine brains and have demonstrated similar fibrils in brain extracts from other species.

Ultrastructure and Freeze-fracture of Airway Epithelium

R.W.A. Godfrey (Department of Lung Pathology, National Heart and Lung Institute, Royal Brompton Hospital, London, SW3 6NP)

Freeze-fracture is a process in which a sample is rapidly frozen and encouraged to fracture along a plane which splits the cell's phospholipid bi-layer membrane apart. While the sample remains in the frozen state the exposed face is "shadowed" and strengthened with carbon to provide a replica of the fractured surface. When the tissue is digested away the replica is examined by the transmission electron microscope. Intra-membrane surfaces, particles and ridges are exposed, thus providing information which is unobtainable by other methods.

Few freeze-fracture studies have been conducted on normal bronchial epithelium. The principal barrier to water, ions and molecules which pass via paracellular channels are the so-called epithelial "tight junctions", in which we currently have an interest. Freeze-fracture replicas of epithelial tight junctions reveal a series of interconnecting strands (fibrils) on the protoplasmic (P) face and complementary grooves on the extracellular (E) face: arranged in belt-form around the apico-lateral borders of the superficial epithelial cells, the arrangement is characteristic of tight junctions in general. The number of strands comprising the tight junction is thought to be related to the permeability of the epithelial paracellular pathway and to the electrical resistance which may be generated by epithelium. There is evidence that in a number of disease states the function of the tight junction is altered; for example in cystic fibrosis we have found a proliferation of tight junctional strands. The process of freeze-fracture allows the application of quantitative methods for analyzing the structural components of the junction including the apical-basal depth of the junction, the number of strands involved in the junction and the complexity of the junction. We have quantified normal human bronchial epithelial tight junctions from main and lobar bronchus (levels I and II respectively). Junctional depth at airway level I was 0.48μ and at airway level II 0.45μ, i.e. slightly but significantly different ($p < 0.01$). There were on average 11 strands per junction; the difference between the two airway levels was not significant statistically. the mean number of interconnections per μm of junctional strand was 1.4 at both airway levels. We have thus established a data pool from which the structure of bronchial epithelial tight junctions in airway diseases can be compared.

All in a Day's Work - From Bugs to Bran

M.L. Parker (AFRC Institute of Food Research, Colney Lane, Norwich, NR4 7UA)

The work of the EM laboratory at the IFR-N is extremely varied and reflects the wide range of research undertaken by the Institute. Work on methanogenic bacteria, phages and wheat-based products will be described to illustrate this diversity.

The food processing industry produces waste organic material often suspended in large volumes of water, and this has to be disposed of economically and inoffensively. Anaerobic bacteria can be used to convert the organic matter to methane, but these microbial communities within the digestors can be extremely troublesome. In most cases, it is the methanogenic bacteria which are most vulnerable, particularly to changes in pH. Interactions between the methanogenic bacterial and other species in chemostat cultures will be described.

In the manufacture of cheese, viral contamination of the *Lactobacillus* bacteria is a difficult industrial problem. Genetic engineers at IFR-N have used the virus, or bacteriophage, to their advantage to develop a way to mature cheese quickly. Electron microscopy has been invaluable in the identification of these bacteriophages.

The proteins of wheat flour play an important role in bread making. During development of bread dough, the proteinaceous gluten network traps bubbles of carbon dioxide produced by yeast fermentation so that when cooked, the characteristic crumb structure is formed. By using monoclonal antibodies to certain gluten components, and immunogold labelling, the behaviour of proteins within the bread can be studied.

The Forensic Scanning Electron Microscope

R. Keeley (Metropolitan Police Forensic Science Laboratory, 109, Lambeth Road, London, SE1 7LP)

Forensic scientists use scientific methods to assist the investigation of crime and to provide evidence for the courts. The nature of the work is as varied as crime itself; during the course of a year the forensic science laboratory is called upon to examine nearly every conceivable type of material in order to answer a variety of questions.

The SEM plays a major part in the examination of a wide range of materials. As an imaging device it is used to examine fingerprints that cannot be visualised by conventional methods, alterations on documents, counterfeit coins and banknotes and surface markings on bullets and cartridge cases. For these tasks the instrument is often used at low magnification (times 2 for fingerprints) because depth of field and atomic number contrast are more important than resolution. On a smaller size scale, X-ray spectrometry is used to analyze small fragments of paint, glass, metals and minerals from clothing and other sources.

The most important application of SEM is the investigation of shootings. Particulate residue in the size range of 1-3μ may be deposited from the percussion primer onto the skin and clothing of the firer of a gun. The particles are sampled with adhesive tape strips and are identified by automatic particle analysis in the SEM. Very small fragments from bullets and bombs can be recovered from wound tissue by enzymic methods and examined in order to identify the type of projectile.

