SOCIETY OF
ELECTRON MICROSCOPE
TECHNOLOGY

THE CUTTING EDGE

SHORT COMMUNICATIONS BY MEMBERS

2.00 p.m. Wednesday 6 December 1995

EASTMAN DENTAL INSTITUTE
Grays Inn Road
London WC1X 8LD

Scanning E M examination of dental microwear
Tania King (Dept of Paleontology, Natural History Museum, London)

Diagnosis of transmissible spongiform encephalopathies by E M
Bill Cooley (Central Veterinary Laboratory, Weybridge, Surrey)

Teething problems: E M at the Eastman Dental Institute
Nicki Mordan (E M U, Eastman Dental Institute, London)

Skeletal ultrastructure in some Cyclostome Bryozoans using F E S E M
Chris Jones (Dept of Mineralogy, Natural History Museum, London)

The importance of E M in the recognition of apoptosis
Catherine Sarraf (Dept of Histopathology, Royal Postgraduate Medical School, London)

The T E M as a measuring tool to locate an anatomical region in tissue
Heather Davies (Biology Dept, Open University, Milton Keynes)

Microscopy Australian style - a look at the CSIRO Microscopy Centre, Canberra
Mark Dominick (Division of Entomology, CSIRO, Canberra, Australia)

THE ANNUAL GENERAL MEETING

(preceded by Tea, Coffee, & Mince Pies)

.....

Please reply to the Secretary - Dr Jill Lewis
19 Bellfield Avenue, Harrow, Middx. HA3 6ST
0181 428 4264 (Telephone, Answerphone, Fax)

I hope to be present at the meeting on 6 December 1995 -

Name .....

Address .....

.....

THE CUTTING EDGE : contributions from members

December 6th 1995

Abstracts

Scanning EM examination of dental microwear

Tania King (Dept of Palaeontology, Natural History Museum)

The examination of microscopic wear marks on the surface of teeth (dental microwear) can give information about the main components of an animal's diet. Inferences can be made about the diet of extinct species by comparing the dental microwear patterns of fossil material with those of present-day groups whose diet is known. A comparison is made of the microwear patterns of a fossil ape, *Griphopithecus alpani,* with those of three present-day apes - chimpanzees, gorillas and orangutans. Findings show that *G.alpani* had a diet similar to that of the orangutan which is known to eat hard fruits and nuts. The results of experimental work to investigate the effects of physical and chemical abrasion on dental microwear during the fossilization process are also reported.

Diagnosis of transmissible spongiform encephalopathies by electron microscopy

Bill Cooley (Central Veterinary Laboratory, Weybridge, Surrey)

The transmissible spongiform encehalopathies (TSE's), or prion diseases, are a complex group of chronic, fatal neurodegenerative disorders. The animal TSE's include scrapie, bovine spongiform encephalopathy (BSE), chronic wasting disease of mule deer and elk, transmissible mink encephalopathy and feline spongiform encephalopathy. The TSE's of man are Creutzfelt-Jakob disease, Gerstmann-Straussler-Scheinker disease and kuru. They are characterised pathologically by vacuolation and astrocytosis, which gives the classical spongiform change within the brain. However, two additional diagnostic criteria for the TSE's, the detection of disease specific fibrils by transmission EM and the detection of the main constituent of the fibrils, an abnormal protease-resistant neuronal membrane glycoprotein by immunoblotting has also been used.

This presentation will concentrate on the involvement of the EM unit at the Central Veterinary Lab with the diagnosis of TSE's by TEM.

The disease-specific structures called scrapie associated fibrils (SAF) were first identified by TEM in brain extracts from affected mice and hamsters experimentally infected with scrapie (Merz 1981 & 1984). Following these publications the EM unit began work on scrapie of sheep and goats (the archetype of TSE's). This work involved the detection of SAF according to the method of Hilmert & Diringer (1984) as an aid to the diagnosis of natural sheep scrapie. This work was quickly expanded following the recognition of BSE, the scrapie-like, or prion disease of domestic cattle (Wells 1987).