The end product of the forensic science laboratory is scientific evidence for the courts. The various applications of SEM in forensic science will be discussed in the context of improvements in the quality of the evidence resulting from the use of the technique.

ABSTRACTS OF POSTERS

Calcium Pyrophosphate Dihydrate Deposition Disease - Morphologic and Microanalytic Features

C.E. Keen[1], P.R. Crocker[2], K. Brady[3], N. Hasan[1] and D.A. Levison[3] (Departments of Histopathology, 1: Lewisham Hospital, London SE13 6LH; 2: St Bartholomew's Hospital, London EC1A 7BE; 3: Guy's Hospital, London SE1 9RT)

On the basis of the number of cases referred to us for the identification of joint crystals in tissue sections, we believe that calcium pyrophosphate dihydrate (CPPD) deposition disease is being under-diagnosed by histopathologists. A critical light microscopic review of 18 cases, all with the diagnosis

confirmed by microanalysis (energy dispersive X-ray spectroscopy in the scanning electron microscope, infrared spectroscopy or both) revealed a distinctive feathery or brush like appearance in all such deposits. This feature was apparent at low power,m while convincing visualisation of crystals within the deposits was difficult even with an oil immersion objective. The sign of birefringence of CPPD crystals is more difficult to demonstrate in tissue sections than synovial fluids, due to factors such as stain, fragmentation and heaping up of crystals, but it can be more readily assessed in unstained sections or following microincineration of the section. In six of our cases the deposits were exclusively within bone; demonstration depended on relative under-decalcification; deposits in this position have not previously been recognised. This study thus provided new information on the histological identification of CPPD deposits in tissues and implies heterogeneity in the pathogenesis of such deposits.

This poster has also been presented at the recent Histopathology meeting at Belfast. A paper will be published in the Journal of Histopathology in December 1991

Biomineralization Pattern in the Otolithic Membrane: Analytical Electron Microscopic Study.

J.A. López-Escámez, F.J. Cañizares, P.V. Crespo and A. Campos (Department of Cell Biology, Faculty of Medicine, University of Granada, E-18071, Granada, Spain)

Different procedures were used to assess the degree of mineralization in the otolithic membrane in the utricle and saccule of the vestibular macula by X-ray microanalysis. The study was carried out in two phases; in the first we selected the most suitable method; in the second, we investigated the pattern of biomineralization in the otoconial layer. Four groups were established to study the otoconia: fixation in 2.5% glutaraldehyde and freeze-drying at -60°C; fixation in 2.5% glutaraldehyde and air-drying; isolated, air-dried otoconia and cryofixation and freeze-drying. All samples were carbon sputter-coated. Ca, P and Ca/P ratios were determined in the otoconia. Statistical analysis was performed by ANOVA 1 and Student's t test.

Chemically fixed specimens showed an increased P level in the utricle that was not observed in the saccule ($p < 0.01$). In the samples where chemical fixation was not used, the Ca, P and Ca/P ratios were not statistically significant. The biomineralization pattern in otoconia can be established by air-drying or cryoprocessing techniques. In the otoconial layer no significant differences were seen between the utricle and saccule.

Scanning Electron Microscopy: Application in the Identification of Diatoms in Cases of Drowning

J. V. Pachar and J.M. Cameron (Department of Forensic Medicine, The London Hospital Medical College, London)

The diagnosis of drowning is one of the most difficult tasks in Forensic Pathology. Among the ancillary investigations, the diatom test is the only one considered reliable because it provides evidence of drowning when no other tests are possible and, also, because it gives evidence of the probable site of drowning.

The study of water and organ samples under SEM, facilitates the identification, photographic record and taxonomic analysis of diatoms making this diagnosis possible under specific conditions.

Demonstration of Iron in Invertebrate Tissues Using a Novel Pyrroline Derivative: Confirmation of Specificity Using X-ray Microanalysis.

J.P. Cassella[1], M.T. Ball[2], I. Ridgers[2], J.K. Sugden[2], S.Y. Ali[1] and J. Hay (1: Department of Experimental Pathology, Institute of Orthopaedics (UCL), Royal National Orthopaedic Hospital, Brockley Hill, Stanmore, Middlesex, HA7 4LP; 2: Department of Pharmacy, Leicester Polytechnic, PO Box 143, Leicester, LE1 9BH).