Since then, the EM unit has developed and modified the extraction procedure in order to develop a diagnostic technique for both scrapie and BSE. This procedure requires the use of unfixed brain material (1 g of CNS tissue: the grey matter from spinal cord plus caudal medulla is ideal). The CNS tissue is then subjected to detergent extraction with N-lauroyl sarcosine, followed by various centrifugation steps at low and high sedimentation forces to pellet the abnormal PrP, before treatment with the enzyme Proteinase K. The "surviving" sedimentable and Proteinase K resistant protein termed $PrPW^{SC}$ can be visualized as SAF following negative staining with 2% phosphotungstic acid in the electron microscope.

Hilmert,H & Diringer,H (1984) Biosciences Reports 4, 165-170
Merz,P.A. et al (1981) Acta Neuropathologica (Berlin) 54, 63-74
Merz,P.A et al (1984) Science 225, 437-440
Wells,G.A.H. et al (1987) Veterinary Record 121, 419-420

Teething problems: E M at the Eastman Dental Institute

Nicky Mordan (EMU Eastman Dental Institute)

A brief insight into the contribution of electron microscopy to some aspects of research undertaken at the Eastman Dental Institute related to periodontitis, dental hypersensitivity and implants. The techniques and strategies required for dealing with hard and difficult dental specimens are discussed..

Skeletal ultrastructure in some Cyclostome Bryozoans using field emission SEM

Chris Jones (EMU, Dept of Mineralogy, Natural History Museum)

Bryozoans are constructed of calcareous crystallites in an organic matrix. There are differences in skeletal ultrastructure between different groups of Bryozoa. In two genera of cyclostome Bryozoa, *Cinctipora* and *Heteropora* , the walls consist initially of imbricated fibres, oriented transversely to the direction of wall growth. Tabular crystallites oriented parallel to the direction of wall growth then develop from overgrowths on the fibres. However, in a third genus of cyclostomes, *Homera*, the initial fibrous layer is absent. The walls are composed of successive layers of tabular crystallites without a preferred growth direction.
This study will contribute towards our understanding of biomineralization, the taxonomic relationships between Bryozoans and the palaeobiological interpretation of fossil Bryozoans.

The importance of electron microscopy in the recognition of apoptosis.

Catherine Sarraf (Histopathology Dept, Hammersmith Hospital)

Apoptosis is a gene directed programme of cell death - that occurs both in the development of normal tissues and in disease states - that is readily recognised by its stereotyped ultrastructural appearance. The morphology has always been recognised in pathology, but it wasn't until the 70's and 80's that it was appreciated (largely by Kerr, Wyllie and Currie in Edinburgh) as a unique process of vital importance in the maintenance and development of tissues. The opposite outcome of cell death, necrosis, is the cumulation of events that occur in cells and tissues after they have sustained mortal damage; apoptosis, conversely is predetermined, active and energy requiring. Early in the process DNA and chromatin are characteristically cleaved into specific size portions by endonucleases, often of the cystine protease family. The resultant fragments can be separated into a typical " ladder pattern" on agarose gels. Subsequent events depend on this initial cleavage, and the presence of fractured DNA is the basis of two light microscope methods for labelling apoptotic cells. Classical apoptotic bodies are readily identified in H & E sections by their chromatin caps and their common location in single "halos" of tissue, but the recognition of fragments that are more difficult to verify is a perpetual problem. Thus, *in situ* end labelling and TUNNEL labelling have been devised for use at LM level and kits are widely available on the market. In both cases labelled complementary nucleotides attach to the fragmented DNA and thus, in theory, label the apoptotic cells (most commonly with immunoperoxidase). However, the labels attach to any broken DNA and do not discriminate between apoptotic and necrotic cells; also there is always a significant number of clearly apoptotic cells present that elicit no label. The only certain way to demonstrate apoptotic bodies is by electron microscopy, in which there is no confusion between condensed electron dense apoptotic bodies with intact organelles as opposed to swollen necrotic cells whose integrity is clearly impaired.