It is generally considered difficult to detect iron in tissue sections of invertebrates using histochemical methods. The conventional Perls iron-hexacyanoferrate reaction is thought to lack sufficient sensitivity for such a demonstration. Using leech (*Hirudo medicinalis*) as a model, it has been possible to overcome this problem by increasing the concentration of mineral acid in the acid-ferrocyanide incubation mixture. However, this approach together with the requirement for acid hydrolysis in order to release the iron from associated protein, is often disruptive to delicate invertebrate tissue architecture.

Use of a novel compound ethyl-1-methyl-4-hydroxy-5-oxo-pyroline-3-carboxylate (1% solution in 70% ethanol), which has considerable sensitivity for iron, in combination with careful use of martius yellow S as counterstain, obviates the requirement for acid hydrolysis, thus preventing any possible acid-associated tissue damage. The specificity of the reaction was confirmed using X-ray microanalysis. The rationale for red to orange-brown gradation in staining associated with pyrroline derivative-iron complex was also determined using this technique.

Detection of Skin Antigens by SEM:P Use of Silver-enhanced Gold Proteins

J.P. Cassella[1], A. Yarwood[2] and J. Hay[3] (1: Department of Experimental Pathology, Institute of Orthopaedics (UCL), Royal National Orthopaedic Hospital, Brockley Hill, Stanmore, Middlesex, HA7 4LP; 2: Jeol (UK) Ltd, Jeol House, Silver Court, Watchmead, Welwyn Garden City, Herts, AL7 1LT; 3: Department of Pharmacy, Leicester Polytechnic, PO Box 143, Leicester, LE1 9BH).

Ultrastructure of skin as determined by TEM is fairly well established. However, surface topography, as assessed by conventional SEM, is not so well defined. Even at relatively low magnification, it is often difficult to orientate around structures such as the basement membrane zone (BMZ) and dermo-epidermal junction (DEJ). Immuno-SEM offers the opportunity to localise and determine the 3-D nature of immune complexes on the surface of the sub-epidermal skin constituents.

Type VII collagen in normal human skin was located *en bloc* using a monoclonal antibody and a rabbit anti-mouse 1nm gold conjugate, followed by silver-enhancement. The specimen was then treated with half strength Karnovsky's fixative, osmicated, alcohol dehydrated and critical point dried. This was followed by carbon-coating; the latter permitted backscattered electron imaging to be performed, a procedure which allows ready visualization of the silver-enhanced gold probes. Silver-enhanced immunogold localized type VII collagen was observed in the backscattered electron imaging (BIE) mode. The method described supplements both LM and TEM studies of skin and provides important information regarding the spatial orientation of skin structure as well as the nature of skin antigens. It should prove useful for the investigation of the pathogenesis of certain skin disorders.

The Use of EM in Vetinary Science

T. Scott

One of the earliest functions was diagnostic virology, e.g. contagious pustular dermatitis of sheep. A virus intermediate in appearance between Orf and the classic pox viruses has been found, apparently peculiar to the red squirrel. Diagnosis is important in neonatal diarrhoea. Herpesvirus has been found in the liver of sick hedgehog.

The spongiform encephalopathies include BSE; Kuru (from Papua New Guinea); Ceuzfeldt2Jacob disease (sporadic form world-wide 1/million, also familial form linked with gene defect); Gerstmann-Straussler (-Scheinker) syndrome. Scrapie of sheep; chronic wasting disease of elk and mule deer; transmissible mink encephalopathy. All characterised by:

neurodegenerative - CNS infection
prolonged incubation period - to 40-50 years in man
chronic, progressive, fatal
no immune or inflammatory response, so no serological diagnosis possible; confirm diagnosis by histopathology of medulla
agent virus-like, contains essential proteinase-K-resistant protein (an abnormally modified host protein)
non-immunogenic
resistant to chemical and physical inactivation, and to normal autoclaving
insensitive to nucleic acid inactivation treatments (so ? a prion)

Merz et al 1981 Acta Neuropathol (Berlin) 54, 63-74
used a very harsh treatment to show the fibrils characteristic of scrapie.

They subsequently correlated EM and histology within a week of the first cattle brains with BSE coming in.

In sheep the fibrils are evenly distributed throughout the brain. In cattle there appeared at first to be fewer fibrils, no false positives but many false negatives by EM. but if the samples are correlated with the histological lesions, numerous fibrils can be found in the brain stem - medulla, mid-brain, basal nuclei.

In 1988 BSE was made a notifiable disease. Even if unfixed material is kept at 37'C for 7 days, the fibrils can still be found. Similarly in e.g. cat. The labels for the fibrils can be decorated with gold label. A collection of antisera is being made, to compare between species. 35-40,000 cases have been reported so far in cattle. The fibrils are glycoprotein - an artefact, but highly specific for this disease.