The TEM as a measuring tool to locate an anatomical region in tissue

Heather Davies (Biology Dept, Open University, Milton Keynes)

In our research it is important to be certain where within a tissue we are taking images from. We have devised a procedure that utilises rotation of the grid, the low magnification capability of a modern TEM and the large screen as a measuring device,
The tissue is initially dissected to a given shape so the position of the area of interest is known. After examination in the light microscope, the block face is cut to a particular shape for orientation purposes in the TEM. In the TEM, the grid is rotated so that the row of cells of interest is in the x or y direction. The grid is then moved a specified number of large screen diameters in either the x or y direction (at right angles to the row of cells) before an image is taken. The section is photographed at low magnification (×120) where, due to volatilization of the resin, the areas photographed are visible.

Microscopy Australian style - a look at the CSIRO Microscopy Centre, Canberra

Mark Dominick (Division of Entomology, CSIRO, Canberra, Australia)

S.E.M.T. 6 December 1995

THE CUTTING EDGE

Scanning EM of dental microwear

Tania King
Dept,o f Paleontology, Natural History Museum

Microwear on teeth can be affected by diet, cooking the food, and weaning. It can be measured as scratches or pits. As test species:
Orang eats hard unripe fruits with husks, producing pits;
Chimp eats softer fruits
Gorilla eats leaves and soft fruit, producing a larger proportion of scratches.

High resolution replicas are made with a dental rubber peel, then cast in epoxy resin. These are examined in low-vacuum SEM by BSE; most workers use higher vacuum and secondary electrons.

An extinct ape (? Geopithecus alpani) had numerous pits on the teeth; the closest match among living primates is the Mangabey, which lives on hard unripe fruits and nuts.

The pattern of abrasion produced during fossilisation looks quite different; it may erode all microwear. Acid etching may also erode microwear, e.g. if the ape has been eaten, by stomach juices. There is no evidence that older animals have much more microwear, and age does not affect the ratio of pits to scratches.

Diagnosis of transmissible spongiform encephalopathies by EM

Bill Cooley
Central Vetinary Laboratory, Weybridge

The spongiform encephalopathies produce a chronic fatal neural degeneration; there is no immune response. The tissues appear vacuolated, with abnormal fibrils 4-6 nm diameter, 100-500 nm long. Type 1 encephalopathy has a single pair of fibrils; Type 2 has two pairs of fibrils; in both cases, helically wound.
The normal isoform PrP^{C} is 33-35 KDa; this converts to the disease-specific form; after proteolytic activation with proteinase K, the resistant core remains.
A complex series of extractions is used, starting with homogenisation of unfixed brain or spinal cord in 10% N-lauroylsarcosine at pH 7.4; then in 1% N-lauroylsarcosine with proteinase K; etc. The final fibril structure varies.
In 10 years they have examined 18,000 cattle, 1700 sheep, a few hamsters, cats & exotics - spongiform encephalopathies have not been found in dogs or cats. The mode of preparation affects the final appearance.
The work is done in a Class 2 Safety Cabinet, in dedicated rooms. The instruments are soaked in chloros afterwards, then autoclaved.

Teething problems: EM at the Eastman Dental Institute

Nicki Mordan
EMU, Eastman Dental Institute

Teeth are embedded in Araldite; sectioned with a diamond saw; demineralised in EDTA for 1 month; re-embedded.
Lemonade, Coca-cola etc are pH about 2.4; if the dentine is exposed, they will erode the tooth.
Sensodyne sealant is quite good, and is painted on; it looks brittle.
Sensodyne toothpaste contains diatoms; it washes off.
Teeth can be implanted into the jaw-bone with a titanium screw; this costs about £1000.

Skeletal ultrastructure in some Cyclostome Bryozoans using FESEM

Chris Jones
Dept. of Mineralogy, Natural History Museum

The Cyclostome Bryozoans are marine, moss-like, colonial corals, going back some 400 million years to the Ordovician. They have a magnesium calcite skeleton on a protein base; this forms flat crystalllites with step-like over-growth. There are 2 different families: hexagonal seeds develop to plates; or rod-like fibres in ordered array develop to slabs.

The importance of EM in the recognition of apoptosis

Catherine Sarraf
Dept, of Histopathology, RPGMS

Apoptosis is programmed and energy-requiring; necrosis is not programmed.
Within the cell, the chromatin usually sits around the edge of the nucleus; then the cell contracts and sits in a halo; then is phagocytosed.
Limbs form initially as a paddle; the digits separate later by cell loss.
Chemotherapeutic agents kill the cells by apoptosis; it is also involved in HIV infection, and in neoplasia.
Necrosis is found where the tissue is far from the blood supply; but there is a good blood supply where apoptosis is occurring.
Nick translation and in situ hybridisation can pick up some instances of apoptosis, and also some other processes; therefore for morphology we need EM.
There are 3 repressor genes for cell death; there are also promotor genes, located in a nematode with exactly 1090 cells, and also found in mammals.

The TEM as a measuring tool to locate an anatomical region in tissue

Heather Davies
Biology Dept., Open University, Milton Keynes

The hippocampus in the brain is used for spatial and perception processes.
Measure by number of screen-diameters from the cell body to the start of myelination.

Microscopy Australian style - a look at the CSIRO Microscopy Centre, Canberra

Mark Dominick
Division of Entomology, CSIRO, Canberra

An endotoxin crystal from Bacillus thruingensis was put into cotton; ~~T~~his reduced the amount of pesticide needed in sprays.
Red-legged earth - mite - its feet cause damage to the leaf of the legume.
Follicle counts on sheep were done by shaving the sheep; making a dental wax impression of the skin - the hairs dig in to the wax; put the pictures in an image analyser for a follicle count. For a good fleece, they want fine hair but a high follicle density.

SEMT Meeting, 6th December, 1995

List of Registrants

Sue Barnes	EMU, Dept of Mineralogy, Natural History Museum
Andrea Boyd	Dept of Oral Medicine, Guy's Hospital, London
Patricia Bland	BBSRC, Institute of Animal Health, Compton, Newbury, Berks
John Bredl	EMU, Physiology Dept., Royal Veterinary College
Judith Brock	Oxford Instruments, Eynsham, Oxon
Bill Cooley	Central Veterinary Laboratory, New Haw, Weybridge, Surrey
Terry Cooper	Taab Laboratories, Aldermaston, Berks.
Barbara Cozens	Dept of Anatomy, University College, London
Linda Davies	Central Veterinary Lab, Weybridge, Surrey
Heather Davies	Biology Dept, Open University, Milton Keynes
Mark Dominic	Division of Entomology, CSIRO, Canberra, Australia
Barry Dowsett	CAMR, Porton Down, Wiltshire
Anne Drewe	Dept of Microbiology, Charing Cross Hospital Medical School
A.Frost	BBSRC, Inst of Animal Health, Compton, Newbury, Berks
Alan Gray	Dept of Oral Pathology, Londiopn Hospital Medical; College
Roger Hockham	Jeol UK Ltd., Welwyn Garden City
Louisa Jones	EMU, Natural History Museum
Chris Jones	EMU, Dept of Mineralogy, Natural History Museum
Mike Kelly	Dept of Oral Pathology, London Hospital Medical College
Tania King	Dept of Palaeontology, Natural History Museum
Jill Lewis	EMU, Eastman Dental Institute
David McCarthy	School of Pharmacy, London
Nicky Mordan	EMU, Eastman Dental Institute
David Patton	Dept of Applied Biology, Univ of West of England, Bristol
Jenny Plummer	BVS, Royal Veterinary College, London
Catherine Sarraf	Dept of Histopathology, Hammersmith Hospital, London
T.Smith	BBSRC, Inst of Animal Health, Compton, Newbury, Berks
Rosemary Suswillo	Bone Unit, Royal Veterinary College, London
Bob Whitenstall	Materials Science Dept., Queen Mary & Westfield College